

SCREENING OF BISPHENOL A AND ITS HALOGENATED ANALOGUES IN SURFACE WATER BY COMBINATION OF PSEUDO-MOLECULARLY IMPRINTED SPE AND LIQUID CHROMATOGRAPHY

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Abstract

This study highlighted the potential of pseudo molecularly imprinted polymer (MIP) for simultaneous extraction of trace bisphenol A (BPA) and its halogenated analogues in surface water. Bisphenol E (BPE) was selected as the pseudo template and the resulting MIP showed appropriate selectivity for other bisphenols, avoiding the interferences originated from the template leakage. Pseudo MISPE-HPLC method was established and then validated with linearity, precision, limit of detection (LOD) and recoveries. LOD for each bisphenol compound was 2.5 -10 ng/L. The proposed method is suitable for highly selective and sensitive detection of trace bisphenols in environmental samples, and a promising approach for environmental monitoring.

Introduction

Bisphenol A (BPA) and its halogenated analogues (Fig. 1) are widely used as additives in manufacturing polymers and printed circuit boards. These bisphenols are the well-known endocrine disrupting chemicals (EDCs) that is ubiquitously found in environment. For instance, tetrabromobisphenol A (TBBPA), a lipophilic halogenated derivatives of BPA, is currently the most utilized brominated flame retardant in the world today with an estimated annual demand of 120,000 tons^{1,2}. Owing to their widespread use, recent studies have shown that bisphenols exist in the environment far from their location of production and

use, and the concentrations of bisphenols, both in the environment and in humans, are rapidly increasing^{3, 4}. Sellstrom⁵ reported that bisphenols could leak from treated products and Osako⁶ reported 620 ng/L of TBBPA in leachate samples from landfill. TBBPA in concentrations of 67 pg/g and 0.3–1.8 ng/g were also found in milk from Norwegian mothers and in human plasma samples, respectively^{7,8}.

Since the concentration of these bisphenols in environmental and biological samples are ultra-low in addition to high levels of many interferences, high-selective and effective sample pretreatment methods are required for sensitive and accurate quantification. Solid-phase extraction (SPE) and liquid-liquid extraction (LLE) are the most utilized sample pretreatment protocols in the preconcentration and cleanup of these bisphenols³⁻⁸. However, a major problem for these conventional extraction protocols is the lack of adequate selectivity, and always being suffered from the various interferences in the sample matrixes⁵⁻¹⁰.

Molecular imprinted solid-phase extraction (MISPE) can improve the accuracy and precision of the analysis due to its remarkable selectivity^{9, 10}. The application of MIP sorbents allows not only preconcentration and cleanup of the sample but also selective extraction of the target analytes. However, because MIPs are prepared by using the analyte as template, leakage of a trace amount of the template remaining in MIP will significantly hinder the accurate and precise assay of the target analyte^{9, 10}.

To overcome this problem, a structural analogue, so-called pseudo template, can be used as an alternative molecule in MIP preparation. In this case, the interested analyte can be separated from the bleeding template in the subsequent highly efficient LC analysis. In this study, a structural analogue of BPA, i.e. BPE, was selected as the pseudo template for preparation pseudo MIP. A pseudo MISPE method was developed for selective extraction of bisphenols in water samples. Coupled with HPLC, it was further employed for selective and simultaneous screening of bisphenols in surface water.

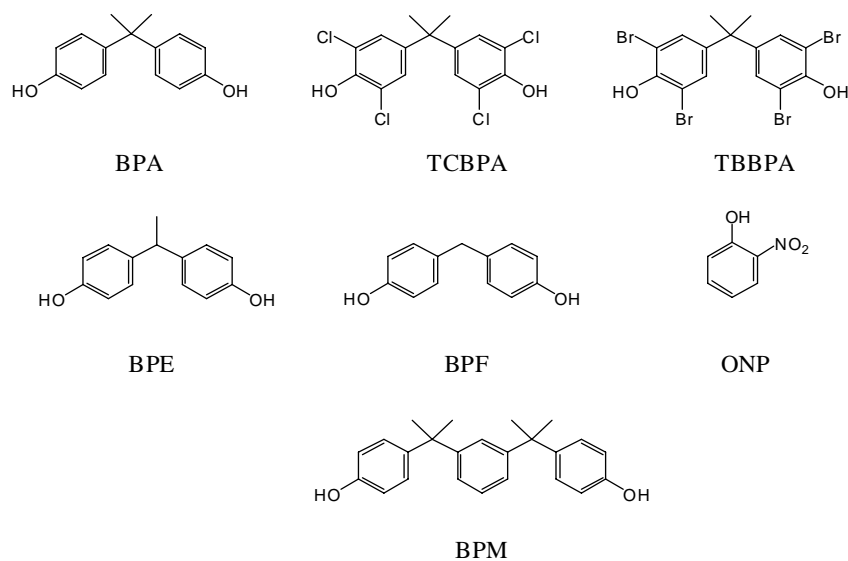


Fig. 1 Bisphenols and related endocrine disrupting chemicals (EDCs)

Materials and Methods

Bisphenols were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ethylene dimethacrylate (EDMA) and 4-vinylpyridine (4-VP) from Acros (NJ, USA) were refined by distillation. HPLC grade methanol was from Fisher (NJ, USA). Chemicals of analytical grade were purchased from Beijing Chemical Reagent (Beijing, China).

Results and Discussion

One merit of MISPE is that MIP sorbent has good selectivity against the template molecule. This makes a challenge for pseudo MISPE protocol that utilizes MIP prepared by a pseudo template. To confirm the selectivity of the pseudo MIP, the recognition properties of each MIP was investigated. As a result, BPE-MIP showed acceptable selectivity for all of bisphenol analogues (Fig. 2). Moreover, it is less used in industry than other bisphenols. Therefore, BPE was selected as the pseudo template for preparation of the desired pseudo MIP.

Besides the high selectivity, for a recommendable MISPE protocol, the high recovery and relatively operational simplicity should also be achieved.

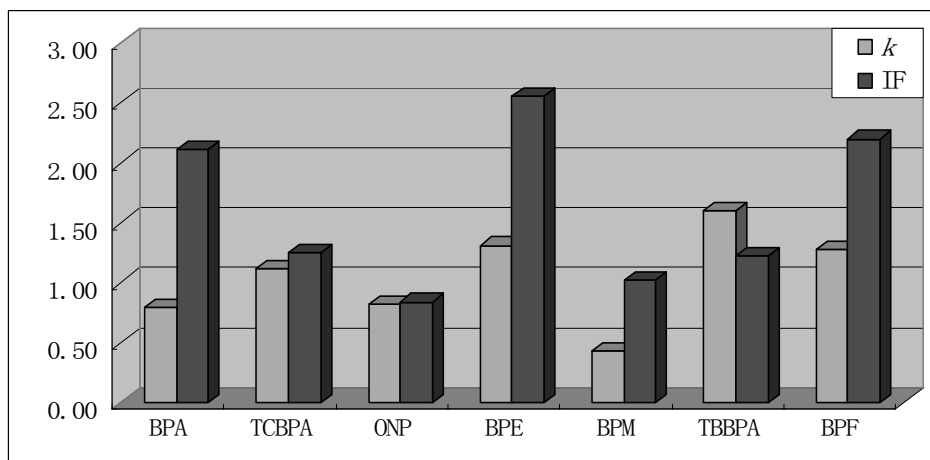


Fig. 2 Retention factor (*k*) and imprinted factor (*IF*) of each EDCs on BPE-MIP

Table 1 shows the recovery of these bisphenols on the BPE-MIP sorbent. At a concentration level of 50 ng/L, BPE has a very high recovery of $338 \pm 87.7\%$, and it decreases to $104 \pm 12.5\%$ at another level of 1000 ng/L. This implies that the template leakage may significantly interfere detection of the target analyte when its concentration is less than 1000 ng/L. In fact, the template leakage problem has been encountered for any other bisphenols when using their own MIP as the MISPE sorbent. As a pseudo MIP, however, BPE-MIP demonstrates good recovery for its structural analogues, i.e. BPA, BPF, TCBPA and TBBPA, at three different concentration levels. The recovery ranges from 59% to 92% for each analyte, and it is greatly enhanced with the increase of the concentration of the bisphenols (Table 1).

Table 1 Recovery of the bisphenols in the MISPE protocol (%)

Bisphenols	Concentration of bisphenols		
	50 ng/L	200 ng/L	1000 ng/L
BPA	59.2 ± 7.82	77.6 ± 4.85	91.4 ± 3.32
BPE	338 ± 87.7	142 ± 21.4	104 ± 12.5
TCBPA	62.5 ± 4.23	69.6 ± 3.48	78.2 ± 5.86
TBBPA	73.6 ± 6.64	81.6 ± 7.36	89.4 ± 3.75
BPF	68.7 ± 8.98	82.8 ± 3.17	85.3 ± 4.24

The proposed MISPE protocol shows highly efficient and selective enrichment of plasma samples, resulting in chromatographic traces with few peaks (Fig. 3). Most of the matrixes were cleaned-up in the pretreating steps. The signals of bisphenol analogues are well separated from interfering matrix component and can be reliably quantified. A single run can be accomplished within 30 min.

MISPE-HPLC method for bisphenols detection was then validated with linearity, LOD and precision. The linearity of the standard calibration curve was 10–1000 ng/L for BPA and BPF, and 20- 1000 ng/L for TCBPA and TBBPA, with a linear regression coefficient more than 0.997. LOD was calculated to be 2.5 ng/L for BPA and BPF, and 10 ng/L for TCBPA and TBBPA, respectively. The intra-assay precision was evaluated by five repeated injections of each spiked samples (50, 200 and 1000 ng/L) and calculated to be between 0.8% and 10.5% RSD. Similar results of 3.2% - 13.5% RSD were obtained for inter-day reproducibility. The values are well within the 20% limits required for the validated assay.

Real samples from Qinghe River were tested by the established pseudo MISPE-HPLC method. Result indicated that the levels of bisphenols in the river water are 127.8, 58.6, 46.5 and 152.5 ng/L for BPA, BPF, TCBPA and TBBPA, respectively.

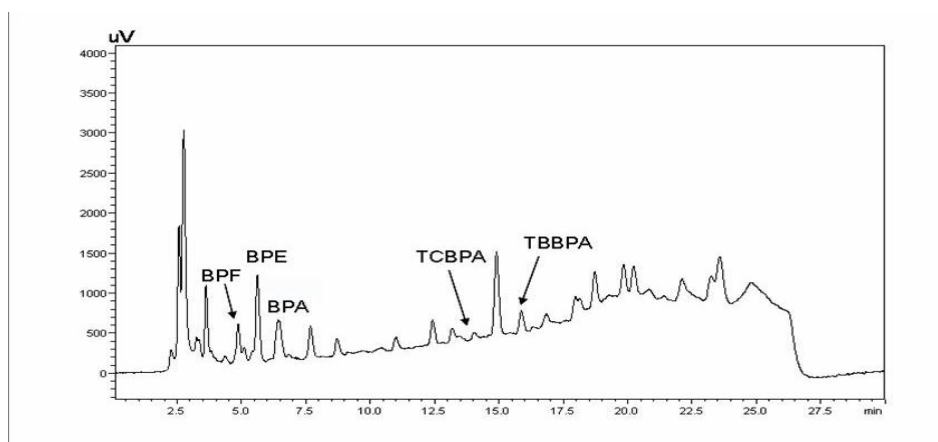


Fig. 3 HPLC chromatogram of the bisphenols spiked blank river water (50 ng/L).

Conditions: column, CAPCELL PAK C18 (4.6 × 250 mm, 5μm, SHISEIDO); mobile phase, 10 mM NH₄Ac buffer (pH 5.8, A) and methanol (B), gradient process: (1) 0- 20 min, form 68% B to 90 B%, (2) 20- 25 min, 90% B, and (3) 25- 30 min, 68% B; detection wavelength, 232 nm.

In conclusion, the pseudo MISPE protocol is more selective than conventional C18 SPE, avoiding time-consuming dilution, pH adjustment and sometimes tedious liquid–liquid extraction steps. Moreover, the high physical and chemical robustness and perfect clean-up effects of MISPE sorbents enabled their good reusability. Those good properties enabled the applications of BPE-MISPE for selective extraction and rapid screening of trace bisphenols in surface water.

Acknowledgements

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