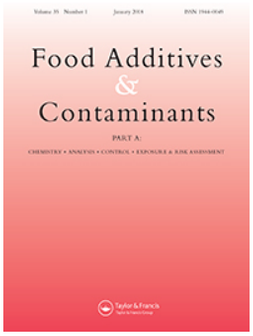

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Solid phase extraction of large volume of water and beverage samples to improve detection limits for GC-MS analysis of bisphenol A and four other bisphenols

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ABSTRACT

Solid phase extraction (SPE) of large volumes of water and beverage products was investigated for the GC-MS analysis of bisphenol A (BPA), bisphenol AF (BPAF), bisphenol F (BPF), bisphenol E (BPE), and bisphenol B (BPB). While absolute recoveries of the method were improved for water and some beverage products (e.g. diet cola, iced tea), breakthrough may also have occurred during SPE of 200 mL of other beverages (e.g. BPF in cola). Improvements in method detection limits were observed with the analysis of large sample volumes for all bisphenols at ppt (pg/g) to sub-ppt levels. This improvement was found to be proportional to sample volumes for water and beverage products with less interferences and noise levels around the analytes. Matrix effects and interferences were observed during SPE of larger volumes (100 and 200 mL) of the beverage products, and affected the accurate analysis of BPF. This improved method was used to analyse bisphenols in various beverage samples, and only BPA was detected, with levels ranging from 0.022 to 0.030 ng/g for products in PET bottles, and 0.085 to 0.32 ng/g for products in cans.

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Bisphenol A; bisphenol AF; bisphenol F; bisphenol E; bisphenol B; SPE; GC-MS; water; beverage

Introduction

Bisphenol A (BPA) is a monomer in the production of epoxy resins which are frequently used in the internal coating for food and beverage cans to protect the contents from direct contact with metal. Residues of BPA in these coatings can migrate into foods, especially at elevated temperatures. Due to consumer concerns regarding the health effects of oestrogenic activity of BPA, industry has already abandoned the use of BPA-containing packaging for foods such as liquid infant formula products (Health Canada 2014) and made efforts to reduce BPA levels in other foods such as canned fish products (Cao et al. 2015). Recently, Soto et al. (2017) assessed the oestrogenicity of tetramethyl bisphenol F (TMBPF) and demonstrated its potential as an alternative for BPA in a new food-contact coating. Thus, BPA levels in foods are expected to decrease, and more sensitive methods are needed to analyse BPA more accurately, especially in foods with already low BPA levels, such as beverage products. Exposure assessment will also benefit from the more sensitive methods, not only for BPA but also for BPA alternatives, since when using detection limits for non-detects (instead of zero) during exposure assessment, the high detection limits from less sensitive methods will overestimate the exposure, while the exposure estimated using the lower detection limits from more sensitive methods will be much more accurate.

Levels of BPA in beverage products are very low in general, at sub-ppb for most canned beverage and even lower (below detection limits) for beverages in plastic and glass containers (Cao et al. 2010). Beverage samples are less complex compared to solid foods, and can be directly concentrated on solid phase extraction (SPE) cartridges followed by LC-MS (Gallart-Ayala et al. 2011; Yang et al. 2014; Regueiro and Wenzl 2015) or GC-MS analysis with derivatisation (Cao et al. 2009; Geens et al. 2010) with sub-ppb detection limits. Since only a small portion of sample (1 – 10 mL) was analysed in the available studies on BPA in beverages, it is feasible to further improve the detection limits of the methods by concentrating more beverage samples on the SPE cartridges, but this has not been investigated previously. Biles et al. (1997) analysed BPA in water by

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concentrating one litre of water sample on an SPE cartridge with detection limit < 0.05 μg/L, but information on method performance is not available. In this study, SPE of large volumes of water and various beverage samples followed by GC-MS analysis was investigated for the analysis of BPA and several other bisphenols including bisphenol AF (BPAF), bisphenol F (BPF), bisphenol E (BPE), and bisphenol B (BPB), and the improved method was used to analyse bisphenols in selected beverage products of cans and plastic bottles.

Materials and methods

Sample collection

Four beverage products (cola, diet cola, ginger ale, iced tea) in 2-L plastic PET bottles and five beverage products (ginger ale, cola A, cola B, diet cola A, diet cola B) in metal cans were collected from a local store in Ottawa in April 2017. The collection of these products was for the purpose of method evaluation only instead of a survey. They were stored at room temperature before analysis.

Materials and reagents

Acetonitrile (HPLC grade), methanol (HPLC grade) were purchased from J.T. Baker (Phillipsburg, N.J.). Toluene (glass distilled), potassium carbonate (ACS grade), iso-octane (pesticide-residue grade), MTBE (methyl t-butyl ether, 99.9%), Na_2SO_4 (anhydrous, ACS grade), 1-pentanol (99%), dodecane (99%) were purchased from Sigma-Aldrich (Oakville, ON). Acetic anhydride (ACS grade) and H_3PO_4 (85% HPLC grade) were purchased from Fisher (Ottawa, ON). Individual 100 μg/mL solutions of unlabelled bisphenols (A, AF, B, E, and F) in acetonitrile and 100 μg/mL solutions of ring-$^{13}C_{12}$ labelled (99%) bisphenols (A, B, AF, and F) in acetonitrile were purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA). The C18 SPE cartridges (500 mg/6 cc) were purchased from Varian (Mississauga, ON.).

Separate composite solutions of labelled and unlabelled bisphenols were prepared in acetonitrile, and stored at 4°C. Calibration standard solutions of derivatised bisphenols were prepared by adding different volumes (2, 10, 20, 30, and 40 μL) of composite bisphenol standard solutions (2.5 ng/μL) and 10 μL composite labelled bisphenol standard solutions (2.5 ng/μL) to 22-mL vials, and by going through the same derivatization procedure as the samples.

Sample extraction, derivatization and analysis

Details of the method for sample extraction and derivatization can be found elsewhere (Cao et al. 2009). Briefly, water or beverage sample was spiked with 10 μL of 2.5 ng/μL labelled composite bisphenol solution, mixed and loaded to the C18 SPE cartridge (500 mg/6 cc) which was conditioned with 13 mL of methanol and 13 mL of H_2O. The C18 cartridge was rinsed with 6.5 mL of H_2O and 13 mL of 30% MeOH/H_2O, and eluted with 6.5 mL of 50% acetonitrile in water. The extract was concentrated and derivatised to di-esters using acetic anhydride, and the di-ester derivatives of bisphenols were extracted with iso-octane followed by MTBE for analysis.

An Agilent 7890 gas chromatograph (GC) coupled to a 5975 mass selective detector (MSD) (Agilent Technologies, Palo Alto, CA) was used for the analysis. The chromatographic separation was achieved on a DB-5MS capillary column (30 m × 0.25 mm × 0.25 μm, Agilent Technologies) using helium as the carrier gas (1.2 mL/min). The injector temperature was 300°C. One microlitre of the extract was injected in splitless mode. The GC oven temperature program was set at an initial temperature of 100°C for 1 min., raised to 300°C at 15°C/min, and held for 4 min, with a total analysis time under 19 min. A solvent delay time of 8 min was used to protect the ion multiplier of the MSD instrument from saturation. The MSD was operated with electron impact ionization in selected ion monitoring (SIM) mode. The GC–MSD interface and MSD source temperatures were 280 and 230°C, respectively. Retention times (RT), the quantification (target) and confirmation (qualifier) ions selected for labelled and unlabelled derivatised bisphenols, and the ion ratios, are summarised in Table 1. Dwell time was 35 ms for each ion.

Identification and confirmation of the bisphenols in the samples were based on the retention times (± 0.05 min) and the peak area ratios (± 25%). Concentrations of the bisphenols in samples were calculated by isotope dilution except for BPE (BPF-$^{13}C_{12}$ was used for internal standard calibration). Each batch

Table 1. Target and qualifier ions selected for the GC-MS SIM analysis of the derivatised bisphenols.

Bisphenols	RT (min)	Target ion (T)	Qualifier ion (Q1)	Ion ratio (Q1/T, %)	Qualifier ion (Q2)	Ion ratio (Q2/T, %)
Bisphenol AF (BPAF)	11.25	267	336	86.8	378	28.0
Bisphenol F (BPF)	12.36	200	107	41.4	242	29.6
Bisphenol E (BPE)	12.62	199	214	40.2	256	20.7
Bisphenol A (BPA)	12.89	213	228	25.8	270	19.6
Bisphenol B (BPB)	13.37	213	119	8.92		
BPAF-$^{13}C_{12}$	11.25	279	348	86.4		
BPF-$^{13}C_{12}$	12.36	212	113	41.7		
BPA-$^{13}C_{12}$	12.89	225	240	26.0		
BPB-$^{13}C_{12}$	13.37	225	125	9.07		

of analysis included three method blanks, spiked and unspiked control samples, and five calibration standard solutions. The results of all samples were corrected for the trace concentrations of the bisphenols in blanks.

Results and discussion

One of the concerns with concentrating large amounts of beverage samples on SPE cartridges is the matrix effect. While more analytes of interests can be concentrated from large amounts of sample, interferences may also be concentrated and eluted at the same time, and thus the method sensitivity may not be increased proportionately to sample volume. It is also very important to make sure breakthrough does not occur for the analytes of interest, which will affect not only the sensitivity of the method but also the accuracy of results if isotope dilution is not used.

Due to the wide varieties of pre-packaged beverage products available on the Canadian market, it was not feasible to investigate each one of them in this study, thus only four types of commonly consumed beverage products were selected instead as examples: cola, diet cola, ginger ale, and iced tea. Water was also included, not only because it is a commonly consumed beverage, but also it has the least matrix effect and thus is suitable as a control. In order to investigate the method performance of SPE of large volumes of beverage products containing bisphenols at lower concentrations, only beverage products in plastic bottles (PET) instead of cans were used in the current study since they usually do not contain BPA at levels above the detection limits of the available methods (using less than 10 mL of samples). SPE of water and the four beverage products was investigated at three sample volumes: 50, 100, and 200 mL, at the same spiking level (0.1 μg/L) for BPA and the other four bisphenols. Method detection limits (MDL) were estimated as 10 times the signal-to-noise (S/N) ratios. The method was also evaluated with 100 and 200 mL of water spiked with 2.5 ng of bisphenols at concentrations of 0.025 and 0.0125 μg/L, respectively. Typical GC-MS ion chromatograms of the standard solution and beverage samples are shown in Figure 1.

As shown in Table 2 and Table 3, good method accuracy and precision were observed for all analytes in the beverages tested at spiking level of 0.1 μg/L, with recoveries averaged for all analytes ranging from 103 to 107% and relative standard deviations averaged for all analytes from 2.6 to 3.7%. For the 100 and 200 mL water spiked with the same amount of bisphenols (2.5 ng), 200 mL of water demonstrated better precision (relative standard deviations from 0.82 to 4.4%) than the 100 mL of water (relative standard deviations from 8.6 to 11.3%). Method detection limits (MDL) are at lower ppt (pg/g) levels in general, even sub-ppt levels with sample volumes of 200 mL for some analytes. No interferences were observed in the analysis of BPA and the other four bisphenols in water; the method detection limits decreased proportionately with the increase of sample volumes from 50 to 200 mL, for example, with 1.2, 0.62, and 0.29 pg/g for BPA in 50, 100, and 200 mL of water, respectively. The method detection limits for all analytes in the other beverage products were also improved with larger sample volumes, though not always in proportion to the volumes. For example, the corresponding method detection limits for BPA at sample volumes of 50, 100, and

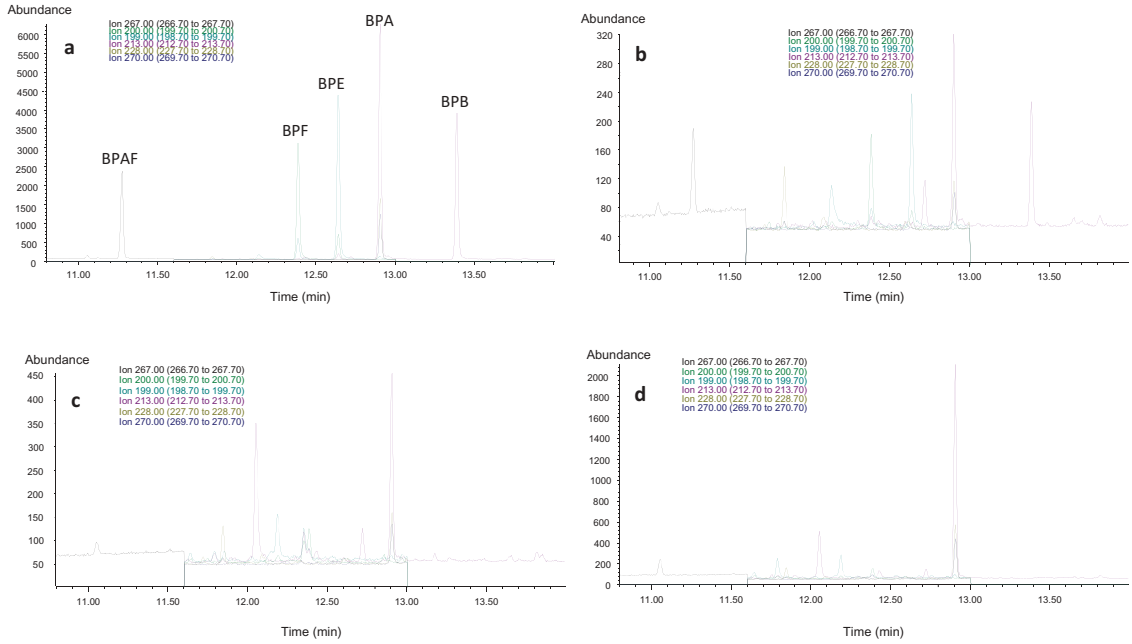

Figure 1. Typical GC-MS chromatograms of bisphenols from standard (a), water spiked at 2.5 ng (b), cola in PET bottle (c), and diet cola in can (d).

200 mL were 1.6, 1.1, 1.0 pg/g ginger ale, respectively; 1.7, 1.4, 1.1 pg/g diet cola, respectively; and 1.4, 1.3, 1.2 pg/g iced tea, respectively. This is likely due to the more complex matrices of these samples. As more analytes are being concentrated with increasing sample volume the background noise levels are also being amplified at the same time. Significant interferences with BPF analysis were observed at sample volumes of 100 and 200 mL for all four beverage products, and accurate quantitative analysis for this particular bisphenol analogue is thus not possible. The higher method detection limits for BPAF in ginger ale at sample volumes of 50 and 100 mL are due to lower absolute recoveries which will be discussed later.

In all the experiments conducted in this study, all samples were spiked with the same amount of labelled bisphenols (25 ng each), with a concentration of approximately 0.2083 ng/μL in the final extract (120 μL). Figure 2 shows the responses of labelled bisphenols from different volumes of water and beverage samples after solid phase extraction. It can be seen that the responses of the four labelled bisphenols (BPAF-^{13}C, BPF-^{13}C, BPA-^{13}C, and BPB-^{13}C) increase with increasing sample volumes of water, diet cola, and iced tea, indicating that SPE of more sample volume of these beverage products improves the absolute recoveries and thus the sensitivity of the method. However, for ginger ale, responses of BPF-^{13}C, BPA-^{13}C, and BPB-^{13}C were found to increase from SPE of 50 mL to 100 mL, and then decrease from 100 mL to 200 mL, indicating that breakthrough may have occurred during SPE of 200 mL of this beverage sample, or SPE of these BPA analogues may have been affected by this sample matrix. This was also observed for BPF-^{13}C during SPE of the cola beverage, where the response of BPF-^{13}C after SPE of a 200 mL sample was only 22% of SPE of a 50 mL sample. Compared to the other beverage samples, the responses of BPAF-^{13}C after SPE of 50 and 100 mL of ginger ale are much lower, possibly due to matrix effect, and this affected the method detection limits of BPAF with SPE of 50 and 100 mL of this sample, as mentioned earlier.

To demonstrate the improved detection limits with SPE of large volumes of beverage samples for the analysis of bisphenols, this method was used to analyse the four beverage products in PET bottles for BPA and the other four bisphenols at three different sample volumes: 50, 100, and 200 mL. One of the approaches to validate method accuracy is to vary the sample weight taken for analysis (Dabeka and Hayward 1993). For a working and reliable method, the results obtained from analysis of different amounts of the same sample should agree well. Using this method, BPAF, BPF, BPE, and BPB were not detected in any

Table 2. Recoveries and method detection limits (MDL) for bisphenols in different volumes of water and beverages spiked at 0.1 µg/L.

Bisphenol	Sample volume (mL)	Amount (ng) of bisphenol spiked	Water		Ginger Ale		Diet Cola		Cola		Iced Tea	
			Recovery (%), mean ± SD (n = 3)	MDL (pg/g)	Recovery (%), mean ± SD (n = 3)	MDL (pg/g)	Recovery (%), mean ± SD (n = 3)	MDL (pg/g)	Recovery (%), mean ± SD (n = 3)	MDL (pg/g)	Recovery (%), mean ± SD (n = 3)	MDL (pg/g)
BPAF	50	5	111.5 ± 0.59	3.1	110.7 ± 3.0	15	107.0 ± 5.5	5.2	108.2 ± 4.8	4.4	104.7 ± 0.2	3.9
	100	10	107.8 ± 3.1	2.1	104.7 ± 4.1	8.5	104.2 ± 1.6	1.2	107.2 ± 3.2	2.5	105.3 ± 2.3	2.2
	200	20	107.8 ± 2.3	1.4	101.4 ± 2.1	0.98	102.7 ± 0.87	0.76	103.6 ± 2.9	1.3	103.3 ± 1.9	1.1
BPF	50	5	113.2 ± 6.2	2.4	110.1 ± 4.7	3.5	92.4 ± 10.0	2.6	109.8 ± 5.8	3.6	111.8 ± 3.4	1.6
	100	10	106.9 ± 3.8	1.1	na		na		na		na	
	200	20	107.4 ± 2.2	0.63	na		na		na		na	
BPE	50	5	104.5 ± 2.7	3.0	102.1 ± 2.3	2.3	98.1 ± 5.5	4.0	100.8 ± 3.5	2.7	101.6 ± 2.5	2.5
	100	10	99.9 ± 4.2	1.3	102.2 ± 2.5	1.5	95.3 ± 1.9	3.1	100.7 ± 2.5	2.1	103.1 ± 1.6	1.5
	200	20	101.6 ± 2.1	0.58	98.5 ± 3.0	1.1	94.8 ± 3.4	1.9	97.0 ± 3.2	1.5	103.2 ± 1.6	1.0
BPA	50	5	113.6 ± 7.3	1.2	93.4 ± 6.5	1.6	106.9 ± 6.6	1.7	121.8 ± 13.0	2.1	108.7 ± 7.2	1.4
	100	10	106.1 ± 4.0	0.62	108.1 ± 5.2	1.1	107.7 ± 2.4	1.4	118.0 ± 2.0	1.2	108.5 ± 3.1	1.3
	200	20	108.7 ± 1.7	0.29	104.7 ± 4.3	1.0	107.2 ± 1.0	1.1	109.9 ± 3.1	0.92	103.9 ± 3.2	1.2
BPB	50	5	108.2 ± 2.1	1.1	111.3 ± 3.3	2.1	107.9 ± 4.2	3.0	107.5 ± 4.3	2.9	106.8 ± 1.3	1.7
	100	10	107.3 ± 4.4	0.85	106.2 ± 0.85	1.9	106.3 ± 0.51	1.3	105.2 ± 2.3	1.8	107.7 ± 4.4	1.1
	200	20	107.2 ± 1.5	0.39	102.4 ± 3.0	0.78	105.9 ± 1.0	1.0	103.9 ± 2.4	1.3	106.5 ± 1.8	0.77

*na: not available due to interference.

Table 3. Method performance for 100 and 200 mL of water spiked at 2.5 ng.

Bisphenol	Sample volume (mL)	Amount (ng) of bisphenol spiked	Recovery (%), mean ± SD (n = 3)	MDL (pg/g)
BPAF	100	2.5	101.7 ± 11.2	2.6
	200	2.5	107.6 ± 2.3	1.3
BPF	100	2.5	95.7 ± 10.8	1.5
	200	2.5	115.1 ± 4.9	0.69
BPE	100	2.5	89.0 ± 7.7	1.9
	200	2.5	94.3 ± 0.77	1.0
BPA	100	2.5	107.8 ± 11.1	4.2
	200	2.5	111.0 ± 4.9	2.1
BPB	100	2.5	99.4 ± 9.8	1.3
	200	2.5	110.5 ± 1.1	0.44

of the PET beverage samples. Therefore, only the results of BPA are shown in Table 4, and each result is the average of three replicate analyses. Levels of BPA in beverage products in PET bottles are usually very low, and below the detection limits of current available methods using fewer samples (< 10 mL). With this improved method, however, BPA was detected in three of the four beverage products in PET bottles. For ginger ale, the results from the analysis of three different sample volumes agreed very well, with an average of 0.022 ng/g. For cola A and iced tea, the results from the analysis of 100 and 200 mL samples also agreed very well, with an average of 0.026 and 0.030 ng/g, respectively. However, the results from the analysis of the 50 mL samples of both cola A (0.011 ng/g) and iced tea (0.014 ng/g) are much lower than those from the analysis of their 100 and 200 mL samples. This demonstrates that for some types of beverages the accuracy of results can be improved with the analysis of larger sample volumes. It should be mentioned that PET bottles do not contain BPA and the traces of BPA in the beverages in PET bottles are very likely from the pre-bottling and subsequent processes. Selection of these samples for analysis is based on the very low levels of BPA and thus to demonstrate the application of the developed method.

Five beverage products in metal cans were also analysed using a 50 mL sample of each. Similar to the beverage products in PET bottles, only BPA was detected, and the average results from three replicate analyses are shown in Table 4. The BPA levels in canned beverage products are about 10 times those in PET bottles, and very similar to the results from our previous study (Cao et al. 2009).

In summary, SPE of large sample volume can improve the detection limits and also the accuracy for the analysis of bisphenols in water and beverage

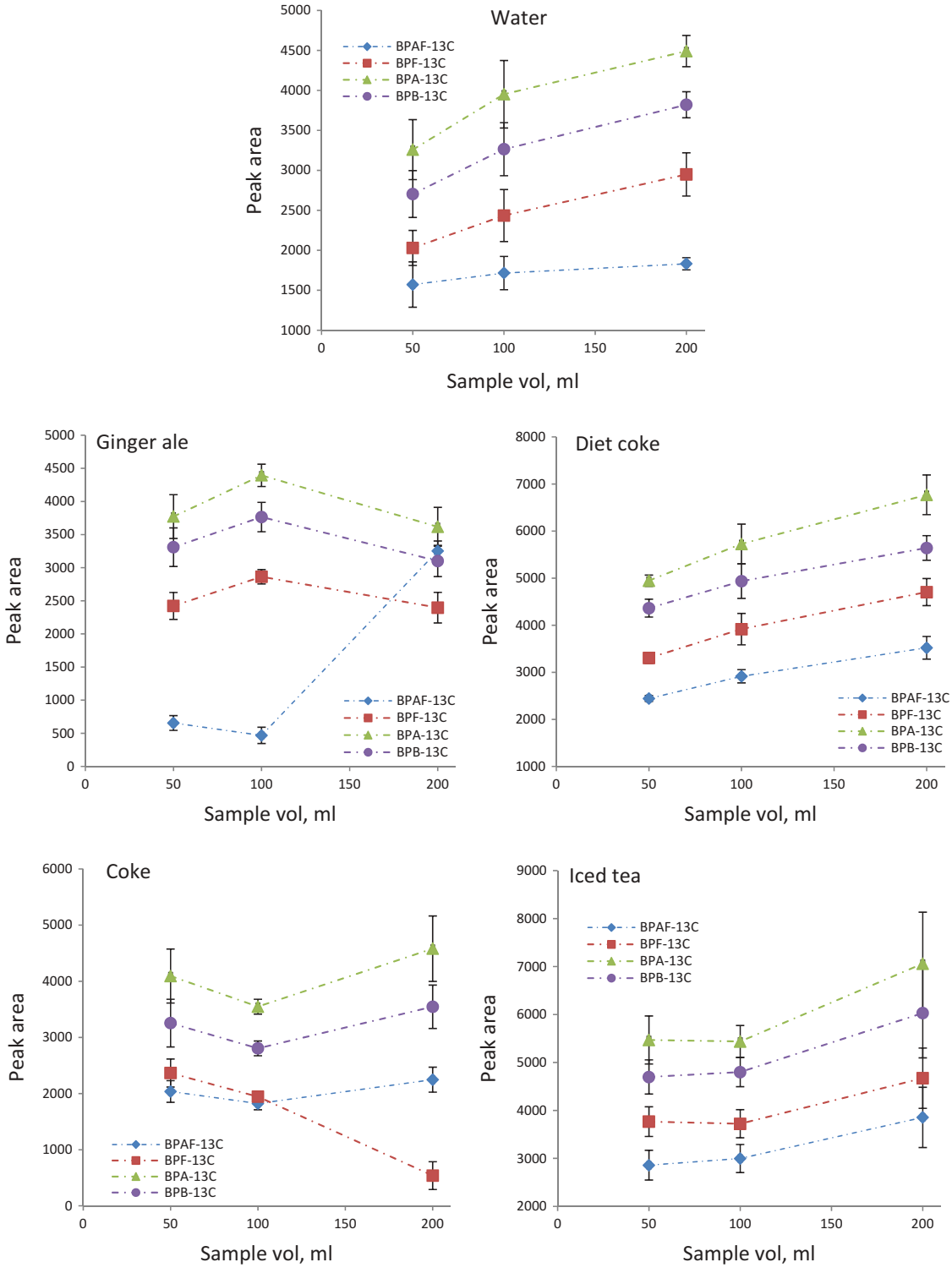

Figure 2. Responses of labelled bisphenols (25 ng) from different volumes of water and beverage samples after solid phase extraction.

products. For a simple matrix like water with almost no interferences, improvement in detection limits is proportional to sample volumes. However, for the more complex beverage products, improvement in detection limits may not necessarily be proportional to sample volumes for all bisphenols depending on the interferences and noise levels around the analytes. Matrix effects and interferences were also observed during SPE of larger volumes (100 and 200 mL) of the four beverage products, and affected the accurate

Table 4. Concentrations (ng/g) of BPA in various beverage samples.

Beverage sample	Container	Sample volume, mL	BPA mass, ng	BPA concentration, ng/g
Ginger ale	PET bottle	50	0.94	0.022
		100	2.17	0.022
		200	4.25	0.021
Cola A, diet	PET bottle	50	nd	
		100	nd	
		200	nd	
Cola A	PET bottle	50	0.56	0.011
		100	2.63	0.026
		200	5.20	0.025
Iced tea	PET bottle	50	0.75	0.014
		100	3.16	0.031
		200	5.99	0.029
Ginger ale	Metal can	50	4.26	0.085
Cola A	Metal can	50	10.0	0.20
Cola A, diet	Metal can	50	7.97	0.16
Cola B	Metal can	50	12.0	0.24
Cola B, diet	Metal can	50	15.3	0.32

nd: not detected.

analysis of some bisphenols (e.g. BPF). Thus this approach should be investigated and fully validated prior to its application to new beverage products.

Disclosure statement

No potential conflict of interest was reported by the authors.

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