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Development of an immunoaffinity column for the highly sensitive analysis of bisphenol A in 14 kinds of foodstuffs using ultra-high-performance liquid chromatography tandem mass spectrometry

Kai Yao[a,b,c,1], Kai Wen[a,b,1], Wenchong Shan[a,b,c], Sanlei Xie[a,b], Tao Peng[a,b], Jianyi Wang[a,b], Haiyang Jiang[a,b,*], Bing Shao[a,c,*]

[a] Beijing Advanced Innovation Center for Food Nutrition and Human Health, College of Veterinary Medicine, China Agricultural University, Beijing 100193, People's Republic of China

[b] Beijing Key Laboratory of Detection Technology for Animal-Derived Food Safety, Beijing 100193, People's Republic of China

[c] Beijing Key Laboratory of Diagnostic and Traceability Technologies for Food Poisoning, Beijing Center for Disease Prevention and Control, 100013, People's Republic of China

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ABSTRACT

An immunoaffinity clean-up material based on a monoclonal antibody (mAb) has been prepared for concentrating and purifying bisphenol A (BPA) in 14 kinds of foodstuffs at trace level. Haptens and immunogen of bisphenol A have been synthesized and comprehensively characterized. An mAb towards BPA was prepared and cross-reactivities with 14 BPA analogues were below 5%. The prepared antibody was coupled to *N*-hydroxysuccinimide-activated Sepharose 4B to manufacture an immunoaffinity column (IAC), which was applied to purify BPA in 14 kinds of foodstuffs. The analyte was then detected by means of ultra-high-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS). Under the optimized conditions, compared with two traditional SPE clean-up methods, the IAC showed better selectivity (matrix effect < 16.8%) and higher sensitivity. The limits of detection for BPA in 14 kinds of foodstuffs ranged from 0.001 μg L^{-1} to 0.01 μg kg^{-1}, and the limits of quantification were in the range from 0.003 μg L^{-1} to 0.04 μg kg^{-1}. The recoveries of BPA from spiked samples ranged from 82.0% to 104.9%, with RSDs below 13.8%. Besides, the IAC exhibited good reusability, with 40% column capacity remaining and no significant loss of recovery after 25 application cycles in real sample detection. These results demonstrated that the developed IAC-UPLC-MS/MS approach has wide applicability for purifying and detecting BPA in various foodstuffs.

1. Introduction

In recent years, bisphenol A (BPA), which is mainly used as a monomer for preparing food packaging materials and coatings on the inside of metal cans [1], has received increasing attention for its potential endocrine-disrupting activities [2–4]. Besides the migration of environmental contamination, food is the major source of BPA exposure to consumers. BPA can leach into food from food contact materials such as packaging (most commonly, from the lining of cans used for high-acidity foods) [5]. Despite some controversy [6], the European food safety authority (EFSA) noted the dietary exposure to BPA to be the highest among children aged 3 to 10 [7], the uses of materials containing BPA in children's products has therefore been forbidden in the European Union and China since 2011 [8,9]. In 2014, the European Union lowered the temporary Tolerable Daily Intake (TDI) from 50 to 4 μg kg^{-1} body weight/day, based on adverse effects on the kidney and mammary gland related to BPA [10]. Currently, the EFSA is awaiting toxicology data on BPA from the U.S. National Toxicology Program for re-evaluation of TDI value [11].

Sensitive and accurate food exposure data are especially critical for the purpose of risk assessment. Currently, the prevalent analytical tool for the detection of BPA is liquid chromatography electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) for its high sensitivity without derivatization [12,13]. However, matrix effects constitute a major problem affecting the quantitative accuracy of LC-ESI-MS/MS data when analyzing food samples due to their inherent

* Corresponding authors at: Beijing Advanced Innovation Center for Food Nutrition and Human Health, College of Veterinary Medicine, China Agricultural University, Beijing 100193, People's Republic of China.

E-mail addresses: haiyang@cau.edu.cn (H. Jiang), shaobingch@sina.com (B. Shao).

[1] These authors contributed equally to this work.

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complexity. Thus, clean-up methods are of great importance for improving the sensitivity of detection methods. Various sample clean-up methods including liquid-liquid extraction (LLE), solid-phase extraction (SPE), solid-phase microextraction (SPME), quick, easy, cheap, effective, rugged, and safe (QuEChERS), and molecularly imprinted polymers (MIPs), have been developed to reduce matrix effects [14–18]. However, retention in most of these clean-up methods (LLE, SPE, SPME, QuEChERS) is based on nonspecific binding, which can lead to co-extraction of BPA and matrix interferences. MIPs are biomimetic polymers that can selectively bind to analytes of interest, but are associated with the challenges of residual leakage and non-specific binding during clean-up procedures due to their incomplete removal of the template and the low efficiency of specific binding sites [19]. These problems can be solved by the use of an immunoaffinity column (IAC), which has been proven to be an efficient way of eliminating the matrix effects through the specific recognition between antibodies and analytes [20]. In previous reports, IACs have been prepared and applied in the purification of BPA in canned fat-containing foodstuffs, beverages, fruits, and vegetables [21,22]. However, the purification effect of these IAC methods was investigated only in a limited number of foodstuff matrices. Furthermore, the reported IAC methods were all developed based on polyclonal antibodies (pAbs). Because of the disadvantage of pAbs, such as large variability between different batches produced in different animals at different times and high potential for cross-reactivity due to recognization of multiple analogues [23], the applicability of previously reported IAC methods has been limited. To meet the requirements of detecting relatively low concentrations of BPA and purifying BPA in complex food matrices, a monoclonal antibody (mAb) with high affinity and specificity has been prepared and used in IAC preparation.

In this study, a highly selective and sensitive analytical method has been developed based on an mAb IAC-UPLC-MS/MS technique. To the best of our knowledge, this is the first report of a specific mAb against BPA that has been used for its IAC purification from different kinds of foodstuffs. The characteristics of the mAb, the performance of the IAC and the stability of the IAC have been investigated. This novel strategy has been evaluated as an effective method for the concentration and purification of BPA from complex food matrices.

2. Materials and methods

2.1. Reagents and materials

Ovalbumin (OVA), bovine serum albumin (BSA), 2,2'-bis(4-glycidyloxyphenyl)propane (BADGE, 95.0% purity), 2-[4-(2,3-dihydroxypropyloxy)phenyl]-2-[4-(glycidyloxy)phenyl]propane (BADGE·H_2O, 95.0% purity), 2,2'-bis[4-(2,3-dihydroxypropoxy)phenyl]propane (BADGE·2H_2O, 97.0% purity), 2,2'-bis[4-(3-chloro-2-hydroxypropoxy)phenyl]propane (BADGE·2HCl, 97.0% purity), 2-[4-(3-chloro-2-hydroxypropyloxy)phenyl]-2-[4-(2,3-dihydroxypropyloxy)phenyl]propane (BADGE·H_2O·HCl, 95.0% purity), bis[4-(glycidyloxy)phenyl]methane (BFDGE, 98.0% purity), bis[4-(2,3-dihydroxypropyloxy)phenyl]-2-[4-(glycidyloxy)phenyl]methane (BFDGE·H_2O, 98.0% purity), bis[4-(2,3-dihydroxypropoxy)phenyl]methane (BFDGE·2H_2O, 98.0% purity), bis[4-(3-chloro-2-hydroxypropoxy)phenyl]methane (BFDGE·2HCl, 97.0% purity) and bis[4-(3-chloro-2-hydroxypropyloxy)phenyl]-2-[4-(2,3-dihydroxypropyloxy)phenyl]methane (BFDGE·H_2O·HCl, 98.0% purity) were purchased from Sigma-Aldrich (St. Louis, MO). *N*, *N*-dimethylformamide (DMF), Ethyl 4-bromobutyrate, 4-bromomethylbenzoic acid methyl ester, *N*-(3-(dimethylamino)propyl)-*N'*-ethylcarbodiimide hydrochloride (EDC·HCl), pyridine, and *N*-hydroxysuccinimide (NHS) were obtained from Aladdin Chemistry Co., Ltd. (Shanghai, China). NHS-activated Sepharose 4B and glass columns with PTFE frits (3 mL) were purchased from Biocomma (Shenzhen, China). Standard BPA (98.5%) was purchased from Dr. GmbH (Augsburg, Germany). Bisphenol F (> 99.0% purity), bisphenol AF (98.0% purity), bisphenol S (98.0% purity), and nonylphenol (98.0% purity) were purchased from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan). The deuterated standard BPA-d_4 (97.8% purity) were purchased from CDN (Québec, Canada). LC-MS-grade methanol (MeOH) and acetonitrile (ACN) were obtained from Merck (Darmstadt, Germany). Oasis HLB cartridges (60 mg, 3 mL; Waters, Milford, MA, USA) and AFFINIMIP cartridges (100 mg, 3 mL; Polyintell, Petit Couronne, France) were also used for sample purification.

Individual stock solutions (10 mg mL^{-1}) were prepared by dissolving 10 mg of standard substance in MeOH (10 mL) and stored at −18 °C in amber glass vials. Working standard solutions (10 μg mL^{-1}) were prepared by diluting the stock solutions with methanol. The working solutions were stored at 4 °C. Glassware was used for all experiments related to BPA. The buffers used in this study were as follows: (1) coupling buffer: 0.2 M $NaHCO_3$, pH 8.3; (2) blocking buffer: 0.2 M $NaHCO_3$, pH 8.3, containing 50 mM ethanolamine; (3) 0.1 M Tris-HCl buffer, pH 7.0; (4) 0.1 M HOAc-NaOAc buffer, pH 4.0; (5) 0.01 M phosphate buffer saline, which consisted of 2.7 mM KCl, 137 mM NaCl, 10 mM Na_2HPO_4, and 2 mM KH_2PO_4, pH 7.4.

Six female BALB/c mice were purchased from Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China), which were raised under aseptic conditions. All animal studies were carried out in accordance with the administration of affairs approved by the animal experimental ethics review committee of China Agricultural University (CAU20160620-2).

2.2. Synthesis of immunogen and coating antigens

BPA hapten 1 and hapten 2 were prepared according to the principle shown in Fig. 1. The procedures were designed according to previous protocols with some modifications [24]. Briefly, BPA (1 g) was dissolved in DMF (7 mL), sodium hydride (300 mg) was added, and the mixture was kept in an ice bath for 40 min. The mixture was then treated with ethyl 4-bromobutyrate (980 mg) (to prepare hapten 1) or 4-bromomethylbenzoic acid methyl ester (980 mg) (to prepare hapten 2) at 80 °C for 7 h. The reaction solutions were diluted with ethyl acetate (50 mL) and then distilled water (50 mL) was added. The organic layer was separated, and the solvent was removed by rotary evaporation. The residue was purified on a silica gel column, eluting with a mixture of ethyl acetate/petroleum ether (1:8, v/v). The eluted fractions containing the pure product were concentrated to dryness in a rotary evaporator. The residue was redissolved in MeOH (7 mL), and hydrolyzed with 2 M sodium hydroxide solution (1 mL) at 40 °C for 50 min. The solution was then adjusted to pH 2.0 with 6 M HCl and extracted with ethyl acetate (2 × 50 mL). The combined organic layers were dried over anhydrous sodium sulfate and concentrated to dryness to obtain the haptens.

The haptens contained carboxyl groups, and could thus be coupled to the carrier proteins (BSA and OVA) using the active ester method to prepare immunogens and coating antigens [25]. In brief, 40 mg of each hapten was dissolved in DMF (1 mL), then NHS (44.7 mg) and EDC (74.5 mg) were added and allowed to react for 2 h. The resulting solutions were slowly added to aliquots (6 mL) of BSA solution (8 mg mL^{-1}) with vigorous stirring. The conjugates were dialyzed against PBS (0.01 M) for 48 h with buffer changes every 5 h and stored at −20 °C prior to use. Hapten–BSA conjugates were used as immunogens, and hapten–OVA conjugates were used as coating antigens.

2.3. Characterization of haptens and immunogen

MS/MS patterns of the haptens were acquired using a Xevo TQ-XS triple-quadrupole tandem mass spectrometer (Waters, Milford, MA, USA). ^1H nuclear magnetic resonance (NMR) spectra of the haptens were recorded on a Bruker DPX-300 NMR spectrometer (Bruker Corp., Germany). Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectra of the immunogens and BSA were obtained on a Bruker MALDI Biotyper TOF instrument (Bruker Corp., Germany).