Determination of bisphenols in beverages by mixed-mode solid-phase extraction and liquid chromatography coupled to tandem mass spectrometry☆

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ABSTRACT

Facing growing restrictions on the use of bisphenol A in food contact materials, several bisphenol analogs are arising as major alternatives to replace this chemical in most of its applications. This work reports a simple and robust method based on mixed-mode solid-phase extraction and stable-isotope dilution liquid chromatography–tandem mass spectrometry for the analysis of bisphenol A and its main analogs – bisphenol S, 4,4′-sulfonylbis(2-methylphenol), bisphenol F, bisphenol E, bisphenol B, bisphenol Z, bisphenol AF, bisphenol AP, tetrabromobisphenol A and bisphenol P – in alcoholic and non-alcoholic beverages. Mixed-mode solid-phase extraction, combining cationic exchange and reversed-phase mechanisms, was optimized to provide a selective extraction and purification of the target analytes. Derivatization of bisphenols with pyridine-3-sulfonyl chloride allowed increasing their ionization efficiency by electrospray ionization. Validation of the proposed method was performed in terms of selectivity, matrix effects, linearity, precision, measurement uncertainty, trueness and limits of detection. Satisfactory repeatability and intermediate precision were obtained; the related relative standard deviations were $\leq 9\%$ and $\leq 12\%$, respectively. The relative expanded uncertainty ($k = 2$) was below 20% for all bisphenol analogs and the trueness of the method was demonstrated by recovery experiments. Limits of detection (LOD) ranged from $1.6\,ng\,L^{-1}$ to $27.9\,ng\,L^{-1}$ for all compounds. Finally, several canned and non-canned beverages were analyzed to demonstrate the applicability of the method. Only bisphenol A and three bisphenol F isomers were detected in any of the samples. Bisphenol A concentration ranged from <LOD to $1.26 \pm 0.09\,\mu g\,L^{-1}$, whereas 4,4′-bisphenol F varied from <LOD to $1.00 \pm 0.08\,\mu g\,L^{-1}$. To the best of our knowledge, 2,2′-bisphenol F and 2,4′-bisphenol F were reported for the first time in beverages, at concentration levels up to 0.12 and $0.51\,\mu g\,L^{-1}$, respectively.

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1. Introduction

Bisphenol A (BPA), a chemical widely used in the manufacture of polycarbonate plastics and epoxy resins, is gathering increasing attention due to its endocrine disrupting potential. BPA-containing materials are employed in a large variety of applications including food and liquid containers, kitchenware, inner linings of metal cans and bottle tops, surface coatings, toys, medical devices, dental fillings and cash register receipts, among others [1–4].

Facing growing restrictions on the use of BPA in food contact materials [5–8], the plastic and canning industries are moving fast to seek alternative chemicals which allow replacing BPA in many of its applications. Thus, over the past few years products labeled as "BPA-free", potentially containing BPA substitutes, are becoming frequent in store shelves [3,9]. These new compounds have been designed to resemble the physicochemical properties of BPA, and most of them belong to the same chemical family of *p,p′*-bisphenols (Fig. S1, supporting information). Among these structural analogs, bisphenol S (BPS), bisphenol F (BPF), bisphenol B (BPB) and bisphenol AF (BPAF) are apparently the major BPA replacements [10,11].

The determination of these emerging contaminants in foodstuffs requires the development and validation of appropriate and robust analytical methods. To date, nevertheless, few methods have been developed for the analysis of BPA analogs in this kind of samples in general, and in beverages, other than canned soft drinks,

in particular. Liquid–liquid extraction and solid-phase extraction (SPE) are the most common techniques for the extraction of BPA from liquid samples, and they have been also applied for the extraction of some BPA analogs from beverages [11–13]. Gallart-Ayala et al. [12] developed an on-line C18 SPE method coupled to liquid chromatography–tandem mass spectrometry (LC–MS/MS) for the determination of BPA, BPF, bisphenol E (BPE), BPB and BPS in canned soft drinks. Recently, molecular imprinted polymers (MIPs) have been used as sorbent for SPE of BPA, BPF, BPS, BPB, BPAF, tetrachlorobisphenol A and tetrabromobisphenol A (TBBPA) from beverages and canned foods [14]. MIPs sorbents normally allow obtaining a high degree of selectivity based on specific recognition of the template molecules. Nevertheless, the presence in some beverages (wine, beer, juices, tea, coffee, etc.) of high amounts of phenolic compounds, such as flavonoids and phenolic acids, presenting similar structures to the template molecules, may reduce the extraction efficiency and selectivity of MIPs. Other techniques, such as dispersive liquid-liquid microextraction [15] and stir bar sorptive extraction [16] have also been applied to the extraction of some bisphenol analogs.

As regards the determination of bisphenols in food and beverages, most of methods are based on gas chromatography coupled to mass spectrometry (GC–MS) following a derivatization step with acetic anhydride [13,15,16], *N,O*-bis(trimethylsilyl)trifluoroacetamide [16] or *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide [17], although some authors have also used LC–MS/MS [11,12,18–20].

In a very recent study conducted by our group [21], a sensitive LC–MS/MS method based on ultrasound assisted extraction preceded by sample disruption with sand and selective clean-up by primary secondary amine (PSA) SPE was proposed for the analysis of thirteen bisphenols in complex solid food samples. Due to the presence of strongly electronegative fluorine atoms on the phenyl ring, pentafluorophenylpropyl HPLC stationary phase was shown to provide an efficient separation of all the studied bisphenols. Indeed, baseline resolution was achieved for the three BPF isomers, which made possible their individual quantification.

The objective of the present work was to develop and validate a robust method based on stable-isotope dilution (SID) LC–MS/MS for the sensitive determination of BPA and its main analogs – BPS, 4,4′-sulfonylbis(2-methylphenol) (DMBPS), BPF, BPE, BPB, bisphenol Z (BPZ), BPAF, bisphenol AP (BPAP), TBBPA and bisphenol P (BPP) – in both alcoholic and non-alcoholic beverages. Mixed-mode SPE, combining cationic exchange and reversed-phase mechanisms, was optimized to provide a selective extraction of the target analytes. Derivatization of bisphenols with pyridine-3-sulfonyl chloride allowed increasing their ionization efficiency by electrospray ionization (ESI), thus improving the limits of detection (LODs). Validation of the proposed method was performed in terms of selectivity, linearity, precision, measurement uncertainty, trueness, LODs and matrix effects. Several canned and non-canned beverages purchased from different supermarkets in Belgium were finally analyzed to demonstrate the applicability of the method to commercial samples.

2. Materials and methods

2.1. Standards, reagents and materials

Bisphenol A (≥99%), bisphenol AF (97%), bisphenol AP (99%), 2,2′-bisphenol F (>98%), 4,4′-bisphenol F (98%), bisphenol P (99%), bisphenol S (98%), bisphenol Z (98%), 4,4′-sulfonylbis(2-methylphenol) (97%), tetrabromobisphenol A (≥97%) and pyridine-3-sulfonyl chloride (95%) were purchased from Sigma–Aldrich (Diegem, Belgium). Bisphenol B (>98%), bisphenol E (>98%) and 2,4′-bisphenol F (>98%) were obtained from TCI (Zwijndrecht, Belgium).

Bisphenol A-$^{13}C_{12}$ (99.2% ^{13}C, 98% chemical purity), 4,4′-bisphenol F-D_{10} (96.8% D, 98% chemical purity) and bisphenol S-$^{13}C_{12}$ (99.6% ^{13}C, 97% chemical purity) were purchased from Toronto Research Chemicals (North York, Canada). Bisphenol AF-3,3′,5,5′-D_4 (99.4% D, 99% chemical purity) was obtained from C/D/N Isotopes (Pointe-Claire, Canada) and $^{13}C_{12}$-tetrabromobisphenol A (99% ^{13}C, 50 μg/mL in methanol) was from Cambridge Isotope Laboratories (Andover, MA,USA). Chemical structures of the analyzed compounds are shown in Fig. S1 (supporting information). Individual stock solutions of each analyte (ca. 1000 mg L^{-1}) and a mixture of them were prepared in methanol. Working standard solutions were made by appropriate dilution in methanol and then stored in amber glass vials at −20 °C.

All organic solvents (acetonitrile, ethyl acetate and methanol) were HPLC or LC/MS grade and all other chemicals were analytical reagent grade. Ultrapure water was produced using a Milli-Q Gradient water purification system from Merck Millipore (Bedford, MA, USA). Formic acid (98–100%), hydrochloric acid (37%), ammonium hydroxide (28–30%), sodium hydroxide and anhydrous sodium carbonate were purchased from Merck (Darmstadt, Germany).

SPE cartridges Oasis HLB (150 mg, 6 mL), Oasis MAX (150 mg, 6 mL) and Oasis MCX (150 mg, 6 mL) were purchased from Waters (Milford, MA). Regenerated cellulose membrane syringe filters (13 mm, 0.20 μm) were purchased from Grace (Lokeren, Belgium).

2.2. Samples

All beverages were purchased from local supermarkets in Belgium between April and December 2014. For method validation, two different pooled samples (alcoholic and non-alcoholic drinks) were prepared from glass bottled drinks, previously analyzed in order to guarantee the absence of bisphenols. The alcoholic pool consisted of malt whisky (40% alcohol by volume), blonde beer (5.2% alcohol) and red wine (13% alcohol) (1:1:1, v/v/v), whereas the non-alcoholic pool was composed by a cola soft drink, mineral water and English breakfast tea (1:1:1, v/v/v). The tea was infused as recommended on the label by manufacturer, i.e. 2 g/100 mL boiling water for 5 min.

2.3. Sample preparation

Carbonated beverages (beer, sparkling wine, sparkling water and soft drinks) were degassed in an ultrasound bath for 1 h prior to the extraction. Under optimized conditions, 10 mL sample were spiked with 2.5 ng of isotope-labeled standards in methanol (100 μL, 25 μg L^{-1}) and then extracted using Oasis MCX SPE cartridges (150 mg, 6 mL). The SPE cartridges were previously conditioned with 5 mL of ethyl acetate followed by 5 mL of methanol and by 5 mL of ultrapure water/formic acid (99:1, v/v).

For non-alcoholic drinks, 10 mL sample were directly loaded onto the SPE cartridges, whereas alcoholic drinks were first diluted 1:1 (v/v) with water/formic acid (99:1, v/v), and the resulting 20 mL of diluted sample were passed through the cartridge. After loading the sample, the cartridge was rinsed with 6 mL of water/formic acid (99:1, v/v) followed by 10 mL of a mixture of methanol/water/formic acid (30:69:1, v/v/v). The sorbent was then dried under moderate vacuum for 2 min and the analytes were finally eluted with 10 mL of a mixture of methanol/ethyl acetate/formic acid (8:91:1, v/v/v).

For juices containing pulp, 10 mL sample were transferred to a 50 mL polypropylene centrifuge tube and 5 mL of acetonitrile/methanol (80:20, v/v) were added. After vortex shaking for 10 s, the tube was immersed in an ultrasonic water bath Branson 2510 (Emerson, Dietzenbach, Germany) and sample was extracted at 40 kHz of ultrasound frequency at 30 ± 3 °C for 20 min. The resulting slurry was centrifuged at 3000 RCF for 5 min at 10 °C (Eppendorf