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A high-throughput screening method of bisphenols, bisphenols digycidyl ethers and their derivatives in dairy products by ultra-high performance liquid chromatography-tandem mass spectrometry

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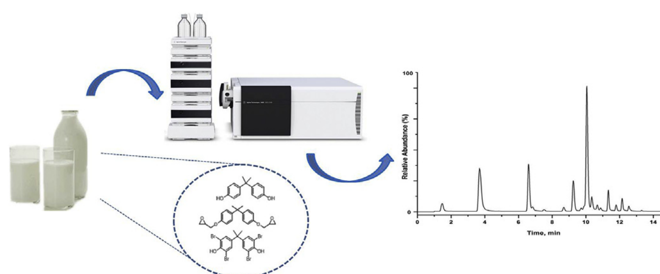
HIGHLIGHTS

- UHPLC-ESI-MS/MS for determination of BPs, bisphenols ethers and their derivatives.
- High throughput method for simultaneous detecting 21 BPs, bisphenols ethers and their derivatives in dairy products.
- An easy, cost-effective, time-saving sample preparation method based on QuEChERS.

GRAPHICAL ABSTRACT



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ABSTRACT

A simple and universal analytical method based on ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) for high throughput screening of 21 bisphenols, bisphenols digycidyl ethers and their derivatives in dairy products was developed. Response Surface Methodology (RSM) was used to optimize sample preparation conditions based on a quick, easy, cheap, effective, rugged and safe (QuEChERS) method. The analytes were extracted by using 15 mL acetonitrile with 1% acetic acid, and the extracts were further purified by using 190 mg of C18 and 390 mg of PSA. The extracts were analyzed by UHPLC-MS/MS with electrospray ionization (ESI) source. Linearity was assessed by using matrix-matched standard calibration and good correlation coefficients ($r^2 > 0.99$) were obtained. The limits of quantitation (LOQs) for the analytes ranged from 0.02 to 5 μg kg^{-1}. The extraction recoveries were in a range of 88.2%–108.2%. Good method reproducibility in terms of intra- and inter-day precision was observed, yielding relative standard deviations (RSDs) less than 8.9% and 9.9%, respectively. The validation method results revealed that the proposed method was sensitive and reliable. Finally, this method was successfully applied to dairy product analysis.

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1. Introduction

Bisphenol A (BPA), which is widely known as one of endocrine disruptors, is an organic compound with two phenol moieties. During the production of plastics, BPA is usually added for

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characteristics of transparency, light weight, durability and excellent impact strength. Bisphenol A has high production volume and is mainly used as functional monomer of polycarbonate plastics andepoxy resins, which are used in electronic equipment, digital media, medical devices, sports safety equipment and food contact materials [1]. For application in food contact materials, polycarbonate plastics are used as reusable bottles (e.g. baby bottles), food storage containers, tableware, water pipes and microwave ovenware. Epoxy resinsare usually used as internal protective lining of metal cans and coating on metal lids [2–5]. Bisphenols diglycidyl ether (BADGE and BFDGE) are also used as monomers ofepoxy resins. Some researchers reported that chlorinated derivatives and hydrolyzed derivatives of bisphenols diglycidyl ether (e.g. BADGE-H_2O, BADGE-$2H_2O$, BADGE–H_2O–HCl, BADGE-HCl, BADGE-2HCl, BFDGE-$2H_2O$, BFDGE-2HCl) may be generated during the thermal stabilization and storage when the coating comes into contact with aqueous and acidic foodstuffs [6]. Some migration studies showed that free bisphenols, bisphenols diglycidyl ether and their derivatives could migrate into packed food in some conditions [7,8].

In recent decades, a series of adverse health issues of BPA have been verified and have raised intense concern. BPA exhibits estrogenic and anti-androgenic effects [9,10], and is related to some metabolic diseases (e.g. heart disease, diabetes, obesity, thyroid and liver function) [11]. Additionally, bisphenols diglycidyl ether and their derivatives also show anti-androgenic and genotoxic effects [12–14]. Moreover, tetrabromobisphenol A (TBBPA) and tetrachlorobisphenol A (TCBPA), which are halogen derivatives of BPA, are flame retardant of large production volume used in polymers, resins and adhesives, and have thyroid hormonal activity and estrogenic activity [9]. In order to protect public health, many countries and organizations began to establish regulations. The European Union (EU) has set food specific migration limits (SML) for BPA (0.6 mg kg^{-1} [15]), BADGE and its hydrolysis products (9 mg kg^{-1} [16]) and chlorinated BADGE (1 mg kg^{-1} [16]). BFDGE was banned to use in food contact materials [16] and BPA was banned in baby bottles [17]. In order to cope with the regulations, bisphenols (BPs), which are structurally similar to BPA, are used to replace BPA in industrial production. Unfortunately, several studies demonstrated that BPs are not safer than BPA [9,18–20], therefore, it is also essential to analyze BPs in some food samples, such as dairy products and infant food. The physicochemical properties and structures of target compounds are shown in Table S1 (supplementary materials).

BPs, bisphenols digycidyl ethers and their derivatives were detected respectively in many studies [21,22]. The chromatographic techniques reported mainly include gas chromatography coupled to mass spectrometry (GC-MS) [23,24], liquid chromatography with fluorescence detection (LC-FLD) [25,26] and liquid chromatography-tandem mass spectrometry (LC-MS/MS) [27]. Although GC-MS has advantages of high sensitivity and capabilities, it has to involve derivative steps before chromatographic separation, which are time-consuming and also increase the possibility of contamination. LC-FLD is well suited to the determination of mixture of BPs, bisphenols diglycidyl ethers and their derivatives; however, a tedious sample preparation is usually needed for removing matrix interferences. And the identification of the target compound which only depends on retention time may lead to false positive results. More important, fluorescence responses of some BPs (e.g. bisphenol A halogenated derivatives) are too low to be detected on account of heavy atom effect. LC-MS/MS is highly sensitive and efficient, thus it was used for identification and quantitative analysis of BPs and bisphenols diglycidyl ethers and their derivatives. There are some reports on the determination of BPs or bisphenols diglycidyl ethers and their derivatives [28,29], however, no high-throughput screening method for simultaneously determining BPs, TBBPA, TCBPA, bisphenols diglycidyl ethers and their derivatives has been reported.

In this work, a high-throughput ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) method has been described for screening 21 analytes, including major BPs, bisphenols ethers and their derivatives, TBBPA and TCBPA. An easy, cost-effective, time-saving sample preparation method based on QuEChERS was applied. Response surface methodology was employed to optimize the critical parameters of sample preparation procedure. The method was successfully applied on determination of the target analytes in dairy products from local market.

2. Materials and methods

2.1. Reagents, materials and standards

Acetic acid (HOAc) and anhydrous magnesium sulfate ($MgSO_4$) were purchased from Xilong scientific (Guangzhou, China). Ammonium formate was purchased from Aladdin (Shanghai, China). Anhydrous sodium acetate (NaAc) was purchased from Alta Aesar (Shanghai, China). HPLC-grade acetonitrile (MeCN) and methanol (MeOH) were sourced from Sigma–Aldrich (St. Quentin Fallavier, France). Cleanert S C18 and Cleanert PSA were obtained from Agela Technologies (Tianjin, China). Ultrapure Water (resistivity, 18.2 MΩ) was purified on a Milli-Q Plus apparatus (Millipore, Brussels, Belgium). Nitrogen with a purity of 99.9999% was used as collision gas, and nitrogen with a purity of 99.995% was used as sheath gas, nebulizing gas and dry gas. Both of them were supplied by Beijing green oxygen science and technology Ltd. (Beijing, China).

Standards of BPA (≥98.5%), BPB (≥99.8%), BPF (≥99.9%), TBBPA (≥99.0%), BFDGE (≥98.0%) and TBBPA-d_{10} (≥98.5%) were purchased from Dr.Ehrenstorfer (Augsburg, Germany). BPA-2, 2′, 6, 6′-d_4 (≥98.6%) was purchased from CDN (Quebec, CANADA). BFDGE-$2H_2O$ (≥96.0%) and BFDGE-2HCl (≥98.0%) were obtained from Toronto Research Chemicals (North York, Canada). BPAF (100%), BPZ (100%) and BADGE (≥99.1%) were purchased from accustandard (New Haven, USA). BPC (≥99.0%), BPP (≥99.0%), BPAP (≥99.0%), BPS (≥98.0%), TCBPA (≥98.0%), BADGE-H_2O (≥95.0%), BADGE-$2H_2O$ (≥97.0%), BADGE-HCl (≥90.0%), BADGE-2HCl (≥97.0%), BADGE–H_2O–HCl (≥95.0%) and DMBPS (≥99.0%) were purchased from Sigma-Aldrich (Steinheim, Germany).

Stock solution of individual analyte was prepared in MeOH (1000 mg L^{-1}). Then, a mixed working standard solution of 21 analytes was prepared at a concentration of 10 mg L^{-1} by combining suitable aliquots of stock solution of each individual standard and diluting them with appropriate volume of MeOH. All of them were prepared weekly and stored in screw capped glass tubes at −20 °C in the dark.

For reducing the approach of laboratorial contamination, glass materials were used in place of plastic materials. And solvents and reagents were also avoided to contact with plastic materials. All glassware was cleaned with methanol prior to the analysis. Moreover, quality control blanks were periodically prepared and analyzed. All solvents were checked for the presence of the target analytes before use.

2.2. Instrumentation and analytical conditions

Chromatographic analyses were performed by an Agilent 1290 Infinity UHPLC system (Agilent, San Jose, CA, USA), consisting of a G4220A binary pump, a G4226A autosampler and G1316A temperature-controlled column manager. The detector was an Agilent 6490 triple quadrupole mass spectrometer with Jet Stream

ESI source. For control and data analysis, Agilent Mass Hunter B.04.01 was used.

Chromatographic separation was carried out on an ACOUITY UPLC BEH C18 (2.1 mm i.d. × 50 mm length, 1.7 μm dia.). The flow rate was 0.2 mL min^{-1}. Mobile phase A and B were 0.001 mM ammonium formate and methanol, respectively. The following linear gradient was used: 0 min, 40%B; 2 min, 40%B; 9 min, 60%B; 12 min, 85%B; 13 min, 85%B; 14 min, 40%B; 15 min, 40%B. The injection volume was 10 μL, and the column temperature was set at 40 °C.To protect the ion source from more contamination, the LC elute was diverted to waste during the first 1 min and the last 1 min of the chromatographic run.

The MS was operated in the both positive and negative ESI mode under the following specific conditions: the dry gas temperature 220 °C, the dry gas flow 14 L min^{-1}, nebulizing gas pressure 20 Psi, sheath gas temperature 300 °C, the dry gas flow 11 L min^{-1}, capillary voltage 3000 V and nozzle voltage 1500 V. Dwell time was set at 20 ms. All quantitative and qualitative data in this study were acquired in multiple reaction monitoring (MRM) mode.

2.3. Sample preparation

5 g of each sample was precisely weighed in glass tube with glass plug (50 mL). For method development and validation, mixed standard solutions were spiked to the blank samples at a concentration range of 0.05–500 μg kg^{-1} and 100 μL of a 1 mg L^{-1}methanol solution of the surrogates (BPA-d_4 and TBBPA-d_{10}) was added. The final concentration of surrogates in samples was 20 μg kg^{-1}. The mixture was vortexed for 1 min and left to stand for 30 min at room temperature in order to do their sufficient combination. 15 mL acetonitrile with 1% acetic acid was added into the mixture, and then, 4 g $MgSO_4$ and 1.48 g NaAc were added to the tube for phase separation. Next, the tube was immediately vortexed for 1 min and stood for layering. After that, the upper layer (10 mL) was transferred to another tube and submitted to a mixture of 1 g $MgSO_4$, 390 mg PSA and 190 mg C18 for clean-up. The tube was vortexed for 1 min and stood for layering again. Finally, 6 mL supernatant was evaporated to nearly dryness under a gentle stream of nitrogen and heating in water bath, and reconstituted with 1.5 mL methanol/water (50:50, v/v). The sample extract was vortexed for 30 s, and then filtered through a 0.22 μm nylon membrane for LC-MS/MS analysis.

2.4. Experimental design for Response Surface Methodology (RSM)

Response Surface Methodology (RSM) was used to optimize the variables and their interactions which were found to be significant in the sample preparation. The Box-Behnken Design (BBD) is a type of RSM and was employed to investigate three independent variables including the amounts of NaAc, PSA and C18 in this work. From the results obtained in preliminary tests, each factor was investigated at three levels: −1, 0 and 1, as shown in Table 1. A total number of design points of $N = 2k(k-1) + C0$ were used. C_0 refers to the number of center point replicates and k refers to the number of variables in this formula. The center point was evaluated in triplicate. Thus, a total of 15 different combinations of random order were employed. The significance of parameters estimates and the goodness of fit regression models were evaluated through appropriate methods. Design Expert trial version 8.0.6.1 was used (Stat-Ease Inc., Minneapolis, MN).

Table 1
Variables and levels evaluated in the Box-Benhnken design to optimize the extraction condition.

Independent variable	Unit	Symbol	Code level		
			−1	0	1
Na Acetate quantity	g	X_1	1	1.5	2
PSA quantity	mg	X_2	200	400	600
C_{18} quantity	mg	X_3	0	200	400

3. Results and discussion

3.1. Optimization of the mass spectrometric parameters

The optimum MS parameters of the 21 analytes were obtained after analyzing single standard solutions using methanol-water (50:50, v/v) as mobile phase. Precursor ions were selected in both positive and negative mode. In agreement with previous literature [30,31], BPs could produce $[M-H]^-$ ions easily in negative mode, and bisphenols digycidyl ethers and their derivatives tend to form $[M+K]^+$, $[M+Na]^+$, $[M+NH_4]^+$ adduct ions in positive mode. Compared with stability of $[M+K]^+$ and $[M+Na]^+$, which could not produce fragment in MS/MS, $[M+NH_4]^+$ ions could produce product ions easily. Therefore, $[M+NH_4]^+$ ions of bisphenols digycidyl ethers and their derivatives were chosen for further fragmentation. The collision energy (CE) was critical parameter which affected sensitivity, so CEs of all transitions of each analyte were optimized. The transitions and CE values of each analyte can be found in Table S2 (supplementary materials). Few data are available about fragment behavior of BPZ, in this study, possible fragmentation pathway of BPZ was displayed. As shown in Fig. 1, the characteristic product ion (*m/z* 223) originated from σ cleavage of hexatomic ring ($[M-H-C_3H_6-H_2]^-$) was observed. The product ion of maximum abundance due to the cleavage of the hydroxyphenyl-alkyl bond and consecutive α-cleavage of hexatomic ring yields the ion at *m/z* 145($[M-H-C_6H_5OH-C_2H_4]^-$).

3.2. Optimization of the performance of chromatography

In a preliminary study, two porous sub-2 μm particle size short columns, ZORBAX SB C18 (2.1 mm i.d. × 50 mm length, 1.8 μm dia.) and ACOUITY UPLC BEH C18 (2.1 mm i.d. × 50 mm length, 1.7 μm dia.), were evaluated for separation performance. The two columns were compared in $MeOH-H_2O$ in their appropriate gradient elution at 0.2 mL min^{-1}. Similar separation performances for total analytes were observed by the two C18 columns; however, BEH C18 column provided better resolution for BFDGE isomers, as shown in Fig. 2. The sum of the three isomers was quantified eventually due to lacking of standard of individual isomer; however, good separation can provide more information about isomer distribution in food samples.

Several mobile phases were tested using BEH C18 column and all of analytes. It can be seen in Fig. 3A and B, the mixture of acetonitrile and water showed stronger eluting power and better separation for BFDGE isomers than the mixture of methanol and water. In spite of this, methanol as organic phase produced higher responses in ESI for most analytes, especially for bisphenols digycidyl ethers and their derivatives. That is probably explained for two reasons. The first one is that methanol has proton donor characteristic which helps to form positive adducts. And better volatility and lower surface tension of methanol could lead to better desolvation of the droplets, which is the second one. Theoretically, acetonitrile as proton acceptor may help to form deprotonated molecular ions $[M-H]^-$ for BPs. However, a remarkable fact was observed that some BPs (e.g., BPZ, BPAP, BPAF, TCBPA, TBBPA), which have high steric hindrance, showed slightly higher responses in the mixture of methanol and water. In agreement with the published studies [32], the elution order changed when methanol

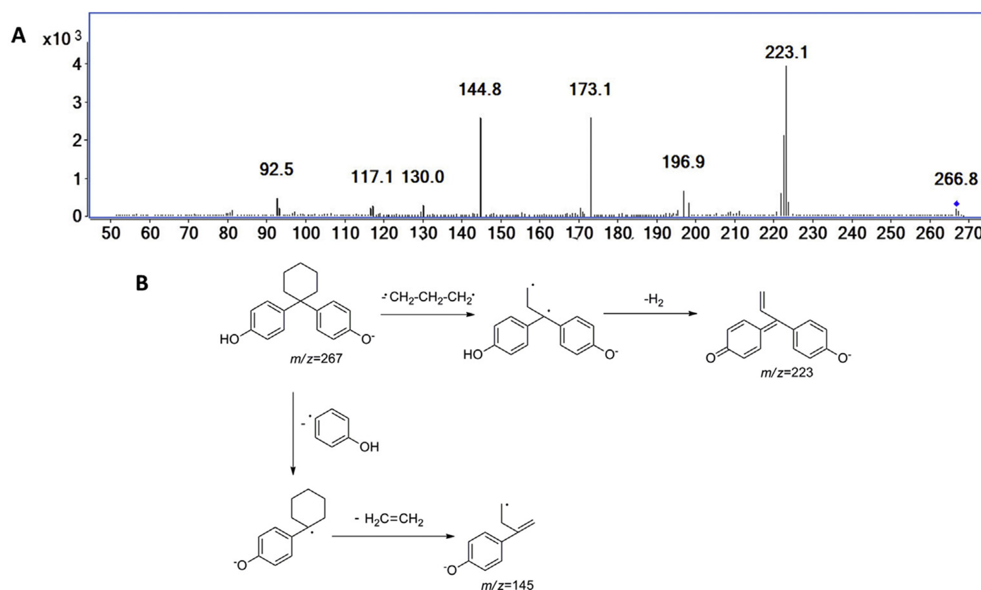

Fig. 1. A) The mass spectrum and B) the fragmentation pathway of BPZ.

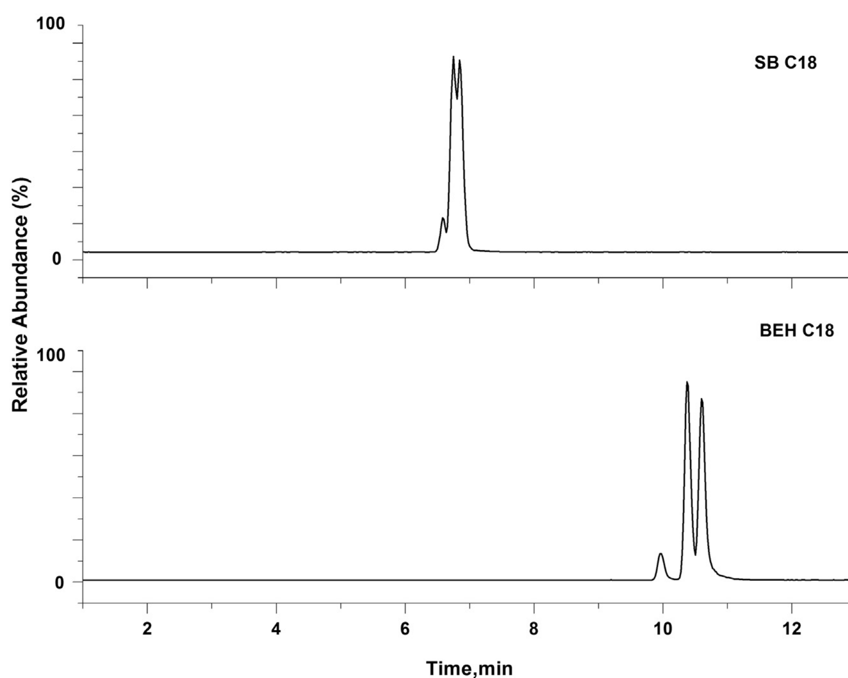

Fig. 2. The effect of the columes on the chromatographic separation of BFDGE isomers.

instead of acetonitrile was used as organic phase. In the mixture of methanol and water, BPS, DMBPS, BPF and BFDGE were eluted early and BADGE–H_2O–HCl was eluted later. That was probably related to proton donor characteristic of methanol.

The response in ESI-MS/MS detection is strongly dependent on the conditions of solutions, so whether it is necessary to use additives was also evaluated. Addition of ammonia to mobile phase may help BPs to produce $[M - H]^-$ precursor ions; however, some literature revealed the step could not improve signals. In addition, the most acidic compound BPS could not be retained by C18 column under basic condition [33]. Formic acid/ammonium formate buffer was usually used to favored the formation of ammonium adduct ions $[M + NH_4]^+$ of bisphenols digycidyl ethers and their derivatives. Here, the effect of ammonium formate concentration on the response of all analytes was explored. The results showed signals of bisphenols digycidyl ethers and their derivatives increased but signals of BPs sharply decreased with addition of ammonium formate concentration. For bisphenols digycidyl ethers and their derivatives, when the concentration of ammonium formate reached 5 mM, response began to fall slowly. Fig. 3C showed peak areas of BADGE and BPA variation tendency corresponding to different concentrations of ammonium formate. Increase of ammonium formate reduces pH value of mobile phase which affects dissociated state of BPA, so ionization efficiency of BPA decreases accordingly. Ammonium formate is helpful for forming $[M + NH_4]^+$ of BADGE; however, high concentration will

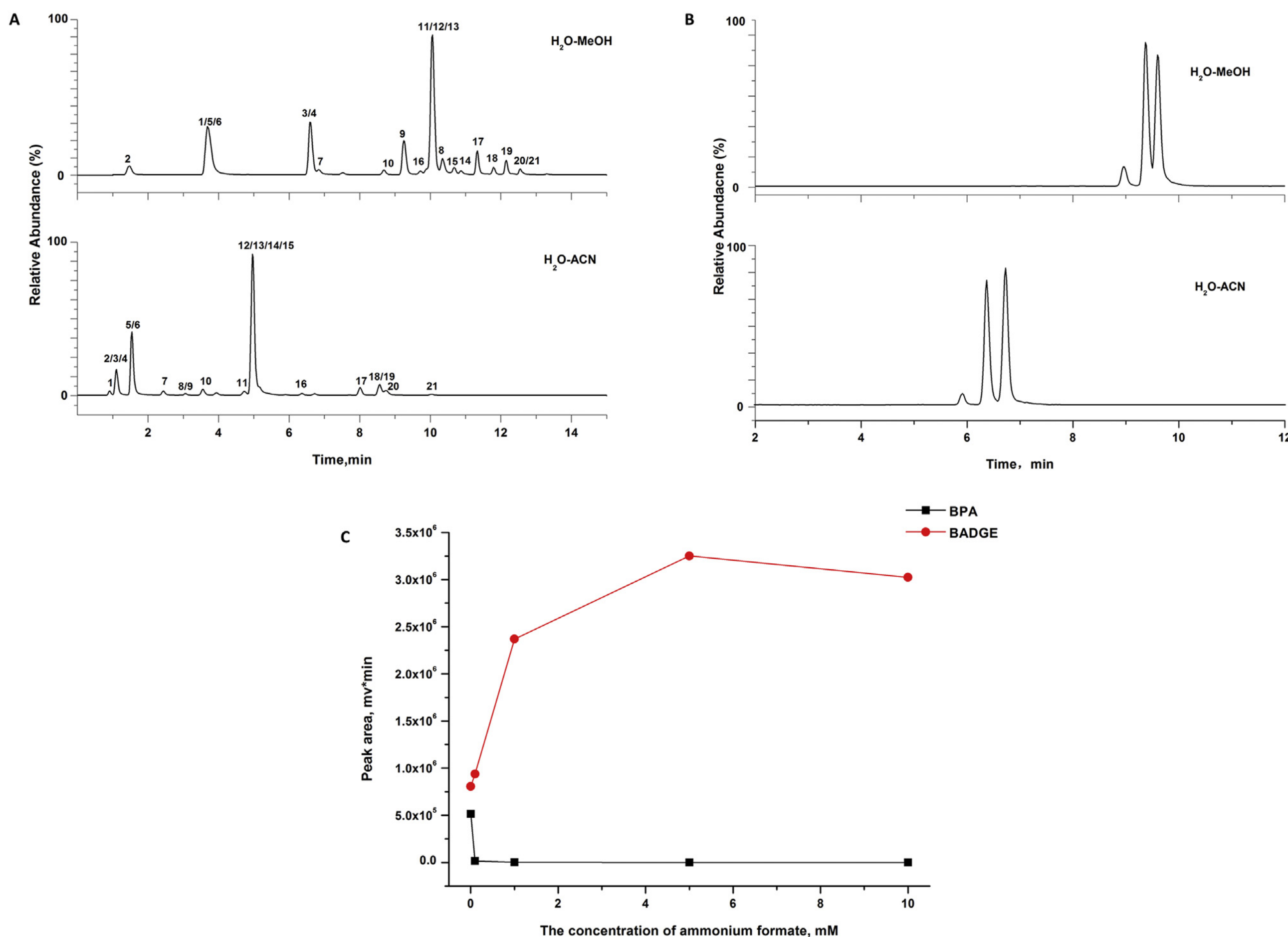

Fig. 3. The effect of two compositions of mobile phase on the chromatographic separation of A) all targeted analytes, B) BFDGE isomers. ACOUITY UPLC BEH C18 (2.1 mm i.d. × 50 mm length, 1.7 μm dia.), linear gradient elution from 40% to 85% MeOH in 15 min and 0.2 mL min^{-1} as flow rate. 1.BFDGE-2H_2O; 2.BPS; 3.BADGE-2H_2O; 4.BADGE-HCl; 5.DMBPS; 6.BPF; 7.BPA; 8.BADGE–H_2O–HCl; 9.BADGE-H_2O; 10.BPB; 11.BPAP; 12.BPC; 13.BPAF; 14.BFDGE-2HCl; 15.BPZ; 16.BFDGE; 17.BADGE; 18.BADGE-2HCl; 19.TCBPA; 20.BPP; 21.TBBPA. C) The effect of the concentration of ammonium formate on the peak areas of BPA and BADGE.

cause competitive inhibition of ionization. For BADGE, 5 mM is the most suitable concentration of ammonium formate. In order to get good responses of all analytes, eventually, 0.001 mM ammonium formate-methanol was selected as the best mobile phase.

3.3. Optimization of the sample preparation procedure

For bisphenol families, methanol and acetonitrile were commonly used as extraction solvents, and the acetic-buffer solvent was used to increase recoveries of some pH-dependent compounds [34]. In this work, different contents of acetate acid (0.1% HOAc and 1% HOAc, v/v) in methanol and acetonitrile were compared for solvent extraction efficiency of a spiked blank sample. Except that the type of solvent varied, the experiments were performed as mentioned in Section 2.3. It can be seen in Fig. 4A, for extraction solvents containing methanol, recoveries were lower, and the recoveries of both BADGE-HCl and BFDGE-2HCl were below 70%. Extraction solvents containing acetonitrile provided better recoveries, however, 0.1% HOAc in acetonitrile could not extract BPP completely, with recovery were only 71%. Compared with others, 1% HOAc in acetonitrile provided good extraction efficiency for all analytes with recoveries over 80%, and was chosen as the extract solvent. The results probably comes out of the fact that acidize acetonitrile has stronger ability of protein precipitation and decreases co-extraction of matrix components. Additionally, extraction efficiencies were affected by pH value due to their acid-base property. Fig. 4B demonstrated that effects of volume of solvent on the recoveries of analytes. It can be observed with the volume of solvent increasing, the recoveries of analytes were higher. When the value of volume of solvent reached 12, the recoveries began to be stable. In order to ensure stability of recoveries, eventually, 15 mL extract solvent was chosen to extract all analytes.

Salts are used to be added to remove water and induce phase separation. $MgSO_4$ has strong ability to bind large amounts of water and provides the most complete liquid-liquid phase separation. (NaAc was chosen for the acetate buffer solution. It could dissolve protein and fat globules, so the amount of NaAc is important for the analytes recoveries. Concerning d-SPE clean-up, different amounts of C18 together with PSA were used to obtain cleaner extracts. C18 is particularly effective to remove lipid matrix components and apolar interferences, and PSA is commonly used to absorb most sugars, organic acids and polar interferences. The amounts of C18 and PSA are critical for the analytes recoveries and method sensitivity.

During optimizing extraction condition, the interaction of different critical factors and the linear relationship between response and variables should be considered. The Box-Behnken Design was used to investigate critical factors included (1) amount of NaAc, (2) amount of C18 and (3) amount of PSA so as to reveal the complicated interaction and relationship. By applying multiple regression analysis to the experimental data, the results of the Box-Behnken Design were fitted to a second-order polynomial

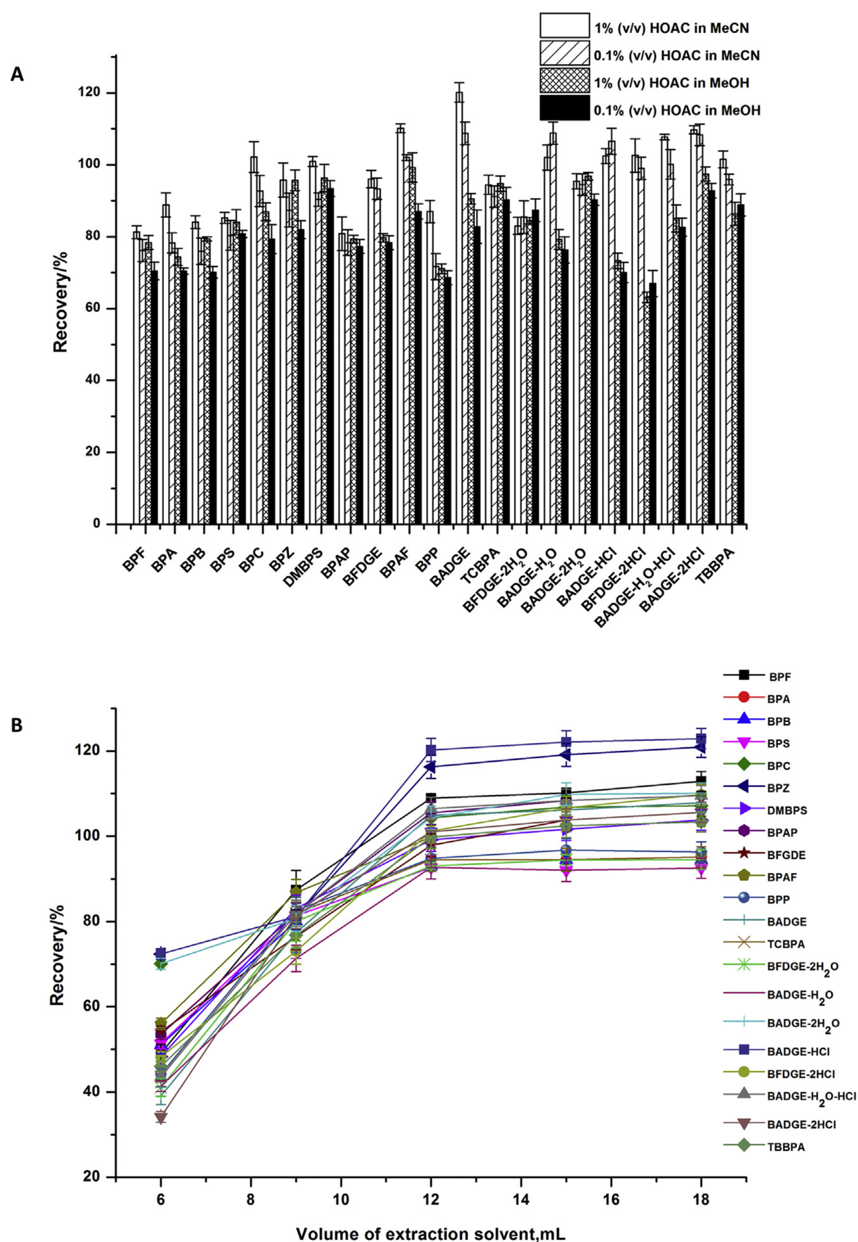

Fig. 4. Effect of extract solvent on the recovery of 21 analytes in milk. A) The type of extract solvent, B) the volume of extraction solvent (n = 3).

equation. The result of ANOVA was shown in Table S3 (supplementary materials). The data were fitted well to the model, since it had high values of R^2, insignificant "lack of fit" *p*-value and significant *p*-value. The Model F-value of 102.67 and $p < 0.0001$ implies the model is significant. Also, X_1, X_2, X_3, X_2X_3, X_1^2, $X_2^2$and X_3^2 were significant model terms for $p < 0.05$. The "Lack of Fit F-value" of 3.43 implies the "lack of fit" is not significant relative to the pure error. The R^2 value indicated the precision and reliability of the model. Thus, a mathematical regression model fitted in the actual factors was given as follows:

$$Y = 39.02 + 29.19X_1 + 0.31X_2 + 0.12X_3 - 8.633 \times 10^{-3}X_1X_2 - 6.839 \times 10^{-3}X_1X_3 - 1.303 \times 10^{-4}X_2X_3 - 9.08X_1^2 - 7.348 \times 10^{-4} X_2^2 - 4.357 \times 10^{-4}X_3^2$$

In order to evaluate the different combinations of the parameters investigated conveniently, the average recovery rate of 21 analytes was used as marker. Fig. 5 showed the corresponding different response plots. It could be observed that the curvature of the response of PSA was more significant than the other two variables, which means that it has the most impact on the extraction efficacy. The response surfaces generated suggested that the best extraction conditions for analytes were 1.48 g NaAc, 390 mg PSA and 190 mg C18. The recoveries of all analytes were tested under the optimized conditions, and satisfying results were observed.

3.4. Validation of the proposed method

3.4.1. Linearity and sensitivity

The linearity of the proposed method was evaluated using matrix-matched spiked samples at six different concentration levels over the range of 0.05–500 μg kg^{-1}. Each concentration level was repeated in triplicate. Calibration curves were resulted from

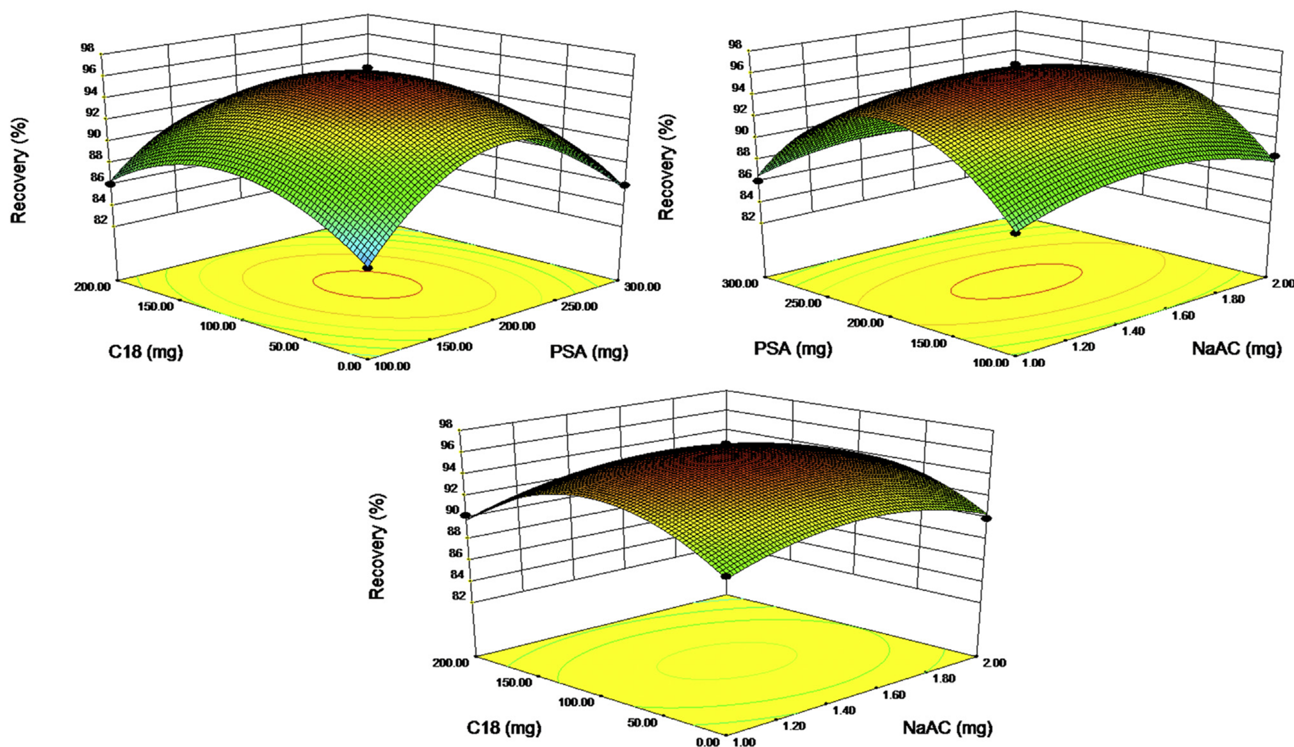

Fig. 5. Response surface plot for average recovery of the target compounds.

the ratios of the peak area of the target compounds to the peak area of the isotope-labeled internal standards. The results revealed that good linearity was achieved for the analyte concentration and the response in the corresponding ranges, indicated by the determination coefficients ($r^2 > 0.99$) and the root mean square error (RMS %<11%).Limits of detection (LOD) and quantification (LOQ) are fundamental parameters that used to evaluate the sensitivity of method. The LODs were determined by the injection of a series of standard solutions until corresponding to a signal-to-noise (S/N) ratio of three. And the limits of quantitation (LOQs) were used to assess the sensitivity by the injection of a series of spiked samples until corresponding to a signal-to-noise (S/N) ratio of ten. They were in the range 0.02–5 μg kg^{-1}, which allows the quantification of analytes presented at low content. Compared with other literature of multiresidue analysis [28], the method showed similar LOQ for most analytes and lower LOQ for BPAF and TCBPA, indicating good sensitivity was obtained. The results are summarized in Table 2.

3.4.2. Trueness

Recovery experiments were performed for evaluating the trueness of the method due to the lack of certified reference materials (CRM). Blank milk samples spiked at three concentration levels (1 × LOQ, 2 × LOQ and 4 × LOQ) were tested for the recovery experiments. Each level was analyzed in five replicates. The results of average recoveries were shown in Table 2. The obtained average recoveries were in the range of 88.2%–108.2% and complied with the routine analytical method which established a range of 80%–120%.

3.4.3. Precision

The precision was evaluated in terms of intra-day precision and inter-day precision, which expressed as RSD. Intra-day precision was performed by spiking blank milk at three concentration levels (1 × LOQ, 2 × LOQ and 4 × LOQ) with five replicates in one day. To evaluate inter-day precision, the same concentration levels were performed during five consecutive days. The RSD values of intra-day precision were in the range of 1.8%–8.9% and for inter-day precision, the RSD values were in the range of 1.7%–9.9%. Both of them were ≦10%, indicating the stability of the proposed method.

3.4.4. Matrix effects

Matrix effect is a common phenomenon in ESI, and suppression or enhancement of the target signal usually occurs, especially in complicated matrix. In this study, the matrix effect was calculated by comparing the slopes of the matrix-free calibration curves to the matrix-matched calibration curves. The percent matrix effect (C %) was calculated according to Eq. (1)

$$C\% = \left(1 - \frac{S_s}{S_m}\right) \times 100 \quad (1)$$

Where S_s is the slope of matrix-matched calibration curve and S_m is the slope of standard solution calibration curve [35]. A positive result represents ionization suppression, and a negative result indicates ionization enhancement. Most analytes did not show significant matrix effects (−50% < C% <+50%) except for BPS and BFDGE-2H_2O. Thus QuEChERS was an effective method of cleanup for milk. Fig. 6 revealed the matrix effects from three types of diary product. A similar ion suppression or enhancement profile was observed. Therefore, milk could be used to construct matrix-matched calibration curves and method validation on behalf of all types of diary product.

3.5. Real samples analysis

Eventually, the UHPLC-MS/MS method developed has been employed to analyze a total of 23 dairy products from local market, including 4 commercial milks, 12 milk beverages and 7 yogurt

Table 2
Validation parameters of the developed method.

Compound	Linear range (μg kg^{-1})	r^2	Matrix effect C (%)	LOD (μg kg^{-1})	LOQ (μg kg^{-1})	Average recovery (%)			Intra-day precision (%) (n = 5)			Inter-day precision (%) (n = 5)		
						Level 1	Level 2	Level 3	Level 1	Level 2	Level 3	Level 1	Level 2	Level 3
BPF	0.5–100	0.9945	6.1	0.08	0.5	93.4	102.6	95.5	3.4	2.5	5.4	6.6	9.0	9.0
BPA	0.5–100	0.9992	14.2	0.08	0.5	95.1	103.8	101.4	5.5	2.1	4.8	9.9	3.1	3.1
BPB	0.5–100	0.9919	17.0	0.08	0.5	88.2	102.3	93.9	6.4	5.1	2.7	8.1	4.9	4.9
BPS	0.1–100	0.9995	69.7	0.004	0.1	92.0	102.5	101.3	3.8	4.7	3.3	2.6	9.4	7.3
BPC	0.5–100	0.9986	7.9	0.2	0.5	88.3	101.5	100.7	2.0	6.2	5.8	6.6	8.2	6.7
BPZ	0.1–100	0.9982	19.0	0.04	0.1	92.4	101.1	103.9	4.0	4.0	2.5	8.2	9.5	8.5
DMBPS	0.1–100	0.9989	−31.2	0.0008	0.1	91.8	101.7	108.2	4.9	4.1	5.9	3.1	7.4	6.4
BPP	0.1–100	0.9982	37.8	0.02	0.1	95.0	99.4	96.2	3.2	4.5	3.6	5.6	4.3	9.4
BPAP	0.1–100	0.9982	19.3	0.02	0.1	91.6	101.6	104.4	7.0	6.3	2.0	7.3	8.4	1.7
BPAF	0.05–50	0.9982	15.7	0.0008	0.02	94.8	99.5	103.0	5.1	3.5	6.3	5.6	7.8	9.5
TCBPA	0.05–50	0.9997	11.4	0.0008	0.02	98.4	93.5	101.4	3.4	4.4	2.1	4.5	6.4	9.8
TBBPA	0.5–100	0.9968	32.0	0.008	0.5	103.7	99.4	100.8	6.7	6.8	5.6	8.5	7.7	9.4
BADGE	0.5–100	0.9923	32.9	0.02	0.5	98.6	96.6	92.1	7.6	7.8	8.9	7.3	5.6	8.6
BFDGE	0.5–100	0.9918	16.9	0.04	0.5	95.7	101.7	96.0	4.4	3.9	4.5	8.4	5.7	9.7
BADGE-H_2O	0.5–100	0.9946	13.9	0.02	0.5	95.5	95.9	97.1	7.1	1.8	7.0	3.5	7.2	7.0
BADGE-$2H_2O$	1–100	0.9974	36.5	0.2	1	103.4	97.4	104.4	2.9	5.5	4.6	9.8	8.3	9.7
BADGE–H_2O–HCl	0.5–100	0.9938	29.4	0.08	0.5	99.8	94.5	97.8	5.6	4.4	5.4	7.3	5.6	6.6
BADGE-HCl	0.5–100	0.9976	32.7	0.2	0.5	99.9	96.2	99.6	6.0	6.0	7.8	9.0	7.7	7.1
BADGE-2HCl	5–500	0.9961	33.6	0.08	5	97.7	104.7	101.6	3.2	2.4	2.2	9.8	6.3	8.7
BFDGE-2HCl	1–100	0.9954	22.5	0.08	1	102.7	94.7	105.3	6.6	4.5	2.6	9.8	5.6	9.6
BFDGE-$2H_2O$	1–100	0.9916	57.4	0.008	1	94.7	99.4	95.0	5.8	4.7	4.3	7.8	6.4	7.1

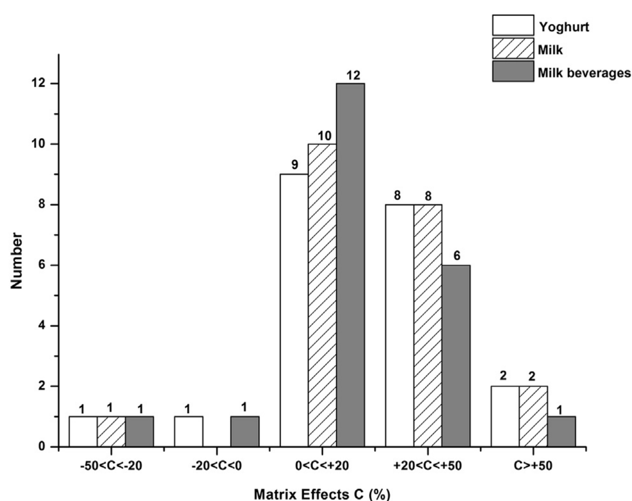

Fig. 6. Matrix effects on different types of dairy products.

products. Three procedural blanks were analyzed in the same sequence. As a result, 7 positive samples were found and 12 target analytes (BPA, BPS, BPF, BPAF, BPAP, BPZ, TCBPA, BADGE, BADGE-2HCl, BADGE-$2H_2O$, BADGE-HCl and BADGE–H_2O–HCl) were detected in these samples. As shown in Table 3, compared with low-level exposure of No.8, No.11, No.15 and No.16, No.19, No.20 and No.23 revealed high exposure level for BPA, BADGE and derivatives. The three milk beverages were all packed with metal cans and metal lids, and BPA, BADGE and derivatives probably released from internal protective lining of their packing. Fig. 7 shows the chromatograms of a spiked blank of milk sample, and the sum of three isomers of BFDGE was used for quantification in this work.

4. Conclusions

In this work, a simple, fast and universal UHPLC-MS/MS method was proposed for high throughput screening 21 BPs, bisphenols digycidyl ethers and their derivatives in dairy products. QuEChERS, which was an inexpensive, efficient, green and time-saving procedure for the extraction and cleanup, was applied to the sample preparation. The sample preparation would be completed in less than 40 min. For eliminating matrix effects, the matrix-matched standard calibration was used. The proposed method achieved the superior selectivity, sensitivity and accuracy by using MRM mode. The performance of the method was demonstrated by its application to real samples and 12 target compounds were

Table 3
Quantification results for target compounds in positive dairy products.

Sample[a]	BPA (μg kg^{-1})±SD[b]	BPF (μg kg^{-1})±SD[b]	BPS (μg kg^{-1})±SD[b]	BPAF (μg kg^{-1})±SD[b]	BPAP (μg kg^{-1})±SD[b]	BPZ (μg kg^{-1})±SD[b]	TCBPA (μg kg^{-1})±SD[b]	BADGE (μg kg^{-1})±SD[b]	BADGE-2HCl (μg kg^{-1})±SD[b]	BADGE-$2H_2O$ (μg kg^{-1})±SD[b]	BADGE–H_2O–HCl (μg kg^{-1})±SD[b]	BADGE-HCl (μg kg^{-1})±SD[b]
No. 8	n.d.	n.d.	0.5 ± 2.6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
No. 11	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	54.8 ± 5.3	n.d.	32.1 ± 2.5	1.8 ± 7.1	24.0 ± 1.0
No. 15	n.d.	n.d.	0.4 ± 6.1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	28.3 ± 9.4	n.d.	24.2 ± 3.3
No. 16	n.d.	n.d.	0.4 ± 1.5	0.4 ± 2.9	0.2 ± 1.6	0.3 ± 7.2	0.1 ± 0.9	n.d.	n.d.	n.d.	n.d.	n.d.
No. 19	33.8 ± 0.3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	199.7 ± 1.7	n.d.	743.6 ± 1.1	4.6 ± 2.3	719.0 ± 5.2
No. 20	76.0 ± 4.2	1.8 ± 3.1	n.d.	n.d.	n.d.	n.d.	n.d.	106.2 ± 4.1	n.d.	1060.3 ± 0.8	357.2 ± 2.9	842.6 ± 2.7
No. 23	127.2 ± 2.8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	20.2 ± 2.3	22.0 ± 3.9	1209.6 ± 2.4	126.2 ± 2.6	910.4 ± 4.4

n.d.: not detected.
[a] Type of dairy products are indicated herein: milk beverages, No.11, No. 15, No. 19, No. 20 and No. 23; yogurt, No. 8 and No. 16.
[b] SD, standard deviation (standard deviation was calculated for triplicate analysis, n = 3).

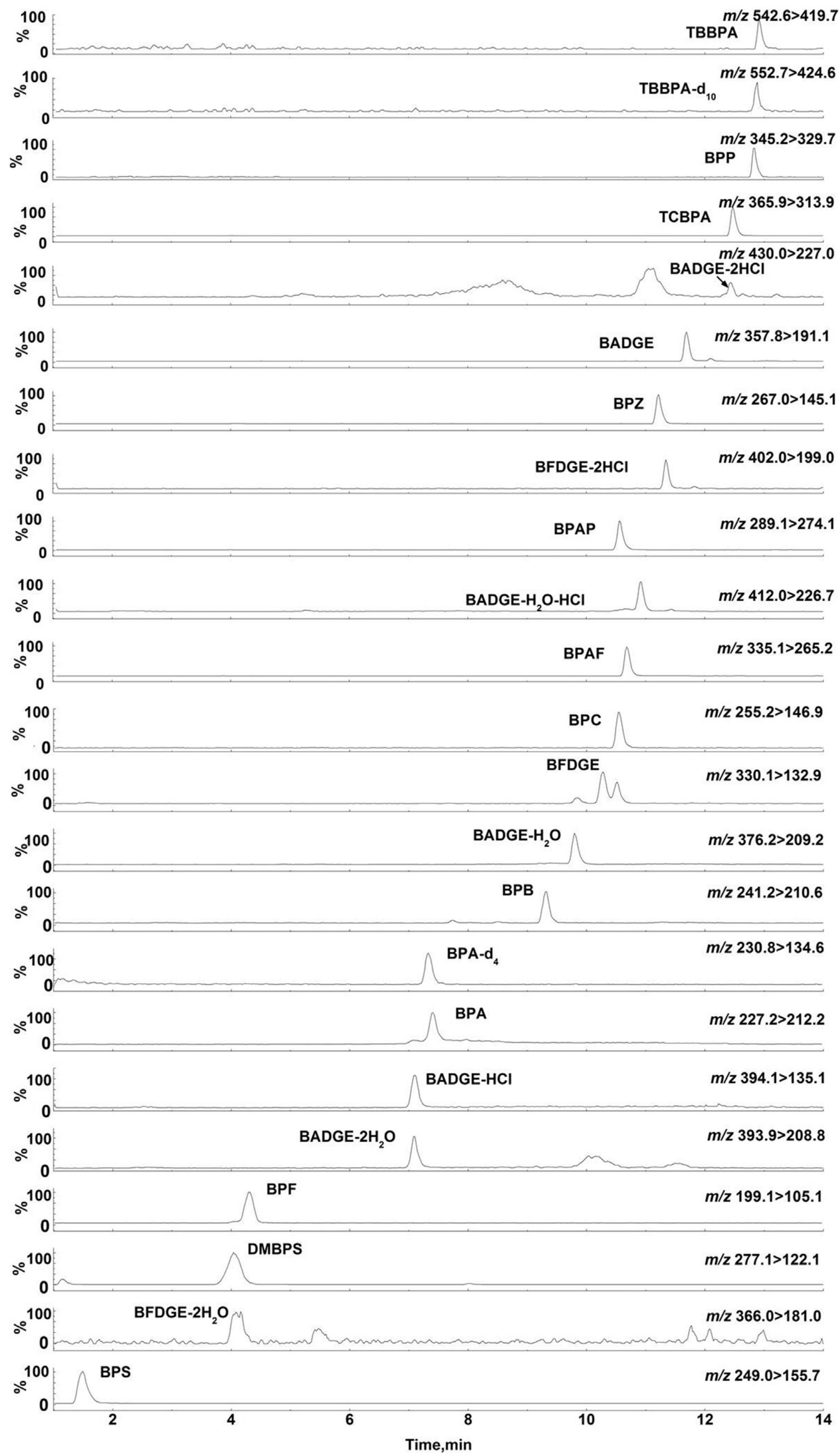

Fig. 7. Chromatograms of a spiked blank of milk sample (5 μg kg^{-1} of each studied analyte).

detected. In summary, a QuEChERS procedure following by UHPLC-MS/MS was a powerful method of screening and quantitative detecting BPs, bisphenols digycidyl ethers and their derivatives in dairy products.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.aca.2016.11.006.

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