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Analytical Methods

# A rapid and simple HPLC–FLD screening method with QuEChERS as the sample treatment for the simultaneous monitoring of nine bisphenols in milk

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## ARTICLE INFO

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Bisphenol B (PubChem CID: 66166)
Bisphenol C (PubChem CID: 6620)
Bisphenol E (PubChem CID: 608116)
Bisphenol F Diglycidyl Ether (PubChem CID: 91511)
Bisphenol F bis (2,3-dihydroxyprophy) Ether (PubChem CID: 3928015)
Bisphenol F Glycidyl 2,3-Dihydroxypropyl Ether (PubChem CID: 71360307)
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## ABSTRACT

A specific, precise and accurate high performance liquid chromatographic (HPLC) analytical method with a fluorescence detector (FLD) was established for the simultaneous determination of nine bisphenols (BPs) in milk samples. Samples were extracted ultrasonically with acetonitrile and cleaned using the QuEChERS technique. Under the optimized conditions, good linearity was obtained for the nine BPs and the correlation coefficients ($R^2$) ranged from 0.9942 to 0.9997. Recovery values for the nine bisphenols in spiked samples were 75.82–93.86% with intra-day and inter-day relative standard deviations (RSDs) from 2.6 to 11.1%. The limits of detection (LODs) and quantification (LOQs) were 1.0–3.1 μg/kg and 3.5–9.8 μg/kg, respectively. The results demonstrated clearly that the approach developed provides reliable, simple, rapid and environmentally-friendly quantification and identification of nine bisphenols in a fatty matrix and could be used for monitoring bisphenols in milk.

## 1. Introduction

Many compounds that are introduced into the environment by human activity can disrupt the endocrine system of higher life forms. The consequences of such disruption can be profound because of the crucial role that hormones play in controlling development. Since hormones affect reproduction and cellular development, and probably alter the risk of carcinogenesis, chronic environmental exposure may have a major impact on health. Bisphenol A (BPA), a high-volume chemical with 2.7 billion kilograms produced annually worldwide (Park et al., 2016), is used to make polycarbonate plastic and epoxy resins for linings in cans and pipes (Staniszewska, Nehring, & Mudrak-Cegiołka, 2016). It can be found, for example, in baby bottles, plastic drinking bottles, microwaveable food products, canned drinks and foods (Zimmers et al., 2014).

Milk is a nourishing food that supplies protein. During milking, transportation, production and drinking, milk inevitably comes into contact with cans, bottles and packing boxes (Niu, Zhang, Duan, Wu, & Shao, 2015). If these containers contain BPA, this hazardous substance can infiltrate the milk.

Environmental pollution is a serious environmental contamination problem and BPA can be found in water and soil from where it can migrate into milk via the food cycle. In more serious cases, BPA can lead to abnormalities of the human reproductive system (Mei, Deng, et al., 2013; Wang, Chen, & Bornehage, 2016). Studies have shown that BPA can decrease sperm count and fertility in males, and increase the rates of breast cancer as well as other hormone-linked diseases in women (Khalil et al., 2014).

Facing growing restrictions on the use of BPA in food contact materials, the plastic and canning industries are seeking alternatives that can replace BPA (Heffernan et al., 2016; Sun et al., 2014). Thus, over the past few years, new compounds have been designed to resemble the

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physicochemical properties of BPA, many of which belong to the same chemical family as p, p-bisphenols (Rocha et al., 2016). Among these structural analogues, bisphenol B (BPB), bisphenol C (BPC) and bisphenol E (BPE), bisphenol F diglycidyl ether (para-para-BFDGE), bisphenol F bis (2,3-dihydroxyprophy) ether (BFDGE·$2H_2O$), bisphenol F glycidyl 2,3-dihydroxypropyl ether (BFGDGE·$H_2O$), bisphenol A bis (2, 3-dihydroxyprophy) ether (BADGE·$2H_2O$) and bisphenol A diglycidyl ether (BADGE) are, apparently, the most important (Fattore, Russo, Barbato, Grumetto, & Albrizio, 2015). Unfortunately, these analogues have potentially harmful toxicological profiles (Schmidt, Kotnik, Trontelj, Knez, & Mašič, 2013; Švajger, Dolenc, & Jeras, 2016).

In the US, Food and Drug Administration (FDA) regulations no longer allow of BPA-based polycarbonate resins in baby bottles. The US Environmental Protection Agency (EPA) has established a BPA reference dose (RfD) of 50 μg/kg/d, based on the lowest observable adverse effect level (LOAEL) (Vandenberg, Maffini, Sonnenschein, Rubin, & Soto, 2009). To reduce the risk of possible health problems associated with BPA, the tolerable daily intake for BPA was established as 10 μg/kg body weight per day by the European Commission's Scientific Committee on Food (SCF) (European Commission., 2002). The European Commission (EC) established a specific migration limit (SML) for BPA of 0.6 μg/g of food or food simulants in Commission European Journal of Communication, 2004/19/EC (Commission European Journal of Communication, 2004). The use of BFDGE has been forbidden (European European European Commission, 2005), and the SMLs fixed by the European Commission are 9.0 mg/kg for BADGE and its hydroxyl derivatives and 1.0 mg/kg for BADGE and its chlorinated derivatives (European Commission, 2005; European European European Commission, 2011).

Current detection methods for BPs are reliant on complex instruments. For example, gas chromatography–mass spectrometry (GC–MS) (Deceuninck et al., 2014), HPLC (Liu et al., 2013; Wu et al., 2014) and liquid chromatography–mass spectrometry (LC–MS) (Vitku et al., 2015). However, major drawback of LC–MS methodology is the charge competition observed in electrospray ionization, leading to questionable results especially where complex biological matrices (such as foodstuffs) are concerned (Gallart-Ayala, Moyano, & Galceran, 2007). GC–MS samples require some derivatization pretreatment prior to chromatographic separation (Deceuninck et al., 2014). More importantly, for non-specialized and primitive laboratories in developing countries, the price of GC–MS and LC–MS instruments is prohibitive. Expensive instrumentation, time-consuming sample pre-treatment and expert personnel for operation limit the practical application of these methods (Wang, Zeng, Wei, & Lin, 2006).

Molecular imprinting (MI) (Wu et al., 2015), enzyme linked immune sorbent assay (ELISA) (Mita et al., 2007), electrochemical analysis method (Xue et al., 2013) and biosensor methods (Mei, Qu, et al., 2013) have also been used for rapid detection of BPs. These methods avoid the drawbacks of instrument-based methods, and are highly sensitive and convenient, but only few BPs like BPA can be tested and multifarious BPs cannot be tested simultaneously. Apart from these methods, HPLC-FLD has great potential for simultaneous determination of multiple BPs due to its simple operation and low cost.

Anastassiades, Lehotay, Stajnbaher, and Schenck (2003) developed an analytical methodology for the simultaneous extraction and isolation of pesticides from food matrices. Analytical methodologies characterized as: quick, easy, cheap, effective, rugged and safe (QuEChERS) have undergone various modifications and enhancements since the introduction of this concept. As a "green" analytical approach (Capela, Homemn, Alves, & Santos, 2016), QuEChERS is used widely to determine pesticide due to its simplicity, low cost and relatively high efficiency (Pérez-Burgos et al., 2012), and has been applied for testing hazardous substance in foods of animal origin recently (Huertas-Pérez et al., 2016; Liu, Lin, & Fuh, 2016). However, as far as we know, there has been no report on the determination of BPs in milk using the QuEChERS method with pre-concentration for sample treatment.

The purpose of this study was to develop a rapid and simple method for the simultaneous determination of nine BPs (BPA, BPB, BPC, BPE, para-para-BFDGE, BFDGE·$2H_2O$, BFGDGE·$H_2O$, BADGE·$2H_2O$ and BADGE) in milk. In the method developed, samples were prepared using the QuEChERS method and analysed with HPLC–FLD. The economic benefits and wide use of HPLC–FLD were combined with the rapid, convenient and environmentally-friendly QuEChERS method for sample pre-treatment. This method could be applied for routine laboratory analysis of nine BPs in large numbers of milk samples.

## 2. Materials and methods

### 2.1. Chemicals, reagents and solution

Analytical standards with high purity were used in the experiment. BPA (Purity 98.5%) and BPB (Purity 99.8%) were provided by Dr. Ehrenstorfer GmbH (Augsburg, Germany); BFDGE·$2H_2O$ (Purity 96%), para-para-BFDGE (Purity 90%), BFGDGE·$H_2O$ (Purity 93.98%), BADGE·$2H_2O$ (Purity 93%) and BADGE (Purity 98%) were provided by Toronto Research Chemical, Inc. (Toronto, Canada); BPC (Purity 98.9%) and BPE (Purity 100%) were provided by AccuStand, Inc. (New Haven, USA). Single standard solutions were obtained by dissolving 10 mg of each standard in methanol (HPLC grade) in a 100 mL volumetric flask, and 1 μg/mL mixed stock standard solutions of nine BPs were prepared by the diluting single standard in mobile phase. This was stored at −20 °C in refrigerator for up to 6 months. The standard curve was based on six serially-diluted concentrations from the 1 μg/mL mixed stock standard solutions. HPLC grade methanol and acetonitrile were obtained from Tedia Company, Inc. (Fairfield, USA). Formic acid was analytical grade and obtained from Sigma-Aldrich (Santa Clara, USA). Primary secondary amine (PSA) (40–63 μm, ultraclean bulk for QuEChERS) and $C_{18}$ (40–63 μm, ultraclean bulk for QuEChERS) were obtained from CNW Technologies GmbH (Duesseldorf, Germany). All other chemicals were of analytical grade, unless stated otherwise, and purchased from Sinopharm (Shanghai, China); they were used without further purification.

### 2.2. Instrumentation and apparatus

Separation was performed using a Waters e2695 separation module with fluorescence detector (Milford, USA) and a Waters Xcharge $C_{18}$ column (250 mm × 4.6 mm, 5 μm, 100 Å) (Milford, USA). An electronic analytical balance BSA224S-CW accurate to 0.1 mg (Gottingen, Germany), homogenizer BÜCHI Mixer B-400 (Flawil, Switzerland), vortex Mixer Labinco L24 (Breda, Netherlands), ultrasonic apparatus Scientz Biotechnology (Ningbo, China), shaker ZP-200 (Taicang, China), membrane filters 0.22 μm Millipore Millex-GV (Massachusetts, USA), centrifugal tube Coring Centristar™ (Coring, USA), Milli-Qultrapure water system Millipore (Molsheim, France), centrifuge Omnifuge 2.ORS (Osterode, Germany) and nitrogen evaporators Peak Scientific N100DR (Glasgow, UK) were used for pretreatment.

### 2.3. Sample collection and preparation

Each milk sample (5 ± 0.02 g) obtained from farms in Lanzhou was placed in a polypropylene centrifuge tube (15 mL). Formic acid (0.1%) acetonitrile (5 mL) solution was added to the milk and mixture ultrasonicated for 10 min. One gram of anhydrous magnesium sulphate and 2.0 g sodium chloride were added, and the mixture was immediately shaken for 5 min, followed by centrifugation at 10,000 rpm for 5 min at 10 °C. Then, the supernatant was transferred to a new tube containing 0.1 mg PSA, 0.1 mg $C_{18}$ and 0.25 mg $MgSO_4$, vortexed for approximately 30 s, and centrifuged at 10,000 rpm for 5 min at 10 °C. The liquid was transferred to another tube, evaporated to dryness under nitrogen at 50 °C and reconstituted with 0.1% formic acid/acetonitrile (55:45, v/v) solution. Finally, the solution was shaken and passed into

an LC vial through a PTFE Millipore filter.

### 2.4. LC operating conditions

The mobile phase, which was degassed and filtered before analysis, consisted of 0.1% formic acid (A) and acetonitrile (B). The eluent flow rate was 1.0 mL/min with the mobile phase initially consisting of 55% A and 45% B, then decreased linearly to 45% A from 0.0 min to 7.0 min, held there for 17.0 min from 7.0 min to 24.0 min, and finally recovered to 55% A and held for 4.0 min. Fluorescence measurements were carried out at 215–245 nm excitation wavelength and 295–315 nm emission wavelength. The injection volume was 20 μL and the temperature of the column oven was maintained at 35 °C.

## 3. Results and discussion

### 3.1. Optimization of developed method

#### 3.1.1. Optimization of HPLC parameters

The correlation between composition of the mobile phase and resolution of the chromatogram was addressed. Parameters A using gradient elution and Parameters B (0.1% formic acid: acetonitrile, 55:45, v/v) were set and compared. Chromatograms of samples spiked with mixed standard solutions of nine BPs at 50 μg/kg were contrasted. Parameters A exhibited a high degree of separation and higher selectivity for analysing BPs and all nine were separated from baseline and exhibited nice peak shapes. With Parameters B, all baseline resolved peaks were obtained, but three peaks after 14.0 min were very smooth, which led to low sensitivity for BPC, para-para-BFDGE and BADGE. So, parameters A were selected for subsequent work.

#### 3.1.2. Optimization of excitation wavelength and emission wavelength

The excitation and emission wavelengths were two factors decisive among the instrument parameters for sensitivity, so they were optimized in single factor experiments. Two experiments to addressing the relationship between peak height, and excitation and emission wavelengths were designed. A standard mixture of the nine BPs (50 μg/L) was filtered directly into a vial and analysed using different excitation and emission wavelengths. Firstly, the excitation wavelength was set at: 215, 220, 225, 230, 235, 240 or 245 nm. Peak heights for the nine BPs were obtained and the chromatograms are shown in Fig. 1. The range of peak heights for the BPs was 4.98–14.69, 7.77–24.80, 10.99–41.04, 12.28–52.52, 10.47–51.96, 7.22–38.07 and 4.55–23.63 * $10^5$ mV. When the excitation wavelength was at 230 nm, all nine chromatograms had the maximum peak height (12.28–52.52 * $10^5$ mV), and maximum sensitivity could be obtained. So, 230 nm was chosen as optimal for excitation.

With the excitation wavelength at 230 nm, the emission wavelength was set at 295, 300, 305, 310 or 315 nm. The range of peak heights for the nine BPs was 8.01–32.41, 9.92–39.12, 10.99–41.04, 9.67–38.20 and 8.22–31.51 * $10^5$ mV. When the emission wavelength was 305 nm, peak heights for the BPs were greatest (10.99–41.04 * $10^5$ mV) and this wavelength (305 nm) was selected for subsequent as optimal for emission. At these wavelengths, the range of peak heights for the nine BPs was at a maximum (12.73–56.02 * 105 mV) and the best sensitivity was obtained.

#### 3.1.3. Optimization of dosage of cleaning agent $C_{18}$ and PSA

$C_{18}$ can remove fat, pigments and vitamins in milk while PSA can remove the carbohydrate and organic acids. Amount of $C_{18}$ and PSA in QuEChERS have a key role in the result, so these were optimized. Conditions for the experiment were kept the same, but the amounts of $C_{18}$ and PSA (1:1, m:m) were varied (0.025, 0.05, 0.1, 0.2 and 0.4 g) before recovery from five spiked samples was determined. The results showed that 70.1–82.4% recovery was achieved for the nine BPs at 0.1 g. When the amounts of $C_{18}$ and PSA were at 0.2 g or 0.4 g, recovery was lower (66.1–80.4%) because superfluous cleaning agent $C_{18}$ and PSA can absorb some target compounds. When the amounts of $C_{18}$ and PSA were less at 0.1 g, recovery was 65.2–80.1% and did not exhibit any significant variation, but there were more peaks, which had a negative effect on sensitivity and accuracy. So, the amounts of $C_{18}$ and PSA used in the final optimised method were both 0.1 g, which was best from an economic point- of-view as well as providing optimal efficiency.

#### 3.1.4. Optimization of ultrasound extraction time

Methods for BP extraction include ultrasonic-assisted extraction (UAE), liquid–liquid extraction (LLE) (Yang, Guan, Yin, Shao, & Li, 2014) and liquid–solid extraction (LSE) (Geens, Neels, & Covaci, 2009; Schmidt, Müller, & Göen, 2013). In contrast, LLE and LSE need plenty of organic solution and take more time, so ultrasonic-assisted extraction (UAE) is the most popular due to its high performance. The dependence of accuracy on recovery for the nine BPs on extraction time was examined at 5, 10, 15, 20 and 25 min as well as shaking (15 min or 30 min) (Fig. 2). When the extraction time was 5 min, incomplete extraction led to minimum recovery (70.1–81.2%) due to insufficient time. When the extraction time was 10 min, recovery increased to 75.7–83.6%. When the extraction time was 15 min, recovery was 75.7–84.1%, and the increase in accuracy was not remarkable. When the extraction time was increased to 20 and 25 min, recovery was less (65.6–82.0%). Shaking for 15 or 30 min produced recovery of 65.2–81.2%. Compared with ultrasound extraction, traditional shaking was inefficient and time-consuming. Thus, 10 min of ultrasound extraction was used in the optimised method.

### 3.2. Purifying effect of QuEChERS method

The effect of $C_{18}$ and PSA on impurities was assessed in three-groups of contrast experiment (Fig. 3): (A) blank milk sample prepared using QuEChERS, (B) blank milk sample not cleaned and (C) 10 μg/kg spiked milk sample prepared using QuEChERS. Both the blank and spiked samples cleaned using QuEChERS contained fewer impurities; their chromatograms were smooth and concise, and no interfering peaks were observed at the retention times for the nine BPs.

### 3.3. The advantages of QuEChERS method compared with other methods

Purification of BPs is based on reversed SPE, solid-phase microextraction (SPME) and dispersive liquid–liquid microextraction (DLLE) (Cunha & Fernandes, 2013). These procedures are time-consuming, expensive and laborious, and often include many processing steps. SPE is the most common technique (Regueiro & Wenzl, 2015). Although traditional SPE sorbents have high capacity and can trap a wide range of analytes, they suffer from low efficiency due to their low selectivity toward specific target molecules (Sun et al., 2014). There is a demand for rapider, simpler and cheaper methods for pretreatment of complex matrices, such as milk, and QuEChERS meets this (Halle, Claparols, Garrigues, Franceschi-Messant, & Perez, 2015). The QuEChERS method developed was compared with the traditional method (Table 1). Only 5 mL acetonitrile was used in the QuEChERS method developed, minimizing the volume of organic solvent used and developing a more environmentally-friendly approach. The QuEChERS method only needed 25 min to prepare samples, and eight steps, the least of any alternative, meaning QuEChERS would reduce milk sample pre-treatment and minimize sources of error. Thus, the QuEChERS method developed was relatively quick, simple and environmentally- friendly.

### 3.4. Method validation

Quality parameters – linearity, accuracy, limit of detection (LOD), limit of quantification (LOQ), precision (repeatability and intermediate precision), and selectivity were evaluated. Bisphenol-free milk samples

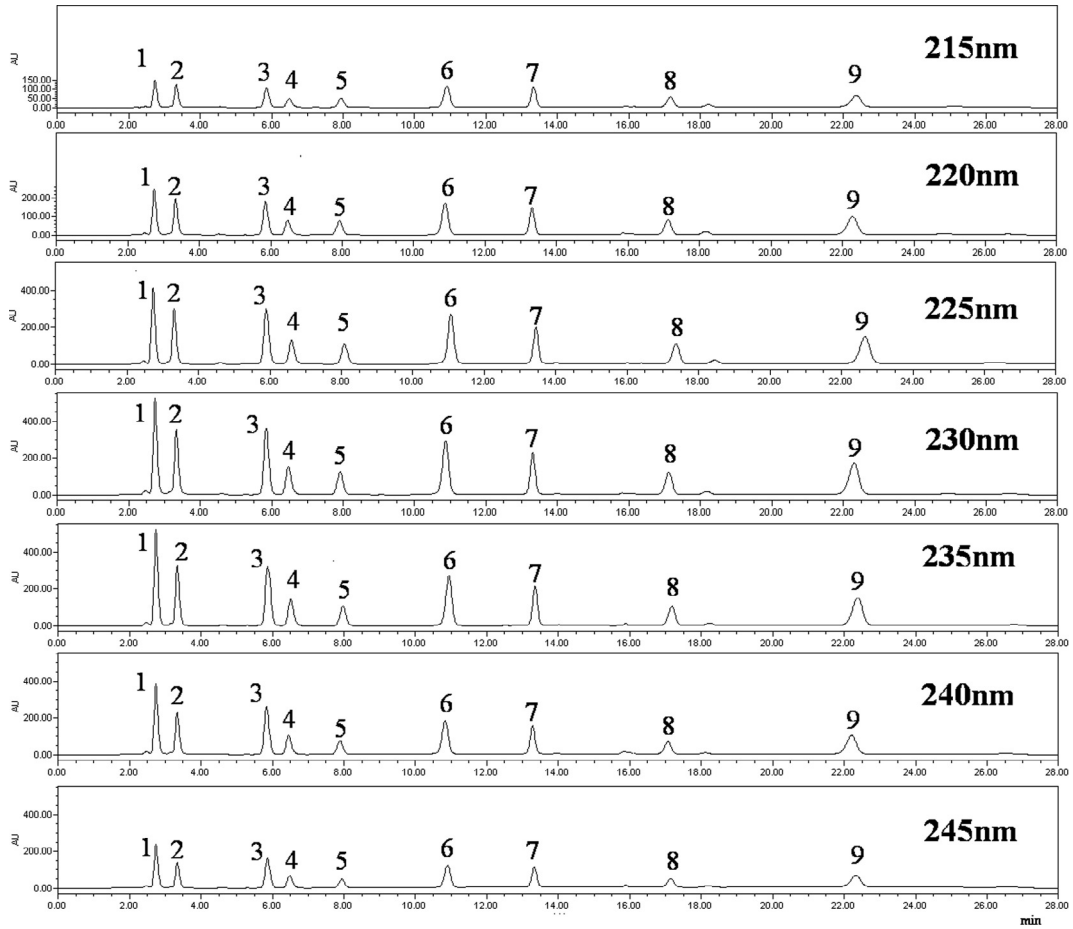


**Fig. 1.** Comparison of 7 chromatograms at 215–245 nm excitation wavelength. (1) BFDGE·2$H_2O$, (2) BADGE·2$H_2O$, (3) BFGDGE·$H_2O$, (4) BPE, (5) BPA, (6) BPB, (7) BPC, (8) para-para-BFDGE and (9) BADGE.

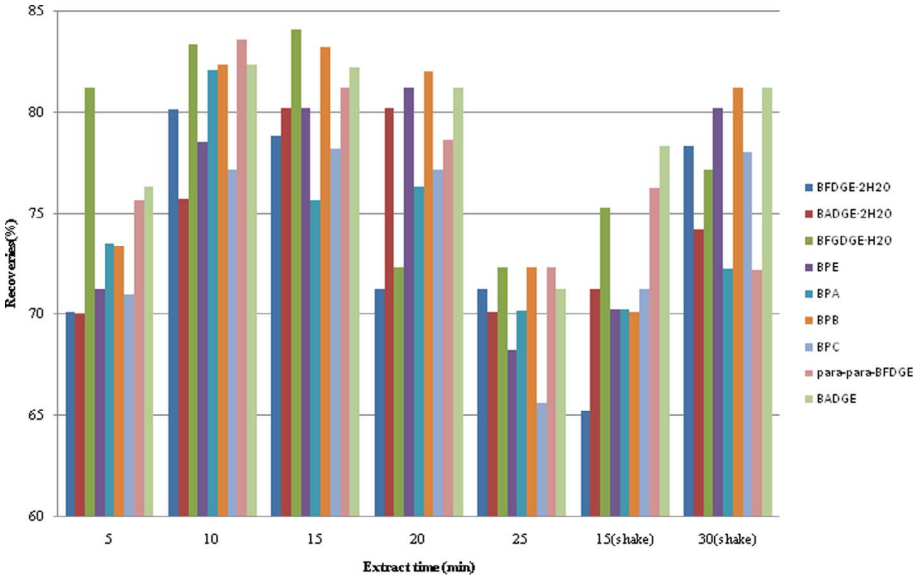


**Fig. 2.** Effect of ultrasound extraction time and shaking extraction time on the recovery rates of nine BPs.

were used as blanks to verify method selectivity and, after spiking, confirm the assignment of peak identities and calculate linearity, precision and accuracy of the method.

### 3.4.1. Linearity

Specificity was tested by analysing a spiked-milk sample. Linearity of the FLD chromatographic response was tested with six calibration points across the range 5–100 μg/kg. The linear regression coefficients for the calibration curves are summarized in Table 2, which shows good results were achieved ($R^2$ 0.9942–0.9997).

### 3.4.2. LODs and LOQs

Method sensitivity was evaluated based on limits of detection (LODs) and quantification (LOQs). LODs and LOQs were calculated

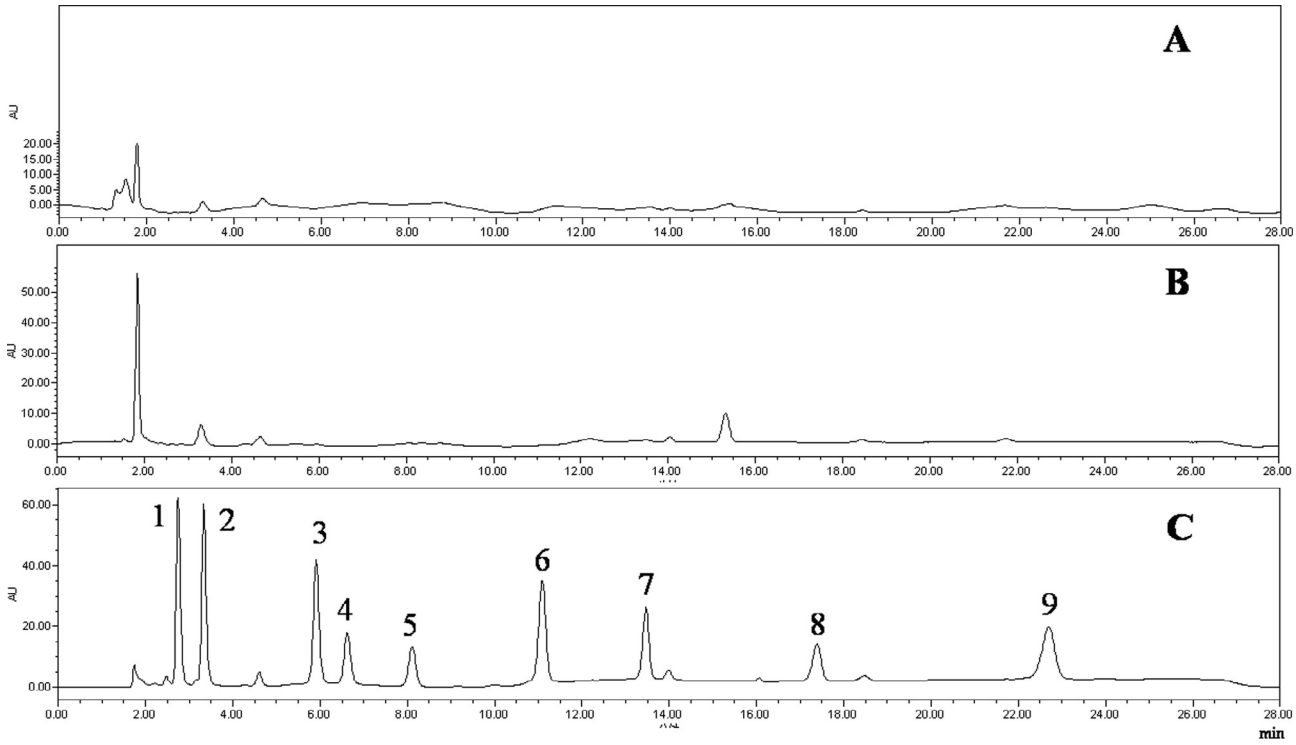


**Fig. 3.** Purifying effect of QuEChERS method on BPs in milk samples. (1) BFDGE·2$H_2O$, (2) BADGE·2$H_2O$, (3) BFGDGE·$H_2O$, (4) BPE, (5) BPA, (6) BPB,(7) BPC,(8) para-para-BFDGE and (9) BADGE.

from the signal-to-noise ratio of chromatograms for milk samples (S/N = 3 for LODs and S/N = 10 for LOQs), as shown in Table 2. LODs for the nine BPs in milk were in the range 1.0–3.1 μg/kg and LOQs were in the range 3.5–9.8 μg/kg. LOQs for the method developed were less 0.6 μg/g, therefore, according to the SML for BPA (0.6 μg/g, Commission Directive 2004/19/EC) for food or food simulant and the SML (9.0 mg/kg of food) for BADGE and its hydroxyl derivatives, adequate for routine monitoring of nine BPs in milk samples.

### 3.4.3. Recovery

To estimate of recovery, blank milk samples were fortified with the mixed standard solution at three concentrations (10, 50 and 100 μg/kg) in intra-day (n = 18) and inter-day experiments (n = 6). 18 spiked samples were analysed in the intra-day experiment (same day), and every day six spiked samples were analysed for three consecutive days. High recoveries were obtained using this simple method, based on QuEChERS extraction with $C_{18}$ and PAS. The results indicated that recoveries for the nine BPs in milk were 75.82–93.86% in the inter-day experiment and 77.60–89.68% in intra-day experiment (Table 3). The results indicated that nine PBs in milk could be quantified using the method developed.

**Table 2**
Linear range, regression coefficient($R^2$), retention time, LOD and LOQ for BPs.

| Compound | Retention time (min) | $R^2$ | Linear range (μg/kg) | LOD (μg/kg) | LOQ (μg/kg) |
|---|---|---|---|---|---|
| BFDGE·2$H_2O$ | 2.742 | 0.9991 | 5–100 | 1.0 | 3.5 |
| BADGE·2$H_2O$ | 3.344 | 0.9942 | 5–100 | 1.3 | 3.8 |
| BFGDGE·$H_2O$ | 5.892 | 0.9979 | 5–100 | 1.2 | 4.1 |
| BPE | 6.539 | 0.9972 | 10–100 | 3.0 | 9.2 |
| BPA | 8.006 | 0.9992 | 10–100 | 3.1 | 9.8 |
| BPB | 10.968 | 0.9997 | 5–100 | 1.5 | 5.0 |
| BPC | 13.367 | 0.9959 | 10–100 | 1.8 | 6.2 |
| para-para-BFDGE | 17.217 | 0.9988 | 10–100 | 2.5 | 8.4 |
| BADGE | 22.444 | 0.9987 | 10–100 | 2.3 | 7.8 |

### 3.4.4. Precision

The precision, expressed as relative standard deviations (RSDs), was determined in the intra- and inter-day experiments. RSDs for the nine BPs in milk are shown in Table 3. The inter- and intra-day RSDs for the

**Table 1**
The QuEChERS developed compared with the traditional methods.

| Pretreatment method(matrix) | Extract organic solvent | Volume (mL) | Elapsed time (min) | Number of processes |
|---|---|---|---|---|
| LLE-SPE (Milk) (Zimmers et al., 2014) | hexane/acetonitrile | 3 + 12 | about60 | 18 |
| UAE-LLE-SPE(Juice) (Regueiro and Wenzl, 2015) | acetonitrile/methanol | 5 | about45 | 9 |
| LLE/LSE-SPE (milk etc.) (Deceuninck et al., 2014) | acetonitrile/cyclohexane | 14 + 15 | about13 | 12 |
| LLE-SPE(Milk) (Park et al., 2016) | methanol/hexane/LMT/acetonitrile | 1.5 + 4+2 + 3 | about60 | 11 |
| LLE-DLLME (edible oils) (Liu etal.,2013) | n-hexane/methanol/ | 4 + 2 | about30 | 12 |
| UAE-LLE-QuEChERS(Milk) (QuEChERS) | acetonitrile | 5 | about25 | 8 |

**Table 3**
Recovery rates and RSDs for nine BPs from milk samples obtained using HPLC-FLD.

| Compound | Spiked level (μg/kg) | Intra-day | | | Inter-day |
|---|---|---|---|---|---|
| | | Recovery(%)/RSDs (n = 6) | | | Recovery (%)/RSD (n = 18) |
| | | 1 day | 2 day | 3 day | |
| BPA | 10 | 79.34 8.6 | 78.34 7.5 | 79.21 7.3 | 77.60 7.0 |
| | 50 | 87.05 8.6 | 87.91 11.2 | 76.26 10.5 | 86.80 9.5 |
| | 100 | 84.43 7.6 | 81.55 8.2 | 81.94 6.3 | 86.97 9.6 |
| BPB | 10 | 85.55 11.6 | 79.05 7.1 | 82.33 5.2 | 88.19 10.5 |
| | 50 | 82.34 8.0 | 90.89 6.9 | 79.53 7.0 | 85.77 10.7 |
| | 100 | 82.74 8.4 | 83.44 2.9 | 88.34 2.6 | 89.68 10.4 |
| BPC | 10 | 82.34 9.9 | 83.11 5.9 | 86.68 5.5 | 84.91 9.2 |
| | 50 | 76.03 5.6 | 89.24 9.0 | 80.46 9.3 | 86.22 10.0 |
| | 100 | 89.28 7.2 | 90.13 3.8 | 81.12 10.8 | 87.32 9.3 |
| BPE | 10 | 84.91 9.5 | 85.49 6.3 | 82.80 5.7 | 85.49 11.1 |
| | 50 | 80.06 4.9 | 90.49 8.4 | 83.06 6.1 | 87.11 10.2 |
| | 100 | 80.21 4.8 | 84.63 7.8 | 79.85 7.5 | 87.69 11.1 |
| para-para-BFDGE | 10 | 85.99 10.7 | 79.82 5.5 | 79.83 9.3 | 84.59 10.9 |
| | 50 | 85.93 5.51 | 93.86 9.4 | 75.82 3.6 | 86.26 7.2 |
| | 100 | 82.32 4.1 | 86.49 7.0 | 83.83 8.9 | 89.39 8.8 |
| BFDGE·2$H_2O$ | 10 | 81.87 9.3 | 86.96 5.3 | 81.30 10.0 | 84.59 10.4 |
| | 50 | 82.32 9.2 | 89.85 7.8 | 85.00 8.1 | 86.60 10.4 |
| | 100 | 86.79 5.7 | 84.62 13.0 | 79.96 6.9 | 87.79 9.8 |
| BFGDGE·$H_2O$ | 10 | 81.25 8.5 | 84.28 6.8 | 85.74 8.1 | 89.31 9.3 |
| | 50 | 82.69 9.9 | 85.46 11.5 | 83.03 9.6 | 88.78 9.2 |
| | 100 | 83.04 8.1 | 86.87 7.6 | 83.61 7.0 | 89.47 7.8 |
| BADGE·2$H_2O$ | 10 | 93.32 11.2 | 82.52 6.7 | 80.10 11.5 | 87.29 8.7 |
| | 50 | 80.81 7.3 | 89.51 9.0 | 83.50 7.4 | 89.19 10.5 |
| | 100 | 87.43 5.8 | 85.12 9.7 | 79.97 7.9 | 78.19 7.9 |
| BADGE | 10 | 91.80 11.1 | 85.20 5.1 | 77.92 9.6 | 87.61 7.9 |
| | 50 | 90.66 3.1 | 89.98 8.5 | 85.16 6.2 | 81.14 8.6 |
| | 100 | 86.44 6.9 | 82.34 11.4 | 81.89 8.6 | 78.44 5.5 |

nine BPs in milk ranged from 2.6% to 13.0% and from 5.5% to 11.1%, respectively, at three concentrations (10, 50, and 100 μg/kg).

### 3.5. Application on real samples

Milk samples were collected from different supermarkets and dairy farms in and around Lanzhou in China. A total of 50 milk samples were collected in 50 mL polypropylene tubes and shipped on ice to the laboratory within 5 h, and stored at −20 °C in refrigerator until analysis. The analytical method was applied successfully for the identification nine BPs in a total 50 milk samples. BPA was found in one at 13.74 μg/kg using the method developed and verified at 14.31 μg/kg (n = 3) using the established LC-MS/MS method (Park et al., 2016). These were below permitted level (0.6 μg/g, Commission Directive 2004/19/EC). BADGE·2$H_2O$ was found in one sample at 15.80 μg/kg and BFDGE·2$H_2O$ was found in two at 16.23 and 17.82 μg/kg, respectively. The amount of BADGE·2$H_2O$ was below permitted level (9.0 mg/kg, European Commission, 2005); the use of BFDGE has been forbidden in Europe (European Commission, 2005), but the limitation requirement of BFDGE has not been set in China. These three positive results were verified using the LC-MS/MS method according to Chinese national standard SN/T 3150–2012 (Chinese national standard, 2012); values were 16.68, 15.15 and 19.22 μg/kg, respectively. Detection rates for BPA, BADGE·2$H_2O$ and BFDGE·2$H_2O$ were 2, 2 and 4%, respectively. The relative tolerances of measurements using the method developed and verified method were 4.06, 5.42, 6.88 and 7.56%, respectively, and less than 10%. These data further illustrates the reliability and accuracy of the method developed.

## 4. Conclusions

An HPLC-FLD method using QuEChERS for preconcentration was established for the simultaneous determination of nine BPs in milk, for the first time. Extraction and purification analysis of nine BPs in milk were examined in depth. Compared with methods published previously, the method developed in this study had many advantages, such as less organic solvent, inexpensive instrumentation, less manual operation and shorter sample preparation time. Good quality results, including recovery, precision, linearity, LOD and LOQ were achieved. Finally, milk samples collected from supermarkets and dairy farms were analysed to demonstrate the applicability of the method. Among nine BPs, BPA, BADGE·2$H_2O$ and BFDGE·2$H_2O$ were detected in some samples. The results were also verified using LC-MS/MS, and the reliability and accuracy of the method developed were verified. The method developed is suitable for rapid determination of trace amounts of BPs in milk in routine analysis and could be used in laboratories and by testing agencies.

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## Conflict of interest

The authors have no conflicts of interest.

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