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On-line solid phase extraction fast liquid chromatography–tandem mass spectrometry for the analysis of bisphenol A and its chlorinated derivatives in water samples

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ABSTRACT

In this study an on-line column-switching fast LC–MS/MS method was developed to analyze bisphenol A (BPA) and its chlorinated derivatives in water. Fast liquid chromatographic separation was performed on a C18 reversed phase column based on fused-core particle technology (2.7 μm particle size) providing analysis times shorter than 3 min and high peak efficiencies. The main benefit of this LC system is that it can easily be hyphenated to a conventional on-line preconcentration device allowing the direct analysis of water samples without any pretreatment at concentrations levels down to 60 ng L^{-1} and preventing contaminations frequently reported in the analysis of BPA. This on-line SPE fast LC system was coupled to a triple quadrupole mass spectrometer operating in enhanced mass resolution mode (Q1 FWHM = 0.7 Th, Q3 FWHM = 0.1 Th) in order to minimize interferences and chemical noise. This highly sensitive and selective method was successfully employed to analyze BPA and its chlorinated derivatives in water samples.

1. Introduction

Bisphenol A (BPA) (2,2-bis[4-hydroxyphenyl] propane) is widely used in the production of polycarbonate plastics and phenolic–epoxy resins, which have a variety of applications, such as plastic food containers and epoxy food-can coatings. Additional applications of BPA include printed circuit boards, composites, adhesives, and tooling. Due to the continuous release of BPA into the environment, in comparison to plasticizers such as phthalates, BPA is commonly detected in aquatic ecosystems. In addition, BPA derivatives are found in the environment. These include some halogenated derivatives of BPA, such as tetrachlorobisphenol A (TeCBPA) (commonly used as flame retardants in polymers), and other chlorinated derivatives, such as monochloro-, dichloro- and trichloro-BPA, which are generated by the chlorination of BPA by the residual chlorine used both as a disinfectant in water treatment plants and as a bleaching agent in paper recycling plants. For instance, these chlorinated derivatives have been found at low μg L^{-1} concentrations in effluents from paper recycling plants [1,2].

Due to the large volumes of BPA produced and the corresponding threat of pollution in the aquatic environment, its toxicity has been intensively studied over the last 20 years. This research has shown that it is not genotoxic or carcinogenic, but only slightly toxic [3]. In contrast, the toxicity of halogenated derivatives of BPA, such as tetrabromobisphenol A (TBBPA) and TeCBPA, is greater than that of BPA. This indicates that halogen atoms may play a role in the toxicity of these derivatives [4]. However, the main environmental concern of BPA and its chlorinated derivatives is not their toxicity, but rather their estrogenic potential, which has been confirmed by numerous studies *in vitro* and *in vivo* [5–9]. Because of these characteristics, BPA is known as an endocrine disruptor [10], and is almost ubiquitous in the environment. In addition the European Commission places BPA in the list of priority substances and has registered it in line with the EU legislation for the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH).

Studies have been conducted to monitor and quantify low levels of BPA and its chlorinated derivatives in water. Gas chromatography coupled to mass spectrometry (GC–MS) with and without derivatization and/or liquid chromatography coupled to mass spectrometry (LC–MS) are commonly used to analyze these compounds [11]. More recently, LC–MS has become a more popular method since it offers advantages such as simple sample treatment without the need for derivatization steps, low detection limits, and good selectivity when dealing with complex matrixes. In LC–MS, tandem mass spectrometry (MS/MS)—mainly using triple quadrupole mass analyzers (QqQ) in selected reaction monitoring (SRM) acquisition mode—is commonly used to improve selectivity and sensitivity in the analysis of BPA and its halogenated derivatives in complex matrices at low concentrations. Nevertheless, other mass analyz-

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ers such as ion trap (IT) and hybrid analyzers have also been used [12–14].

Since BPA and its chlorinated derivatives are found in the environment at very low concentration levels (ng L^{-1}) preconcentration and clean-up procedures are mandatory. Off-line solid phase extraction (SPE) is commonly used for the analysis of these compounds in water samples and large-sample volumes followed by solvent evaporation steps are always required [15,16]. Most of these procedures are time-consuming and error-prone, for instance Inoue et al. [17] reported that the leaching of BPA from the cartridges used in off-line SPE is similar to the BPA concentration in water environmental samples. Developing on-line sample preparation procedures is a good way to reduce procedural errors, contamination, and analysis time. With such benefits, several papers have been published using on-line SPE coupled to conventional LC (5 μm particle size columns) to analyze BPA in environmental samples (water and sediments) and biological fluids (milk and urine) [18–23] with analysis times higher than 10 min.

To reduce analysis time, the integration of high speed LC columns into on-line SPE-LC systems is generally recommended. Until now, on-line SPE has been hyphenated to short analytical LC columns to analyze water samples [24]. Turbulent-flow chromatography (TFC) and short monolithic columns have also been used on-line with fast chromatographic separation to analyze priority pesticides and emerging contaminants in surface and drinking water [25,26].

Despite the common application of these methods, there is a clear attraction to develop methods that couple on-line SPE systems to ultra high performance liquid chromatography (U-HPLC), which would provide fast and ultra fast run times simultaneous with ultra high efficient chromatographic separations. This would then give high sample throughputs. However, the direct hyphenation of an SPE sample device with a U-HPLC system using a sub-2 μm particle column is challenging. This is mainly due to two factors [27]: firstly, since U-HPLC uses sub-2 μm particle columns combined with high flow rates (up to 1.0 mL min^{-1} with 2.1 mm i.d. columns), high backpressures of up to 1000 bar—which are not directly compatible with the commercially available on-line extraction systems that operate at backpressures <400 bar—can be generated. This is relevant because switching from a low pressure to a high pressure system can produce band broadening and distorted peaks. Secondly, large amounts (several mL) of a strong solvent such as methanol, typically used in the SPE elution step, cannot be directly introduced into the U-HPLC systems without producing band broadening and interfering with retention.

The aim of this research was to evaluate the capacity of an on-line column-switching fast LC–MS/MS method used to analyze BPA and its chlorinated derivatives in water samples. We achieved this using a chromatographic column, based on fused-core technology. These columns provide fast and highly efficient separations under standard LC backpressures (<400 bar). This is because the particles with a 0.5 μm radius shell of porous stationary phase surrounding a 1.7 μm non-porous core exhibit reduced diffusion mass transfer, which allows for high mobile phase flow rates with a similar efficiency and peak capacity to that achieved in columns with sub-2 μm porous particles [28]. The on-line SPE LC–MS/MS method developed in this study was used to analyze BPA and its halogenated derivatives at low concentrations in water samples.

2. Experimental

2.1. Chemicals and consumables

Bisphenol A, 2,2-bis(4-hydroxyphenyl)propane (BPA), bisphenol A-d_{16} (BPA-d_{16}), and tetrachlorobisphenol A, 2,2-bis(3,5-dichloro-4-hydroxyphenyl)propane (TeCBPA) were obtained from Sigma–Aldrich (Steinheim, Germany). Monochlorobisphenol A (MCBPA), dichlorobisphenol A (DCBPA), and trichlorobisphenol A (TCBPA) were synthesized and purified in our laboratory [2]. Methanol (MeOH), acetonitrile (ACN), and water grade LC–MS were purchased from Riedel-de Haën (Seelze, Germany). Ultra high quality (UHQ) water was obtained by purification in an Elix-Milli-Q system (Millipore Corp. Bedford, MA). Stock standard solutions of individual compounds and BPA-d_{16} (10 mg L^{-1}) were prepared in methanol and stored at 4 °C. Intermediate solutions were prepared weekly from the stock standard solutions by appropriate dilution in MeOH:water (10:90). Calibration standard solutions prepared in methanol:water (10:90) ranging from 50 ng L^{-1} to 1000 ng L^{-1} of each compound were prepared daily with 200 ng L^{-1} of the labelled compound BPA-d_{16}. Mobile phases were filtered using 0.45 μm nylon filters (Whatman, Clifton, NJ, USA). In addition, to analyze these compounds precautions had to be taken during the sample treatment to prevent and minimize sample contamination and/or to avoid the loss of the analytes during treatment.

Nitrogen (99.8% pure) supplied by a Claind Nitrogen Generator N_2 FLO (Lenno, Italy) was used for the MS atmospheric pressure ionization (API) source. High-purity argon (Ar_1) purchased from Air Liquide (Madrid, Spain) was used as a collision-induced gas (CID gas) in the triple quadrupole mass spectrometer.

In this study, two columns were used to perform the chromatographic separation of BPA and its chlorinated derivatives: an Ascentis Express C18 (fused-core) column (50 mm × 2.1 mm, 2.7-μm particle size) (Supelco, Sigma–Aldrich) and an Aquity BEH C18 column (50 mm × 2.1 mm, 1.7-μm particle size) (Waters Corp.).

2.2. On-line and chromatographic conditions

A Summit® x2 Dual-Gradient System (Dionex, Sunnyvale, CA) equipped with a Summit P680 dual ternary gradient pump, a TCC-100 Thermostatted Column compartment with a 10-port switching valve and an ASI-100T automated sample injector with a 2500-μL injection loop was used for both on-line preconcentration and chromatographic separation. Chromatographic separation was performed on the Ascentis Express C18 (fused-core) column at 30 °C using a ternary mobile phase (ACN/MeOH/water) and gradient elution mode.

A Hypersil Gold C18 column (20 mm × 2.1 mm, 12 μm particle diameter, 175 Å pore size) (Thermo Fisher Scientific, Whatman, MA) was used for the fully automated SPE on-line trace enrichment procedure. The optimal on-line preconcentration conditions were as follows: 1 mL of sample was loaded onto the SPE column, which had been previously preconditioned with MeOH:water (5:95, v/v), at a flow rate 1 mL min^{-1}; the analytical column was equilibrated to the initial conditions of the chromatographic separation 30:20:50 (acetonitrile:methanol:water) at a flow rate of 600 μL min^{-1}; the SPE column was then washed with 10:90 (v/v) MeOH:water and after 4.3 min the analytes were eluted into the analytical column in back-flush mode at 600 μL min^{-1} after the gradient elution program had started (at 0 min, 30:20:50; from 0 min to 1 min, a linear gradient elution up to 80:20:0 and finally an isocratic step of 5 min at these conditions); finally, 5 min after the back-flush elution of the analytes, the switching valve was switched to the inject position and the SPE column was equilibrated with MeOH:water (5:95, v/v) while the chromatographic analysis was running. A general scheme of the whole procedure is detailed in Fig. 1.

2.3. Mass spectrometry conditions

The on-line solid phase extraction (SPE) liquid chromatography system was coupled to a triple quadrupole mass spectrometer TSQ Quantum Ultra AM (Thermo Fisher Scientific) equipped with

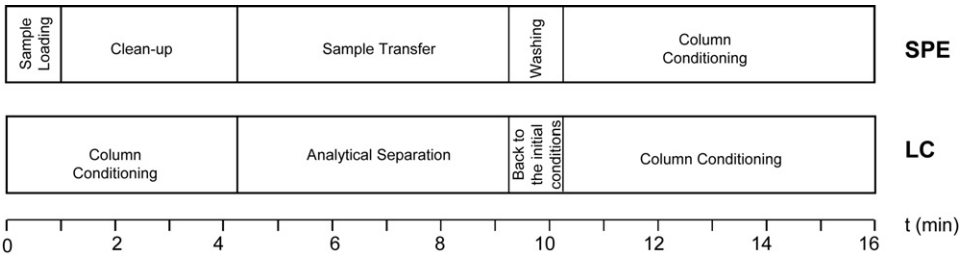

Fig. 1. Time schedule of the on-line SPE column switching LC procedure.

Table 1
Tandem mass spectrometry transitions for the acquisition mode.

Compound	Precursor ion (m/z)[a] $[M-H]^-$	Quantitation		Confirmation		Ion ratio ± SD[d] ($n=5$)
		Product ion (m/z)[b]	CE[c] (V)	Product ion (m/z)[b]	CE[c] (V)	
Bisphenol A	227.1	212.08	20	133.07	26	3.2 ± 0.08
Bisphenol A-d_{16}	241.2	223.10	22	142.00	25	2.8 ± 0.10
Monochlorobisphenol A	261.1	210.07	26	182.07	30	0.83 ± 0.11
Dichlorobisphenol A	295.0	244.03	25	216.04	32	1.0 ± 0.07
Trichlorobisphenol A	328.9	277.99	33	249.99	26	1.9 ± 0.15
Tetrachlorobisphenol A	364.9	313.95	28	285.96	33	1.3 ± 0.1

[a] Mass resolution: 0.7 Th (FWHM).
[b] Mass resolution: 0.1 Th (FWHM).
[c] Collision energy.
[d] Standard deviation.

hyperbolic quadrupoles and an electrospray ionization source (ESI) operating in negative mode. Nitrogen (purity > 99.98%) was used as a sheath gas, ion sweep gas, and auxiliary gas at flow rates of 60 a.u., 20 a.u., and 40 a.u. (arbitrary units), respectively. The ion transfer tube temperature was set at 375 °C and the electrospray voltage was −4 kV. Tandem mass spectrometry data were acquired in high-selective selected reaction monitoring (H-SRM) mode using the deprotonated molecule $[M-H]^-$ as a precursor ion.

Two transitions for each compound were monitored using a dwell time of 20 ms and 1 μscan (Table 1). For the low resolution method, both Q1 and Q3 operated at 0.7 Th FWHM (full width half maximum). For the enhanced mass resolution method on Q1, Q1 operated at 0.1 Th FWHM and Q3 at 0.7 Th, while for enhanced mass resolution on Q3, Q1 operated at 0.7 Th FWHM and Q3 at 0.1 Th FWHM. Argon was used as a collision-induced-disociation (CID) gas at 1.5 mTorr and the optimum collision energy (CE) for each transition was optimized. The results are summarized in Table 1. The Xcalibur software version 2.0 (Thermo Fisher Scientific) was used to control the LC/MS system and to process data.

To optimize the source working conditions and to perform the tandem mass spectrometry experiments, 1 mg L^{-1} standard solutions prepared in methanol were infused at a flow rate of 3 μL min^{-1} using the syringe pump integrated into the TSQ Quantum Ultra AM instrument, and mixed with the mobile phase (600 μL min^{-1}, ACN:MeOH:water (50:20:30)) using a Valco zero dead volume tee piece (Supelco, Alcobendas, Spain).

2.4. Samples

Several water samples from different sources were analyzed. These sources included a paper recycling plant (effluent), a wastewater treatment plant (WWTP) from an important industrial area close to Barcelona (influent), a river water and a drinking water treatment plant (DWTP) (influent and samples collected at different points during the water treatment taking into account the hydraulic retention times, HRT) sited in the river Llobregat (Catalonia, Spain). The treatment at the DWTP consisted of the following: prechlorination to break-point, the addition of coagulants and flocculants, sand filtration, and dilution with groundwater in variable amounts to improve water quality. Following this, ozonation, granular activated carbon (GAC) filtration, and a final post-chlorination took place, to ensure the chlorine residual was applied. Water samples were collected in 1-L glass bottles and 1 mL of ascorbic acid (0.1 M) was added to avoid chlorination from the residual chlorine during storage (4 °C). Prior to analysis, 1 mL of MeOH was added to the water samples (1 L) and the particulate matter was removed by centrifugation at 4000 r.p.m.

Bisphenol A was determined using the isotope dilution method. The deuterated standard (BPA-d_{16}) was added at the beginning of the sample treatment. BPA-d_{16} was also used as an internal standard to quantify the chlorinated derivatives since no labelled standards are actually available for these chlorinated compounds. Nevertheless, matrix-matched calibration using surface water free of BPA and its chlorinated derivatives spiked at different concentration levels were used to quantify the samples. The results were calculated including the expanded uncertainty within a 95% of level of confidence.

3. Results and discussion

3.1. Liquid chromatography–tandem mass spectrometry

To analyze BPA and its chlorinated derivatives with LC–MS a reversed phase column (SunFire C18 column, 150 mm × 2.1 mm ID and 3.5 μm particle size, Waters) and a MeOH:water in gradient elution mode (300 μL min^{-1}) were used. Methanol was selected since it provides higher BPA responses in ESI than acetonitrile [2,29,30]. Moreover, any buffer was added to the mobile phase since it has been reported that additives produce ion suppression of BPA and its chlorinated derivatives when ESI in negative mode is used [2,14,29,31,32].

This liquid chromatography separation was coupled to the triple quadrupole mass spectrometer using an ESI source in negative mode. The ESI (negative) full-scan MS spectrum of BPA and MCBPA showed only the isotopic cluster corresponding to the deprotonated molecule $[M-H]^-$ as had been observed previously [2]. Nevertheless, the spectra of the highly chlorinated BPAs showed a double-charged ion at lower m/z values corresponding to the dou-

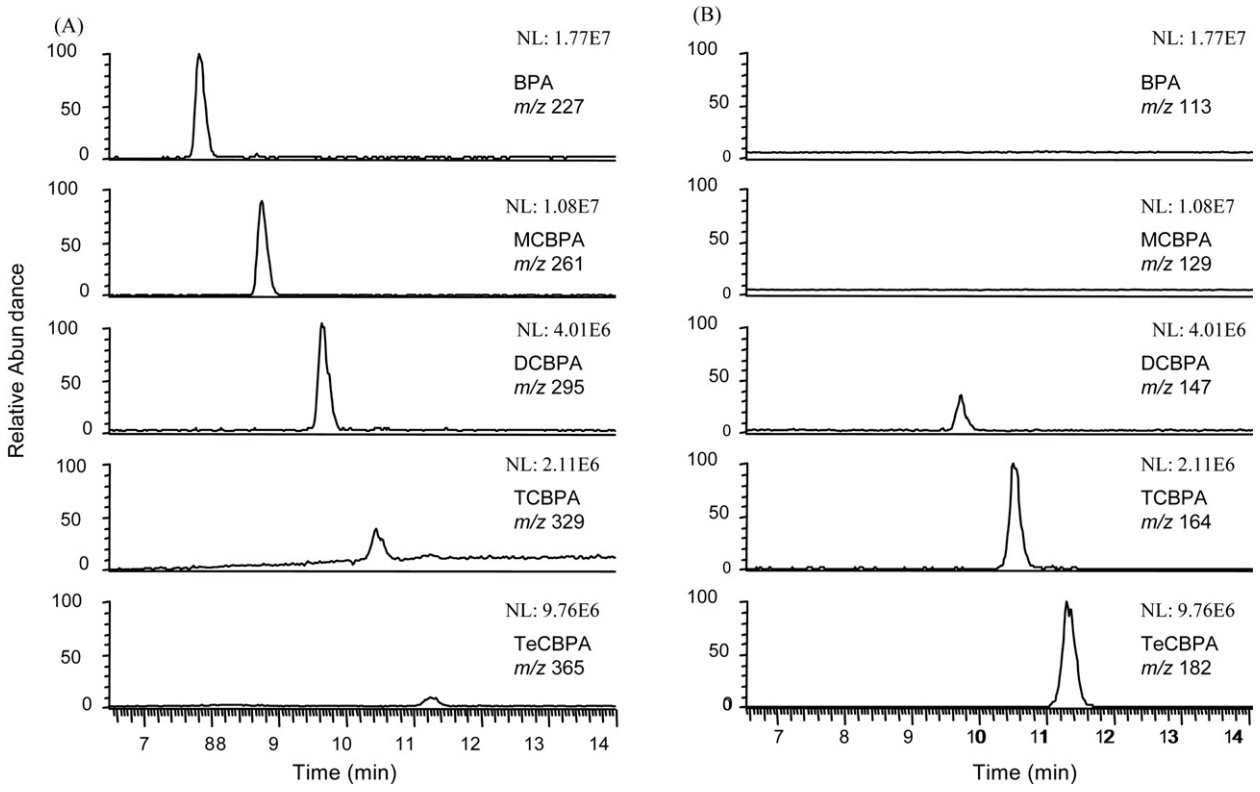

Fig. 2. Full-scan LC–MS chromatograms of BPA and its chlorinated derivatives using a SunFire C18 (150 mm × 2.1 mm ID and 3.5 μm particle size) (Waters) and MeOH:water (60:40) as a mobile phase at flow rate of 300 μL min^{-1}. Ion monitored (A) $[M-H]^-$ and (B) $[M-2H]^{2-}$.

ble deprotonation of both hydroxyl groups $[M-2H]^{2-}$. The relative abundance between the mono-charged and double-charged ions depended on the chlorination degree. An increase on the number of chlorine atoms produced an increase on the signal of the double-charged ion, probably favoured by the lower p*K*a values of the highly chlorinated derivatives of BPA that made easy the double deprotonation in the liquid phase. Fig. 2 shows the LC–MS chromatogram acquired in full-scan mode using a MeOH:water mobile phase. As Fig. 2 shows poor responses were obtained for the chlorinated derivatives of BPA when the mono-charged ion $[M-H]^-$ was monitored. In contrast, responses were more prominent for the highest chlorinated derivatives when the double-charged ions $[M-2H]^{2-}$ were recorded. Furthermore, when using ACN instead of MeOH only the mono-charged ions were observed while the responses for BPA and MCBPA decreased (from 1.5 to 2 times). Therefore, to obtain the best responses for BPA and MCBPA and to avoid the formation of the double-charged ions for the highly halogenated derivatives, ternary mobile phases ACN:MeOH:water were tested and the best separation and detection was obtained using a mobile phase of 30:20:50. In order to improve the efficiency of the separation and reduce the analysis time two columns were evaluated, one based on fused-core particle technology (Ascentis Express C18) and the other a sub-2 μm particle size column (Aquity BEH C18). Both provided a base line separation for all the chromatographic peaks with high efficiencies, which were similar or slightly better for the fused-core column with the additional advantage of lower backpressure (300 bar in front of 725 bar) and obtaining in both cases the separation in less than 3 min at a flow rate of 600 μL min^{-1}.

Under these conditions, tandem mass spectrometry conditions were optimized (collision energies, CID gas pressure, and spray voltage) using the highest ion on the isotopic cluster as a precursor ion. The product ion scan spectrum of BPA showed two abundant ions, one at *m*/*z* 212—the base peak—and the other at *m*/*z* 133 corresponding to the fragments $[M-H-CH_3]^-$ and $[M-H-C_6H_6O]^-$, respectively. For the chlorinated derivatives of BPA (MCBPA, DCBPA, TCBPA, and TeCBPA) the two most abundant product ions resulted from the loss of a CH_4Cl and from the loss of C_2H_4OCl, in agreement with those observed in an ion trap mass analyzer [2]. These product ions were selected for quantification and confirmation of the chlorinated BPA derivatives in water samples (Table 1).

3.2. On-line SPE procedure

There are several critical factors that may contribute to poor results in the analysis of BPA. Special care must be taken into account during sample manipulation due to background contamination at ng L^{-1} mainly coming from solvents, SPE cartridges and plastic ware [17,20]. In this work, in order to minimize sample manipulation and to prevent sample contamination an on-line solid phase extraction liquid chromatography method was developed. For this purpose the two fast LC separations commented above in Section 3.1 have been coupled to an on-line SPE method. The coupling of the sub-2 μm particle size column to the SPE was not possible due to the big difference between the backpressure of both systems (<400 bar for the SPE and >750 bar for the LC). In contrast the lower backpressure obtained with the fused-core column (300 bar) allowed the direct hyphenation, So this last method has been used for further studies.

To achieve the highest level of sensitivity and maximize recoveries we ensured optimal working conditions (e.g. sample load flow rate, loaded sample volume, washing solvent) during the preconcentration step. Sample load flow rate is limited by the highest pressure admissible by the SPE column (130 bar) and by the lowest flow rate required to ensure the equilibration of the enrichment column, improving the capacity for retaining analytes. Based on these factors, a flow rate of 1 mL min^{-1} was selected to load the water

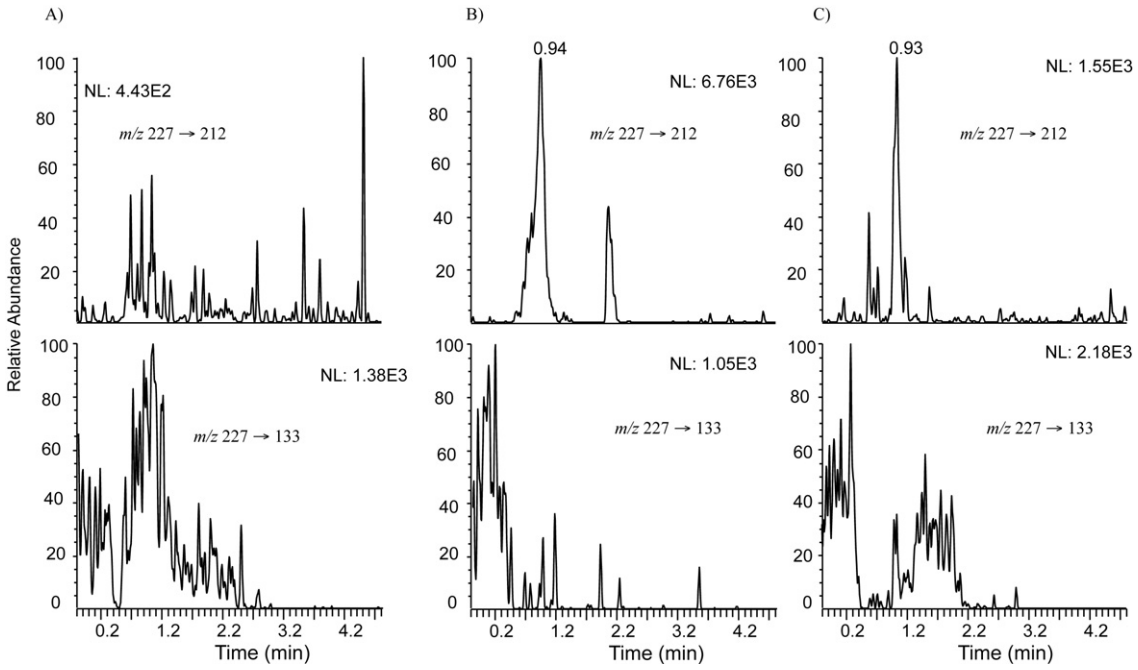

Fig. 3. On-line SPE LC–MS/MS of: (A) UHQ LC–MS water, (B) UHQ LC–MS water filtered through a nylon filter, and (C) UHQ LC–MS water filtered through a regenerate cellulose filter.

samples into the SPE-column. The sample volume to be loaded was also limited by the volume of the vials (4 mL) and the loop volume (2.5 mL). A sample load of 1 mL was enough to ensure sufficient sensitivity to enable the detection of these compounds at concentrations as low as ng L^{-1} and over a reasonable sample loading time.

When analyzing water samples, we aimed to reduce the interfering compounds that could affect both the selectivity and sensitivity of the method, by adding a washing step after sample loading and before the introduction of the analytes into the analytical column. Several water:MeOH mixtures were tested to evaluate the effect of MeOH concentration in the washing solvent. For this reason a sample of surface water from a mountain river (river Garonne, Vall d'Aran, Spain) free from the studied compounds was used and was spiked with 200 ng L^{-1}. The best solvent composition was 10:90 MeOH:water at which level we were able to ensure a balance between the reduction of interferences and the recovery of analytes. Finally, on-line SPE recoveries were estimated by comparing the signal obtained by the direct injection into the analytical column (10 μL) of the surface mountain river water spiked at 10 μg L^{-1} (100 pg injected) with the signal obtained after loading 1 mL of the same sample spiked at 100 ng L^{-1} in order to load the same absolute amount (100 pg injected) into the SPE column. The recoveries ranged from 85% to 100%. Sample carry over from sample-to-sample is a major problem in on-line processes and in order to prevent it in the on-line SPE system, the system was flushed with 100% MeOH (5 min) after the complete elution of the analytes and again after the SPE column was conditioned with 5:95 MeOH:water. Analyses of blank samples were performed for every batch of samples to control carry over and background contamination. No significant signals were observed in these blank samples after a full working day.

BPA can be found at ng L^{-1} in (ultra high quality) UHQ water leached from plastic and epoxy resins used in the purifying equipments [20]. Therefore, to guarantee no BPA contamination in the UHQ water used as a solvent in this method, purified water samples obtained with an Elix-Milli-Q system and an LC–MS grade water samples were analyzed. BPA was found at concentrations ranging from 20 ng L^{-1} to 200 ng L^{-1} in the UHQ water obtained from a Milli-Q system. This range of contamination is particularly varied, which makes it difficult to use blanks to overcome the problem. For instance, a decrease in the concentration of BPA (from 200 ng L^{-1} to 25 ng L^{-1}) was observed throughout the day as UHQ water was produced. Since BPA was not detected in the LC–MS grade water, the UHQ water was used as a solvent to develop the method.

Filtration is currently used as preliminary step in water analysis to eliminate particulate matter. Since the BPA analysis revealed problems of background contamination due to the ubiquity of this compound in the laboratory environment, this step was also evaluated. Two types of membrane filters with a pore size of 0.45 μm were tested, one made of nylon and the other of regenerated cellulose. UHQ water samples free from BPA and spiked at 500 ng L^{-1} with the analytes were filtered using both types of membrane filters and then analyzed.

When nylon membrane filters were used up to 90% of BPA and its chlorinated derivatives disappeared, probably by adsorption onto the membranes. The addition of small amounts of an organic solvent such as methanol (10%) to the water sample before the filtration step prevented this adsorption. Regenerated cellulose membrane filters did not produce this adverse effect, but unfortunately such filters, as also occurred with the nylon ones, released some compounds that interfered chromatographically with BPA making BPA quantification difficult. Fig. 3 shows the chromatogram corresponding to the analysis of 1 mL of UHQ LC–MS water blank (Fig. 3(A)) and the same water sample (5 mL) after being filtered through the nylon filter (Fig. 3(B)) and the regenerated cellulose filter (Fig. 3(C)). In both cases, impurities were eluted at the same retention time as the BPA, and as a result could not be determined. To avoid adsorptions and to prevent the any interference, 10% of MeOH was added to the water sample and to eliminate the particulate matter, the water samples were centrifuged at 4000 r.p.m. rather than filtered.

3.3. On-line SPE fast liquid chromatography–enhanced mass spectrometry

It is well known that in the analysis of complex matrices, increasing the mass resolution power reduces interferences. Nev-

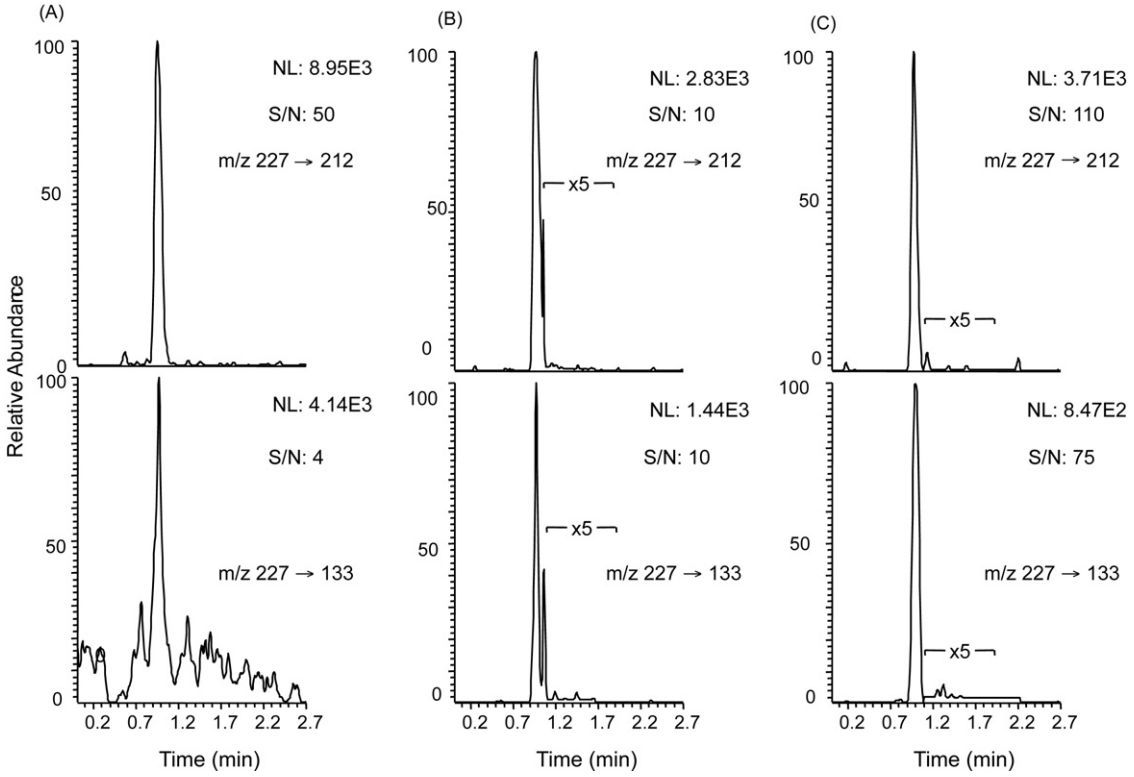

Fig. 4. On-line SPE LC–MS/MS chromatogram of a surface water sample spiked with bisphenol A at 100 ng L^{-1}. Acquisition mode: (A) SRM, (B) H-SRM on Q1, and (C) H-SRM on Q3.

ertheless, this approach forces the use of high resolution mass spectrometry systems such as time-of-flight analyzers but sacrificing sensitivity and linearity. In this study enhanced mass resolution capabilities of a hyperbolic triple quadrupole were used to determine BPA and its derivatives in water samples. To evaluate the performance of the enhanced mass resolution mode, a surface water sample free from the studied compounds spiked at 100 ng L^{-1} was analyzed using the enhanced mode on Q1 (Q1 FWHM = 0.1 Th, Q3 FWHM = 0.7 Th) and on Q3 (Q1 FWHM = 0.7 Th, Q3 FWHM = 0.1 Th). The results were compared with those obtained at low resolution (Q1 FWHM = 0.7 Th, Q3 FWHM = 0.7 Th). When the m/z window was reduced to 0.1 Th FWHM the mass resolving power increased to *ca.* 3.000 for this family of compounds (MW ≈ 300 Da) and was enough in most cases to avoid potential interferences with endogenous compounds in the water samples.

As can be seen in Fig. 4 where the on-line SPE LC–MS/MS chromatograms using the three acquisition modes are shown, the cleanest chromatograms and the best signal-to-noise ratio (S/N) were obtained for the H-SRM mode with enhanced mass resolution in Q3. Enhanced mass resolution on the precursor ions (Q1 at 0.1 Th FWHM) has been generally used [33–37] showing a good performance, but in our case enhanced mass resolution on Q3 showed the best selectivity and sensitivity for BPA (Fig. 4(C)) and it was selected as acquisition mode. Nevertheless, for the chlorinated derivatives of BPA no differences using enhanced mass resolution on Q1 or on Q3 were observed.

To evaluate the performance of the on-line SPE fast LC–MS/MS (H-SRM) method, quality parameters such as limit of quantitation (LOQ), run-to-run precision, ion-ratio precision, and linearity were studied. To estimate the method limits of quantitation (MLOQs) based on a signal-to-noise ratio of 10, UHQ LC–MS water free from BPA was spiked at very low level (down to 10 ng L^{-1}) and 10% MeOH was also added to each sample before the analysis. The values obtained are summarized in Table 2. The developed method provided similar MLOQs for all the compounds ranging from 57 ng L^{-1} to 60 ng L^{-1}, which was enough to determine the presence of BPA and its chlorinated derivatives in water samples.

Calibration standards between 50 ng L^{-1} and 1000 ng L^{-1} were prepared by diluting the stock standard solutions in water and adding 10% MeOH to each calibration standard before analysis using on-line SPE LC–MS/MS (H-SRM). The calibration curves based on the peak area ratio ($A_{compound}/A_{labelled\ compound}$) showed good linearity for all compounds ($r^2 > 0.9996$) in the working range. Run-to-run precision was estimated from the data obtained when

Table 2
Method limits of quantitation and repetitivity of the on-line column switching LC–MS/MS.

Compound	Method limits of quantitation (ng L^{-1})			Run to run, %RSD ($n = 5$)	
	LC–MS water	Surface water	Wastewater	Low concentration[a]	Medium concentration[b]
BPA	57	57	115	13	11
MCBPA	57	57	176	13	5
DCBPA	60	60	183	11	8
TCBPA	60	60	180	14	3
TeCBPA	57	57	140	14	3

[a] Surface water spiked at 50 ng L^{-1}.
[b] Surface water spiked at 300 ng L^{-1}.

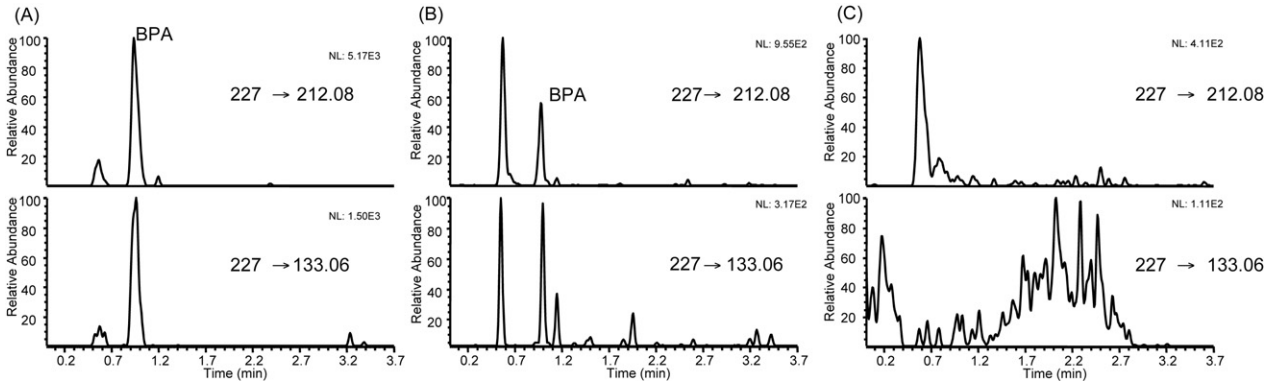

Fig. 5. On-line SPE LC–MS/MS chromatogram of water samples: (A) river water sample, (B) water sample after prechlorination step, and (C) water sample after sand filtration.

analyzing five replicates of a surface water sample spiked at two concentration levels, 100 ng L^{-1} and 500 ng L^{-1}. Precision values expressed as relative standard deviations (RSDs) based on concentration were slightly higher at low concentration levels (50 ng L^{-1}) but always below 15% (Table 2).

To confirm the identity of an analyte, an error in the ion ratio between both quantitative and confirmatory transitions had to be lower than 20%. The ion ratio for bisphenols was evaluated (Table 1). The results ranged from 0.83 to 3.2 with a relative standard deviation below 10% ($n = 5$).

3.4. Analysis of water samples

To evaluate the feasibility of the method for the analysis of BPA and its chlorinated derivatives in water, a surface water sample and a wastewater sample, were analyzed. The surface water was collected from the Garonne River (Vall d'Aran, Spain) and the wastewater was from the influent of a wastewater treatment plant (WWTP). Both samples were analyzed and no BPA or chlorinated derivatives were detected. They were therefore used to estimate the method limit of quantitation (MLOQs) by spiking these blank samples at very low concentration levels. Surface water provided MLOQs ranging from 57 ng L^{-1} to 60 ng L^{-1}, as the instrumental ones estimated using UHQ LC–MS water (Table 2). However, MLOQs estimated from the wastewater sample (115–183 ng L^{-1}) were only 2–3 times higher than those obtained for surface water. This is probably due to the slight matrix components that affected the electrospray ionization efficiency.

Finally, the developed method was used to analyze BPA and its chlorinated derivatives in several water samples: river water, samples from a drinking water treatment plant (DWTP) collected at different sampling points during the water treatment process and an effluent from a paper recycling plant. BPA was detected in two river water samples at 101 ± 22 ng L^{-1} (October 2008) and 322 ± 70 ng L^{-1} (September 2008) collected at the entrance of the DWTP. To study the effectiveness of the treatments performed in the DWTP, several sampling points after each step of the water treatment were collected and analyzed. The majority of BPA was eliminated (>85%) during the prechlorination step due to its high reactivity with chlorine. This is in agreement with the results

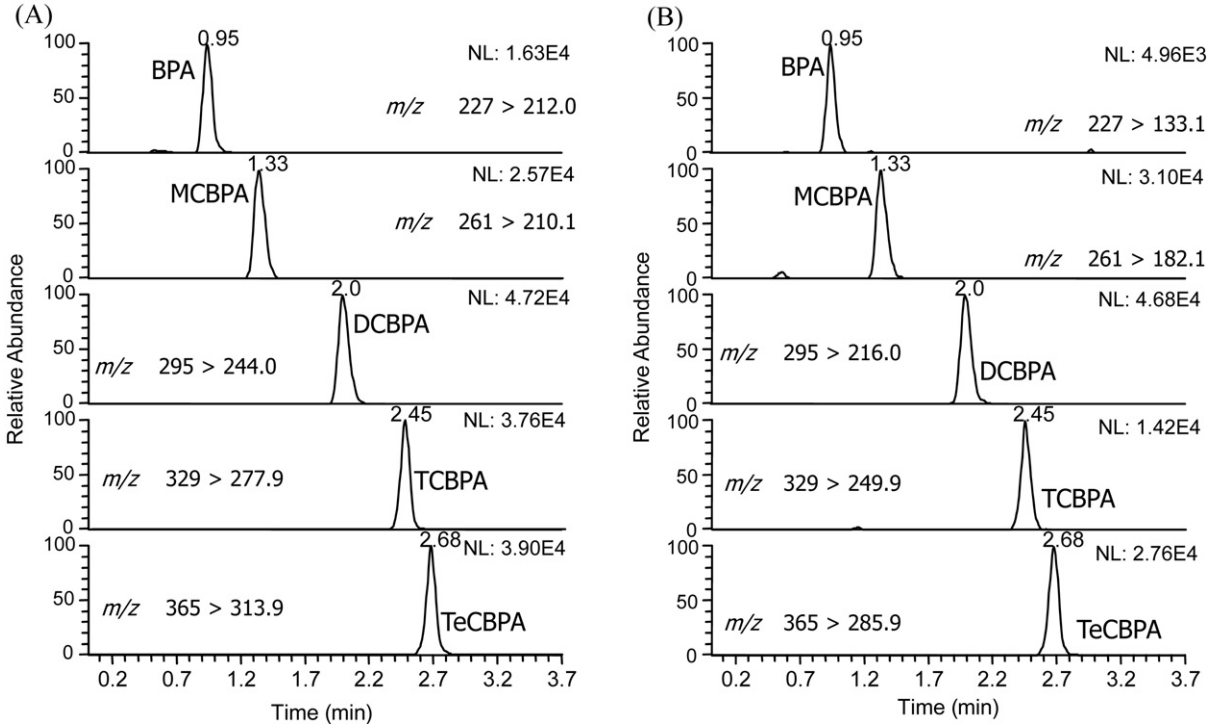

Fig. 6. On-line SPE LC–MS/MS chromatogram of an effluent wastewater sample from a paper recycling plant: (A) quantitation transitions and (B) confirmation transitions.

obtained by Gallard et al. [38], who studied BPA degradation by chlorination in water containing humic substances and observed that it was eliminated due to the formation of its chlorinated derivatives. Chlorinated derivatives were not detected in the samples collected in the DWTP, probably because of their low concentration. The residual BPA was eliminated during the sand filtration step. For example, Fig. 5 shows the BPA extracted chromatograms of river water (Fig. 5(A))—where BPA was detected at $322 \pm 70\ ng\ L^{-1}$—and also that of the same water collected after the prechlorination (Fig. 5(B)) and sand filtration steps (Fig. 5(C)).

The chlorinated derivatives of BPA were detected in the effluent wastewater of the paper recycling plant (Fig. 6) at concentrations of MCBPA $739 \pm 80\ ng\ L^{-1}$, DCBPA $836 \pm 134\ ng\ L^{-1}$, TCBPA $460 \pm 90\ ng\ L^{-1}$, and TeCBPA $530 \pm 72\ ng\ L^{-1}$. These results are in agreement with those reported by other authors in studies of water samples from paper recycling plants in Japan [39], in which BPA was also detected at $679 \pm 100\ ng\ L^{-1}$.

4. Conclusions

In this study an on-line SPE fast LC–MS/MS method was developed for the analysis of BPA and its chlorinated derivatives in water. The low backpressure provided by the use of a fused-core column in the chromatographic separation allowed the direct hyphenation of a conventional on-line SPE system with U-HPLC obtaining high peak efficiencies and base line separation of the studied compounds in less than 3 min. Additionally, the use of enhanced mass spectrometry (H-SRM) working at 0.1 Th FWHM on Q3 provided good sensitivity and selectivity for the analysis of these compounds at a concentration of $ng\ L^{-1}$ in water samples.

With this methodology we did not experience problems of contamination caused by the release of BPA during sample treatment and by the presence of interfering compounds. It can therefore be considered a valuable and attractive tool for use in the routine monitoring of these compounds in water. The method developed was used to the analysis BPA and its chlorinated derivatives in different types of water samples. BPA was detected at low concentrations in river water at the entrance of a DWTP but was eliminated during the two first steps of the treatment (prechlorination and sand filtration). The chlorinated derivatives of BPA, however, were detected in the effluent wastewater of a paper recycling plant.

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