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Short communication

Contamination of ultrapure water with bisphenol A leached from polysulfone ultrafilter☆

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ABSTRACT

Ultrapure water produced by a water purification system is one of the most essential and widely used reagents in laboratories. However, its quality is usually the least well-characterized and often overlooked. Here we investigate the contamination of ultrapure water by bisphenol A (BPA) leached from a polysulfone (PS) ultrafilter in a water purification system. To evaluate the level of BPA in ultrapure water, we used an offline solid-phase extraction (SPE) coupled with liquid chromatography mass spectrometry (LC–MS). Initial BPA level leached from a new PS ultrafilter was 0.70 ± 0.06 ng/mL. The concentration of BPA decreased gradually with continuous dispensation of purified water and was 0.20 ± 0.02 ng/mL at 33.5-L dispensation. The total amount of extractable BPA was 64.4 ± 1.4 μg per PS ultrafilter. The cumulative amount of BPA leached during dispensation of 33.5-L water was 1.2 ± 0.1 μg, which only accounts for 2% of the total amount of extractable BPA.

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1. Introduction

Environmental contaminants have recently been of significant concern to scientists and clinicians due to their potential to interfere with analytical assays and techniques [1–3]. For example, McDonald et al. observed anomalous kinetics with human monoamine oxidase-B, which was later found to be caused by bioactive contaminants that leached out of disposable laboratory plasticwares [2]. Howdeshell et al. also reported that the use of polycarbonate cages induced unusual estrogenic activity in a human breast cancer cell proliferation assay [3]. The identification and elimination of these contaminants in the laboratory is often time-consuming and difficult, typically involving the use of highly sensitive analytical instruments, which may not be readily available to all researchers.

Within the last decade, a substantial body of literature has linked bisphenol A (BPA) to potential health hazards to humans owing to its ability to disrupt endocrine signaling in the body [4–11]. The research has drawn considerable attention in both the scientific and regulatory communities due to the potential hazards posed to society [4,8,10,12]. Major leachable sources of BPA are plastics made of polycarbonate, polysulfone (PS), and epoxy resins [9]. These polymers have been widely used to manufacture electronic components, construction materials, drinking bottles, food containers, lab-wares, and medical devices [13,14]. Thus, BPA has now become a ubiquitous contaminant not only in the human environment, but also in research laboratories. Although there is a significant body of literature focused on the adverse effects of BPA at low dosages [15–18], there are discrepancies in the relevance and reliability of the published results which make it difficult to properly evaluate the hazards of BPA [7,9].

Ultrapure water is one of the most essential and widely used reagents in laboratories and clinical practices. For example, in the case of hemodialysis treatment, hundreds of liters of ultrapure water are required for ultrapure dialysate [19]. Furthermore, many scientists and clinicians often rely on a water purification system to obtain ultrapure water without properly testing the water to ensure its quality or purity. However, all water purification systems are prone to contamination by byproducts released from the plastic materials which come into direct contact with the water. In particular an ultrafilter, which is often filled with porous PS hollow fibers [20], can be a significant source of BPA contamination. This concern led us to perform qualitative and quantitative analyses of ultrapure water generated by a typical laboratory-based water purification system that utilizes a PS ultrafilter.

2. Experimental

2.1. Chemicals and materials

A PS ultrafilter was obtained from Thermo Scientific (Dubuque, IA). All solvents, including HPLC-grade water, ethanol, and

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acetonitrile were HPLC grade if not specified otherwise, and were purchased from Fisher Scientific (Raleigh, NC). BPA was obtained from Sigma–Aldrich (St. Louis, MO). $^{13}C_{12}$- and D_{16}-BPA were from Cambridge Isotope Laboratories (Andover, MA).

2.2. Sample extraction and preparation

To study the elution profile of BPA released from a new PS ultrafilter, we used a readily available Type I water purification system (Nanopure Diamond, Dubuque, IA; others may show similar trends). A volume of 33.5-L ultrapure water (18 MΩ cm in resistivity) was dispensed with this system, and 100 mL fractions were collected seven times at 0, 2.25, 4.5, 8.75, 17, 25.25, and 33.5 L. In addition, 100 mL of feed water was also collected as a control. Commercial HPLC-grade water was also analyzed as an additional control for comparison purposes. A surrogate was added to all collected solutions to account for any user errors associated with sample handling. For this purpose, each sample was spiked with D_{16}-BPA (0.5 ng/mL). Then, the samples were enriched by offline solid-phase extraction (SPE) using a Waters symmetry shield C_{18} column (3.9 mm width × 20 mm length, 3.5 μm particle size). BPA and D_{16}-BPA retained in the C_{18} cartridge were eluted with acetonitrile. The eluted solution was dried under a stream of nitrogen and reconstituted in 100 μL of 50% aqueous acetonitrile before analysis. In separate experiments, the maximum amount of extractable BPA from the PS-based ultrafilter was also determined after Soxhlet extraction. The extraction was completed on the individual PS hollow fibers in the ultrafilter. Pre-cut PS hollow fibers of 0.7-g mass were placed in the Soxhlet apparatus, and the extraction was performed at 110 °C for 20 h using 70 mL of 50% aqueous ethanol as solvent.

2.3. LC–MS analysis

Samples were directly analyzed without chemical derivatization using liquid chromatography (LC) coupled with single quadrupole mass spectrometry (MS) (Alliance 2695-ZQ4000; Waters, Milford, MA). An internal standard was spiked into all samples directly prior to analysis that helped compensate for run-to-run variations in instrumental performance. $^{13}C_{12}$-BPA was an excellent choice considering the similar chemical profile to BPA, and was introduced at 500 ng/mL concentration immediately before the LC–MS experiments. The separation of BPA was achieved using a Waters Xterra MS C_{18} column (3.0 mm × 150 mm, 3.5 μm) at a flow rate of 250 μL/min with a linear gradient from 54% to 75% of acetonitrile (solvent A) relative to HPLC water (solvent B). The column was re-equilibrated at least 10 min before each analysis. BPA, $^{13}C_{12}$-BPA, and D_{16}-BPA were ionized by negative ion electrospray ionization. The deprotonated molecules of BPA and its isotope-labeled internal standards were measured in a selected ion monitoring mode at *m/z* 227, 239, and 241, respectively.

3. Results and discussion

3.1. Enrichment by offline SPE

The limit of detection (LOD) and the limit of quantification for the LC–MS system were 5 and 15 ng/mL, respectively. Offline SPE using the C_{18} column we developed was well suited for the enrichment of BPA in water due to its hydrophobic characteristic. The column accommodated for more than 100 mL of the total sample volume without exceeding the analytical capacity of the column. Using this method, BPA was concentrated to a level of 800 times the initial solution. The resultant LOD and limit of quantification were 0.007 and 0.02 ng/mL, respectively. To determine the recovery yield of BPA, each sample was spiked with D_{16}-BPA (0.5 ng/mL).

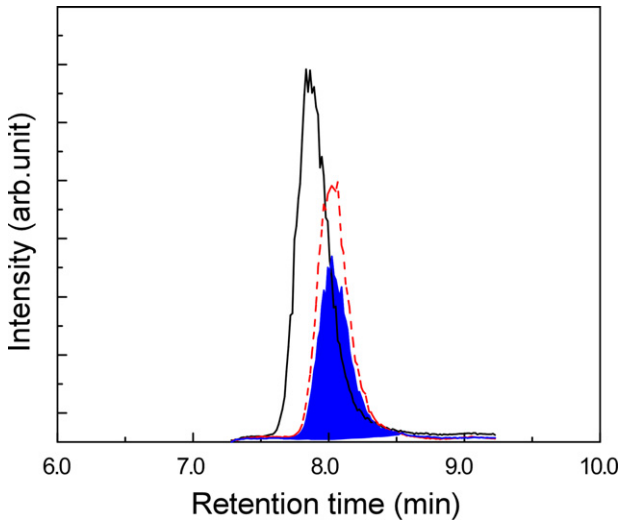

Fig. 1. Typical LC–MS chromatogram for BPA enrichment and separation using offline SPE-LC–MS method. BPA (solid line; blue-shaded), $^{13}C_{12}$-BPA (dashed line; un-shaded), and D_{16}-BPA (solid line; un-shaded) were ionized by negative ion electrospray ionization. The deprotonated molecules of BPA and its isotope-labeled internal standards were measured in a selected ion monitoring mode at *m/z* 227, 239, and 241, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

The sample was mixed well and then concentrated as described. All experiments were run in triplicate. The average recovery yield for all of the samples was 90 ± 3%.

3.2. Qualitative analysis and background level of BPA

The analytical confidence of selective BPA detection was established in qualitative experiments. As shown in Fig. 1, the LC–MS analysis of water samples gave a deprotonated BPA (solid line, blue-shaded) at *m/z* 227 with 8.02 min retention time. Water samples spiked with BPA internal standard yielded identical retention times. Ion signal intensity at *m/z* 227 was higher in the spiked sample than in the unspiked sample, as expected. In addition, tandem MS further confirmed that BPA was detected in the water samples (data not shown). For quantitative analysis, both $^{13}C_{12}$- and D_{16}-BPA were spiked into water samples. LC–MS analysis showed that $^{13}C_{12}$-BPA (dashed line, un-shaded) and D_{16}-BPA (solid line, un-shaded) were eluted at 8.02 and 7.86 min, respectively. To determine the background level of BPA, both the commercial HPLC-grade water and feed water, which was supplied from an in-house pretreatment system to the water purification system, were analyzed. BPA was not detected in the HPLC-grade water with a 0.007 ng/mL detection limit (Table 1). However, a small amount of BPA was measured at 0.02 ± 0.01 ng/mL in the feed water. This BPA background was subtracted in subsequent experiments analyzing the elution profile of BPA released from PS ultrafilters.

Table 1
BPA levels in various samples.

Sample	BPA level
HPLC-grade water	<LOD[a]
Feed water (ng/mL)	0.02 ± 0.01
PS hollow fibers (μg/g)	8.80 ± 0.18
PS ultrafilter (μg)	64.4 ± 1.4
33.5 L ultrapure water[b] (μg)	1.2 ± 0.1

[a] LOD was 0.007 ng/mL.
[b] Cumulative amount of BPA leached from a water purification system for a new PS ultrafilter.

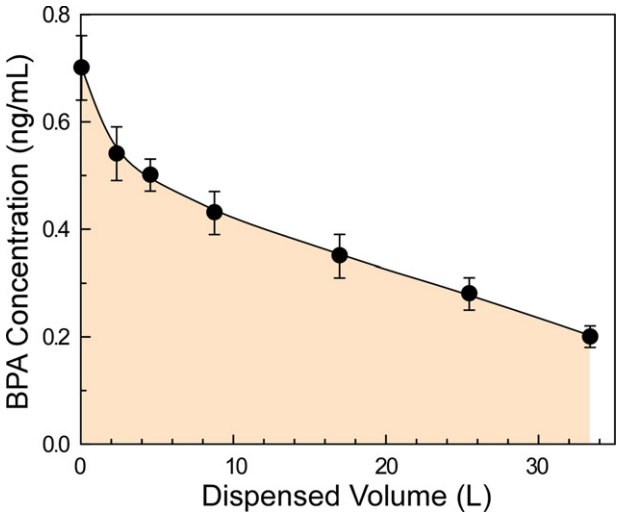

Fig. 2. Elution profile of BPA released from a PS-based ultrafilter. The background level of BPA detected in the feed water was subtracted. The solid line was added for clarity. The shaded area gives the cumulative amount of leached BPA.

3.3. BPA release from PS ultrafilter and total BPA content

The elution profile of BPA released from a PS ultrafilter is shown in Fig. 2. The newly installed PS ultrafilter released this compound in a sub-ppb level of 0.70 ± 0.06 ng/mL at the beginning. Fig. 2 also reveals that the amount of leached BPA decreased with continuous use of the ultrafilter and exhibited a concentration of 0.20 ± 0.02 ng/mL after 33.5-L water was dispensed.

The maximum amount of extractable BPA was also measured for the PS ultrafilter. Soxhlet extraction was performed on the fibers of the filter because of its experimental convenience for controlled continuous extraction. The unit maximum amount of BPA was determined to be 8.80 ± 0.18 μg/g in the PS hollow fibers. This unit maximum was multiplied by the total weight of the PS fibers in the ultrafilter (7.3 g) to obtain the maximum amount of BPA, which could be extracted from the system (64.4 ± 1.4 μg). The cumulative amount of BPA released during the 33.5-L dispensation was also calculated by measuring the area under the curve of Fig. 1 and was 1.2 ± 0.1 μg. This corresponds to 2% of the maximum amount of extractable BPA from the PS ultrafilter. It is expected that the remaining BPA is continuously released with use over time. Further analysis showed that BPA leached at a concentration of 0.05 ng/mL after 65-L of water dispensation, which supports the notion that BPA is released in a continuous fashion from the PS ultrafilter.

Typical levels of BPA in animal and human samples, including serum, urine, and breast milk, are in the range of 0.05–3.0 ng/mL [13,21]. Our data from this experiment has shown that PS-based ultrafilters have the potential to leach similar level of BPA into purified water. Thus, unexpected high background or fluctuation is possible in these analyses when ultrapure water, obtained right after installation of a new PS-based ultrafilter, is used as extraction or sampling media.

4. Conclusion and outlook

In a Type I water purification system, BPA was found to leach out of a PS ultrafilter and contaminate the final product of nominally ultrapure water. Because a PS ultrafilter is not used in all water purification systems, however, BPA content in ultrapure water is expected to vary depending on the water purification method. This may have the potential to complicate the understanding of some toxicological data and challenge the validity of investigational results. Thus, it is imperative to be aware of the type of materials used not only in research laboratories, but in the clinical environment as well.

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