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Determination of nine bisphenols in sewage and sludge using dummy molecularly imprinted solid-phase extraction coupled with liquid chromatography tandem mass spectrometry

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ABSTRACT

This paper describes the determination of bisphenol A (BPA), bisphenol S (BPS), bisphenol F (BPF), bisphenol E (BPE), bisphenol B (BPB), bisphenol AF (BPAF), bisphenol AP (BPAP), bisphenol Z (BPZ) and tetrabromobisphenol A (TBBPA) in sewage and sludge samples. A highly class-selective dummy molecularly imprinted polymer was used for solid phase extraction (SPE) and clean-up of the samples. Bisphenols was quantified by high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS). The developed method had acceptable recoveries (43.6–101%), precision (RSDs: 1.5–15%) and matrix effects (−6.7 to 28%). The method limits of quantitation (MLOQs) for nine bisphenols in sewage and sludge samples were 0.0007–16.3 ng L^{-1} and 0.0004–8.28 ng g^{-1} dry weight (dw), respectively. The method was applied to a survey of a municipal wastewater treatment plant (WWTP) in Dalian. All of the tested bisphenols, except BPB and BPZ, were presented in the analyzed samples. BPA, BPS, and BPF with the concentrations 412, 109 and 66.4 ng L^{-1} in the WWTP influent, respectively, were the predominant bisphenols. The results demonstrated that BPS and BPF have become the most frequently used substitutes of BPA.

1. Introduction

Bisphenols (BPs) are a class of chemicals with similar structure widely used in the plastics manufacturing industry, mainly including bisphenol A (BPA), bisphenol S (BPS), bisphenol F (BPF), bisphenol E (BPE), bisphenol B (BPB), bisphenol AF (BPAF), bisphenol (BPAP) and bisphenol Z (BPZ). The release of BPs into the food and environmental has caused wide concern due to its potential health risks [1–5]. The adverse effects of BPA on reproductive [6,7], immune [8,9] and central nervous [10,11] systems have been well documented. Comparative estrogenic activities have also been reported for BPS, BPF, BPE, BPB and BPAF [12–14]. Recent studies shown that BPF and BPS (the most important substitutes of BPA [15,16]) are not necessarily safer and there is a need to remove all of the bisphenols from consumer merchandise [17–20].

The occurrence of BPA in environmental matrices, human samples and foodstuffs has been abundantly reported [21–24]. BPA was found in 92.6% of the 2517 participants in the United States (U.S.) [25]. Limited studies have shown that other bisphenols were also detected in matrices such as river water [26], sediment [15], wastewater [27], indoor dust [28], milk [29] and soft drink [30]. BPS, BPF and BPAF has been found not only in river water and sediment [26,31] but also in indoor dust [28]. BPB was detected in canned foods in European countries [32,33]. High concentration of BPS was identified in currency bills and paper products [34]. BPF was reported to be the predominant substitute of BPA in foodstuffs [35].

Due to the ultra-low concentration of bisphenols in environmental samples, high sensitive detection instruments and efficient sample pretreatment techniques are both indispensable. At present, solid-phase extraction (SPE) coupled with

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LC–MS/MS detection is the most frequently used method. The (U) HPLC–ESI–MS/MS with MRM mode provided a highly effective method for the quantitative determination of bisphenols [26,31,36]. Sample preparation based on hydrophilic–hydrophobic balance (HLB) [27], mixed-mode anionic exchange (MAX) and mixed-mode cationic exchange (MCX) [31,37] sorbents were previously reported as effective clean-up methods before LC–MS/MS analysis of BPs. However, significant signal suppressions for BPs detection were observed when HLB or HLB + MAX were used for sample preparation of sewage and sludge samples [26]. Such signal suppressions were caused by co-elution of matrix components which have influences on the ionization efficiency of BPs. Therefore, highly selective sample preparation method able to remove or minimize the co-elution components was needed.

Molecularly imprinted polymers (MIPs) are tailor-made materials with high affinity and selectivity for template molecules [38,39]. Molecularly imprinted solid-phase extraction (MISPE) is the most widely used area of MIPs. The high selectivity of the MIPs made them suitable for enriching of ultra-trace analytes in complex matrices. Until now, MISPE used in sample pretreatment of food, biological and environmental samples were extensively reported [40,41]. However, inherent drawbacks of MIPs such as template bleeding and low class-selectivity, limited theirs application in real sample analysis.

In our previous work, highly class-selective MIPs for bisphenol analogues were prepared by using 1,1,1-Tris(4-hydroxyphenyl)ethane (THPE) and phenolphthalein (PP) as dummy templates [42,43]. Sample preparation methods based on THPE-DMISPE were demonstrated to have great potential in complex sample pretreatment including sediment and human urine. Since dummy templates were used, the THPE-DMISPE method was free from template bleeding problem, and can be used in the routine analysis of trace BPs. The previous works, however, focused on the methodology, and BPs cannot be detected in real samples due to the low sensitivity of HPLC detection.

In this work, the THPE-DMISPE procedure was first used for sample pretreatment of real sewage and sludge samples in the HPLC–MS/MS analysis. Matrix effects in the detection of BPs were carefully studied and compared with the commercial SPE sorbents. The linearity, accuracy, precision, and sensitivity of the developed DMISPE-HPLC–MS/MS method were evaluated. Finally, the optimized method was applied to the quantitation of nine BPs in sewage and sludge samples collected from a WWTP in Dalian, China.

2. Experimental

2.1. Chemicals and reagents

Bisphenol A (BPA), bisphenol S (BPS), bisphenol F (BPF), bisphenol E (BPE), bisphenol B (BPB), bisphenol AP (BPAP), bisphenol AF (BPAF), bisphenol Z (BPZ), 1,1,1-Tris(4-hydroxyphenyl)ethane (THPE), trifluoroacetic acid (TFA) and ethylene dimethacrylate (EGDMA) were supplied by J&K Chemical Ltd. Chemical Reagent Co. (Beijing, China). Methacrylic acid (MAA), 4-Vinylpyridine (4-VP) and 2,2′-azobisisobutyronitrile (AIBN) were purchased from Acros (NJ, USA). $^{13}C_{12}$-labeled BPA and TBBPA were obtained from Cambridge Isotope Laboratories (Andover, MA). The methanol, acetonitrile and water used were LiChrosolv® hypergrade for LC–MS (Merck KGaA, Germany) and formic acid was purchased from Fisher Scientific (NJ, USA).

2.2. Preparation of SPE column

Dummy molecularly imprinted polymer was synthesized by the method described previously [43]. Briefly, THPE was used as the dummy template, with 4-vinylpridine and acetonitrile as functional monomer and polymerization solvent, respectively. SPE cartridges with a 3 mL volume were packed with 200 mg of the THPE-DMIP sorbents and used for sample pretreatment.

2.3. Sample collection

Four sewage samples (Sewage 1–4) and one sludge sample (Sludge 1) were collected from a WWTP in Dalian, China. The sampling locations in the WWTP are shown in Fig. S1. To inhibit the microbial activity, Formaldehyde (1%, v/v) was added to each sample immediately. Samples were then sealed in glass jars and transported to the laboratory at room temperature within a short time (5–10 min) for further processing. Water samples were filtered with a 0.45 μm glass membrane and stored at −20 °C. Sludge sample was freeze-dried, homogenized, and held at −20 °C until analysis.

2.4. Sample pretreatment

2.4.1. Sewage samples

After adjusting to pH 9.0 using sodium hydroxide solution, sewage samples (100 mL of Sewage 1, 300 mL of Sewage 2/3/4) were spiked with 20 ng of $^{13}C_{12}$-BPA and $^{13}C_{12}$-TBBPA internal standards and percolated through the THPE-DMIP cartridges (preconditioned with 3 mL acetonitrile and 3 mL water) at a flow rate of 3 mL min^{-1}. The cartridges were then vacuum-dried for 30 min and selectively washed with 3 mL acetonitrile to remove interferences. Bisphenols were finally eluted using 12.0 mL of methanol. The eluate was evaporated under a stream of high purity nitrogen gas and reconstituted in 1.0 mL with methanol–water (50:50, v/v) for HPLC–MS/MS analysis.

2.4.2. Sludge sample

Freeze-dried sludge sample (0.2 g) was spiked with 20 ng of $^{13}C_{12}$-BPA and $^{13}C_{12}$-TBBPA internal standards and allowed to stand at room temperature (~15 °C) for 24 h (in dark). The sludge sample was then extracted with 5 mL methanol–water (pH = 12.0) mixture (5:3, v/v) by ultrasound for 5 min and shaking for 30 min. After centrifugation at 4500*g* for 5 min, the supernatant was collected and transferred into a glass tube. The extraction process was repeated twice. The extracts were combined and evaporated to ~4 mL under a stream of N_2. After diluted to 10 mL with water, the extract was adjusted to pH 9.0 and loaded onto the THPE-DMIP column. The column was then rinsed with 3 mL water and vacuum-dried for 30 min. After drying, the column was further washed with 3 mL acetonitrile and eluted with 12 mL methanol. The eluate was dried under a stream of nitrogen and reconstituted in 1.0 mL with methanol–water (50:50, v/v) for HPLC–MS/MS analysis.

2.5. Instrument and analytical conditions

Sample analysis was performed using a TSQ Quantum Access MAX mass spectrometer coupled with an Accela HPLC System (Thermo Fisher System, San Jose, CA, USA). HPLC separation was conducted on a Hypersil GOLD C18 column (150 × 2.1 mm; 3 μm). The mobile phase consisted of methanol (A) and water (B). The gradient program with a flow rate of 200 μL min^{-1} was as follows: 35% A to 100% A (25 min), held for 5 min. The injection volume was 10 μL.

The mass spectrometric analysis was performed in negative ion mode (ESI) with multiple-reaction monitoring (MRM). The capillary voltage was maintained at −2.5 kV. Source and de-solvation chamber temperature were both at 300 °C. Nitrogen gas was used as the cone and de-solvation gas at 5 psi and 20 psi, respectively. The MRM transitions, collision energy and tube lens value used for