Research Article

Simultaneous determination of nine bisphenol migrations in products related to sanitary and safety of drinking water by auto-solid phase extraction and ultra-performance liquid chromatography with photodiode array and fluorescence detector

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Abstract
Bisphenol analogues in drinking water and their migrations from products related to sanitary and safety of drinking water are potential threat to human health. In this study, a robust method for simultaneous determination of bisphenol A and its eight analogues was developed by an auto-solid phase extraction and ultra-performance liquid chromatography coupled with photodiode array and fluorescence detector. The efficiency of sample preparation was largely improved by optimizing the loading rate of solid phase extraction to 100 mL/min without reducing recovery. The nine bisphenol analogues were separated on an Acquity UPLC BEH C_{18} column in 13 min with methanol and water as the mobile phase. The limit of quantification for the nine bisphenol analogues ranged from 20 to 40 ng/L. The recoveries of the method for the target compounds ranged from 77.8 to 121.5% at three spiked levels (40, 200 and 400 ng/L) with the relative standard deviation of 0.54–7.22%. The established method was successfully applied to analyze 62 water samples including bottled drinking water, tap water and soak water of products related to sanitary and safety of drinking water. The developed method was fast, robust and suitable for simultaneous determination of multiple bisphenol analogues in batch samples.

Keywords Bisphenol A · Bisphenol analogue · Drinking water · Solid phase extraction · Ultra performance liquid chromatography

1 Introduction

Bisphenol A and its analogues are widely used phenolic substance in industry. They widely occurred in various environments [1–6], drinking water and products used in the water supply facilities [7], food packaging materials [8] and foodstuffs [9, 10]. They have attracted great public attention due to their endocrine disruptor effect on human health and environment. The results of in vitro and in vivo studies have shown that bisphenol analogues (BPs) have carcinogenic, mutagenic and genotoxic capabilities and they might cause considerable damage to male and female reproductive systems [11–14]. The major sources of human exposure to bisphenol analogues were foodstuffs, drinking containers, tap water, water pipes and food packaging materials [7, 8, 15, 16]. It is worth noting that bisphenol analogues could result in indirect contamination of drinking water and foodstuffs by migrating from plastic

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materials. Therefore, it is necessary to improve the analytical methods for determination of the potential occurrence of various bisphenol analogues.

Various sample preparation techniques including solid phase extraction (SPE), solid phase microextraction (SPME) [2], on-column large volume injection [7], liquid–liquid extraction [17], electro-enhanced solid-phase microextraction [18], disk extraction [19, 20], liquid phase microextraction [21], molecularly imprinted polymer [5, 22–24] and QuEChERS extraction [25, 26] have been employed for purification and concentration of bisphenol A (BPA) in water samples and foods. Serials analytical techniques including high performance liquid chromatography with ultraviolet detector (HPLC–UV) [3, 22], high performance liquid chromatography with fluorimetric detector (HPLC–FLR) [8], high performance liquid chromatography with ultra-violet absorption and fluorescence detection (HPLC–UV–FLR) [9], liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) [5, 6, 10, 14, 18, 21], gas chromatography with mass spectrometry (GC–MS) or gas chromatography with tandem mass spectrometry (GC–MS/MS) [1, 4, 7, 10, 17], electrochemical sensor [23], fluorescence sensor [27] and Enzyme-Linked Immunosorbent Assay [28] have been used for the analysis of BPA in recent years.

Compared with HPLC method, GC–MS, GC–MS/MS or LC–MS/MS methods have better qualitative effect. However, the MS-based methods depending on expensive apparatus are prone to endure matrix effect and require isotope internal standards to improve the quantification accuracy. GC–MS or GC–MS/MS methods require further derivatization after sample preparation, which increases the risk of background introduction and sample loss. When the water samples were purified and enriched using the solid phase extraction (SPE) cartridges, their work efficiency was largely limited by their loading flow rates at no more than 10 mL/min. Furthermore, the reported analytical methods mainly focused on the detection of BPA, but multiple bisphenol analogues are rarely reported in drinking water-related products.

The present study aimed to develop a rapid method for simultaneous determination of BPA and its eight analogues in drinking water, and soak water of products related to sanitary and safety of drinking water by ultra-performance liquid chromatography coupled with both photodiode array and fluorescence detector (UPLC–PDA–FLR). Compared with the conventional HPLC, UPLC could enhance the characteristics regarding analysis speed, resolution, sensitivity and more environmental friendly consumption of organic reagents. To improve the extraction efficiency and avoid the risk of background introduction during the sample preparation, the different loading flow rate and conditions for the concentration and purification using SPE cartridges by an automatic apparatus were optimized very painstaking for nine bisphenol analytes. Finally, the developed and optimized method was employed to analyze different water samples including bottled drinking water, tap water and soak water of products related to sanitary and safety of drinking water.

2 Experimental

2.1 Chemicals and materials

HPLC-grade acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). Ultrapure water was obtained from a Milli-Q system (Bedford, MA, USA). Oasis HLB were purchased from Waters (MA, USA). Glass microfiber filters GF/C were obtained from Whatman (GE Healthcare, UK). Analytical standards of bisphenol A (BPA), bisphenol B (BPB) and bisphenol F (BPF) were provided by Dr. Ehrenstorfer GmbH (D-86119 Augsburg, Germany). Bisphenol S (BPS), bisphenol AF (BPAF), bisphenol E (BPE), bisphenol Z (BPZ), bisphenol P (BPP) and bisphenol AP (BPAP) were obtained from Sigma-Aldrich (St. Louis, Mo, USA). Sodium hypochlorite (5%), sulphuric acid, anhydrous calcium chloride and anhydrous sodium bicarbonate were offered by local suppliers.

2.2 Preparation of standard solutions

All the stock standard solutions of individual compound were prepared in methanol and stored at − 24 °C in the dark. The mixture standard solution of bisphenols was prepared at the concentration of 10 µg/mL in 50% methanol aqueous solution. All the working solutions were freshly prepared for spiking procedures and calibration curves.

2.3 Preparation of simulated tap water

Simulated tap water was prepared by mixing 25 mL 0.04 mol/L sodium bicarbonate solution and appropriate 0.025 mol/L chloride storage solution. The mixture was diluted to 1 L with distilled water and adjusted pH to 8.0 with 1 mol/L sulphuric acid. The final residual chlorine and hardness were 2.0 mg/L and 100 mg/L, respectively.

2.4 Sample collection and preparation

Five samples of bottled drinking water were collected from local markets (Zhejiang). Ten samples of tap water were collected from local waterworks (Zhejiang). Forty-seven samples of products related to sanitary and safety of drinking water were obtained from different manufacturers in China. These products include plastic pipes, storage tanks,

gooseneck faucet, reverse osmosis membrane/ultrafiltration membrane filter element of water quality processors using tap water as raw water and some materials in contact with drinking water.

Soak water of products related to sanitary and safety of drinking water was prepared according to the guidelines of Chinese Sanitary Standard for Drinking Water as follows: samples were successively cleaned with tap water and distilled water for 30 min, and then soaked using simulated tap water or distilled water at 25 ± 5 °C in dark for 23–25 h. Samples such as reverse osmosis equipment were soaked with distilled water because they could be damaged by residual chlorine. The excessive residual chlorine in tap water and soak water was removed using 0.2 g/L of ascorbic acid as reductant after the water samples passed through the GF/C Glass microfiber filters. The filtered water samples were concentrated and purified by Oasis HLB SPE cartridges using automatic solid phase extraction apparatus (Reeko Instrument Company, China). HLB SPE cartridges of different specification and filler content (60 mg/3 mL, 200 mg/6 mL and 500 mg/6 mL) were compared at different sample loading flow rates (5, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100 mL/min). The different ratios of methanol aqueous solution (0%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100%) were used as washing solution. HLB cartridge was conditioned by 10 mL methanol and 5 mL ultrapure water. After 500 mL sample was loaded at 100 mL/min, the cartridge was successively washed by 10 mL ultrapure water at 100 mL/min, and 5 mL 50% methanol aqueous solution at 1 mL/min. The cartridge was finally eluted using 1 mL methanol. The eluent was mixed with 1 mL ultrapure water. The mixture was passed through a 0.22 μm syringe filter prior to UPLC analysis.

2.5 UPLC–PDA–FLR

The UPLC–PDA–FLR system consisted of Waters Acquity™ UPLC and PDA detector, FLR detector (Waters, Manchester, UK). Chromatographic separation was conducted on a Waters ACQUITY™ UPLC BEH C18 (150 mm × 2.1 mm, 1.7 μm) with column temperature at 30 °C. The flow rate was 0.25 mL/min and the injection volume was 10 μL. Mobile phase A (ultrapure water) and phase B (methanol) were applied in a binary gradient: 20% B (initial mobile phase), 20–65% B (0–3.0 min), 65–70% B (3.0–6.0 min), 70% B (6.0–7.5 min), 70–80%B (7.5–8.5 min), 80% B (8.5–11.5 min), 80–20% B (11.5–11.6 min) and 20% B (11.6–13.0 min). Bisphenol S was scanned from 190 to 400 nm and accumulated at 259 nm using PDA detector. The other eight bisphenol analogues were detected using FLR detector with excitation wavelength at 228 nm and emission wavelength at 301 nm. Data acquisition and processing was performed using Waters Empower 2 software.

2.6 Method validation

The methodological characteristics were evaluated, including linearity, limit of detection (LOD), limit of quantification (LOQ), selectivity, precision and accuracy. The linearity was evaluated by preparing working curve in the concentration range from 40 to 600 ng/L. LOD and LOQ were determined as the lowest concentration of individual standard with signal-to-noise ratio (*S/N*) of 3 and 10, respectively. The selectivity of method was assessed by PDA wavelength scan spectrum. Precision and accuracy were evaluated by spiked real samples in six replicates (intra-day) at three different concentration levels (40, 200 and 400 ng/L).

3 Results and discussion

3.1 Optimization of UPLC condition

According to the paper published [8], when using FLR detector, the excitation wavelength and emission wavelength for BPA were 275 nm and 305 nm, respectively. After optimization, 228 nm was selected as excitation wavelength; the emission wavelengths for bisphenols except BPS without fluorescence reaction were 295 nm for BPAF, 306 nm for BPZ and 301 nm for BPA, BPB, BPE, BPF, BPAP and BPP. Thus, 228 nm and 301 nm were selected as excitation wavelength and emission wavelength for FLR detector, respectively. For BPS analysis, PDA at 259 nm was finally optimized and employed.

Serial preliminary experiments were carried out to obtain the optimal UPLC conditions, specifically the selection of liquid chromatographic column, composition of mobile phase and sample solution. Methanol and acetonitrile are commonly used as organic phases in UPLC methodology. Compared with methanol, acetonitrile could provide higher sensitivity and better separation. Methanol was finally selected as the organic phase because it could separate BPAF and BPAP but accetonitril could not. Then several common reversed-phase columns including ACQUITY UPLC BEH C18 (2.1 × 100 mm, 1.7 μm), ACQUITY UPLC BEH C18 (2.1 × 150 mm, 1.7 μm), ACQUITY UPLC HSS T3 (2.1 × 100 mm, 1.7 μm), and ACQUITY UPLC Phenyl (100 mm × 2.1 mm, 1.7 μm) were compared. The satisfactory separation and symmetrical peak of all target compounds were acceded when ACQUITY UPLC BEH C18 (2.1 × 150 mm, 1.7 μm) was used (Fig. 1) and 50% methanol aqueous was selected as sample solution.

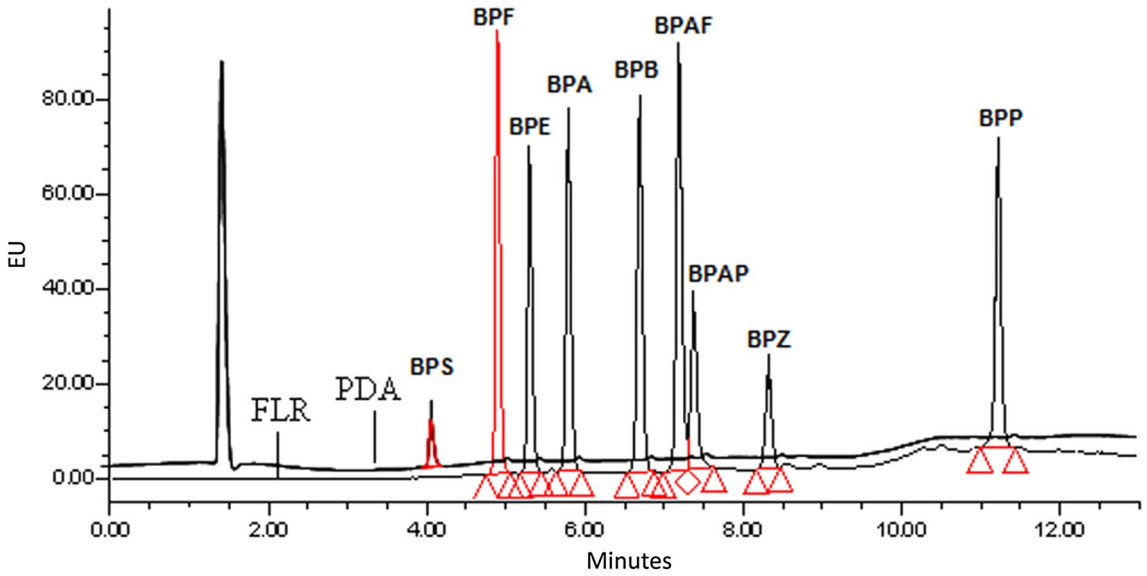

Fig. 1 UPLC–FDA–FLR chromatograms of nine bisphenol analogues

3.2 Optimization of SPE condition

In this study, serial preliminary experiments were carried out to obtain the optimal SPE conditions, including the selection of SPE cartridge, composition of elution solution, elution volume, washing solution and sample loading flow. Methanol was selected because of its more efficient elution capacity for all targeted bisphenol analogues. HLB SPE cartridges of different specification and filler content could successfully enrich nine kinds of bisphenol analogues. Especially, the HLB SPE cartridge of 60 mg/3 mL could easily elute all the nine target compounds with 1 mL methanol with the recovery rates of higher than 90%. By using this kind of SPE cartridge, the eluent could be directly filtered and analyzed after diluted with 1 mL ultrapure water, which might decrease the loss during evaporation under nitrogen flow. BPS was eluted when the methanol ratio reached 60%. BPF, BPE, BPA, BPB and BPAF were eluted at 70% methanol, while BPAP, BPZ and BPP were eluted at 80% methanol (Fig. 2). When the flow rate of 50% methanol aqueous solution was higher than 1 mL/min, the recovery could decrease. After repeated optimization, 10 mL ultrapure water and 5 mL 50% methanol aqueous solution were used as washing solution at 100 mL/min and 1 mL/min flow rate, respectively. When loading flow high at 100 mL/min by automatic solid phase extraction apparatus, the recovery also could keep higher than 90%. Thus, 100 mL/min was selected as sample loading flow rate to greatly enhance the efficiency of sample concentration and purification.

3.3 Effect and removal of excessive chlorine

The excessive chlorine in simulated tap water and tap water might affect the enrichment of bisphenols by SPE cartridge because the excessive chlorine could oxidate or chloridize bisphenols [7]. When samples were directly enriched by SPE cartridges after passing through the filter, all the recoveries of nine bisphenol analogues were under 5%. When 0.2 g ascorbic acid was added to per liter simulated tap water and tap water before loading on SPE cartridge, the recoveries of the nine bisphenol analogues were enhanced obviously and even reached higher than 90% (except BPP). It was considered that the addition of ascorbic acid could affect the dissociation status of the bisphenols and improve their extraction by changing the solution pH.

3.4 Linearity, sensitivity and accuary

The nine bisphenol analogues showed good linearity in the range of 40–600 ng/L, and the correlation coefficients were 0.999 (Table 1). The LOD and LOQ of the established method for nine bisphenol analogues were determined as 6–12 ng/L and 20–40 ng/L, respectively. The average recovery of nine bisphenols at three different spiking levels ranged from 77.8 to 121.5% with RSD ranged from 0.54 to 7.22% (Table 2).

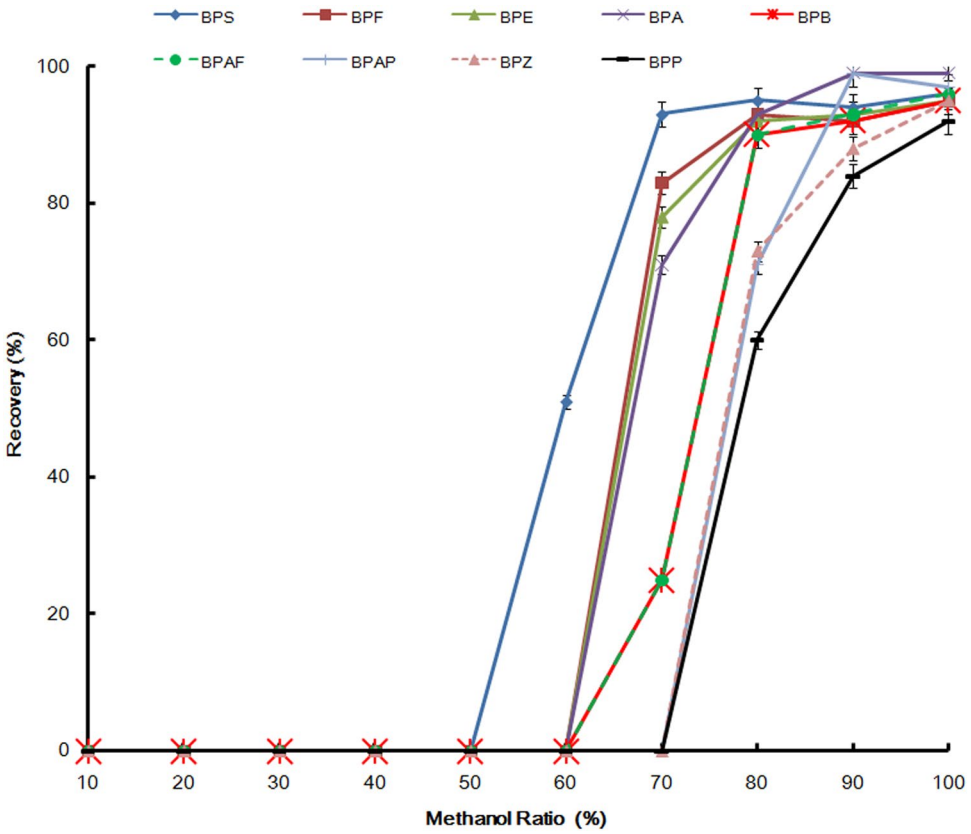

Fig. 2 Elution curve of nine bisphenol analogues with different methanol aqueous solutions

Table 1 Regression equation, coefficient, LOD and LOQ of nine bisphenol analogues using the UPLC–PDA–FLR method

Compound	Regression equation	Correlation coefficient (R^2)	LOD (ng/L)	LOQ (ng/L)
BPS	$Y=1.56\times10^2X+2.12\times10^1$	0.999	12	40
BPA	$Y=3.35\times10^3X+8.72\times10^3$	0.999	6	20
BPB	$Y=4.12\times10^3X$-1.92×10^3	0.999	6	20
BPE	$Y=2.74\times10^3X+1.92\times10^3$	0.999	6	20
BPF	$Y=3.57\times10^3X+6.03\times10^2$	0.999	6	20
BPAF	$Y=4.34\times10^3X$-4.14×10^3	0.999	6	20
BPAP	$Y=1.88\times10^3X+3.05\times10^4$	0.999	12	40
BPP	$Y=3.06\times10^3X$-5.70×10^3	0.999	12	40
BPZ	$Y=1.60\times10^3X+8.33\times10^2$	0.999	12	40

3.5 Blank level

For bisphenol analogues analysis, special attention has to be given to the cross contamination from different sources. Special precaution should be taken regarding the experimental control, including avoiding the usage of plastic vessles. All laboratory glasswares were heated at 400 °C. The SPE cartridge was conditioned with methanol of 3 times column volume to decrease the background of bisphenol analogues. The blank values should be lower than the lowest detection limitations. In this experiment, the results of the blank level evaluation experiment demonstrated that the blank levels of nine bisphenols were all below the LOD of 6 ng/L and 12 ng/L.

3.6 Method application

Five samples of bottled drinking water, ten samples of tap water and 47 samples of soak water from products related to sanitary and safety of drinking water were analyzed by the developed UPLC–PDA–FLR method. The results showed that all the nine bisphenol analogues were not detected in the five bottled drinking water. The determined concentrations of BPA were 43.1 ng/L and 16.3 ng/L

Table 2 Recovery of nine bisphenol analogues using the UPLC–PDA–FLR method ($n = 6$)

Sample	Compound	Spiking level 1 (40 ng/L)		Spiking level 2 (200 ng/L)		Spiking level 3 (400 ng/L)	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Tap water	BPA	95.9	0.93	100.9	2.03	96.2	1.17
	BPB	96.9	1.84	99.0	2.05	94.9	0.90
	BPE	121.5	7.12	104.9	1.69	96.1	0.93
	BPF	97.6	1.33	102.5	1.51	100.0	0.54
	BPAF	96.4	1.40	96.9	1.27	95.0	0.90
	BPAP	107.9	3.66	102.6	1.13	96.6	0.90
	BPP	96.9	4.69	83.9	3.80	77.8	0.80
	BPS	101.9	1.13	100.9	2.39	99.7	1.02
	BPZ	120.0	4.09	101.1	2.08	94.6	1.24
Soak water	BPA	104.9	2.02	100.8	2.14	98.0	2.20
	BPB	90.8	3.40	100.4	1.89	100.7	2.00
	BPE	92.6	6.20	98.8	0.81	98.2	2.50
	BPF	94.0	5.28	98.7	0.93	98.2	2.11
	BPAF	105.3	4.56	93.9	2.55	100.4	2.97
	BPAP	91.0	7.22	100.8	3.46	105.4	2.30
	BPP	91.8	4.58	98.1	2.18	91.3	1.33
	BPS	96.2	2.96	99.2	1.50	102.6	1.96
	BPZ	87.1	3.36	100.6	2.42	100.2	2.25

Table 3 The contents (ng/L) of detected bisphenol migrations in tap water and products related to sanitary and safety of drinking water

No	Sample	BPA	BPS	BPAF
1	Tap water	43.1	ND[a]	ND
2	Tap water	16.3	ND	ND
3	Reverse osmosis membrane filter element	160	ND	ND
4	Ultrafiltration membrane filter element	40.2	ND	ND
5	Gooseneck faucet	90.2	ND	ND
6	Plastic pipe	30.8	ND	ND
7	Plastic pipe	31.5	ND	60.2
8	Plastic pipe	30.1	ND	61.0
9	Plastic storage tank	21.3	ND	20.4
10	Plastic storage tank	20.7	ND	50.3
11	Color masterbatch	20.1	ND	ND
12	Polyethylene pellet	ND	249	ND

[a]ND, not detected or lower than the limit of detection

in two samples of tap water, respectively (Table 3). The BPA migration was detected in ten samples of soak water from products related to sanitary and safety of drinking water at the concentrations ranged from 20.1 to 160 ng/L. The highest value of BPA migration was detected in a reverse osmosis membrane filter element. The BPS migration was detected in one soak water of polyethylene pellet at 249 ng/L. The BPAF migration was measured at the concentration of 20.4–61.0 ng/L in four soak waters from two plastic pipes and two plastic storage tanks. In the BPAF-positive soak waters, the BPA migration was simultaneously detected in the range of 20.7–31.5 ng/L. Other six kind of bisphenol analogues were not detected in all the analyzed samples.

4 Conclusions

In this work, a robust UPLC–PDA–FLR method was developed for the simultaneous determination of nine bisphenol analogues in bottled drinking water, tap water and soak water of products related to sanitary and safety of drinking water. The efficiency of sample preparation was greatly improved by optimizing the sample loading flow to 100 mL/min on an automatic solid phase extraction apparatus. The methanol eluent of 1 mL after diluted with 1 mL ultrapure water was directly injected into UPLC–PDA–FLR system for analysis, avoiding the risk of background introduction and sample loss during the sample concentration process by blowing nitrogen. All the validation results demonstrated that the established method was simple, fast, robust and suitable for the simultaneous determination of BPA and other eight analogues (BPB, BPE, BPF, BPAF, BPAP, BPS, BPP and BPZ) in bottled drinking water, tap water and soak water of products related to sanitary and safety of drinking water. The presented investigations could be valuable for risk assessment considering the

release of bisphenol analogues from materials that have contact to consumer products like drinking water.

5 Supplementary materials

The typical chromatograms of bisphenol analogues-positive samples were shown in the supplementary materials.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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