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Contamination status of bisphenol A and its analogues (bisphenol S, F and B) in foodstuffs and the implications for dietary exposure on adult residents in Zhejiang Province

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ABSTRACT

An effective method has been developed for the simultaneous determination of four bisphenols (bisphenol A, S, F and B) in various foodstuffs. The contaminants were extracted by QuEChERS-based strategy and subjected to ion-exchange solid-phase extraction for further clean-up. The critical variables were screened by Plackett-Burman design and then optimized by central composite design. Under the optimized conditions, satisfactory accuracy (recoveries 76%–116%) and precision (RSDs < 12%) were achieved. The established method was then used to assess the contamination status of 379 real samples. Bisphenol A was demonstrated to be the predominant bisphenol with highest incidence (79.7%) and average concentration (14.3 μg/kg). The positive rates (mean concentration) of bisphenol S, F and B were 37.7% (1.6 μg/kg), 26.9% (1.4 μg/kg) and 0.0% (not detected), respectively. Finally, the daily dietary intakes of $\sum_4$ bisphenolsfor adult residents were estimated (55.9–76.1 ng/kg bw/day) according to the contamination concentrations and the daily recommended intakes.

1. Introduction

Bisphenol A (2,2-bis(4-hydroxyphenyl)-propane, BPA), is a primary additive in polycarbonate, epoxy resins and polyacrylate etc, which are widely used in the industrial production of plastic food containers and metal can linings (Liao & Kannan, 2011). Nowadays, the annual production of BPA reaches over 7.7 million metric tons worldwide. The characteristic of lightweight, durability and strong impact resistance of the plastic products are significantly improved with the addition of BPA. But at the same time, BPA can be released from the plastic products and then migrated to the packaged foodstuffs and the surrounding environment due to the following reasons: (a) incomplete reaction of the polymeric monomers; (b) come in contact with the acid during daily use, or experience washing and heating operations; (c) degradation of the polymeric materials caused by heat sterilization, ultraviolet radiation, severe shake, or improper use; (d) natural aging of the packaging materials (McCombie & Biedermann, 2019; Zhou et al., 2018).

Although the toxicities exerted by BPA have been extensively studied and the estrogenic activity is its best-known characteristic, controversies still exist among the scientific community over the adverse effects of chronic exposure to BPA (Chen, Ike, & Fujita, 2002). The long-term contact with BPA can decrease sperm count and impair male fertility, and can also lead to other adverse effects, like endometriosis, sexual dysfunction, cardiovascular disease etc (Schecter et al., 2010). In other serious cases, BPA may even stimulate the breast cancer progression (Pupo et al., 2012).

Abbreviations: BPA, bisphenol A; BPB, bisphenol B; BPF, bisphenol F; BPS, bisphenol S; CCD, central composite design; FA, formic acid; GC-MS, gas chromatography-mass spectrometry; HPLC-MS/MS, high performance liquid chromatography tandem mass spectrometry; IEDI, International Estimated Daily Intake; LOD, limit of detection; LOQ, limit of quantification; ME, matrix effect; MRM, multiple-reaction-monitoring; RSM, response surface methodology; SPE, solid-phase extraction; UFLC, ultra-fast liquid chromatography

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Taking into account the above worrisome effects, the use of BPA has been restricted or banned in many countries. For instance, the U.S. Food and Drug Administration and the European Food Safety Authority do recommend adopting precautionary actions on the use of BPA in consumer products that come into contact with food (Cunha & Fernandes, 2013). Structural analogues, like bisphenol S (4,4′-sulfonyldiphenol, BPS), bisphenol F (4,4′-dihydroxydiphenylmethane, BPF) and bisphenol B (4,4′-(1-methylpropylidene)-bisphenol, BPB), were thus employed as substitutes in response to the growing restrictive regulations. Unfortunately, the estrogenic potencies (BPB > BPA > BPF > BPS) have also been proven for these alternatives via animal (rats) experiments (Ahsan, Ullah, Ullah, & Jahan, 2018; Russo et al., 2018).

Diet is considered the primary source of BPA exposure for the general population. The analysis of bisphenols in food is almost invariably accomplished by means of chromatographic techniques, like gas chromatography-mass spectrometry (GC–MS), high performance liquid chromatography tandem optical detectors or mass spectrometry (HPLC-MS/MS). For instances, BPA in edible marine biota was determined using GC-MS (Santhi, Hairin, & Mustafa, 2012), 21 bisphenols in dairy products were analyzed by HPLC-MS/MS (Cheng et al., 2017), BPA and tetrabromobisphenol A in seafood were measured by HPLC-MS/MS (Cunha, Oliveira, & Fernandes, 2017). By comparison, the advantages of HPLC-MS/MS lies in its applicability to polar, high-boiling and heat-labile analytes. Moreover, the derivatization reaction that requires the use of weakly polar organic solvents (such as acetone, *n*-hexane, etc) is avoided through this technique, and thus reducing the possibility of exudation of bisphenols from plastic consumables; on the other hand, preferable sensitivity and anti-interference capacity also overcome the limitations of optical detectors.

Although much effort has been put forth to assess the contamination degree of bisphenols in foodstuffs, the majority of methods only focus on BPA or some specific matrices like milk and energy drinks (Gallo et al., 2017; Xiong et al., 2018). Sample preparation schemes remain the primary challenge in determining the concentrations and profiles of bisphenols in food, in view of the matrix complexity and wide concentration spans (Cunha & Fernandes, 2013). To date, liquid-liquid extraction and solid-phase extraction (SPE) are the two most widely employed pretreatment techniques in this regard (Koestel et al., 2017; Zimmers et al., 2014). Other preparation approaches, e.g. dispersive liquid–liquid microextraction and matrix solid-phase dispersion, have certain advantages in terms of enrichment factor, analysis speed or solvent consumption (Ballesteros-Gómez, Rubio, & Pérez-Bendito, 2009). Nevertheless, the practical applications of these techniques are usually actualized on the premise that the instrument possesses high sensitivity and anti-interference capability or the sample composition is relatively simple (Vela-Soria, Ballesteros, Zafra-Gómez, Ballesteros, & Navalón, 2014; Ye et al., 2015). QuEChERS (quick, easy, cheap, effective, rugged and safe) strategy is another reasonable alternative for multi-component analysis, which was originally proposed by Anastassiades, Lehotay, and tajnbaher, & Schenck, (2003) for the extraction of pesticide residues, and has been subsequently expanded to the analysis of veterinary drugs and mycotoxins (Anastassiades et al., 2003). Typical QuEChERS application generally consists of two steps of salting out and dispersive SPE. However, the purification capacity of this methodology is very limited due to the high-throughput characteristic (Arroyo-Manzanares, Huertas-Pérez, Gámiz-Gracia, & García-Campaña, 2013; Xiong et al., 2018).

The present study aimed to combine QuEChERS-based extraction with ion-exchange SPE technique, and thereby developing a rapid and accuracy detection system, which can meet the requirement for the simultaneous analysis of four bisphenols (BPA, BPS, BPF and BPB) in various food matrices. To maximize the extraction efficiencies, the statistical methodology called Plackett-Burman design was initially applied to screen out the critical factors, single-factor experiment and response surface methodology (RSM) were then conducted for further optimization. Finally, the profiles and concentrations of four bisphenols in various foodstuffs (n = 379) were measured, followed by a dietary exposure assessment to adult residents.

2. Experiment

2.1. Sample collection

Sampling approach was primarily based on the risk-monitoring scheme of Zhejiang Province (from June 2017 to April 2018) and the dietary characteristics of local residents had been fully considered. In this way, the study can reflect the actual exposure levels of the population concerned to a large extent. In total, 379 real samples were collected from the local markets and divided into twelve categories: (1) water; (2) beverages; (3) rice; (4) wheat flour; (5) shellfish; (6) fish; (7) fresh meat; (8) vegetables; (9) canned cereal; (10) canned fish; (11) canned meat and (12) others: edible oil, egg, honey, etc. Further information on samples, including the packaging materials and the specific amount are available in Table S1 (Supplementary information). The sealed samples including bottled water, beverages and canned products were stored at room temperature (20 °C) in the dark and were not opened until analysis. All the solid foodstuffs (edible portions) were homogenized thoroughly using an electric triturator. Hereinto, an aliquot was weighed for analysis and the remaining contents were stored at −20 °C.

2.2. Standards and chemicals

Acetonitrile and methanol (mass spectrum grade) were purchased from Fisher Chemical (Geel, Belgium). Formic acid (FA) and ammonium hydroxide (chromatographic grade) were purchased from Merck KGaA (Darmstadt, Germany) and Anaqua Chemicals (Wilmington, America), respectively. Anhydrous magnesium sulfate and sodium chloride (analytical grade) were purchased from the local suppliers. Ultra-pure water was obtained through Milli-Q system (Dubuque, America). Analytical standards of four bisphenols with purity over 97% were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). $^{13}C_{12}$-labeled BPS, BPA-d_4 and BPF-d_{10} were purchased from Cambridge Isotope Laboratories (Massachusetts, America), CDN Isotopes (Quebec, Canada) and Toronto Research Chemicals (Toronto, Canada), respectively. SPE cartridges including ProElut Carb Glass cartridges (6 cc, 500 mg), Oasis HLB (6 cc, 150 mg) and Oasis MAX cartridges (6 cc, 150 mg) were purchased from DIKMA (Shanghai, China) and Waters (Massachusetts, America), respectively. The standard stock solution was prepared individually in acetonitrile to yield a final concentration of 1.0 mg/mL and stored at −20 °C. The standard working solution (0.3 μg/mL of BPS, 1.0 μg/mL of BPA, BPB and BPF) and the mixed internal standard solution (1.0 μg/mL of $^{13}C_{12}$-BPS, BPA-d_4 and BPF-d_{10}) were prepared by serially diluting the corresponding stock solutions with appropriate volume of acetonitrile.

2.3. Analytical procedure

Depending on the differences of physical forms of samples, the extraction protocol could be classified into “water”, “beverages” and “others”, and described as follows:

For “water”: 20.0 mL of water sample was precisely transferred using a glass pipette. 10 μL of the mixed internal standard solution (1.0 μg/mL) and 0.4 mL of ammonia were added and the mixture was then well mixed.

For “beverages”: 10.0 mL of sample was weighed into a 50-mL centrifuge tube with the addition of 10 μL of internal standard solution, and subsequently extracted using 10.0 mL of acetonitrile containing 2.2% of FA (v/v). The tested tube was shaken with an oscillator (Heidolph, Schwabach, Germany) at 2000 rpm for 10 min. To the extractant were added 4.0 g of anhydrous magnesium sulfate and 1.0 g of sodium chloride with immediate vortexing for 3 min to enhance the

partition of the bisphenols into the organic layer. The tube was then centrifuged at 10,000 × g for 5 min (4 °C). 5.0 mL of the supernatant organic layer was transferred into a 20-mL glass tube and diluted with 14.1 mL of 4.1% ammonium (*v/v*).

For "others": 2.0 g of representative portion was weighed into a 50-mL centrifuge tube with 10 μL of internal standard solution. The spiked samples were left at room temperature for 30 min, 10.0 mL of water and 10.0 mL of acetonitrile containing 2.2% of FA (*v/v*) were added sequentially. The remaining operation steps were essentially the same as "beverage" category.

The purification was mainly based on ion-exchange SPE, using an Oasis MAX cartridge, which was initially conditioned with 12 mL of acetonitrile and then equilibrated with 6 mL of water. After all the diluent was loaded on the cartridges, and before elution using 5 mL of FA/water/acetonitrile (2/10/88, *v/v/v*), the sorbents were washed with 6 mL of water and 6 mL of acetonitrile in turn. The eluent was collected into a new glass tube and evaporated to dryness under a gentle nitrogen flow at 45 °C. The residue was reconstituted with 1.0 mL of ammonium/water/acetonitrile (1/79/20, *v/v/v*) and the obtained sample solution was then transferred to a 2-mL polypropylene centrifugation tube. Prior to injection, the tube was subjected to high-speed freeze centrifugation (14,000 × g, 10 min, 4 °C) for further purification and only the supernatant was analyzed.

2.4. Quality assurance and control

Given the ubiquitous presence of bisphenols in the laboratory environment, precautions must be taken to control the blank background values. During the whole course of experiment, the plastic consumables including pipette tips and centrifuge tubes were made of high-quality polypropylene while the chemical reagents were newly opened, which had been confirmed containing no or extremely low level of BPA. On the other hand, all the glassware was washed with appropriate amount of methanol and then pyrolysed for 3 h at 500 °C to destroy the potential organic interferences before use. As for quality control, one duplicate sample and one spiked sample were inserted in each interval of ten samples. Meanwhile, the procedural blanks (1 blank and 3 samples) were conducted to monitor whether abnormal background values occur during the whole pretreatment in which ultra-pure water was used as a substitute for the investigated samples. The concentrations measured in all tested assays were subtracted from the mean values of procedural blanks.

2.5. Instrumentation and software

Shimadzu LC-20AD ultra-fast liquid chromatography (UFLC, Kyoto, Japan) interfaced with AB Sciex QTrap® 5500 mass spectrometer (California, America) was employed for chromatographic separation, MS detection, and data acquisition. Chromatographic separation was carried out using a gradient elution with eluent A being water and eluent B being acetonitrile on an Acquity BEH C_{18} column (2.1 mm × 100 mm, 1.7 μm) with column temperature at 40 °C. The detailed gradient program was described as follow: 20% B (initial mobile phase), 20%-40% B (0.00–1.00 min), 40%-70% B (1.00–5.00 min), 70%-95% B (5.00–5.02 min), 95% B (5.02–7.02 min), 95%-20% B (7.02–7.05 min) and equilibrated for another 3 min. The flow rate was 0.3 mL/min and the injection volume was set at 10 μL. Electrospray ionization in the negative mode was performed in multiple-reaction monitoring (MRM) conditions. The MRM transitions, declustering potential and collision energy were optimized by direct infusion of standard solutions into mass spectrometer and summarized in Table S2. Other ionization source parameters were also optimized and set as follows: ionspray voltage maintained at −4.5 kV; source cone temperature 500.0 °C; nitrogen was used as curtain gas and collision gas at 20.0 psi and 7.0 psi, respectively.

The design of experiment matrix and statistical analysis were performed using Design-expert 8.0.6.0 (Stat-Ease Inc., Minneapolis, USA), IBM SPSS Statistics 19 (SPSS Inc., Chicago, USA) and Minitab 17.1.0 (Minitab Inc., USA).

2.6. Method validation

Validation of the established methodology in this study concerned linearity, matrix effect (ME), selectivity, accuracy, repeatability, limit of detection (LOD) and quantification (LOQ). These methodological parameters were validated in eleven representative matrices including mineral water, milk, peanut oil, egg, cabbage, rice, crucian carp, canned tuna, pork, canned beef and wheat flour. All sample vials were injected in duplicate and averaged the response values.

Two sets of calibration curves, i.e. the pure solvent curve and the matrix-matched curve were constructed. The solvent curve was obtained through appropriate dilutions of working solution while the latter one was achieved by spiking blank extract with specific amounts of standards. Both these two curves were freshly constructed at eight concentration levels each batch: 0.03, 0.15, 0.75, 3.0, 6.0, 15.0, 30.0 and 120.0 ng/mL for BPS; 0.1, 0.5, 2.5, 10.0, 20.0, 50.0, 100.0 and 400.0 ng/mL for other three bisphenols. Signal suppression or enhancement effect, i.e. ME was assessed by comparing the slope of matrix-matched curve versus the slope of solvent curve. The selectivity was investigated by checking whether interference peaks existed around the retention time as targets between the chromatograms of standard solutions and blank extracts.

Given the potential presences of BPA and BPS in procedural blanks, the detection limits were determined according to the procedure that proposed by International Union of Pure and Applied Chemistry, specifically, mean + (3 × SD) as LOD, and mean + (10 × SD) as LOQ. Using this criterion, there is < 1% probability that a signal measured at mean + (3 × SD) or greater would be the result of a random fluctuation of the blank signal (Long & Winefordner, 1983). On the other hand, the detection limits of BPB and BPF were calculated from the spiked blank chromatograms based on the signal-to-noise ratio of 3:1 (LOD) and 10:1 (LOQ), respectively.

Accuracy including relative recovery and absolute recovery was studied by spiking blank samples at three concentration levels and processing six replications each level (repeatability). Hereinto, the relative recovery was calculated by comparing the calculated results versus the concentrations of pure solvent standards, and the absolute rates were the peak area ratios of spiked sample to spiked blank extractant. The setting of spiking concentration was primarily based on the natural content and matrix complexity of the sample. Specifically, the levels were set as follows: 100 ng/mL, 200 ng/mL and 500 ng/mL for water samples and milk, 2.0 μg/kg, 5.0 μg/kg and 10.0 μg/kg for peanut oil, egg, cabbage and rice, 5.0 μg/kg, 10.0 μg/kg and 20.0 μg/kg for animal-derived matrices and wheat flour.

3. Results and discussion

3.1. Selection of extraction and purification approaches

The purification effects of conventional extraction techniques, like liquid-liquid extraction and single SPE, were usually limited by sample complexity, which is more pronounced in animal foods. Multiple SPE combination, like GCB-NH_2 can improve the purification capacity (especially for the seafood), but at the same time it involved overmuch processing steps, which may increase the possibility of introducing contamination. In this study, QuEChERS-based extraction was proposed as a prelude to remove some polar interferences and proteins, owing to the salting-out effect. Hereinto, the selection of organic solvent played an important role in the partition of analytes during the phase separation (Zhou et al., 2018). In this context, methanol and acetonitrile were investigated as possible candidates. As the experimental result shown, unsatisfactory phase separation degree and protein

precipitation effect were observed in methanol-water system after adding the salting-out agents, the acetonitrile-water was therefore selected. On the other hand, the appropriate amounts of FA helped transfer the bisphenol compounds into molecular forms and thus enhanced the distribution ratio of bisphenols in organic phase.

However, as mentioned above, the high-throughput characteristic of dispersive SPE limits its purification ability. After the QuEChERS-based extraction, there were still a great deal of co-extracted components, especially the weak-polar interferences, which could affect the sensitivity of electrospray ionization source and cause irreversible contamination to equipment with the increase of injection numbers. To get rid of this dilemma, SPE has been a relatively mature alternative. In this respect, great consideration must be taken regarding the affinity between sorbents and analytes in order to produce favorable recovery and selectivity. Both non-selective (Oasis HLB and Carb) and ion-exchange sorbents (Oasis MAX) were assessed by passing the pure standard solution (1.0 ng/mL, 10 mL) through the cartridges. Although high withdrawal capacities were observed on all tested sorbents (with overall recoveries > 85%), the non-selective sorbents suffer from low efficiency due to the poor selectivity toward specific molecular structures. Nevertheless, the ionized phenolic hydroxyl groups on bisphenols could be steadily bound to the anion-exchange sorbent under alkaline condition and thus the ion-exchange SPE cartridges were preferred. Under this premise, the composition of eluent used in SPE process should be specified. As known, acidic eluents are commonly used to eliminate the binding between the target compound and the anion-exchange sorbent. However, in this regard, the combination of pure acetonitrile and anhydrous FA does not work actually due to the insufficient ionization of FA. It is necessary to add appropriate water to improve this situation. After optimization, favorable elution effects were observed with the proportion of FA ranging from 3% to 10%, and the upper-bound value was finally selected for the consideration of full ionization.

Besides, high-speed freeze centrifugation was applied as a supporting approach of purification as well. Low-temperature (freezing-out) effect promoted the precipitation of some weak polar interferences. Validation tests were performed in blank pork samples with spiking concentration at 0.5 μg/kg, and the results (Fig. S1) indicated that the centrifugation procedure does play a role in reducing the matrix suppression effect (except for BPS).

3.2. Optimization strategy for preparation procedure

Throughout the whole pretreatment process, many experimental parameters could be found to affect the results in various extents. The optimal preparation conditions were commonly achieved using the single-dimensional optimization (i.e. one-factor-at-a-time). This experimentation, however, it ignores the interaction effects among variables and cannot identify the importance of different factors, which results in defective depiction of the parameter effects on the response (Homem, Alves, Alves, & Santos, 2016). In this context, a multi-response chemometrics optimization involving Plackett-Burman design, single-factor experiment and RSM was implemented to overcome these problems.

Screening experiment was performed at the beginning of the establishment of methodology as a prelude to a detailed optimization. Its purpose is to make sure that the factors being optimized do indeed significantly contribute to the responses and thus narrowing the investigation range of candidate variables. In this respect, Plackett-Burman design is an efficient way to explore multiple factors and screen out the significant ones without concerns about interacting and nonlinear effects.

After the critical factors had been identified, single-factor experiments were designed to determine the central levels for these factors, which is of significant importance, probably more so than the design itself. However, this method is not suitable for further optimization due to the ignorance of interdependence and interaction among the factors. As for the RSM methodology, it is an optimization technique that has been widely used to overcome this dilemma. Among the various classes of RSM designs, central composite design (CCD) is one of the most popular methods to explore the relationship between the responses and the levels of the effective factors, due to its simple structure and high efficiency. In this study, CCD was applied for further optimization, which combined a two-level factorial design with star designs (level ± α) and center points (level 0). The optimization involves estimating the coefficients by fitting the experimental data to the response functions, checking its goodness-of-fit, identifying important interactions and searching the optimum conditions (Zhou et al., 2018). CCD also ensures that the designed experiments provide the maximum amount of relevant information with a minimum number of runs. As a result, a second-order polynomial equation describing the relationship between responses and variables is educed according to Eq. (1) (Bashiry et al., 2016):

$$Y = \delta_0 + \sum_{i=1}^{f} \delta_i X_i + \sum_{i=1}^{f} \delta_{ii} X_i^2 + \sum_{i=1}^{f} \sum_{j=1}^{f} \delta_{ij} X_i X_j + \varepsilon \quad (1)$$

where, Y represents the predicted response, X_i and X_j are independent variables, δ_0 is the compensation term while ε is the experimental error. The coefficients, δ_i, δ_{ii} and δ_{ij} represent the linear, interaction and quadratic item, respectively. After deriving the formula from the experimental data, analysis of variance is employed to identify whether the variations of responses are interpreted by pretreatment experiments or by random errors. Actually, this can be achieved by the Fisher distribution (F-test). Also, the Student's t-test was used to determine whether the independent, interaction and quadratic effects have significant effects on responses. In this context, if the t-probability is < 0.05, the effect is considered significant.

Finally, a desirability function was applied to mitigate the conflicts and reconcile the responses, and thus to reach the optimal conditions for all the investigated factors. The role of this function determines that the quality of experimental model that has many features is infeasible as long as one is outside of the desirable limit (Homem et al., 2016):

$$d_i^{\max} = \begin{cases} 0 \quad \text{if } f(x) < A \\ \left(\frac{f(x)-A}{B-A}\right)^w \quad \text{if } A \le f(x) \le B \\ 1 \quad \text{if } B < f(x) \end{cases} \quad (2)$$

where, d_i is the desirability value for each variable, $f(x)$ represents the recovery value in this study, A is the minimum recovery value while B is the maximum one. With respect to w, it is the weight that is used to identify the importance. Subsequently, the achieved multiple responses were substituted into a composite function followed by its optimization:

$$d = (d_1 \times d_2 \times \ldots \times d_n)^{\frac{1}{n}} \quad (3)$$

where d represents the overall desirability, which allows the analyst to find the experimental combinations to reach the optimum responses for all the investigated factors simultaneously. In this case, the optimization assays were performed in 10.0 mL of ultra-pure water with the addition of 5 ng of BPA, BPB, BPF and 1.5 ng of BPS. Sample preparation was applied according to the category of "beverage", and the absolute recovery values were treated as responses. Moreover, equal weights were given to the response of each target.

3.2.1. Screening design

A Plackett-Burman design was implemented considering seven suspected factors on the basis of the designed matrix (Table 1): volume of water in QuEChERS-based extraction (X_1), concentration of FA in acetonitrile (X_2), concentration of ammonia in diluent solution (X_3), volume of ammonia solution used for dilution (X_4), proportion of acetonitrile in washing solution (X_5), concentration of FA in eluent (X_6) and concentration of ammonia in reconstitution solution (X_7).

Table 1
The variables, coded levels, screening runs and experimental results of Plackett-Burman design.

No.	Factor	Coded level	
		Level −1	Level +1
X_1	volume of water in QuEChERS-based extraction (mL)	5.0	7.5
X_2	concentration of FA in acetonitrile (%, *v/v*)	1.0	2.0
X_3	concentration of ammonia in diluent solution (%, *v/v*)	2.0	3.0
X_4	volume of ammonia solution used for dilution (mL)	5.0	10.0
X_5	proportion of acetonitrile in washing solution (%)	75	100
X_6	concentration of FA in eluent (%)	2.0	4.0
X_7	concentration of ammonia in reconstitution solution (%)	1.0	2.0
X_8	fictitious factor	−1	1

Run	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	Response values ± SD (%)			
									BPS	BPF	BPA	BPB
1	+1 (7.5)[a]	−1 (1.0)	+1 (3.0)	+1 (10.0)	−1 (75.0)	−1 (2.0)	−1 (1.0)	−1 (−1.0)	93.2 ± 0.9	82.1 ± 1.9	81.3 ± 5.6	76.9 ± 3.5
2	+1 (7.5)	+1 (2.0)	−1 (2.0)	+1 (10.0)	+1 (100.0)	−1 (2.0)	−1 (1.0)	−1 (-1.0)	44.3 ± 2.0	50.5 ± 1.0	45.9 ± 1.3	58.1 ± 1.0
3	−1 (5.0)	+1 (2.0)	+1 (3.0)	−1 (5.0)	+1 (100.0)	+1 (4.0)	−1 (1.0)	−1 (-1.0)	21.5 ± 1.6	21.1 ± 0.1	20.7 ± 0.3	30.0 ± 1.0
4	−1 (5.0)	−1 (1.0)	+1 (3.0)	+1 (10.0)	−1 (75.0)	+1 (4.0)	+1 (2.0)	−1 (-1.0)	87.3 ± 2.8	78.9 ± 2.7	82.6 ± 3.5	73.8 ± 3.4
5	+1 (7.5)	−1 (1.0)	−1 (2.0)	+1 (10.0)	+1 (100.0)	−1 (2.0)	+1 (2.0)	+1 (1.0)	92.0 ± 1.6	81.7 ± 0.5	82.5 ± 4.0	76.6 ± 4.3
6	+1 (7.5)	+1 (2.0)	−1 (2.0)	−1 (5.0)	+1 (100.0)	+1 (4.0)	−1 (1.0)	+1 (1.0)	18.3 ± 1.7	1.6 ± 0.1	5.1 ± 0.2	9.0 ± 0.6
7	+1 (7.5)	+1 (2.0)	+1 (3.0)	−1 (5.0)	−1 (75.0)	+1 (4.0)	+1 (2.0)	−1 (-1.0)	63.2 ± 8.3	52.0 ± 0.9	66.3 ± 1.2	75.5 ± 4.6
8	+1 (7.5)	+1 (2.0)	+1 (3.0)	+1 (10.0)	−1 (75.0)	−1 (2.0)	+1 (2.0)	+1 (1.0)	76.2 ± 0.9	82.8 ± 2.4	84.9 ± 4.1	79.1 ± 7.1
9	−1 (5.0)	+1 (2.0)	+1 (3.0)	+1 (10.0)	+1 (100.0)	−1 (2.0)	−1 (1.0)	+1 (1.0)	75.2 ± 4.2	67.4 ± 0.8	88.4 ± 5.9	80.3 ± 6.8
10	+1 (7.5)	−1 (1.0)	+1 (3.0)	+1 (10.0)	+1 (100.0)	+1 (4.0)	−1 (1.0)	−1 (-1.0)	79.2 ± 4.2	82.3 ± 2.4	86.0 ± 5.2	81.3 ± 5.3
11	−1 (5.0)	+1 (2.0)	−1 (2.0)	+1 (10.0)	+1 (100.0)	+1 (4.0)	+1 (2.0)	−1 (-1.0)	44.3 ± 4.0	30.5 ± 1.4	22.9 ± 1.7	31.5 ± 2.0
12	+1 (7.5)	−1 (1.0)	+1 (3.0)	−1 (5.0)	+1 (100.0)	+1 (4.0)	+1 (2.0)	+1 (1.0)	68.3 ± 0.4	52.5 ± 1.0	59.6 ± 2.4	71.5 ± 2.8
13	−1 (5.0)	+1 (2.0)	−1 (2.0)	+1 (10.0)	−1 (75.0)	+1 (4.0)	+1 (2.0)	+1 (1.0)	98.9 ± 2.0	77.8 ± 2.0	92.9 ± 7.2	86.5 ± 6.1
14	−1 (5.0)	−1 (1.0)	+1 (3.0)	−1 (5.0)	+1 (100.0)	−1 (2.0)	+1 (2.0)	+1 (1.0)	55.1 ± 1.4	51.5 ± 0.8	57.1 ± 3.1	70.1 ± 4.2
15	−1 (5.0)	−1 (1.0)	−1 (2.0)	+1 (10.0)	−1 (75.0)	+1 (4.0)	−1 (1.0)	+1 (1.0)	70.6 ± 4.9	66.1 ± 1.5	76.2 ± 2.9	77.4 ± 1.7
16	−1 (5.0)	−1 (1.0)	−1 (2.0)	−1 (5.0)	+1 (100.0)	−1 (2.0)	+1 (2.0)	−1 (−1.0)	46.8 ± 2.2	45.1 ± 1.0	50.1 ± 2.3	64.5 ± 1.7
17	+1 (7.5)	−1 (1.0)	−1 (2.0)	−1 (5.0)	−1 (75.0)	+1 (4.0)	−1 (1.0)	+1 (1.0)	70.6 ± 6.8	45.1 ± 0.3	52.1 ± 1.4	63.3 ± 3.2
18	+1 (7.5)	+1 (2.0)	−1 (2.0)	−1 (5.0)	−1 (75.0)	−1 (2.0)	+1 (2.0)	−1 (-1.0)	26.1 ± 0.6	15.8 ± 0.4	22.7 ± 0.6	33.6 ± 0.4
19	−1 (5.0)	+1 (2.0)	+1 (3.0)	−1 (5.0)	−1 (75.0)	−1 (2.0)	−1 (1.0)	+1 (1.0)	61.3 ± 5.2	43.2 ± 0.4	50.7 ± 3.1	65.2 ± 0.9
20	−1 (5.0)	−1 (1.0)	−1 (2.0)	−1 (5.0)	−1 (75.0)	−1 (2.0)	−1 (1.0)	−1 (−1.0)	60.5 ± 2.1	44.0 ± 0.4	51.2 ± 3.3	63.9 ± 1.2

[a] Actual values are given in the brackets.

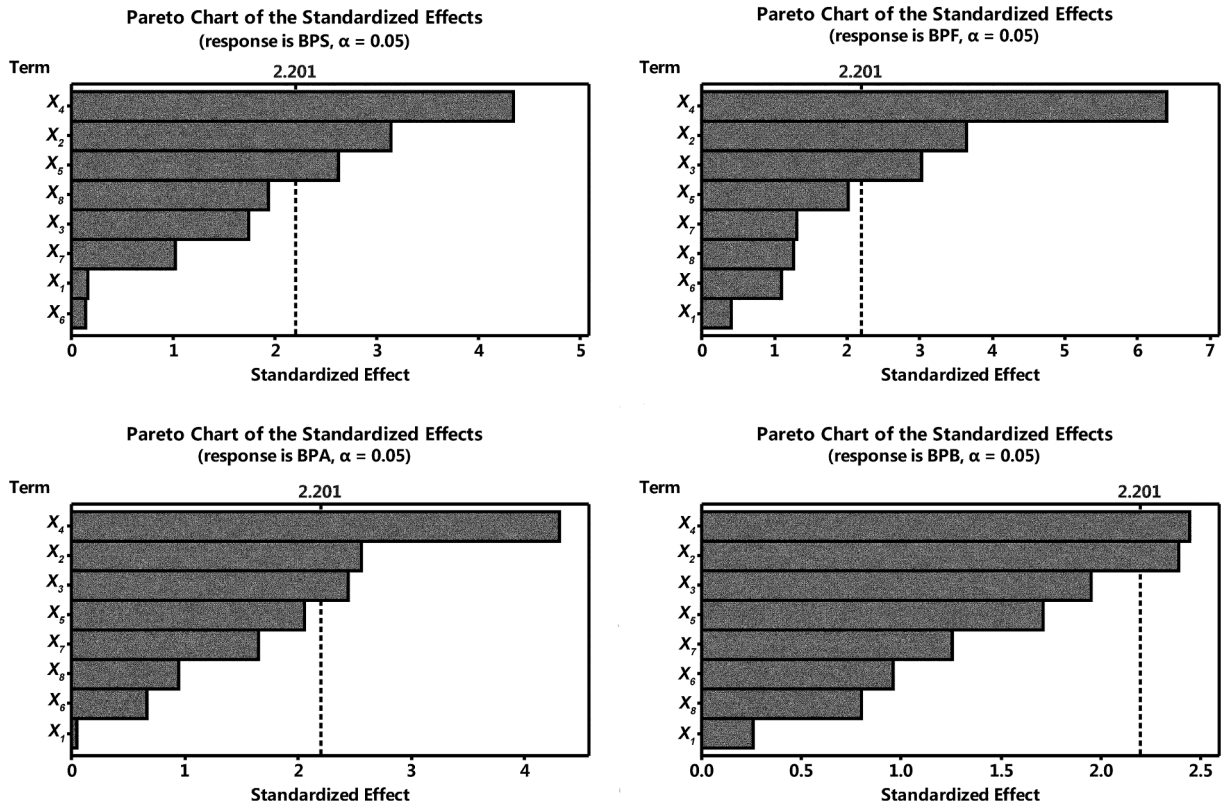

Fig. 1. Pareto Chart of standardized effects (X_1-volume of water in QuEChERS-based extraction; X_2-concentration of FA in acetonitrile; X_3-concentration of ammonia in diluent solution; X_4-volume of ammonia solution used for dilution; X_5-proportion of organic phase in the washing solution; X_6-concentration of FA in eluent; X_7-concentration of ammonia in the reconstitution solution; X_8-control group) of four bisphenols.

Furthermore, a fictitious factor (X_8) has also been introduced to determine whether there is systematic error or unknown variable affecting the results. A total of 20 runs with two investigation levels for each factor were involved in the screening design. As a result, the effects of factors are shown in the form of Pareto charts (see Fig. 1). Detailed results of variance analysis of Plackett-Burman design were summarized in Table S3 (Supplementary information). The bar lengths are proportional to the absolute effects while the reference line represents 95% of confidence interval. When the standardized effect of investigated factor exceeds the reference line, it indicates a significant impact on the results with 95% probability. Analyzing the figure, it could be found that the extraction efficiencies of all four bisphenols were affected by X_4 and X_2 in a significant way. The importance of X_3 followed, which exhibited significant effect on two bisphenols (BPA and BPF). These three factors were selected for further optimization taking into account above results. On the other hand, the rest four factors did not exhibit significant effects on the extraction efficiencies in the studied range (except X_4 on BPS) and were thus set at fixed levels based on the signs in Pareto charts. Additionally, the effects of control group never exceed the threshold line indicating that no systematic error has occurred.

3.2.2. Single-factor experiment

After the critical factors had been determined, single-factor experiments ($n = 3$) were subsequently carried out to search the approximate range of the optimum value, so as to ensure that it falls within the designed range of CCD. The above-mentioned critical factors were assessed individually while the other two held at central levels. Specifically, in this case, the levels of X_A (concentration of FA in acetonitrile) were set to 0.0%, 1.0%, 2.0%, 3.0% and 4.0%, X_B (concentration of ammonia in diluent solution) were 0.0%, 1.0%, 2.0%, 3.0% and 4.0%, X_C (volume of ammonia solution used for dilution) were 0.0 mL, 5.0 mL, 10.0 mL, 15.0 mL and 20.0 mL. The experimental results were presented as Supplementary data in Fig. S2. Analyzing the figure, the optimum levels of X_A and X_B were determined to be 2.0% and 4.0%, respectively. With respect to X_C, although the recovery rates increased slightly when the volume of ammonia solution was at higher levels (15.0 mL or 20.0 mL), meanwhile more time must be devoted to finish the assays. Finally, as a compromise, the volume of 10.0 mL was set as the central level in the following CCD.

3.2.3. CCD experimentation

After the central levels of critical factors had been determined by single-variable experiment, response surface method based on CCD was designed to investigate the influence of these factors on multiple responses. Totally, twenty experimental runs were designed, including six replications at the center point of cubic domain. In a rotatable matrix of CCD (presented in Table S4), each factor was studied at five levels ($\pm\alpha$, ± 1, 0) to reduce the uncontrollable influences. The numerical values of α depend on the number of experimental factors investigated, and for two, three and four factors, they are assigned to 1.41, 1.68 and 2.00, respectively. Thus, under the current experimental conditions, level α is equivalent to level 1.68. After performing the designed experiments, a quadratic model was fitted to the response data, for instance, the polynomial model for BPA was educed as follow:

$$\begin{aligned} Y = & -692.84 + 132.88 \times X_A + 267.84 \times X_B + 44.20 \times X_C - 0.97 \times X_A \\ & \times X_B + 4.01 \times X_A \times X_C - 0.21 \times X_B \times X_C - 41.22 \times X_A^2 - 31.57 \\ & \times X_B^2 - 1.78 \times X_C^2 \end{aligned} \quad (4)$$

The analysis of variance was performed to evaluate the data of the optimization experiments (detailed data in Table S5). Summary statistics with a maximum P-value of 0.0002 indicated that the generated model was not aliased for further analysis. Meanwhile, the F-value of model (12.81–23.64) implied that the variations of responses were associated with the polynomial model rather than the pure errors. "Adeq Precision" measures the signal to noise ratio and a ratio > 4.0 is desirable. As a result, a minimum ratio of 10.485 (for BPA) was obtained, which indicated the signal intensity is adequate and the conducted models can be used to navigate the design space. The coefficient of determination (R^2) was calculated by least square regression and applied to evaluate the overall variation in the data accounted by the model. According to Ranjbari' study (Ranjbari & Hadjmohammadi, 2015), the R^2 should be at least 0.800 to verify the favorable consistency between the actual data and theoretical predictions. As a result, satisfactory R^2 values (varied from 0.920 to 0.955) were achieved for all four bisphenols, which indicated that the established polynomial models were capable of explaining 92.0% to 95.5% of the variation in responses. The lack of fit test has shown that P-value (varied from 0.0869 to 0.1103) is > 0.05. Therefore, this model could fully explain the variation in the response. The significant effects were identified by the Student's t-test. If the Prob > |t| is < 0.05, the effect is considered very significant while Prob > |t| is between 0.05 and 0.10, it is considered to be relatively significant. As the results shown in Table S5, the independent variable of X_C and all quadratic effects exhibited very significant effects on the responses of all bisphenol compounds. Among the interactions, only X_A*X_C affected the recoveries of BPS and BPB in a significant way. The three-dimensional surface plots were highly recommended to visualize the relationships between the levels of factors and the response values. Hereinto, The effects of two factors can be visualized simultaneously while the third one is set to its central level. As an example, the response surface and contour plots of BPS were exported and synthesized together by Design-expert software in Fig. 2, which mapped against different combination of two investigated factors. The diagrams of three other bisphenols were presented in Fig. S3. Fig. 2A and B depict the interactions of X_A versus other two factors. According to the plotted surfaces, the recovery reaches to the maximum when the term of X_A is approximately at the center level. Response surface plots for the concentration of ammonia in diluent solution (X_B) are shown in Fig. 2A and C. According to these plots, lower level of X_B (between level −1 and level 0) are proven to help improve the extraction efficiency. In the same way, the increase in the volume of ammonia solution used for dilution (X_C, between level 0 and level +1)

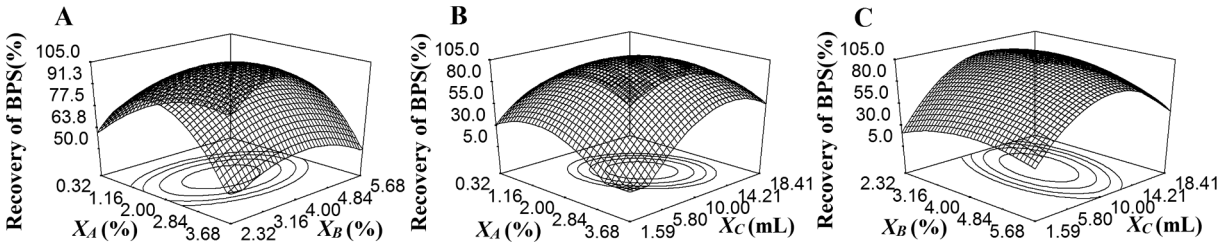

Fig. 2. The estimated response surface plots of BPS response versus: (A) Concentration of FA in acetonitrile-Concentration of ammonia in diluent solution; (B) Concentration of FA in acetonitrile-Volume of ammonia solution used for dilution; (C) Concentration of ammonia in diluent solution-Volume of ammonia solution used for dilution.

were welcomed. As mentioned above, in the course of extraction, the presence of FA helps the bisphenols keep molecular forms and thus enhances the partition ratios in the organic extractant. But on the other hand, excessive acidic contents contradict the usage of anion-exchange SPE, which will certainly lead to a reduction in the adsorption capacity. In this regard, the employment of ammonia is necessary to neutralize FA and adjust the pH conditions, thereby improving the ionization efficiency of bisphenols. Moreover, the volume of diluent plays an important role in controlling the organic proportion of sample solution. Appropriate dilution proportion can save time and ensure high withdrawal capacities, in the process of loading the diluent on SPE columns.

Considering the high number of variables and responses, a desirability function was conducted to identify the optimum experimental condition. Finally, the experimental combination (2.2% of FA, 14.1 mL of 4.1% ammonium) with the highest desirability value of 0.873 ($d = 0.873$) was chosen as the optimum solution. At this experimental point, the absolute recovery rates were predicted to be 66.8% for BPS, 79.3% for BPB, 79.6% for BPA and 74.8% for BPB. A series of supplementary experiments ($n = 3$) were subsequently conducted to validate the consistency of the actual extraction efficiency and the theoretical predictions under the optimum condition. As a result, deviations between the obtained results (mean ± RSD, 56.6% ± 2.9% for BPS, 69.6% ± 1.4% for BPF, 74.1% ± 5.0% for BPA and 69.1% ± 4.7% for BPB) and the predicted values are slight (< 15%), which indicated that the prediction accuracy of the established model was acceptable (Homem et al., 2016).

3.3. Optimization of UFLC-MS/MS conditions

The pure standard solutions were infused directly into the mass spectrometer in full-scan mode to get the accurate precursor parameters. De-protonated products, i.e., $[M-H]^-$, were normally obtained under the negative mode. The type of additive in aqueous phase plays an important role in the separation and ionization of analytes. Ammonia was usually added at 0.1% to enhance the ionization efficiencies, and therefore, ammonia solution and pure water were compared. Despite the slight enhancement on the responses of bisphenols (Fig. S4), the retention of BPS on chromatographic column was weakened under alkaline condition and tailed peaks were observed as well, which reduced the sensitivity of this method. Thus, water was preferred considering the sufficient sensitivity and chromatographic repeatability. But at the same time, the acidic contents (approximately 2% FA) in the final SPE eluent were incompatible for direct injection under negative mode, and the employment of ammonia was still necessary to reconcile the chromatographic behavior and ionization efficiency. Finally as a trade-off, the reconstitution solution involved the addition of ammonia to regulate and control the pH conditions, and the final proportion was set to 1%.

3.4. Validation of the established method

3.4.1. Background contamination

All necessary precautions should be taken to control the concentrations of free bisphenols within reasonable ranges, which helps improve the methodological detectability and decrease the probability of false positive. In our case, bisphenols (BPS and BPA) were mainly found to stem from two aspects of the preparation procedure: (a) SPE cartridges; (b) chemical reagents, plastic wares and other laboratory consumables. The exudation of bisphenols from SPE cartridges could be effectively reduced by pre-washing with sufficient volume of acetonitrile. As usual, the cartridges were conditioned with 3–6 mL of acetonitrile and with this condition, the free BPA concentration was approximately 0.45 ng/mL. However, the background values could be controlled below 0.3 ng/mL when the volume of acetonitrile increased to 12 mL. Therefore, adequate pre-washing intensity was considered to be an essential element in maintaining a low-level background concentration. This experimental phenomenon was consistent with the conclusion previously reported (Ballesteros-Gómez et al., 2009).

Under the optimized conditions, the background concentrations of BPA in the representative matrices were determined to be 0.29 ± 0.05 ng/mL (mean ± SD) in water and 0.44 ± 0.03 ng/mL in crucian carp. As for BPS, the highest background value was estimated to be 0.03 ± 0.02 ng/mL in crucian carp. It could be inferred that the number of preparation steps and the natural properties of sample are the major reasons for the differences in background contamination. After conversion, the contamination degrees in actual samples were approximately within the range of 0.0145 ng/g (water) to 0.44 ng/g (crucian carp). In summary, the concentrations of free bisphenols in sample extractants, especially for BPA, could be controlled at low levels (BPA < 0.5 ng/mL; BPS < 0.2 ng/mL), which were acceptable or negligible comparing with the natural contents.

3.4.2. Selectivity, MEs and linearity

The representative MRM chromatograms of pure solvent standard (10 ng/mL) and canned beef were illustrated together in Fig. S5. By comparison, no interference peaks were observed near the retention time of analytes implying that the method selectivity under the established conditions was satisfactory. As known, the co-elution components could compete with the interests during the ionization process, particularly in electrospray ionization source, thereby affecting the determination accuracy of trace-level contaminants. Although the isotopic internal standards were employed to correct the signal suppression or enhancement effects, MEs were still estimated as a criterion for the assessment of purification effects of extractant (except water). As shown in Table S6, the MEs in ten typical matrices were determined ranging from 38% to 74% for BPS, 25% to 85% for BPF, 11% to 87% for BPA and 32% to 92% for BPB, respectively. Hereinto, BPS was strongly suppressed in most cases, which could be attributed to the massive coexistence of strongly polar interferences during the same elution period. The other three bisphenols were generally suppressed in animal-derived matrices due to the medium and weak polarity interferences. As for the linearity, satisfactory results were obtained both in pure solvent ($R^2 > 0.9998$) and blank matrix extract ($R^2 > 0.9900$) throughout all the analyzed concentrations.

3.4.3. Accuracy, precision and sensitivity

Accuracy was evaluated through the spiking experiments (detailed data in Table S7). The relative recoveries ranged from 87% to 112% for BPS, 81%-110% for BPF, 87%–116% for BPA and 76%–112% for BPB in all tested assays, with the associated RSDs not exceeding 12%. Absolute recoveries were used to estimate the withdrawal effects of the established method, and acceptable results (over 40%, RSDs ≤ 23%) were obtained in most matrices. However, it must be noted that fairly low absolute recoveries (from 22% to 40%) were observed in flour matrix. The poor performance might be related to the fact that the sorbents on cartridge were encapsulated by the superfine wheat powder, therefore reducing the contact areas between the analytes and sorbents considerably.

LODs and LOQs were investigated and the results ranged from 4 ng/L to 55 ng/L and 14 ng/L to 120 ng/L in aqueous samples, and from 0.05 μg/kg to 1.2 μg/kg and 0.1 μg/kg to 4.0 μg/kg in other matrices. The highest detection limit appeared in wheat matrix, which was blamed on the poor withdrawal effects. For comparison, some typical reports on the determination of bisphenol analogues in food samples were summarized, showing in Table 2. As shown, very few methods are available for the simultaneous determination of multiple bisphenols in various foodstuffs. The analytical performance of this methodology, including the scope of application, purification capacity, control of background values and accuracy of data, were comparable to or better than these previous methods.

Table 2
Comparison of the proposed method with some previously reported methods for the determination of bisphenols in food matrices.

Reference	Sampling quantity	Target compound	Preparation approach	Matrix species	Background contamination of BPA	Detectability (LOQs)	Quantitative approach
Cunha and Fernandes (2013)	40	BPA, BPB	QuEChERS-based extraction & DLLME[a] & derivatization	++[b] (canned vegetables and fruits)	not mentioned	0.1–0.4 μg/kg	GC-MS (IS[c])
Liao and Kannan (2013)	267	eight bisphenols	extraction & SPE	+++++++++	0.06 ng/mL	0.01–0.05 μg/kg	LC-MS/MS (IS)
Errico et al. (2014)	16	BPA	extraction & normal-phase SPE	+ (canned tomatoes)	not mentioned	0.26 μg/kg	HPLC-UV/FLD
Zimmers et al. (2014)	21	BPA	Liquid-liquid partitioning & SPE	+ (breast milk)	0.10 ng/mL	0.22 μg/kg for LOD	UPLC-MS/MS (IS)
Sungur, Köroğlu, and Özkan (2014)	68	BPA	immunoaffinity column	++++	not mentioned	not mentioned	HPLC-PAD
Niu et al. (2015)	12 groups	BPA	gel permeation chromatography or on-line SPE	+++++++++++++	not mentioned	2.0 μg/kg	LC-MS/MS (IS)
Gallo et al. (2017)	40	BPA, BPB, BPF, BADGE, BFDGE	molecularly imprinted SPE	+ (energy drink)	not mentioned	0.5 μg/kg	UPLC-FLD
Xiong et al. (2018)	50	nine bisphenols	QuEChERS methodology	+ (milk)	not mentioned	3.5–9.8 μg/kg	HPLC-FLD
This study	379	BPS, BPF, BPA & BPB	QuEChERS-based extraction & SPE	+++++++++++++	0.29–0.44 ng/mL	0.004–4.0 μg/kg	UFLC-MS/MS (IS)

[a] Abbreviation for dispersive liquid-liquid microextraction.
[b] One "+" represents one kind of matrix, more "+" indicate favorable applicability of the method.
[c] Internal standard method.

Table 3
Contamination status of bisphenols in twelve investigated food categories.

Category of matrix	BPS				BPF				BPA			
	frequency (%)	measurable range (μg/kg or μg/L)	mean/median (μg/kg or μg/L)	95th percentile (μg/kg or μg/L)	frequency (%)	measurable range (μg/kg or μg/L)	mean/median (μg/kg or μg/L)	95th percentile (μg/kg or μg/L)	frequency (%)	measurable range (μg/kg or μg/L)	mean/median (μg/kg or μg/L)	95th percentile (μg/kg or μg/L)
water	30.3	0.007–0.13	0.02/0.0	0.13	15.2	0.01–0.03	0.004/0.0	0.03	21.2	0.05–0.08	0.01/0.0	0.08
beverages	23.9	0.06–0.18	0.01/0.0	0.05	54.4	0.05–0.23	0.04/0.03	0.12	80.4	0.13–36.4	2.7/0.2	11.3
rice	25.8	0.3–1.5	0.11/0.0	0.5	-[a]	–	–	–	64.5	0.5–3.8	0.9/0.8	1.9
wheat flour	–	–	–	–	–	–	–	–	39.4	1.0–1.0	0.4/0.0	1.0
shellfish	100.0	0.3–25.0	2.1/0.5	3.8	–	–	–	–	90.0	1.3–13.4	2.6/1.6	3.6
fish	100.0	0.4–65.8	9.2/3.9	38.8	–	–	–	–	66.7	1.1–12.5	3.0/1.7	11.8
meat	75.0	0.3–104.0	9.0/2.3	37.0	–	–	–	–	75.0	1.1–59.8	5.4/1.7	32.5
vegetables	62.5	0.5–3.2	0.5/0.4	1.4	–	–	–	–	75.0	0.6–4.5	1.3/1.0	3.2
canned cereal	–	–	–	–	61.3	0.7–9.3	1.1/0.4	4.6	100.0	3.9–148.0	33.4/17.2	117.5
canned fish	50.0	0.3–1.4	0.3/0.1	1.2	73.3	1.1–22.9	5.1/2.1	14.3	100.0	5.4–243.0	51.8/32.6	163.0
canned meat	13.3	0.3–0.5	0.05/0.0	0.4	83.3	0.7–36.4	10.6/6.7	33.4	100.0	10.3–326.0	73.5/59.5	213.0
Others	7.3	0.1–0.4	0.05/0.0	0.03	14.6	0.5–0.6	0.11/0.0	0.4	63.4	1.0–13.6	2.4/0.8	10.3

[a] No available results were achieved for this item.

3.4.4. Application of the validated method

A total of 379 samples involving in twelve different categories were subjected to analysis in this study and all the obtained results are sketched in the form of box plots in Fig. S6 (detailed data in Table 3). To our knowledge, very few studies have investigated the contamination status of bisphenols on such a large sample size. Although BPA had been gradually replaced by its analogues, it was still demonstrated to be the predominant bisphenol contaminant with highest incidence (79.7%) and average concentration (14.3 μg/kg). 37.7% of the samples were contaminated with BPS (average 1.6 μg/kg) while the overall detection rate of BPF was 26.9% (average 1.4 μg/kg). The maximum contamination concentration could be reached up to 104.0 μg/kg of BPS in pork, 36.4 μg/kg of BPF in canned meat and 326.0 μg/kg of BPA in canned meat, respectively. Furthermore, BPB was not detected in any of the samples.

After analyzing the data, the contamination conditions in beverages were found to be more serious comparing with water samples. Two abnormally high results of BPA were detected in two energy beverages at 35.8 μg/L and 36.4 μg/L, respectively. Both of them were packaged in hard metal cans with plastic linings, which might be the responsible for the release of BPA. 64.5% and 25.8% of rice samples were positive for BPA (0.5 μg/kg-3.8 μg/kg) and BPS (0.3 μg/kg-1.5 μg/kg) whereas only 39.4% of flour samples were detected containing BPA at the level around LOD (1.0 μg/kg). The relatively higher detection limits in flour must be one of the main causes of low positive rates, and frankly, the practical application in flour matrix is still defective. Although these two kinds of cereals are usually available in unpackaged or bulk form, bisphenol compounds were still found. There are two likely conjectures to explain the presence of bisphenols in them: (a) rice and wheat are in contact with the processing parts (used for shelling and milling), packaging materials and plastic containers during the whole circulation process, which may be involved the contamination of bisphenol compounds; (b) the crops may absorb and concentrate the bisphenol compounds from their surroundings, such as soil, air and dust. In the author's opinion, both the conjectures are reasonable to a certain extent. But the determination results of fresh vegetables, in which positive rates of BPA and BPS were found to be 75.0% and 62.5%, ranging in levels up to 4.5 μg/kg and 3.2 μg/kg, pointed more to the latter one (b). The incidences (mean concentration and measurable range) of BPA were determined to be 90.0% (2.6 μg/kg, 1.3 μg/kg–13.4 μg/kg) in shellfish, 66.7% (3.0 μg/kg, 1.1 μg/kg–12.5 μg/kg) in fish and 75.0% (5.4 μg/kg, 1.1 μg/kg–59.8 μg/kg) in meat. On the other hand, the corresponding results of BPS were 100.0% (2.1 μg/kg, 0.3 μg/kg–25.0 μg/kg), 100.0% (9.2 μg/kg, 0.4 μg/kg-65.8 μg/kg) and 75.0% (9.0 μg/kg, 0.3 μg/kg–104.0 μg/kg), respectively. Although no correlation was found between the types of animal-derived food (fresh meat, fish and shellfish) and BPA levels (*Sig.* = 0.972), the contamination contents of BPS in fish were far exceeding the shellfish and meats (*Sig.* = 0.024).

All canned products contained BPA with concentrations varying from 3.9 μg/kg-326.0 μg/kg. The occurrences of BPS in canned fish, meat and cereal products were determined to be 50.0%, 13.3% and 0.0%, respectively. Compared with the fresh food of the same category (meat and fish), significantly higher contents of BPA were found in the processed canned matrices. Nevertheless, the contamination scenarios of BPS were on the contrary, which could be partially explained by the fact that the strong polar contaminant was lost during the whole production process. On the other hand, the occurrence of BPF was mainly reported in the canned foodstuffs. Measurable range (detection rate) were determined from 0.7 μg/kg to 9.3 μg/kg (61.3%) in canned cereals, 0.7 μg/kg to 36.4 μg/kg (83.3%) in canned meats and 1.1 μg/kg to 22.9 μg/kg (73.3%) in canned fish, respectively. It could be inferred from the above experimental data that BPF had been used as an additive in the industrial production of lining materials for metal cans. In the category of “others”, BPA was also widely distributed in edible oil, peanut butter, honey and eggs (overall incidence of 63.4%) while only two peanut butter samples that packed in plastic-lined containers were contaminated with BPF.

3.4.5. Dietary exposure assessment

In 2015, European Food Safety Authority lowered the tolerable daily intake of BPA from 50 μg/kg bw/day to a temporary value of 4 μg/kg bw/day, based on the uncertainties surrounding health effects after exposure (Husøy et al., 2015). It is of great significance to compare the exposure estimates such as International Estimated Daily Intake (IEDI) with the proposed toxicological criteria, which can reflect the dietary exposure status and risk characteristic of bisphenols (Osman, Al-Humaid, Al-Rehiayani, & Al-Redhaiman, 2011). The target population in the present study is adult residents in Zhejiang Province (18–45 years old). The chronic evaluation models were constructed by multiplying the average contamination concentrations and daily recommended intakes according to Eq. (5):

$$IEDI_{mean} = \frac{\sum_{k=1}^{n} X_{k,mean} \times C_{k,mean}}{bw_{mean}} \quad (5)$$

where, $X_{k,mean}$ represents the daily recommended intakes of food *k*, and the data originated from the 2016 version of Chinese Residents' Dietary Guidelines (http://dg.cnsoc.org/article/04/8a2389fd5520b4f30155be1475e02741.html). $C_{k,mean}$ is the mean concentration of the analyzed samples of category *k*. In the course of data analysis, the protocol for assigning concentration values to negative results (below LODs) is critical to the dietary exposure assessment. This issue has been extensively studied and there are no international guidelines on the need to report both the LOD and LOQ in a standardized manner (FAO/WHO, 2009). The criteria used in this study are primarily based on “Principles and Methods for the Risk Assessment of Chemicals in Food. Chapter 6: Dietary Exposure Assessment of Chemicals in Food”. Furthermore, the bisphenol compounds were classified as the chemicals that unlikely to be present unless specifically added. More specifically, if < 60% of results are less than the LOD, then a reasonable estimate of the mean can probably be obtained by setting all negative results to LOD/2; on the other hand, a lower-bound value of 0 is assigned to the non-detected samples when the negative rate is considerable (over 60%). The average body weight (bw_{mean}) was set as 63 kg on the basis of a previous study reported by Niu, Zhang, Duan, Wu, and Shao (2015) in which nearly 4,000 individuals were investigated (Niu et al., 2015). Additionally, high exposure scenario was also assessed using the 95th percentile concentrations instead of the mean concentrations as follow:

$$IEDI_{95th} = \frac{\sum_{k=1}^{n} X_{k,mean} \times C_{k,95th}}{bw_{mean}} \quad (6)$$

Finally, the estimated dietary intakes of bisphenols were summarized in Table 4. Among these data, $IEDI_{mean}$ of BPS, BPF and BPA were determined to be within 12.5–22.4 ng/kg bw/day, 3.0–3.0 ng/kg bw/day and 40.4–50.7 ng/kg bw/day while $IEDI_{95th}$ were 48.5–86.4 ng/kg bw/day, 6.3–6.4 ng/kg bw/day and 117.2–153.7 ng/kg bw/day, respectively. By comparison, exposure evaluation results of bisphenol compounds in this experiment were somewhat lower than several previous reports. The estimates proposed by Scientific Committee of European Commission were at 110–370 ng/kg bw/day for adults (EC, 2002) while a comparable level (185 ng/kg bw/day) was also reported by U.S. Food and Drug Administration from the cumulative *IEDI* database. Moreover, higher intake level of BPA at 400–1400 ng/kg bw/day was proposed at the joint meeting of Food and Agriculture Organization and World Health Organization (FAO/WHO, 2010). But for BPS, the exposure level in our case was significantly higher than the results reported previously (Liao & Kannan, 2013), which was only 1.66 ng/kg bw/day.

Although the canning operations and canned foods are the primary sources of bisphenols (especially BPA and BPF), the poor annual per

Table 4
Estimated daily dietary intakes of bisphenols for adult residents in Zhejiang Province.

Category	Daily recommended intake	mean (ng/kg bw/day)			95th (ng/kg bw/day)		
		BPS	BPF	BPA	BPS	BPF	BPA
Water	1500–1700 mL/day	0.47–0.54	0.10–0.11	0.24–0.27	3.1–3.5	0.71–0.81	1.9–2.2
Milk[a]	300 g/day	0.0	1.0	7.1	0.0	1.2	10.5
Grain[b]	250–400 g/day	0.22–0.35	0.0–0.0	2.6–4.1	1.0–1.6	0.0–0.0	5.8–9.2
Aquatic products[c]	40–75 g/day	3.6–6.7	0.0–0.0	1.8–3.3	13.7–25.7	0.0–0.0	4.9–9.2
Meat	40–75 g/day	5.7–10.7	0.0–0.0	3.4–6.4	23.5–44.0	0.0–0.0	20.6–38.7
Vegetable	300–500 g/day	2.4–4.0	0.0–0.0	6.2–10.3	6.7–11.1	0.0–0.0	15.2–25.4
Egg[d]	40–50 g/day	0.10–0.12	0.0–0.0	0.50–0.62	0.28–0.35	0.0–0.0	0.70–0.87
Canned cereal	2667 g/year	0.0	0.16	3.8	0.0	0.56	13.6
Canned fish	2667 g/year	0.032	0.56	6.0	0.16	0.96	19.2
Canned meat	2667 g/year	0.008	1.2	8.8	0.048	2.9	24.8
IEDI		12.5–22.4	3.0–3.0	40.4–50.7	48.5–86.4	6.3–6.4	117.2–153.7

[a] Data derived from milk samples only (mean: BPA: 1.5 μg/L; BPF: 0.22 μg/L; 95th: BPA: 2.2 μg/L; BPF: 0.26 μg/L).
[b] Data derived from both rice and wheat flour categories, and were calculated by each half of the daily consumption.
[c] Data derived from both shellfish and fish categories, and were calculated by each half of the daily consumption.
[d] Data derived from eggs only (mean: BPS: 0.15 μg/kg, BPA: 0.78 μg/kg; 95th: BPS: 0.44 μg/kg, BPA: 1.1 μg/kg).

capita consumption of this kind of food (approximately 8 kg per year, data come from Analysis Report on the Current Situation of China's Canning Industry in 2018) restricted the dietary intakes. This is also the major reason for the difference in research results from different regions. Overall, the upper limit of $IEDI_{mean}$ for total bisphenols (76.1 ng/kg bw/day), and even the high exposure scenario (246.5 ng/kg bw/day) accounted for < 10.0% of tolerable daily intake. However, it must be highlighted that the projected intake is an underestimation of the actual status since some specific types of food were not involved during the course of assessment, e.g. fast food, fruits, etc. Combined with the results in the present study and the U.S. funded Clarity-BPA project, little risk potential for health effects to adult population were observed. But even so, there are still controversies about the safety of BPA at trace concentrations given it may be following a non-monotonic dose-response. For instance, some reports had indicated that trace level of BPA (1.2 μg/kg) exhibit significantly higher binding affinity for estrogen receptor gamma, and the combination was found to protect the estrogen receptor from being deactivated (Li et al., 2018).

4. Conclusion

This study developed a rapid and universal method for the simultaneous analysis of four bisphenols in various foodstuffs. QuEChERS-based extraction and ion-exchange SPE purification were employed as the major sample preparation procedure. The chemometrics approaches, i.e. Plackett-Burman design and CCD, were implemented to reach the optimal experiment conditions with less workload. In comparison to the methods reported previously, the established method exhibited benefits in its applicability, sensitivity and accuracy. Regardless of the assessment result in this study, continuous concerns were essential to control the potential health risk posed by bisphenols through diet, especially for the protection of sensitive populations like pregnant women, adolescents, etc. Furthermore, the scope of investigation and sample size need to be enlarged in order to generate more reliable and representative data. At the same time, there are still some deficiencies in the practical application of the established method, which needs further exploration and optimization.

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Declaration of Competing Interests

The authors declare no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2019.05.022.

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