Full length article

Dispersive micro solid phase extraction (DMSPE) using polymer anion exchange (PAX) as the sorbent followed by UPLC–MS/MS for the rapid determination of four bisphenols in commercial edible oils

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ABSTRACT

The present work presents a novel and rapid analytical method for the simultaneous analysis of bisphenol A (BPA), bisphenol B (BPB), bisphenol F (BPF) and bisphenol S (BPS) in edible oil based on dispersive micro solid phase extraction (DMSPE) for the first time followed by isotope dilution-ultra high performance liquid chromatography tandem mass spectrometry (UPLC–MS/MS). The edible oil sample was dispersed by n-hexane and extracted with ammoniated methanol-water solution. Then the target analytes were dispersedly absorbed using the polymer anion exchange (PAX) as the sorbent and eluted by acidic methanol. After that, four bisphenols were separated on a C18 column by gradient elution with methanol and 0.05% ammonium hydroxide in water as mobile phase, detected by MS/MS under multiple reactions monitoring (MRM) mode and quantified by internal standard method. The PAX amounts, adsorption time, concentrations of formic acid in the elution solvent and volume of elution solvent for the DMSPE technique were optimized. The limit of detection and quantitation (LOD and LOQ), matrix effect, recovery and precision of the developed method were investigated. Results indicated that BPS and the rest three bisphenols displayed excellent linearity in the concentration ranges of 0.1–50 µg/L and 0.5–250 µg/L, respectively, with correlation coefficients (R^2) all larger than 0.998. Achieved MLODs ($S/N = 3$) varied between 0.1–0.4 µg/kg for all bisphenols. The mean recoveries at three spiked levels in edible oil were in the range of 87.3–108%. Intra-day precision ($n = 6$) and inter-day precision ($n = 5$) were <9% and <11%, respectively. This method is of rapid-and-simple pretreatment, accurate and sensitive, and suitable for the simultaneous determination of bisphenols in edible oil.

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1. Introduction

Bisphenol A (BPA), an important industrial chemical substance, has been widely used as a monomer in the manufacture of polycarbonates (PC), phenol resins, polyesters and epoxy resins due to its chemical stability, high temperature resistance, good ductility and low production cost. It is attracting growing concern recently because of its extensive use in a large variety of applications especially in food containers and food contact materials

[1,2]. Unfortunately, BPA has been reported to have estrogenic activity and endocrine disrupting properties, and chronic low-level exposure to this compound has adverse effects on human development and reproductive health [3–7]. As an endocrine disruptor, the migration of BPA from food contact materials into food or food simulants especially during storage and processing at elevated temperatures has also been reported [8–13].

However, as the exposure to BPA has a wide range of adverse issues on human health, BPA has been prohibited in the use of baby bottles and the coating of infant formula packaging by several organizations of European Union and some other countries. Nevertheless, a group of chemicals which also consist of two phenolic rings joined through a bridging carbon, known as BPA-related compounds or bisphenols, has been increasingly adopted to replace BPA for similar use [1,2]. Among these analogues, bisphenol B (BPB),

bisphenol S (BPS) and bisphenol F (BPF) are the major BPA replacements [14–16]. Several researches have shown that the toxicities such as genotoxicity and endocrine disrupting properties, even teratogenicity and carcinogenicity of BPB, BPS and BPF are very similar to those of BPA [14,16–18].

For decades, these bisphenols have been strictly restricted by many regulations and countries. For example, the Commission Directive 2004/19/EC established the specific migration limit (SML) of 0.6 mg/kg and 0.05 mg/kg in foods or food simulants for BPA and BPS, respectively, while the use of BPF is prohibited. Even so, bisphenols have been ubiquitously found in a variety of foodstuffs including ready-made meals [1], beverages [2], milk [8], food crops [19], canned vegetables and fruits [20], etc. Hence, determination of these emerging contaminants in foodstuffs is of great importance and requires the development and validation of appropriate and robust analytical methods.

As regards the determination of bisphenols in food matrices, most of analytical methods are mainly based on liquid chromatography (LC) [9,12], gas chromatography coupled to mass spectrometry (GC–MS) [19,20] and LC–MS/MS [2,21–23]. However, the accuracy and sensitivity of LC mainly depends on the detector, some of which may not meet the requirements. In most cases, a further solvent evaporation step to concentrate the analytes and a derivatization procedure are required in GC–MS analysis, and these procedures are laborious, often requiring large amounts of potentially hazardous solvents and besides, can lead to severe analyte losses [2,20,24]. Because of its high selectivity, sensitivity and anti-interference ability, LC–MS/MS has been widely used in the trace detection of harmful substances in foodstuffs [25–27]. Previous studies about determination of bisphenols are mainly focused on BPA, BPF, tetrabromobisphenol A (TBBPA), tetrachlorobisphenol A (TCBPA) and their DGEBA derivatives in beverages, crops, meat products and canned foods. Nevertheless, to the best of our knowledge, few research studies [12,21] have been reported to simultaneously determine the concentrations of bisphenols of edible oil. Niu et al. [21] reported an analytical method, on the basis of the gel permeation chromatography (GPC) clean-up followed by LC–MS/MS, to simultaneously determine BPA and alkylphenols in plant oil [21], but GPC procedure is gradually being phased out due to its disadvantages such as time-consuming, solvent-consuming, costly and environmentally unfriendly. Liu et al. [12] adopted two novel sample extraction methods including liquid–liquid extraction followed by dispersive liquid–liquid microextraction (LLE-DLLME) and reversed-phase dispersive liquid–liquid microextraction (RP-DLLME) for the analysis of BPA in edible oils, however, only one target bisphenol (BPA) was analyzed in their work.

In recent years, the dispersive solid phase extraction (DSPE) was introduced by Anastassiades et al. [28] for analyzing the pesticide residues in fruits and vegetables and has now been widely used for composition analysis in dairy products, soy sauce and other complex food matrix due to its quick, easy, cheap, effective, rugged and safe advantages [25–27,29]. The dispersive micro solid phase extraction (DMSPE), a simple and miniaturized modification of DSPE, has also been developed for the clean-up of target analytes [30]. DMSPE is commonly performed by trapping the target analytes in the sorbents. After extraction, the sorbent containing the target analytes is isolated by centrifugation or filtration. The target analytes can then be eluted or desorbed by an appropriate desorption solvent [31,32].

Therefore, the aim of the present study was to develop a selective and sensitive method, on the basis of a novel DMSPE and UPLC–MS/MS, to simultaneously determine BPA, BPS, BPB and BPF in edible oil. The extraction agents, extraction and purification parameters, instrumentation conditions were optimized. The linearity, selectivity, accuracy and precision were evaluated in order to assess the performance of the proposed method, which was

then applied to the analysis of four bisphenols in several commercial edible oils collected from local markets. Moreover, the kinetic migration rule of BPA from PC bottles to oils was first reported.

2. Materials and methods

2.1. Chemicals, reagents and standards

Methanol and *n*-hexane (HPLC grade) were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Ammonia water (mass concentration, 25%) was of analytical pure and obtained from Guangzhou Chemical Reagent Factory (Guangzhou, China). Ultra-pure water (18.2 MΩ cm) was prepared from a Milli-Q ultra-pure water system (Millipore, Bedford, MA, USA).

Standards of BPA (purity ≥98%) and BPF (purity ≥98%) were purchased from Dr. Ehrenstorfer GmbH (Ausburg, Germany). Standards of BPS (purity ≥98%) and BPB (purity ≥95%) were obtained from Sigma-Aldrich (Steinheim, Germany) and CNW Technologies (Düsseldorf, Germany), respectively. The isotope internal standards of BPA-d_4 (purity ≥99%) and BPS-$^{13}C_{12}$ (purity ≥97%) were purchased from CDN Isotopes Co. (Pointe-Claire, Canada) and Toronto Research Chemicals (North York, Canada).

Individual standard stock solutions (100 mg/L) were prepared by dissolving an appropriate amount of each standard and internal standard in methanol and storing at −20 °C in amber glass vessels. Mixed standard solution (0.5 mg/L of BPS, 1.0 mg/L of BPA, BPB and BPF) was prepared by combining appropriate amount of each individual standard stock solution and diluting in methanol. Mixed internal standard solution (0.25 mg/L for BPS-$^{13}C_{12}$ and 0.5 mg/L for BPA-d_4) was also prepared by combining appropriate amount of each individual internal standard stock solution and appropriate dilution with methanol. All the working standard solutions were stored at 4 °C. A series of mixed standard working solutions all containing 5.0 μg/L of BPS-$^{13}C_{12}$ and 10.0 μg/L of BPA-d_4 20 μg/L were prepared by diluting the mixed standard solution and mixed internal standard solution with methanol-water-formic acid (49.8/49.8/0.4, *v/v/v*) before use.

2.2. Apparatus

A MS3 basic vortex mixer (IKA GmbH, Germany) was applied for the vortexing of sample mixture. The 5418 high speed centrifuge and 5804R centrifuge (Eppendorf Corp., Germany) were used for centrifugation of sample solution. An ACQUITY™ ultra high performance liquid chromatography and Waters Xevo™ TQ tandem triple quadrupole mass spectrometer (Waters Co., USA) were used for sample analysis. Polymer anion-exchange (PAX) sorbent was purchased from Boehner Agela Co. (Tianjin, China) and was used for the adsorption and purification of the target analytes.

2.3. Samples and sample preparation

A total of fourteen edible oil samples with different manufacturing processes, packaging materials and brands, including one olive oil sample, three sunflower seed oil samples, two rape seed oil samples, one sesamum indicum oil sample, one corn oil sample, three peanut oil samples, one palm oil sample and one blend oil sample, were purchased from the local supermarkets in Guangzhou, China (numbered from S1 to S14). A pooled blank edible oil sample (numbered S$_{mix}$) was made of 14 samples and was used for method development, method blanks and quality assurance sample. Two used PC bottles (detected with BPA by preliminary experiments) were collected on a street market, into which 50 g of sunflower oil and rape seed oil (free of BPA shown by preliminary experiments), respectively (S3 and S6), were stored at room temperature (approx-