



Determination of BPA, BPB, BPF, BADGE and BFDGE in canned energy drinks by molecularly imprinted polymer cleaning up and UPLC with fluorescence detection



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ABSTRACT

A new method for simultaneous determination of five bisphenols in canned energy drinks by UPLC with fluorescence detection, after clean up on molecularly imprinted polymers, is herein described. The method was validated at two concentration levels, calculating trueness, repeatability and within-laboratory reproducibility, specificity, linearity of detector response, the limits of quantifications and the limits of detection for each bisphenol. The method is specific, reliable and very sensitive, allowing for determination of bisphenol F diglycidyl ether (BFDGE), bisphenol A (BPA), bisphenol B (BPB), bisphenol F (BPF) and bisphenol A diglycidyl ether (BADGE) down to 0.50 ng/mL; it was employed to determine contamination levels from these bisphenols in forty energy drinks of different brands, collected from the market in Naples. BPA was detected in 17 out of 40 samples (42.5%); in some energy drinks also BPF, BADGE and BFDGE were determined.

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1. Introduction

Bisphenol A (BPA) is used primarily as a monomer in the production of polymers (like polycarbonate and epoxy-resins), as antioxidant in some plasticizers, and as an inhibitor of polymerization of vinyl chloride in the production of soft PVC (Geens, Goeyens, & Covaci, 2011). When this compound is used in polymer production, residual monomers of BPA remain after incomplete chemical reaction or, more rarely, as the result of a chemical degradation of the polymer (Geens et al., 2011). When used as additive in thermal paper and PVC, BPA is not covalently bound with the

polymer macromolecules, therefore this compound may easily migrate into the surrounding medium, such as food and beverages (Geens et al., 2011). Bisphenol A Diglycidyl Ether (BADGE) and Bisphenol F Diglycidyl Ether (BFDGE) are used as starting substances for epoxy-resins and as additives in PVC organosol resins, in order to remove excess of hydrochloric acid during reaction (Cao, Corriveau, & Popovic, 2009; Satoh, Ohyama, Aoki, Iida, & Nagai, 2004). Except for the occupational exposure, food, drinking water and beverages are the possible route of human exposure (Kang, Kondo, & Katayama, 2006). Endocrine disruptor chemicals (EDCs) are substances that influence synthesis, transport, secretion, action, binding or elimination of endogenous hormones in the body (García-Arevalo et al., 2014; Jeng, 2014; Le Corre, Besnard, & Chagnon, 2015); endocrine disruptors include persistent organic pollutants, heavy metals and plastic component such as phthalates and bisphenol A, among others, that are presently

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emerging (García-Arevalo et al., 2014). The bisphenolic compounds could migrate from the internal coating of food and beverage cans into food (Cao et al., 2009); in fact, the beverages in cans are more contaminated than those packed in glass (Fasano et al., 2015).

The migration limits set by the European Commission are 0.6 mg/kg of food for BPA, 9.0 mg/kg of food for BADGE and its hydroxyl-derivatives and 1.0 mg/kg of food for BADGE and its chlorinated derivatives (European Commission, 2005, 2011a). Moreover, the use of BFDGE has been forbidden (European Commission, 2005); no tolerable limit has been set for BPB. In 2015, EFSA has published a re-evaluation of BPA exposure from diet and other sources (European Food Safety Authority, 2015). EFSA experts have reduced the “tolerable daily intake” (TDI) of BPA from 50 µg per kilogram of body weight per day (µg/kg of bw/day) to 4 µg/kg of bw/day, assessing that, on the basis of collected data, the exposure risk to consumers is low.

Following the recent restrictions on the use of BPA in food contact materials (European Commission, 2011b; French Republic, 2012; Food and Drug Administration, 2012, 2013), the plastic and the canning industry are moving to seek alternative chemicals that allow the replacement of BPA in many of its applications. In recent years some products labeled as “BPA-free”, potentially containing BPA substitutes, are becoming frequent in can (Simoneau, Valzacchi, Morkunas, & Van den Eede, 2011; Simoneau, Van den Eede, & Valzacchi, 2012; Scherer, Maynard, Dolinoy, Fagerlin, & Zikmund-Fisher, 2014). These alternative chemical compounds, mainly belonging to the class of *p,p'*-bisphenols, have physical and chemical properties similar to BPA and show variable toxicity and a high estrogenic activity (Chen, Ike, & Fujita, 2002); among these structural analogues, Bisphenol S (BPS), Bisphenol F (BPF), Bisphenol B (BPB) apparently are the main BPA replacements; therefore, the industrial use of BPF and BPS has gradually increased, presumably as safer alternatives to BPA. Both compounds nowadays can be detected in soft drinks and canned food, as well as in thermal paper receipts (Becerra & Odermatt, 2012; Gallart-Ayala, Moyano, & Galceran, 2011; Liao & Kannan, 2013; Viñas, Campillo, Martínez-Castillo, & Hernández-Córdoba, 2010). In addition, BPS has been detected in human urine samples (Liao et al., 2012). Nowadays, scarce toxicological data regarding BPF and BPS do not allow to evaluate related exposure risks to consumers. BPF and BPS have endocrine modulation capabilities, and showed toxicological profiles generally similar to BPA, exhibiting genotoxic and carcinogenic potency and effects on metabolism and oxidative stress (Rosenmai et al., 2014; Roelofs, van den Berg, Bovee, Piersma, & van Duursen, 2015). In the literature, there are data about the presence of BPA in some soft drinks (Fasano et al., 2015), in infant formula (Cirillo et al., 2015), and in energy drinks (Cao et al., 2009; Russo, Barbato, & Grumetto, 2016; Geens, Apelbaum, Goeyens, Neels, & Covaci, 2010; Gallart-Ayala et al., 2011; Cunha, Almeida, Mendes, & Fernandes, 2011).

The term “energy drinks” refers to a category including a variety of non-alcoholic beverages containing active substance such as carbohydrates, amino acids, caffeine, and vitamins (Alford, König, Aufricht, & Verster, 2011) which is often combined with taurine, D-glucuronono-γ-lactone, guaraná, maltodextrin, ginseng, carnitine, creatine and ginkgo biloba; other common ingredients are vitamins and, usually, artificial and natural sweeteners (which are added also to “mask” the unpleasant flavour of some ingredients) (Friis, Lyng, Lasgaard, & Larsen, 2014).

The energy drinks are drunk mainly for their stimulating effects and for energizing by young people and adults, but also by practitioners of intense exercise and sport activities before competitions (Alford, Cox, & Wescott, 2001; Ivy et al., 2009). The use of these drinks, also in combination with alcoholic beverages, is spreading in the last years, in order to increase the stimulatory effect (Oteri, Salvo, Caputi, & Calapai, 2007; Marczyński & Fillmore,

2014; Alford et al., 2011; Reissig, Strain, & Griffiths, 2009; Arria et al., 2011).

In a study commissioned by EFSA to the Consortium Nomisma-Areté, with the aim to collect consumption data for energy drinks in specific consumer groups (adults, adolescents and children) in the EU, the highest prevalence of consumption was observed for the adolescent consumers group (68%). The prevalence for consumption was 30%, and 18% for adults and children, respectively. The study found out that among respondents, teenagers belong to the age group with the highest probability (68% of total respondents) of consuming energy drinks (56% in Italy). About 12% (7% in Italy) of adolescent consumers were identified as “high chronic” consumers, i.e. consuming energy drinks 4–5 times per week or more (Zucconi et al., 2013).

In addition to the risk due to the active ingredients in energy drinks there might be the risk of the presence of bisphenols that migrate from the can to the drink. Considering energy drink consumption especially among young people, who represent a population particularly interested by pubertal hormonal activity, the intake of substances with endocrine disrupting activity could represent a subtle health problem.

The determination of these emerging contaminants in beverages requires the development and validation of reliable analytical methods, but a few methods have been described in the literature for the analysis of BPA and its analogues in beverages other than canned soft drinks; recently, a HPLC method for fluorescence detection of eight bisphenols in soft drinks down to limits of quantification in the range 5.52–21.37 ng/mL has been described (Russo et al., 2016).

In this study, we describe an original multi-residue method developed for simultaneous determination of BPA, BPB, BPF, BADGE and BFDGE in energy drinks. The method is based on clean up using specific molecularly imprinted polymers designed for selective binding of BPA, that is Affinimip® Bisphenol A SPE cartridges, that resulted effective also for other bisphenols; determination of all analytes was performed by ultra-performance liquid chromatography with fluorescence detection (UPLC-FLD). A molecularly imprinted polymer is a selective chromatographic solid stationary phase, synthesized starting from functional monomers assembled around a template molecule, that has a chemical structure similar to the compound to be purified (target molecule) and polymerized in the presence of a cross-linker; often the compound itself is used as a template. Once the polymer is synthesized, template molecule is extracted; this way, an “active site” is created, that is a three-dimensional network with high binding selectivity for the target molecule, allowing for its purification by affinity chromatography. The molecularly imprinted polymers can be packed in a column for solid phase extraction (SPE). Affinity chromatography on molecularly imprinted polymers is usually more effective than other SPE techniques, such as reversed phase, adsorption and normal phase chromatography, for purification from complex matrices, thanks to a high degree of specificity in binding of the analyte(s) to the stationary phase.

The method we developed was validated at 2.0 ng/mL and 10.0 ng/mL, evaluating trueness as mean recoveries, and precision in terms of relative standard deviations for repeatability and within-laboratory reproducibility; the limit of quantification (LOQ) and the limit of detection (LOD) were also evaluated for each compound.

2. Materials and methods

2.1. Chemicals and reagents

Bisphenol A (BPA, 99% analytical purity), bisphenol F (BPF, 99.7% analytical purity), bisphenol F diglycidyl ether (BFDGE, 98%

analytical purity) and bisphenol A diglycidyl ether (BADGE, 97% analytical purity) were purchased from Sigma-Aldrich (St. Louis, MO, USA); bisphenol B (BPB, 99% analytical purity) was purchased from TCI Europe (Zwijndrecht, Belgium). HPLC grade methanol (MeOH) and acetonitrile (ACN) were from Romil (ROMIL Ltd, UK). Glacial acetic acid 99.8% and ammonium acetate supplied by Carlo Erba (Milan, Italy) were all analytical grade reagents. HPLC grade water was in-house produced using a MilliQ laboratory system (Millipore, Bedford, MA, USA). The Affinimip[®] SPE Bisphenol A cartridges in glass tube were from Polyntell (Polyntell SA, Paris, France).

2.2. Standard reference materials and quality control samples

Standard stock solutions of BPA, BPF, BFDGE, BADGE and BPB were prepared dissolving 10.0 ± 0.1 mg of each compound in 10.0 mL of methanol (concentration 1.0 mg/mL). A mixture standard stock solution at 10.0 μ g/mL containing all these compounds, was prepared in methanol from single standard solutions at 1.0 mg/mL. Both individual and mixture standard stock solutions were stored in dark glass vials at -18 °C until use. Standard calibration curves were calculated injecting mix standard solutions at 1.0–5.0–10.0–25.0–50.0 ng/mL of each bisphenol; to this aim, a standard mix solution at 50.0 ng/mL was prepared diluting with methanol the standard solution at 10.0 μ g/mL. From the standard mix at 50 ng/mL, the standard mix solutions at 1.0–5.0–10.0–25.0 ng/mL were prepared daily by serial dilution with methanol.

2.3. Glassware washing

To avoid the possible contamination due to bisphenols deriving from plastics, only glassware was used during analysis; furthermore, all the glassware had been previously thoroughly washed with appropriate detergent, rinsed with distilled water, then twice with both acetonitrile and methanol and dried in oven at 100 °C.

2.4. Sample clean up

After sample gentle mixing, about 20 mL of energy drink were poured in a glass tube, degassed for 60 min in an ultrasonic bath, then 5.0 mL were transferred in a 10 mL centrifuge glass tube, added with 1.0 mL of 0.2 M aqueous ammonium acetate and mixed by vortex for 30 s to adjust pH at about 4.0. The sample was loaded onto an Affinimip[®] Bisphenol A SPE cartridge, previously conditioned with 3.0 mL of 2% acetic acid solution in methanol, 3.0 mL acetonitrile and 3.0 mL MilliQ water, according to the manufacturer instructions. After sample loading, the cartridge was washed with 9.0 mL MilliQ water, 6.0 mL MilliQ water/acetonitrile 60/40 v/v and dried for 30 s applying vacuum. Elution was carried out with 3.0 mL methanol, then 3.0 mL acetonitrile; eluent phases were gathered up, mixed by vortex and evaporated at room temperature under a gentle nitrogen stream. The residue was dissolved in 1.0 mL methanol and transferred into a glass vial for UPLC-FLD analysis.

2.5. UPLC-FLD analysis

Analyses were performed using an Acquity UPLC H-Class system (Waters Corp., Milford, MA, USA), equipped with fluorescence detector, an autosampler and an oven for column temperature control.

Chromatographic separation was performed on a 2.7 μ m particles, 75×4.6 mm Ascentis Express RP-Amide stainless steel column (Supelco, Bellefonte, PA, USA) at 25 °C temperature. Chromatography was run at 0.500 mL/min flow rate, injecting 5.0 μ L of sample. A linear gradient elution was applied, using MilliQ water

(A) and acetonitrile (B) as mobile phases. The analysis started from 50% B for 0.50 min, followed by a linear gradient from 50% to 95% of B in 5.5 min, then holding at 95% of B for 3.0 min. Finally, phase B was decreased to 50% in 2.0 min and equilibrated at 50% for further 4.0 min. Fluorescence detection of BPA, BPB, BPF, BADGE and BFDGE was carried out at 275 nm excitation wavelength and 305 nm emission wavelength.

The concentration of each compound in ng/mL was determined by external calibration curve method, calculating linear regression of the chromatographic peak areas vs standard solution concentrations; interpolation of the linear regression curves was employed for quantification of each bisphenol. The linearity of detector response was assessed during each working session, by correlation coefficients of $r^2 \geq 0.998$. The presence of each analyte was confirmed on the basis of relative retention times (t_r), with a tolerance $t_r \pm 2.5\%$ in respect to the reference standard peaks. The results in ng/mL of energy drink were calculated corrected for analyte recovery during each working session.

2.6. Method validation

Method performance was evaluated by analyzing energy drink samples spiked at 2.0 ng/mL and at 10.0 ng/mL of all target bisphenols; two working sessions and six replicates were carried out for each spiking level in different days. For each spiking level and all compounds we calculated method trueness, in terms of mean percentage recoveries, and precision, as relative standard deviation (RSD) of both repeatability (within-day) and laboratory-reproducibility (between days). The limit of quantification (LOQ) of each bisphenol was measured analyzing energy drink samples spiked at decreasing concentrations, down to the lowest content showing chromatographic peaks with a signal-to-noise ratio $>10/1$ in respect to the baseline bias of a blank sample; the limit of detection (LOD) for each compound was calculated according to the equation: $LOQ = 3.3 \times LOD$.

Reagent and process blanks showed no significant interference in the diagnostic area of the chromatogram, accounting for method specificity.

2.7. Survey of contamination from bisphenols in canned energy drinks

Forty canned energy drinks from different brands were collected from local supermarkets in Naples and its province (Southern Italy) between June and November 2015. For each sample tested, the pH value was measured, to verify possible correlation with bisphenol migration into the energy drink. In addition, whenever possible, the country of production and/or bottling plant for each sample tested were recorded. During each working session, precision and trueness quality controls were introduced, analyzing a sample spiked at 10 ng/mL, as well as reagent and process blanks.

2.8. Statistical analysis

Data analysis was performed through software R version 3.2.3 in order to evaluate Pearson's correlation between studied compounds and other variables such as pH values and contact surface of drink with internal coating of cans (R Core Team, 2015).

3. Results and discussions

3.1. Clean up of bisphenols and UPLC-FLD analysis

The Affinimip[®] SPE we used for cleaning up was intended by the manufacturer only for purification of BPA from cola soft drinks (see application notes from Polyntell). For the first time, we have tested

the Affinimip[®] Bisphenol A SPE cartridges for cleaning up of other bisphenols from canned energy drink, because we hypothesized the three-dimensional network of the molecularly imprinted polymer synthesized for BPA could recognize and bind even other similar molecular structures. Indeed, in preliminary experiments we observed that also BPB, BPF, BADGE and BFDGE were bound by the cartridges and recovered with significant yields from different energy drinks; the pH value of the energy drinks tested was about 3, except for 3 samples, and was adjusted to about 4 to improve binding to the Affinimip cartridge. This clean up procedure introduces 5-fold sample concentration, removing at the same time most of the interfering compounds, thanks to the affinity binding based on the specific interactions between the molecular structure and the stationary phase of the cartridge. This is a significant improvement in respect to less specific interaction mechanisms, characteristic of other SPE techniques, such as adsorption and C-18 or C-8 reversed phase.

Then, we developed a chromatographic method for separation of these five bisphenols by UPLC with fluorescence emission detection. We selected a stainless steel column containing a stationary phase with an amide group embedded in an 18-carbon chain, linked to 2.7 μm fused-core particles, that allowed for good retention and improved selectivity of phenolic acidic compounds. The gradient elution of the bisphenols showed a complete separation of all compounds in about two minutes, with sharp peaks (ca. 0.2 min base peaks); in Fig. 1a the fluorescence chromatogram of a standard mix at 5.0 ng/mL is reported. BFDGE showed a distinctive three peak pattern in all chromatograms, because of three diastereomers, that is *o,o'*-BFDGE, *o,p'*-BFDGE, and *p,p'*-BFDGE; the peak in the middle is the highest and was selected for quantitative analysis.

3.2. Method validation

The method was in-house validated by cleaning up and analyzing uncontaminated energy drink samples spiked at two different levels, that is 2.0 ng/mL and 10.0 ng/mL. Three replicates were carried out during each working session, in two different days, for all spiking levels; the samples spiked at 10.0 ng/mL were diluted 1:1 in volume with methanol prior to UPLC-FLD analysis, to fall within calibration curves.

In Fig. 1b is shown a process blank, obtained by cleaning up a water sample on Affinimip[®] Bisphenol A SPE during a working session, to evaluate interference from the solvents, reagents and cartridge; as can be seen, the area where the single bisphenol peaks are eluted is not interfered, accounting for method specificity. The linearity of fluorescence detector was verified within the concentration ranges of 1.0–50.0 ng/mL. The chromatogram of a sample spiked at 2.0 ng/mL is reported in Fig. 1c; all bisphenols are recovered from the matrix, showing resolved chromatographic peaks. The results regarding method performance are reported in Table 1. Mean recoveries ($n = 6$) ranging from 78% to 94% prove the trueness is very high, even at low concentrations, apart from BPF that showed recovery rates at 52–58%. This proves the molecularly imprinted polymer Affinimip[®] is specific for BPA, but is effective for multi-residue purification of structurally related compounds such as BPF, BPB, BADGE and BFDGE. Precision data were very satisfactorily even at low concentrations; RSD values from 1.4 to 10.8% account for very good method repeatability ($n = 3$). Likely, within-laboratory reproducibility RSD values ranging from 4.7 to 9.9% ($n = 6$) account for very good precision between days. The LOQ for each compound was determined analyzing three uncontaminated energy drink samples spiked at 0.50 ng/mL and 1.0 ng/mL, respectively, and evaluating the presence of the corresponding chromatographic peak showing a signal-to-noise ratio

>10/1. We found out the LOQ = 0.50 ng/mL for all bisphenols studied; we calculated the LOD for each compound is 0.15 ng/mL.

3.3. Survey of contamination from bisphenols in canned energy drinks

The method developed and validated was applied for monitoring the contamination from bisphenols in canned energy drinks, collected from different supermarkets in Naples and its province (Southern Italy). During each working session an energy drink sample spiked at 10.0 ng/mL was analyzed, to verify the mean recoveries were comparable to the performance parameters of the method as calculated during validation.

Forty energy drink samples were analyzed; the results are reported in Table 2, showing that 22 samples (55.0%) were contaminated above the LOQs by one, two or three bisphenols. BPA was the most abundant contaminant, present in 17 energy drinks (42.5% of samples) at contamination levels ranging from 0.50 to 3.3 ng/mL; BADGE was determined in 9 energy drinks (22.5% of samples) in a wide range of concentrations, from 0.50 to 19.4 ng/mL. BPF was detected in 6 energy drinks (15.0% of samples), at lower concentrations, from 0.50 up to 1.3 ng/mL. BFDGE, characterized by a three-shaped chromatographic peak, was detected only in 4 samples (10.0% of samples) at concentrations close to the LOQ, while BPB was not detected at all. In 8 energy drinks we determined the simultaneous presence of a couple of bisphenols, that is BPA/BPF (3 samples), BPA/BFDGE (2 samples) and BPA/BADGE (3 samples). Two energy drinks were simultaneously contaminated by BPA, BPF and BADGE, for a total bisphenol content from 16.2 up to 22.0 ng/mL, and 1 sample contained BPA, BPF and BFDGE, for total bisphenol content at 5.2 ng/mL. There was no significant relationship between the concentrations of contaminants and the brand of energy drink, the pH values and the content of beverage in the can (500 mL or 250 mL). Two samples (Energy drink #16 and #17) from the same brand, purchased in one supermarket and bearing the same lot number, contained BADGE at high concentrations; however, other three samples from the same brand, purchased on different days and bearing different lot numbers, contained lower concentrations of BADGE, confirming that contamination does not depend on the brand. Probably, the level of contamination may be caused by production lines, or incorrect transport and storage conditions of that product batch. The frequency of contaminated samples, that is the percentage ratio between the number of contaminated samples and the number of analyzed samples, the mean concentrations, the median and the minimum and maximum values determined for each bisphenol in 40 samples of energy drinks, are reported in Table 3. For BPA, detected in 42.5% of samples, we determined mean contamination level at 1.2 ng/mL, with median value at 0.70 ng/mL in a concentration range between 0.50 – 3.3 ng/mL. For BADGE, contaminating 22.5% of samples, mean level at 4.7 ng/mL and median value at 1.4 ng/mL in a concentration range between 0.50 and 19.4 ng/mL, were observed. BPF and BFDGE were detected in a fewer energy drinks (15.0% and 10.0%, respectively). BPF was determined at 0.82 ng/mL mean level (median value at 0.65 ng/mL in a concentration range between 0.50 and 1.3 ng/mL), while BFDGE was measured at 0.55 ng/mL mean level (median at 0.55 ng/mL in a range between 0.50 and 0.60 ng/mL). There are few data in the literature about the contamination in energy drinks from bisphenols. In a study carried out in the USA (Liao & Kannan, 2013), BPA and other analogue bisphenols, such as BPF, BPS, BPP were detected in canned food and a few drinks, but no information was reported about energy drinks. The BPA contamination levels we measured in our survey are comparable to those determined by Geens in four energy drinks from Belgium, that ranged from 0.16 ng/mL to 4.79 ng/mL with a mean value of 2.29 ng/mL (Geens et al., 2010). In a survey to determine only BPA levels in canned beverages from

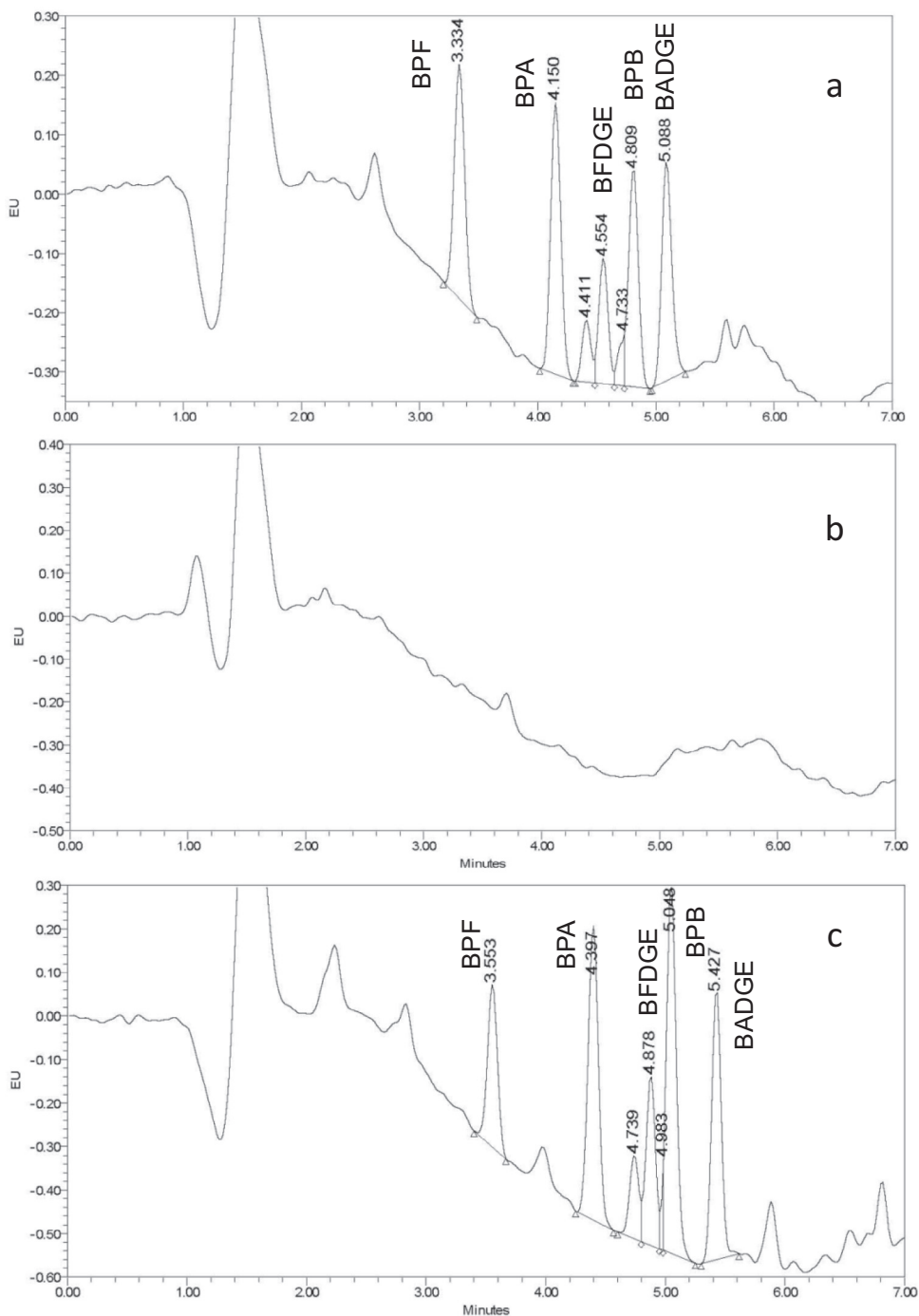


Fig. 1. The UPLC-FLD chromatograms of: (a) a standard mix of bisphenols at 5.0 ng/mL in methanol; (b) a process blank; (c) an energy drink sample spiked at 2.0 ng/mL.

Table 1
Method mean percentage recoveries, repeatability and within-laboratory reproducibility.

Compound	Spiking level, ng/mL	Mean percentage recovery, % (n = 6)	Repeatability RSD, % (n = 3)	Within-laboratory reproducibility RSD, % (n = 6)
BPF	2.0	58	4.8–1.9	6.0
	10.0	52	6.3–4.3	8.6
BPA	2.0	93	3.1–10.4	9.9
	10.0	78	10.8–2.5	7.7
BFDGE	2.0	82	8.1–5.5	6.3
	10.0	89	7.8–4.0	9.0
BPB	2.0	88	3.8–8.1	7.0
	10.0	94	7.9–2.0	8.1
BADGE	2.0	87	2.3–1.4	4.7
	10.0	91	4.2–2.2	7.0

Table 2

Contamination levels of bisphenols measured in 40 samples of energy drink from Southern Italy market.

Sample	BPF, ng/mL	BPA, ng/mL	BFDGE, ng/mL	BPB, ng/mL	BADGE, ng/mL
Energy drink #1	<LOQ	0.7	<LOQ	<LOQ	<LOQ
Energy drink #2	0.5	0.5	<LOQ	<LOQ	<LOQ
Energy drink #3	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Energy drink #4	<LOQ	<LOQ	<LOQ	<LOQ	0.7
Energy drink #5	<LOQ	0.6	0.5	<LOQ	<LOQ
Energy drink #6	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Energy drink #7	<LOQ	0.7	0.5	<LOQ	<LOQ
Energy drink #8	<LOQ	0.9	<LOQ	<LOQ	<LOQ
Energy drink #9	<LOQ	1.3	<LOQ	<LOQ	<LOQ
Energy drink #10	<LOQ	<LOQ	0.6	<LOQ	<LOQ
Energy drink #11	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Energy drink #12	0.5	0.7	<LOQ	<LOQ	<LOQ
Energy drink #13	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Energy drink #14	<LOQ	<LOQ	<LOQ	<LOQ	0.5
Energy drink #15	<LOQ	0.5	<LOQ	<LOQ	2.5
Energy drink #16	0.6	2.2	<LOQ	<LOQ	13.4
Energy drink #17	0.7	1.9	<LOQ	<LOQ	19.4
Energy drink #18	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Energy drink #19	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Energy drink #20	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Energy drink #21	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Energy drink #22	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Energy drink #23	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Energy drink #24	<LOQ	<LOQ	<LOQ	<LOQ	1.4
Energy drink #25	<LOQ	<LOQ	<LOQ	<LOQ	3.1
Energy drink #26	<LOQ	1.2	<LOQ	<LOQ	0.7
Energy drink #27	<LOQ	1.2	<LOQ	<LOQ	0.6
Energy drink #28	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Energy drink #29	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Energy drink #30	1.3	2.6	<LOQ	<LOQ	<LOQ
Energy drink #31	1.3	3.3	0.6	<LOQ	<LOQ
Energy drink #32	<LOQ	0.6	<LOQ	<LOQ	<LOQ
Energy drink #33	<LOQ	0.7	<LOQ	<LOQ	<LOQ
Energy drink #34	<LOQ	0.5	<LOQ	<LOQ	<LOQ
Energy drink #35	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Energy drink #36	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Energy drink #37	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Energy drink #38	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Energy drink #39	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Energy drink #40	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

Table 3

The frequency of contaminated samples, the mean concentration, median and minimum and maximum values for BPA, BADGE, BPF and BFDGE in energy drinks.

	Mean, ng/mL	Median, ng/mL	Min–Max range, ng/mL	Frequency of contaminated samples
BPA	1.2	0.70	0.50–3.3	42.5%
BADGE	4.7	1.4	0.50–19.4	22.5%
BPF	0.82	0.65	0.50–1.3	15.0%
BFDGE	0.55	0.55	0.50–0.60	10.0%

Canadian market by gas-chromatography/mass spectrometry (GC/MS) analysis, Cao et al. (2009) reported this compound was detected in 11 out of 12 samples of energy drinks; contamination levels ranged from 0.072 µg/L to 4.5 µg/L with a mean value at 1.07 µg/L. More recently, Cunha et al. (2011) described a survey to monitor levels of BPA and BPB in infant formulas and canned beverages from Portuguese market by GC/MS analysis; they reported BPA contaminated 7 energy drinks in a concentration range from 0.15 µg/L to 3.24 µg/L (mean value at 1.28 µg/L) while BPB was determined in 3 samples at 0.08 µg/L mean level.

4. Conclusion

An effective and sensitive multi-residue method was developed and validated to analyze five bisphenols in energy drinks, by clean up on molecularly imprinted polymers cartridge and UPLC-FLD determination. For the first time, the Affinimip® Bisphenol A SPE cartridges were successfully used for simultaneous purification of

several bisphenols, suggesting they could be tested also for other food and beverages. The UPLC separation of all compounds in 15 min is very effective, because all the analytes are resolved and can be quantified; moreover, very low LOQs were attained, down to 0.50 ng/mL for BPA, BPF, BPB, BFDGE and BADGE.

The survey showed that 42.5% of energy drinks from the local market in Naples and its province were contaminated by BPA, but at very low concentrations. Also BADGE contaminated several samples (22.5%), showing maximum level at 19.4 ng/mL; anyway, no sample resulted not compliant to the migration limits set by the European Union at 0.6 mg/kg and 9.0 mg/kg, respectively. The mean contamination levels we determined are similar to those reported by other authors. These results suggest that a low intake of bisphenols can derive from energy drinks; a risk assessment should consider also the contribution from this kind of beverage in habitual consumers. The concern about possible cocktail effects due to the contemporary contamination by BPA and its analogues has not yet been addressed.

Conflict of interest statement

The authors declare they have any financial or personal conflict of interest, neither actual neither potential, that could influence this work.

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