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Analysis of bisphenols in soft drinks by on-line solid phase extraction fast liquid chromatography–tandem mass spectrometry

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ABSTRACT

In this study, an automated on-line solid-phase extraction coupled to fast liquid chromatography–tandem mass spectrometry (on-line SPE fast LC–MS/MS) method was developed for the simultaneous analysis of bisphenol A (BPA), bisphenol F (BPF), bisphenol E (BPE), bisphenol B (BPB) and bisphenol S (BPS) in canned soft drinks without any previous sample treatment. A C18 (12 μm particle size) loading column was used for the SPE on-line preconcentration before the liquid chromatography baseline separation of bisphenol compounds using a C18 Fused-Core™ (50 mm × 2.1 mm i.d.) column, which took less than 3 min. Gradient elution and heated electrospray were used to reduce matrix effect and improve ionization efficiency. To select the most intense and selective transitions, fragmentation studies were performed by multiple-stage mass spectrometry in an ion trap mass analyzer and tandem mass spectrometry in a triple quadrupole instrument, this latter instrument being used for quantitation in SRM mode. Quality parameters of the method were established and we obtained a simple, fast, reproducible (RSD values lower than 10%) and accurate (precision higher than 93%) method for the analysis of bisphenols in canned soft drinks at the ng L^{-1} level using matrix-matched calibration.

1. Introduction

Food and beverage cans are internally coated with epoxy-based lacquers to prevent food coming into direct contact with the metal of the can. Bisphenol A (BPA) is currently used in the synthesis of these epoxy resins. Therefore, foodstuffs may be expected to contain residual BPA due to migration from the epoxy resin coatings. European legislation has established 0.6 mg kg^{-1} as the migration limit for BPA in articles intended to come into contact with foodstuffs [1]. Both the tolerable daily intake (TDI) set by the EU Commission [2] and the reference dose (RfD) established by the U.S. Environmental Protection Agency (US EPA) [3] are 0.05 mg BPA/kg body weight/day, whereas Health Canada established a provisional TDI for BPA at 25 μg kg^{-1} of body weight/day [4]. Due to these restrictions, other bisphenol compounds such as bisphenol F (BPF), bisphenol B (BPB), bisphenol E (BPE) and bisphenol S (BPS), considered as substitutes for BPA in industrial applications [5], are starting to be used for the production of epoxy resins. However, no maximum residue levels or migration limits have been established to date for these compounds in food. As regards toxicity, abundant data for BPA are available, although less information has been published on the other compounds. BPF, BPE and BPB have shown moderate to slight acute toxicity and an estrogenic activity similar to BPA [5], whilst BPS exhibited higher estrogenic activity, probably due to its polarity and the presence of sulfur in the structure [6].

BPA has been detected in canned food at relatively high concentrations ranging from 95 μg kg^{-1} to 842 μg kg^{-1} [7–14], as well as in lower amounts in canned soft drinks (0.032–4.5 μg L^{-1}) [13,15]. In contrast, there are few data on the concentrations of the other BP compounds in canned food; Grumetto et al. [16] determined BPB (27.1–85.7 μg kg^{-1}) in canned peeled tomatoes and Vinas et al. [17] analyzed BPS in canned food, 11.5–175 μg L^{-1} in the supernatant and <LOD – 36.1 μg kg^{-1} in the food.

For the determination of BPs in foodstuffs, liquid chromatography or gas chromatography coupled to mass spectrometry (LC–MS and GC–MS) is generally used. When these compounds are analyzed by GC–MS, a derivatization step is recommended in order to increase the volatility of the compounds and to improve sensitivity in mass spectrometry. Since derivatization in GC–MS requires additional sample manipulation, thus increasing analysis time and reducing reproducibility, LC–MS has been used as an alternative technique in recent years for the analysis of this compound [18,19]. Until now, no more than two BPs have been analyzed simultaneously, BPA and BPB by liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS) [16] and BPA and BPS by GC–MS [17].

Methods for analyzing BPA in food samples have been reviewed recently [18], showing that liquid–liquid extraction (LLE) and solid

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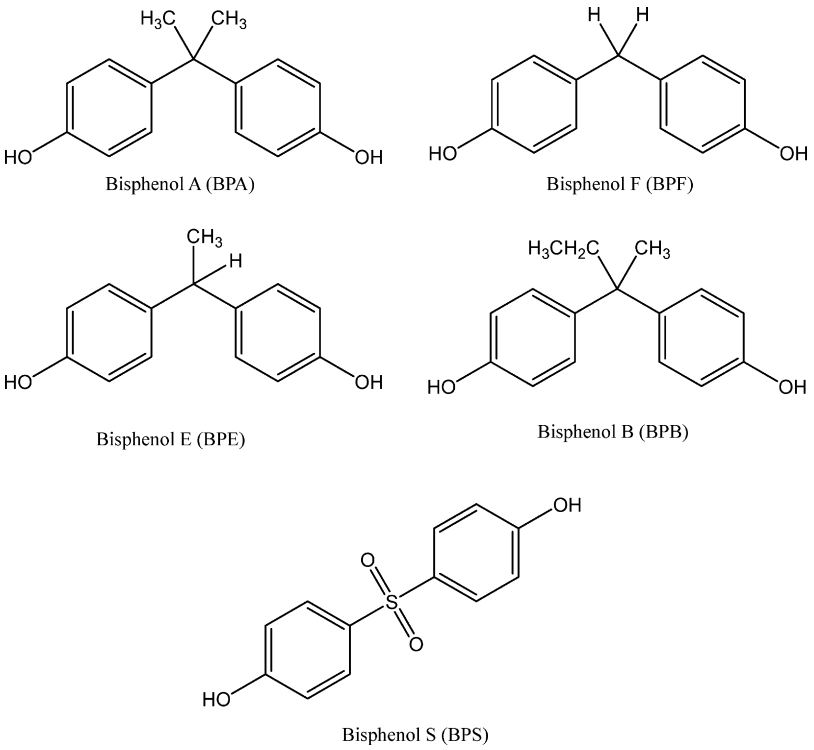

Fig. 1. Chemical structures of bisphenols (BPs).

phase extraction (SPE) are the most common sample preparation treatments. However, background contamination by BPA released from laboratory plasticware can generate significant errors in analyses at very low concentration levels [20,21]. A good alternative method which avoids this problem and minimizes sample manipulation is the use of on-line SPE.

The aim of this study was the simultaneous analysis of five bisphenolic compounds (BPA, BPF, BPE, BPS and BPB) in beverages by updating the SPE fast LC–MS/MS method recently developed by our research group for the analysis of BPA and its halogenated derivatives in water [22]. Matrix effects were evaluated in order to propose a method for the routine analysis of these compounds in canned soft drinks.

2. Experimental

2.1. Chemicals and reagents

Bisphenol A [2,2-bis(4-hydroxyphenyl)propane, BPA], bisphenol A-d_{16} (BPA-d_{16}), bisphenol S [bis-(4-hydroxyphenyl)sulfone; BPS], bisphenol F [bis(4-hydroxyophenyl)methane, BPF] and bisphenol E [bis(4-hydroxyophenyl)ethane, BPE] were obtained from Sigma–Aldrich (Steinheim, Germany). Meanwhile, bisphenol B [bis(4-hydroxyphenyl)butane, BPB] was purchased from TCI Europe (Zwijndrecht, Belgium). The structures of the studied compounds are given in Fig. 1.

Methanol (MeOH), acetonitrile (ACN) and water LC–MS grade were purchased from Riedel-de Haën (Seelze, Germany). Stock standard solutions (10 mg kg^{-1}) were individually prepared by weight in methanol and stored at 4 °C. Intermediate solutions were prepared weekly from the stock standard solution by appropriate dilution in water. Calibration standard solutions ranging from 50 ng L^{-1} to 10 μg L^{-1} of each bisphenol compound were prepared daily containing 400 ng L^{-1} of the internal standard (BPA-d_{16}). Mobile phases were filtered using 0.22 μm membrane nylon filters (Whatmann, Clifton, NJ, USA) and samples were centrifuged at 4000 r.p.m. before analysis by LC–MS/MS.

Nitrogen (99.98% pure) supplied by Claind Nitrogen Generator N_2 FLO (Lenno, Italy) was used for the API source. High-purity Argon (Ar_1) purchased from Air Liquide (Madrid, Spain), was used as a collision-induced-dissociation gas (CID gas) in the triple quadrupole instrument.

2.2. On-line SPE and chromatographic conditions

The system used for both on-line preconcentration and chromatographic separation was a Summit® x2 Dual-Gradient System (Dionex, Sunnyvale, CA) equipped with a Summit P680 dual ternary

Table 1
Tandem mass spectrometry transitions for the acquisition mode.

Compound	Precursor (m/z), $[M-H]^-$	Quantitation		Confirmation		Ion ratio ± SD
		Product (m/z)	CE (eV)	Product (m/z)	CE (eV)	
Bisphenol A	227	212	20	133	26	3.2 ± 0.08
Bisphenol A-d_{16}	241	223	22	142	25	2.8 ± 0.10
Bisphenol F	199	93	23	105	22	1.3 ± 0.11
Bisphenol E	213	198	20	197	29	1.8 ± 0.07
Bisphenol B	241	212	19	226	28	12.5 ± 0.15
Bisphenol S	249	108	27	156	23	1.9 ± 0.01

CE: collision energy, SD: standard deviation (n: 5).

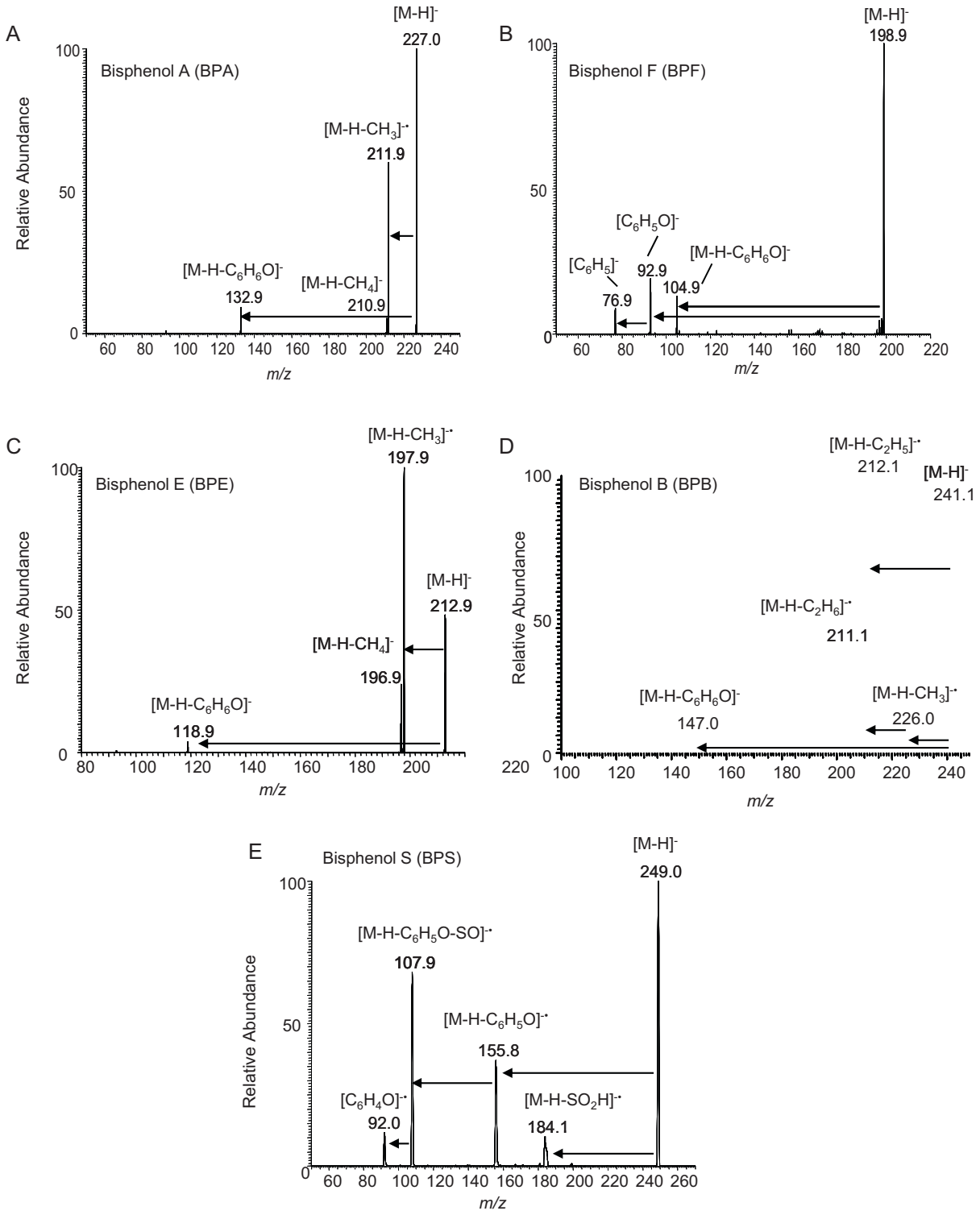

Fig. 2. MS/MS spectra of bisphenols. Operating conditions: 0.7 *m*/*z* FWHM on Q1 and 0.1 *m*/*z* FWHM on Q3. A) BPA. B) BPF, C) BPE, D) BPB and E) BPS.

gradient pump and a TCC-100 thermostated column compartment that includes a 10-port switching valve and an ASI-100T autosampler with a 2.5-mL injection loop. The chromatographic separation was performed on a Supelco Ascentis Express C18 (Fused-Core™) column (Sigma–Aldrich) of 50 mm × 2.1 mm i.d. and 2.7 μm particle size, at 50 °C column temperature using a MeOH/water gradient elution. For the fully automated on-line trace enrichment a Hypersil Gold C18 column (20 mm × 2.1 mm, 12 μm particle diameter, 175 Å pore size) (Thermo Fisher Scientific, Whatmann, MA) was used. This column was previously conditioned with MeOH:water (5:95 v/v) at 1 mL min^{-1}. During this step, the analytical column was equilibrated at initial conditions of the chromatographic separation 15:85 (MeOH:water). The soft drink sample (1 mL) was then preconcentrated on-line in the SPE column using MeOH:water (5:95) (1 mL min^{-1}) as loading solvent. Then it was sequentially washed with 2 mL MeOH:water (15:85 v/v). After washing, the analytes were backflushed and transferred to the analytical column to perform the chromatographic separation using the following gra-

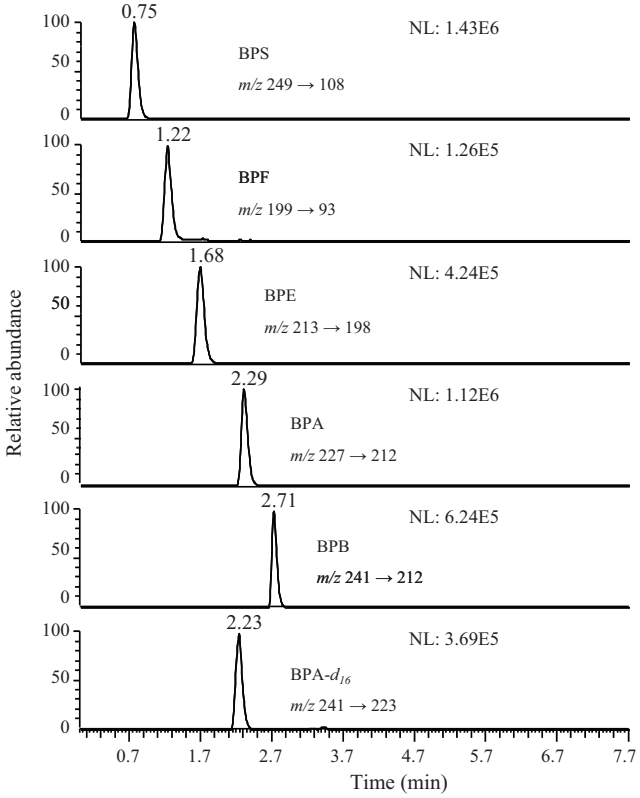

Fig. 3. On-line SPE LC–MS/MS chromatograms of a standard solution (1 mL, 10 μg L^{-1}). Working conditions as indicated in Section 2.

dient elution program: 0 min 15% MeOH; from 0 to 3 min a linear gradient elution up to 80% MeOH followed by a isocratic step of 3.5 min. Standards prepared in cola matrix used for calibration were also preconcentrated on-line by the same SPE-LC procedure as for samples.

To prevent sample carry-over 100% MeOH (600 μL min^{-1}, 5 min) was used. Moreover, water blank samples were injected between sample batches to control carry-over and background contamination.

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, San Jose, CA) equipped with a heated electrospray ionization source (H-ESI I) operating in negative mode (−4 kV) and vaporizer temperature of 300 °C. Nitrogen (purity > 99.98%) was used as a sheath gas, ion sweep gas and auxiliary gas at flow rates of 60, 20 and 40 a.u. (arbitrary units), respectively, and the ion transfer tube temperature was set to 375 °C. For tandem mass spectrometry, the deprotonated molecule $[M-H]^-$ was used as precursor ion. Two transitions for each compound were monitored using a dwell time of 20 ms and 1 μscan/scan (Table 1). When working at SRM mode (low resolution), both Q1 and Q3 operated at 0.7 *m/z* FWHM, whilst highly selective selected reaction monitoring (H-SRM) mode on Q3, Q1 operated at 0.7 *m/z* FWHM and Q3 at 0.1 *m/z* FWHM. Argon was used as collision-induced-dissociation (CID) gas at 1.5 mTorr and the collision energy (CE) for each transition was optimized (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 carry out the tandem mass spectrometry experiments, a 1 mg L^{-1} stock standard methanol solution of each compound was infused at a flow-rate of 3 μL min^{-1} using the syringe pump integrated into the TSQ instrument and a Valco zero dead volume tee piece (Supelco, Alcobendas, Spain) to mix the standard solution with the mobile phase (600 μL min^{-1}, 60:40 MeOH:water). Tandem mass spectrometry spectra were acquired between *m/z* 30 and *m/z* 300 in enhanced mass resolution mode on Q3 (0.1 *m/z* FWHM) in profile mode selecting the $[M-H]^-$ as precursor ion.

2.4. Soft drink samples

Eleven canned soft drinks including soda, beer, cola, tea and energy drinks were collected in Barcelona supermarkets (July 2009). All samples were carbonated drinks except the tea drink products. The samples were stored unopened until analysis at 4 °C. Twenty milliliter aliquots of carbonated soft drink samples were degassed by sonication for 20 min and centrifuged at 4000 r.p.m. Then, 1 mL was loaded into the on-line SPE LC–MS/MS system for analysis.

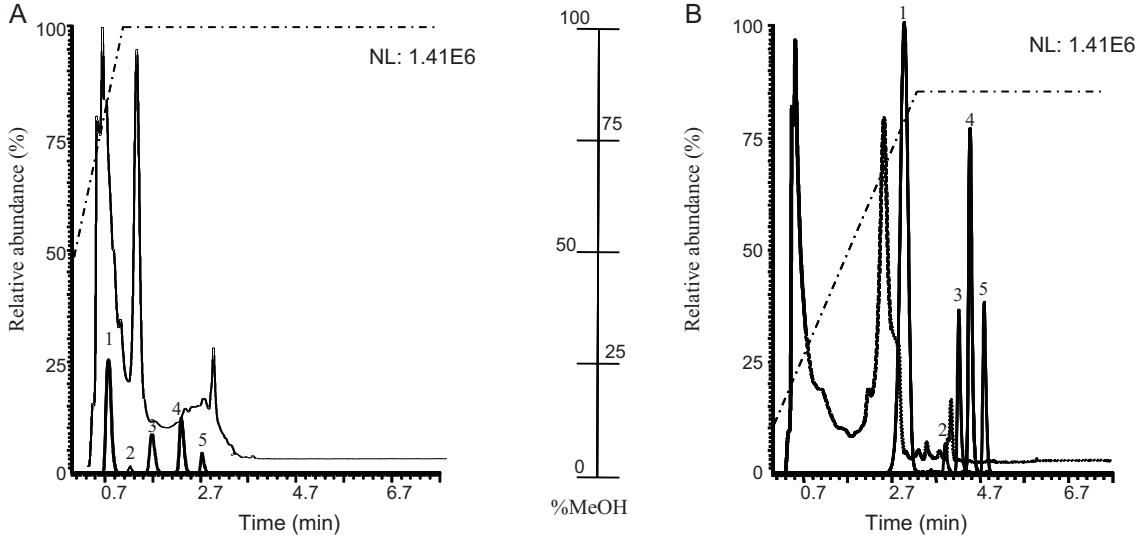

Fig. 4. On-line SPE LC–MS/MS and LC–UV at 228 nm chromatograms of a glass cola sample spiked at 10 μg L^{-1} A) ESI at ambient temperature, gradient elution 0 min, 50:50 MeOH:water; from 0 to 1 min, linear gradient up to 100% MeOH and B) H-ESI at 300 °C, gradient elution 0 min 15% MeOH; from 0 to 3 min a linear gradient elution up to 80% MeOH, isocratic step (3.5 min). Compounds: 1. BPS, 2. BPF, 3. BPE, 4. BPA and 5. BPB.

Bisphenol A was quantified using isotope dilution method adding the deuterated standard (BPA-d_{16}) before the centrifugation step. Matrix-matched calibration using a cola blank sample was used for quantitation of the other bisphenols, since isotopically labeled compounds were not commercially available and matrix effects were observed. Results include expanded uncertainty within a 95% confidence level.

3. Results and discussion

3.1. Liquid chromatography–tandem mass spectrometry (LC–MS/MS)

Only BPA has been extensively studied by LC–MS/MS, whereas few data are available about the LC–MS/MS behavior of the other BPs (BPF, BPE, BPB and BPS). In this study, ESI was used to ionize BPs in negative mode using MeOH:water 50:50 (v/v) as mobile phase. The $[M-H]^-$ was the only ion observed in the full scan mass spectrum of all the studied compounds. The MS/MS spectra of bisphenols that contain an alkyl chain in the central carbon (BPA, BPE and BPB) showed the product ion originated from the radical loss of an alkyl group ($[M-H-CH_3]^{-\bullet}$) for BPA and BPE (Fig. 2A and C) and $[M-H-C_2H_5]^{-\bullet}$ for BPB (Fig. 2D), as a base peak. Furthermore, the loss of a methyl was also observed in the MS/MS spectrum of BPB (Fig. 2D), since this compound has two different alkyl chains, methyl and ethyl, at the central carbon. Additionally, in the MS/MS spectra of bisphenols the characteristic product ion resulting from the cleavage of the hydroxyl–benzyl group, $[M-H-C_6H_6O]^-$ for BPA, BPF, BPE and BPB and $[M-H-C_6H_5O]^{-\bullet}$ for BPS (Fig. 2A–E) were also observed. Moreover, some of these compounds showed a characteristic product ion due to the cleavage of the hydroxyphenyl–alkyl bond that yields the ion at m/z 93 $[C_6H_5O]^-$ for BPF and the product ion at m/z 92, $[C_6H_4O]^{-\bullet}$ for BPS. For this last compound a product ion at m/z 184 that can be related to the loss of $^{\bullet}SO_2H$ (Fig. 2E) and additionally a characteristic abundant product ion at m/z 108, due to the consecutive losses of the hydroxyphenyl group and the sulfur oxide group $[M-H-C_6H_5O-SO_2]^{-\bullet}$ were observed. These consecutive losses were confirmed by multiple-stage mass spectrometry on an ion-trap (IT) mass analyzer. Table 1 summarizes the two transitions selected for each compound and the optimum collision energies that maximize the intensity of the product ions used for both quantitative and confirmatory purposes.

3.2. On-line SPE LC–MS/MS method

The use of a C18 (Fused-Core™) column provided a highly efficient chromatographic separation of BPs with a rapid analysis time (less than 3 min) working at low backpressure (<400 bar), conditions which are compatible with the on-line SPE system. A base line chromatographic separation was achieved using MeOH:water in gradient elution mode at 600 μL min^{-1} and 50 °C. As an example, Fig. 3 shows an on-line SPE LC–MS/MS chromatogram of a standard solution (1 mL, 10 μg L^{-1}).

For matrix effect evaluation, different soft drink samples including cola, lemon soda and tonic contained in glass bottles and free of BPs were spiked with BPs (10 μg L^{-1}) and analyzed by on-line SPE LC–MS/MS using ESI in negative mode as the ionization source. For these preliminary analyses, samples were loaded using MeOH:water (5:95) and the analytes were transferred in backflush mode by gradient elution (MeOH:water (50:50) and a linear gradient up to 100% of MeOH in 1 min). Under these conditions, the responses observed were 80–95% lower than those obtained for a standard solution at the same concentration level, probably due

Table 2
Method limits of detection (MLDs), method limits of quantitation (MLQs) and run-to-run precision of the developed on-line SPE LC–MS/MS.

Martix sample																				
Compound	Cola[a]						Lemon soda[a]						Tonic[a]							
			Concentration level						Concentration level						Concentration level					
			500 ng L^{-1}		200 ng L^{-1}				500 ng L^{-1}		200 ng L^{-1}				500 ng L^{-1}		200 ng L^{-1}	
	MLD (ng L^{-1})	MLQ (ng L^{-1})	%RSD (n: 5)	Bias (%)	%RSD (n: 5)	Bias (%)	MLD (ng L^{-1})	MLQ (ng L^{-1})	%RSD (n: 5)	Bias (%)	%RSD (n: 5)	Bias (%)	MLD (ng L^{-1})	MLQ (ng L^{-1})	%RSD (n: 5)	Bias (%)	%RSD (n: 5)	Bias (%)
BPS	25	84	4.5	97	3	97	15	50	3.5	97	4	96	5	15	2.5	97	2.5	98
BPF	50	167	8	96	10	93	32	106	5	96	8	94	42	140	4	96	5	96
BPE	25	84	6	97	6	94	12	40	5	95	5	97	18	60	6	95	9	93
BPA	25	85	2.5	98	3	98	15	50	4	97	5	96	25	85	3.5	97	5	94
BPB	50	167	3	97	5	96	40	132	5	95	10	95	50	167	7	96	10	93

[a] Glass beverage soft-drink.

to the presence of matrix components that cause ion suppression in the ESI source. As an example of co-elution of matrix components with analytes, Fig. 4A shows the chromatogram obtained for a cola sample acquired in SRM mode and also the UV (228 nm) chromatogram, where it can be observed that BPs eluted in a dirty area of the chromatogram. Several strategies were evaluated to reduce the matrix effect observed in the analysis of beverages and to improve both the selectivity and sensitivity of the method. First a clean-up step was added before transference of the analytes from the SPE column to the analytical column, using 15:85 MeOH:water to remove interferences. In addition, ionization efficiency was improved by increasing ESI temperature to 300 °C, providing extra desolvation. Under these conditions, the responses of BPs increased ~2.5 times. Since matrix effect was still observed, gradient elution was modified by reducing the amount of organic solvent and the gradient slope in order to increase retention of the analytes and to force their elution into a cleaner chromatographic area, thus minimizing the co-elution with matrix components in the eluting front. Fig. 4B shows the chromatograms obtained for the cola sample spiked at 10 μg L^{-1} analyzed using heated ESI and the new gradient conditions. An important reduction of matrix effect was observed, improving the responses by up to ~7 times.

The best way to compensate matrix effects in quantitative analysis is the use of isotope-labeled internal standards; however, for most of the bisphenols analyzed in this study (BPF, BPE, BPB and BPS), these standards were not available. For this reason matrix-matched calibration was evaluated for the analysis of BPs in canned soft drink beverages. Calibration curves, obtained from matrix-matched standards prepared for three soft-drink beverages (cola, lemon soda and tonic) were established for all the studied compounds (see Table S1, supporting material). These curves displayed good linearity over the selected concentration range (50 ng L^{-1}–10 μg L^{-1}) with linear regression correlation coefficients better than 0.996. Moreover, a statistical paired-sample comparison analysis was performed using the slopes of the matrix-matched calibration curves prepared for the three soft drink beverages. For a 95% confidence level, the slopes were not significantly different (p-value of 0.23), showing that these matrices would provide similar quantitation results. In this study, for a routine analysis, cola from a glass bottle was used for matrix-matched calibration. For this matrix, on-line SPE recoveries were estimated by comparing the signal obtained by direct injection into the analytical column (10 μL) of a cola sample spiked at 25 μg L^{-1} (250 pg injected) with the signal obtained after loading 1 mL of beverage spiked at 250 ng L^{-1} in order to load the same absolute amount (250 pg injected) into the SPE column. Good recoveries from 85 to 100% were obtained for the studied compounds.

Table 3
Canned soft-drinks analysis using a LC–MS/MS.

Sample	Concentration (ng L^{-1} ± SD)				
	BPS	BPF	BPE	BPA	BPB
Orange soda	n.d.	218 ± 15	n.d.	607 ± 25	n.d.
Lemon soda 1	n.d.	141 ± 11	n.d.	433 ± 13	n.d.
Lemon soda 2	n.d.	n.d.	n.d.	232 ± 17	n.d.
Energy drink 1	n.d.	n.d.	n.d.	561 ± 22	n.d.
Energy drink 2	n.d.	n.d.	n.d.	MLD[a]	n.d.
Tonic	n.d.	n.d.	n.d.	44 ± 2	n.d.
Tea lemon	n.d.	n.d.	n.d.	MLD[a]	n.d.
Apple soda	n.d.	n.d.	n.d.	503 ± 19	n.d.
Soda	n.d.	n.d.	n.d.	MLD[a]	n.d.
Cola	n.d.	n.d.	n.d.	522 ± 22	n.d.
Beer	n.d.	n.d.	n.d.	n.d.	n.d.

n.d., not detected using the H-SRM on Q3 acquisition mode.
[a] Detected using H-SRM on Q3 acquisition mode (MLD for BPA 5 ng L^{-1}).

To evaluate the performance of the on-line SPE LC–MS/MS method for the analysis of bisphenols in canned soft drinks, quality parameters such as limit of detection (MLD), limit of quantitation (MLQ), run-to-run precision and ion ratio precision were studied in three soft drink beverages (cola, lemon soda and tonic). To estimate the limits of detection (MLD) and limits of quantitation (MLQ) based on a signal-to-noise ratio of 3 and 10, respectively, the three soft drink beverages were spiked at a very low concentration level (down to 200 ng L^{-1}). Similar MLDs and MLQs were observed for most of the compounds in the three matrices (Table 2), showing that the method proposed is sensitive enough to quantify bisphenols in canned soft drinks down to ng L^{-1} (60–167 ng L^{-1}). Only for BPS in the tonic sample were these values lower. This can be explained by the fact that this compound eluted with the lowest retention time and could be sensitive to matrix changes. To obtain accurate quantitation measurements for this compound at concentrations lower than the cola LDQ (84 ng L^{-1}), matrix-match with the self-matrix is recommended. Reproducibility and bias were estimated from the data obtained from analyzing five replicates of each sample at two concentration levels, 200 ng L^{-1} and 500 ng L^{-1}. Precision values expressed as standard deviations (SDs) based on concentration were always good with relative standards deviations (%RSD) lower than 10% for both concentration levels (Table 2) and

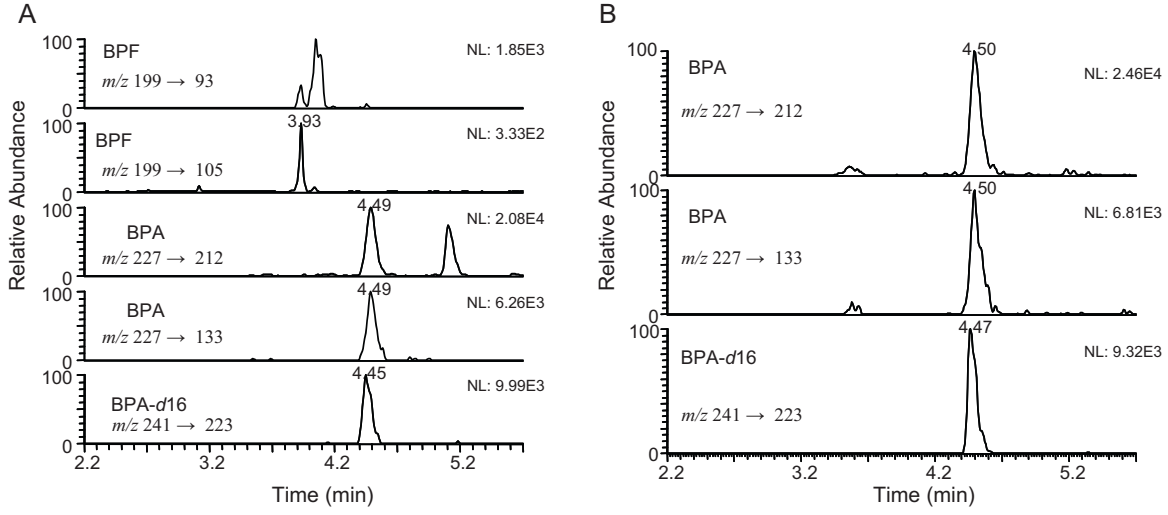

Fig. 5. LC–MS/MS chromatograms of two canned soft-drink beverages: A) lemon soda 1, and B) energy drink 1. Working conditions as indicated in Section 2.

the bias values expressed as relative error were higher than 93% at both low and medium concentration level (Table 2). To confirm the identity of the analyte, the ion ratio (quantitation-to-confirmation) for bisphenols was also evaluated (Table 1), obtaining an error below 10% for the three samples at the two concentration levels.

3.3. Sample analysis

The on-line SPE LC–MS/MS method was used for the simultaneous analysis of bisphenols (BPA, BPF, BPE, BPB and BPS) in eleven canned soft drinks and the results obtained are summarized in Table 3. Bisphenol A was detected in most of the analyzed samples at concentrations ranging from 44 ng L^{-1} to 607 ng L^{-1}. These values are consistent with the results published by Cao et al. [13]. Bisphenol F (BPF) was detected in only two samples, orange and lemon soda at a concentration of 218 ng L^{-1} and 141 ng L^{-1}, respectively, whilst other BPs were not detected in the analyzed samples. As an example, Fig. 5 shows the LC–MS/MS chromatogram corresponding to the lemon soda (Fig. 5A) where BPF and BPA were detected and the energy drink beverage where only BPA was identified (Fig. 5B).

Additionally, in this study enhanced mass resolution was used to minimize interferences and background noise when dealing with complex matrices. H-SRM on Q3 (Q1 0.7 m/z FWHM, Q3 0.1 m/z FWHM) provided lower limits of detection (5–10 times lower) than those obtained using SRM acquisition mode. This fact allowed us to confirm the presence of BPA at MLD level (5 ng L^{-1}) in three samples (energy drink 2, tea lemon and soda) that were considered to be negative under SRM mode working conditions. Moreover this acquisition mode in combination with the ion ratio confirmed the results obtained for the positive samples analyzed using SRM mode and prevented false positives.

4. Conclusions

This paper reports the development of an on-line SPE fast LC–MS/MS method for the simultaneous and direct analysis of bisphenols (BPA, BPF, BPE, BPB and BPS) in canned soft drinks. Good chromatographic separation in less than 5 min was obtained using a Fused-Core™ particle column at 600 μL min^{-1} at low backpressure that enabled the on-line SPE system to be coupled with LC–MS/MS. In tandem mass spectrometry most of these compounds showed the loss of the alkyl group from the central carbon atom and the cleavage of the hydroxyl–phenyl alkyl bond. Only BPS showed different behavior related to the SO_2 group in the molecule.

The use of a clean-up step in the on-line SPE preconcentration in combination with a gradient elution that forced the compounds to elute in a cleaner chromatographic area and the use of heated electrospray (300 °C) enabled matrix effects to be minimized. Under these conditions, BPs can be analyzed at concentrations as low as 100 ng L^{-1} using matrix matched calibration. Selectivity and sensitivity can be improved using H-SRM with the advantage of preventing false negatives. This fast, robust, sensitive and selective method can be proposed for routine analysis of BPs in soft-drinks.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.aca.2010.10.034.

References

[1] Commission Directive 2004/19/EC of 1 March 2004 amending Directive 2002/72/EC relating to plastic materials and articles intended to come into contact with foodstuffs, Off. J. Eur. Communities 2004 L71 (2010) 8–21.
[2] European Food Safety Authority (EFSA), Opinion of the scientific panel on food additives, flavourings, processing aids and materials in contact with food (AFC) related to 2,2-bis(4-hydroxyphenyl)propane, EFSA J. 2006 428 (2010) 1–6.
[3] U.S. Environmental Protection Agency (U.S. EPA), Bisphenol A. (CASRN 80-05-7), Integrated Risk Information System (IRIS), 1993 (2010) Available from http://www.epa.gov/iriswebp/iris/subst/0356.htm.
[4] Health Canada, Survey of bisphenol A in canned drink products (2010) http://www.hc-sc.gc.ca/fn-an/securit/packag-emball/bpa/bpa_survey-enquete-can-eng.php.
[5] M.Y. Chen, M. Ike, M. Fujita, Environ. Toxicol. 17 (2002) 80.
[6] A. Rivas, M. Lacroix, F. Olea-Serrano, I. Laios, G. Leclercq, N. Olea, J. Steroid Biochem. Mol. Biol. 82 (2002) 45.
[7] A. Goodson, W. Summerfield, I. Cooper, Food Addit. Contam. 19 (2002) 796.
[8] B.M. Thomson, P.R. Grounds, Food Addit. Contam. 22 (2005) 65.
[9] E.M. Munguia-Lopez, S. Gerardo-Lugo, E. Peralta, S. Bolumen, H. Soto-Valdez, Food Addit. Contam. 22 (2005) 892.
[10] J. Sajiki, F. Miyamoto, H. Fukata, C. Mori, J. Yonekubo, K. Hayakawa, Food Addit. Contam. 24 (2007) 103.
[11] J. Yonekubo, K. Hayakawa, J. Sajiki, J. Agric. Food Chem. 56 (2008) 2041.
[12] X.L. Cao, J. Corriveau, S. Popovic, G. Clement, F. Beraldin, G. Dufresne, J. Agric. Food Chem. 57 (2009) 5345.
[13] X.L. Cao, J. Corriveau, S. Popovic, J. Agric. Food Chem. 57 (2009) 1307.
[14] T. Yoshida, M. Horie, Y. Hoshino, H. Nakazawa, Food Addit. Contam. 18 (2001) 69.
[15] R. Braunrath, M. Cichna, J. Chromatogr. A 1062 (2005) 189.
[16] L. Grumetto, D. Montesano, S. Seccia, S. Albrizio, F. Barbato, J. Agric. Food Chem. 56 (2008) 10633.
[17] P. Viñas, N. Campillo, N. Martinez-Castillo, M. Hernandez-Cordoba, Anal. Bioanal. Chem. 397 (2010) 115–125.
[18] A. Ballesteros-Gomez, S. Rubio, D. Perez-Bendito, J. Chromatogr. A 1216 (2009) 449.
[19] H. Gallart-Ayala, E. Moyano, M.T. Galceran, Mass Spectrom. Rev. (2009).
[20] K. Inoue, K. Kato, Y. Yoshimura, T. Makino, H. Nakazawa, J. Chromatogr. B: Biomed. Sci. Appl. 749 (2000) 17.
[21] Y. Watabe, T. Kondo, H. Imai, M. Morita, N. Tanaka, K. Hosoya, Anal. Chem. 76 (2004) 105.
[22] H. Gallart-Ayala, E. Moyano, M.T. Galceran, J. Chromatogr. A 1217 (2010) 3511.