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Reducing Bisphenol A Contamination from Analytical Procedures To Determine Ultralow Levels in Environmental Samples Using Automated HPLC Microanalysis

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A new high-performance liquid chromatography (HPLC) method has been developed to detect ultralow concentrations, below 1 ng/L (ppt), of bisphenol A (BPA) using column switching, and electrochemical detection. This HPLC method provided a detection limit of 0.36 ppt BPA and repeatability of 9.3% with a relative standard deviation at 1 ppt (r = 0.999 with a linear calibration curve over a concentration range of 1−100 ppt.). BPA is inherently ubiquitous in the environment, including the very tools and solvents used for its analysis, so to obtain meaningful results, the overall BPA contamination concentration must be below the detection limit for BPA using the analytical system. Therefore, purified water for preparing the standard BPA solution must be filtered with a hydrophobic membrane to suppress BPA background levels of contamination. In addition, all of the glassware used to prepare the standards and samples for analysis must be treated carefully to eliminate residual BPA contamination. Although BPA-free water and heat-treated glassware were used as precautionary measures for analysis, manual preparation and injection resulted in considerable BPA levels that will confound the results. Furthermore, the use of manual injection syringes with a fixed cemented needle also contributed to substantial BPA contamination, even if fluorescence detection was employed. However, manual injection syringes with a removable needle gave rather good results compared to that of the cemented needle type. By employing these precautionary measures and procedures to reduce BPA contamination from the analytical procedure, 1−10 ppt of BPA in environmental water samples was accurately determined using a column-switching HPLC system. This paper describes a systematic procedure and solution for reducing BPA contamination introduced by methods and procedures to determine a full range of BPA concentrations in environmental samples, such as lake water, even at very low concentrations.

Bisphenol A (BPA) is frequently detected in environmental water and is now attracting attention as an endocrine disrupter, because it has rapidly entered into the environment, the food chain, and therefore, human consumption. It has recently been proposed that trace amounts of BPA also show estrogenic activity, even at concentrations below 1 ppt.[1−3] The challenge is that BPA is almost ubiquitous in the environment, and therefore, accurate determination of such ultralow concentrations of BPA that have potentially tremendous impact is interfered by many factors used for analysis, such as contamination from glassware, manual injection syringes, or any other materials that come in contact with the analytical samples or standards. Furthermore, purified water from water purification systems contains certain amounts of BPA. Therefore BPA-free water is essential to prepare standard solutions and it is critical that all the glassware and components must be clean and essentially BPA- free to obtain accurate results.

In this study, the BPA concentration in environmental water samples was analyzed by high performance liquid chromatograph (HPLC). Although GC/MS is commonly utilized for BPA determination, the drawback to this method is that pretreatment of the samples requires ∼1 day to be completed. On the other hand, the HPLC-electrochemical detection (ECD) method only requires simple sample pretreatment followed by immediate analysis. In fact, this method has also been applied to the practical applications such as the determination of BPA in serum.[4,5]

In this study, factors affecting BPA contamination were addressed, and the way for suppressing or eliminating its contamination for analysis was investigated. Among the various sources of BPA contamination, manual syringes and water

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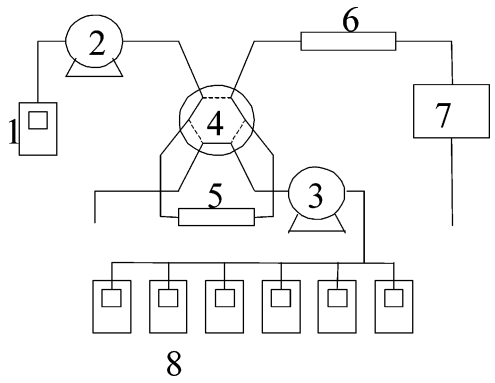

Figure 1. Flow diagram of the column switching HPLC system: (1) mobile phase, (2, 3) pump, (4) high-pressure flow switching valve, (5) pretreatment column, (6) analytical column, (7) ECD, and (8) samples (one for rinsing solvent).

purification systems were the primary focuses to reduce confounding BPA contamination introduced by sample preparation and analysis. In this study, commonly used manual syringes with the fixed cemented needle caused considerable BPA contamination. In addition, the glassware utilized in these analyses to accurately detect trace amounts of BPA had to be treated at high temperature to eliminate background contamination. In addition, in order to detect very low levels of BPA in samples, it was necessary to concentrate the samples prior to analysis. This was done in several ways.

Concentration of samples for trace analysis of BPA is essential to achieve sensitivity at the parts-per-trillion level. BPA samples were concentrated using membrane concentration and column switching techniques as pretreatment procedures. The on-line HPLC column switching method (see Figure 1) was also applied to the actual determination of BPA in river water and lake water. Both methods were then compared.

EXPERIMENTAL SECTION

Instrumentation and Chemicals. Manual injection syringes with a cemented needle were purchased from Hamilton (Reno, NV) and Terumo (Tokyo, Japan) and manual syringes with a removable needle were also purchased from Terumo.

A lab-scale water purification system was purchased from Millipore (Bedford, MA), which consisted of Elix-UV and Milli-Q Gradient. Water for analysis was purified by passing through RO membrane, ion exchanger, and UV lamp. A large-scale water purification system utilized in the study was equipped at the National Institute for Environmental Studies, Tsukuba, which consisted of 10-, 5-, and 1-μm mesh filters, an 800-L storage tank made with titanium, a large scale charcoal bed (two 2120 $\times$ 260 mm i.d. titanium containers were connected in series), a UV lamp, and a 0.45-μm mesh filter for the final filtration system.

Empore disks (47 mm o.d. of SDB-XD type consisting of styrene−divinylbenzene copolymer), a membrane stationary phase, was purchased from 3M (St. Paul, MN). Acetonitrile and methanol (HPLC grade) were purchased from Wako pure chemicals (Osaka, Japan). The HPLC system, including a Shim-pack VP-ODS column (150 $\times$ 4.6 mm i.d., silica-based C18 stationary phase for analysis) and a SPC-RP3 (30 $\times$ 4 mm i.d., polymer-based reversed-phase stationary phase for pretreatment), was purchased from Shimadzu (Kyoto, Japan). The HPLC system consisted of a LC-10Avp solvent delivery pump, LC-6A sample concentration pump, CTO-10Avp column oven, FCV-12AH two-position flow changeover valve, FCV-13AL six-port flow selection valve, SIL-10AXL automatic injector, Rheodyne 7725 manual injector (Cotati, CA) with a 100-μL loop, RF-10AXL fluorescence detector (FLD), SCL-10A system controller, and Class-VP work station software. A Coulochem II electrochemical detector (ECD) was purchased from ESA (Chelmsford, MA). The HPLC mobile phase composition was 20 mM (sodium) phosphate buffer (pH 7.0)−acetonitrile (60:40 (v/v)) for standard evaluation, whereas (65:35 (v/v)) for the actual sample determination was used (isocratic elution). The flows rates for analysis and pretreatment were 0.8 and 2.5 mL/min, respectively. Excitation and emission wavelengths were set at 275 and 310 nm, respectively, for fluorescence detection (FLD). The electrode potential for ECD was +0.55 V, and the column oven temperature was maintained at 40 °C. Purified water utilized to prepare standard solutions of BPA was filtered with the Empore disk to remove the trace amounts of BPA prior to use.

All the glassware except volumetric vessels was treated with heat at 400 °C.

Contamination from Manual Syringe. The column was equilibrated with the mobile phase. After the baseline had stabilized, 50 μL of mobile phase was injected several times by several different manual syringes with the cemented needle and the removable needle. Detection was compared using both FLD and ECD.

Membrane Concentration of Samples. The Empore disk was set into a dedicated filtration apparatus consisting of a vacuum pump, glass-attachment, glass-concentrating tube, and stainless steel manifold with a three-way cock. Two hundred milliliter portions of BPA solutions or environmental water samples were then filtered. After filtration, the membrane (dried by excess aspiration) was washed with 2 mL of methanol, then 5 mL of ethyl acetate was added to elute the retained components. The extract was evaporated to dryness and then reconstituted with mobile phase for HPLC analysis to make 1 mL of sample solution, then 50 μL of it was injected onto the HPLC by autosampler.

HPLC Column Switching. To accomplish suppression of BPA contamination and determine the BPA concentration in water samples, a special technique was required. A column-switching HPLC with a pump injection system was one of the solutions. Figure 1 shows a flow diagram of the column-switching HPLC system used in this study. The pump delivered 50 mL of BPA standard solutions or environmental water samples, and the BPA was concentrated on the pretreatment column. Then the mobile phase was delivered via a six-port switching valve, and then the concentrated BPA was directed to the analytical column and detected by the ECD after the separation on the analytical column.

Contamination from Water Purification System. Purified water obtained from the commercially available water purification system, Milli-Q, and a large-scale water purification system were also analyzed with column-switching HPLC method. These analyses were performed several times over a course of several days to evaluate day-to-day variation of BPA concentrations in purified water.

RESULTS AND DISCUSSIONS

Contamination from Manual Syringe. During the course of this experiment, strong BPA contamination was observed under

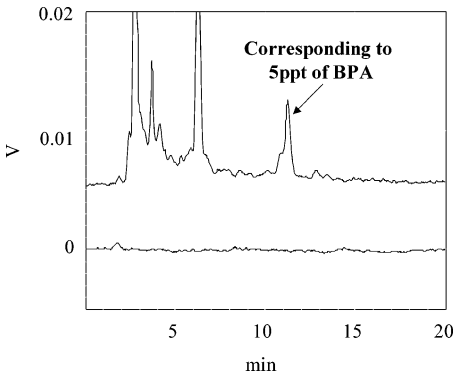

Figure 2. Comparative chromatograms of mobile phase injected with cemented (upper) and removable (lower) needle syringes. HPLC conditions: mobile phase, 20 mM sodium phosphate buffer (pH 7.0)−acetonitrle (60:40 (v/v)); flow rate, 0.8 mL/min; column, Shim-pack VP-ODS (150 × 4.6 mm i.d.); detection, florescence ex 275 nm, em 310 nm; temperature, 40 °C; injection volume, 50 μL.

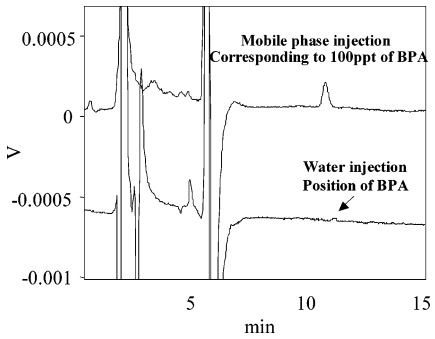

Figure 3. Comparative chromatograms of mobile phase and water injected with removable needle syringe. HPLC conditions were the same as Figure 2, except for detection of electrochemical detector at +0.55 V.

several conditions. Figure 2 (upper part) is a chromatogram of just mobile phase injected by the syringe with a cemented needle. The detected peak area of BPA corresponded to ∼5 ppb. This determination was based on using the results obtained by an automatic injector, which met the criteria or below the detection limit of BPA contamination level. This phenomenon was observed even if newly purchased and completely washed syringes with methanol, acetonitrile, hexane, and chloroform were used. On the other hand, the syringe with a removable needle provided an acceptable baseline in this sensitivity range with FLD, as is shown in Figure 2 (lower part)

When the ECD was used to obtain a higher sensitivity range than that of FLD, even the manual syringe with a removable needle could not satisfy the required BPA contamination criteria. Figure 3 shows a chromatographic difference between 50 μL of mobile phase and water as samples. These chromatograms show that a manual syringe, which has a removable needle, can be used for trace amounts of BPA analysis only when the sample solvent does not contain organic solvent.

It might be suggested that the source BPA contamination with both manual syringes originated from the adhesive or resinous parts. The syringes with a cemented needle have adhesive. Among the types of adhesive, epoxy−resin adhesive provides the best possible origin of BPA contamination in the analytical procedure.[9] The syringes with a removable needle that were used had only a small amount resin attachment that would explain the difference observed between the two syringe types.

In traditional pretreatment of BPA samples in environmental water for HPLC analysis, sometimes the samples are subjected to multiple redissolving or desorption with organic solvents, such as methanol or acetonitrile. This means that the final sample solvent contains quite a lot of organic solvent in which BPA can easily be dissolved and, therefore, BPA contamination, even if the syringe with a removable needle is employed. If the contamination originated from the syringes, injection with the organic solvent would promote BPA elution from the syringes.

Membrane Concentration. When the 20 and 5 ppt of standard BPA solutions were treated five times, the RSD values for reproducibility of the whole procedure were 3.9 and 9.9%, respectively, and overall BPA contamination was higher than 1.4 ppt. These results show that membrane pretreatment is not suitable for BPA determination when an expected concentration is below 10 ppt. As was described in the latter paragraph, the Empore disk can retain BPA in water completely at low concentrations, such as at the 1 ppt level. This means that the filtrate is almost BPA-free. BPA will be contaminated in subsequent procedures, such as washing with ethyl acetate, reconstitution with mobile phase, transfer from the evaporator to the sample vial, and so on. Organic solvent in the glassware can also adsorb BPA from the atmosphere. Organic solvent is easily evaporated naturally, and residual BPA would be a source of contamination. For preventing this contamination, heating of all glassware used for the analysis procedure and preparation above the boiling point of BPA (250 °C) is necessary. In the manual pretreatment of biological samples for BPA determination with GC/MS, heating glassware before sample preparation reduced BPA contamination to one-third of that obtained with non-heat-treated glassware. To minimize this contamination, the operator must have manipulative skills..

HPLC Column Switching. A column-switching HPLC system has two major benefits for microanalysis. One is automated pretreatment, and the other is automated sample concentration. By using this system, some manual procedures, such as evaporation or sample transfers from glassware are eliminated. In addition, the loss of actual sample can be minimized.[6] When the sample volume is large enough, on-column concentration is possible.[7] Proper combinations of column pretreatment and sample solvent can provide a >1000-fold concentration of sample for analysis.

Using this column-switching HPLC method, the pump delivers the sample solution. Consequently, accuracy and precision of injection volume depend on the flow rate and the time program for the pumps and switching valves. Considering these matters, the sample delivery pump was previously operated and the starting point of pretreatment was programmed to automatically change

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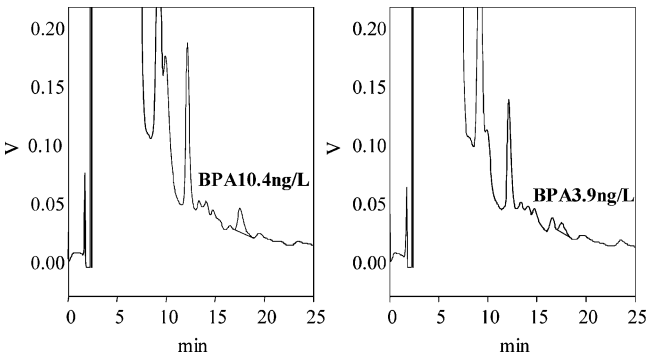

Figure 4. Chromatograms of lake water obtained with column-switching HPLC. A 50-mL portion of lake water was concentrated onto a pretreatment column packed with molecularly imprinted polymer. HPLC conditions were the same as Figure 3, except for the mobile acetonitrile concentration of mobile phase. (See Experimental Section.)

the position of the two-position flow switching valve. This sleek, sophisticated method provided rather impressive results.

Standard aqueous solutions of BPA at 1, 10, and 100 ppt were injected repeatedly ($n = 5$) to determine the repeatability; the results were 9.3, 3.4, and 0.5% RSD, respectively, and a linear calibration curve with a correlation coefficient >0.999 was obtained. The detection limit estimated on the basis of standard deviation (σ) of the y intercept of the calibration curves was 0.36 ppt, which was obtained as [3.3(σ of y intercept)/(slope value of calibration curve)]

The equation of the calibration curve was $y = 78665x - 47475$, where x is the concentration of BPA, and y is the peak area and σ of y intercept estimated on the basis of the residual sum of squares.

By using this HPLC system coupled with molecularly imprinted polymer[8] as a pretreatment column, trace amounts of BPA were reliably determined in actual environmental samples or purified water. Figure 4 shows chromatograms of BPA in actual lake water samples. Low concentrations of BPA were resolved without serious peak overlapping. In fact, the level of 1 ppt can be easily determined using this method. It was observed that the detection limit using this HPLC, even if combined with the column-switching system and FLD, has been as low as 50−10 ppt so far;[10,11] therefore, this method is quite reliable and easy. The columns used are commercially available, as well.

Contamination from Water Purification System. Both water purification systems provided certain levels of BPA contamination.[12] The contamination varied daily, as is shown in Figure 5. Consequently, there is no reliable way to correct for a certain degree of contamination by calculation. BPA-free water that was used for preparing BPA standard solutions must be used; otherwise, reliable calibration curves cannot be generated. BPA-free water can be obtained by filtering purified water with the Empore disk, SDB-XD or C18 type. Both disks show strong BPA retention if applied with just water as the solvent. Then the filtrate can be used as BPA-free water for creating the calibration curve. Figure 6 shows a comparison of chromatograms of purified water

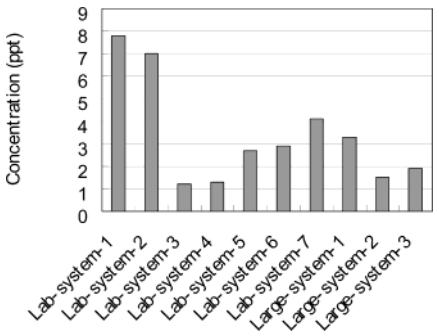

Figure 5. Day-to-day variation of contaminated BPA in pure water. The concentrations in Lab-system 1−7 were obtained by the same milli-Q system but in different days. The concentrations of Large-system 1−3 were obtained by large-scale water purification system on different days.

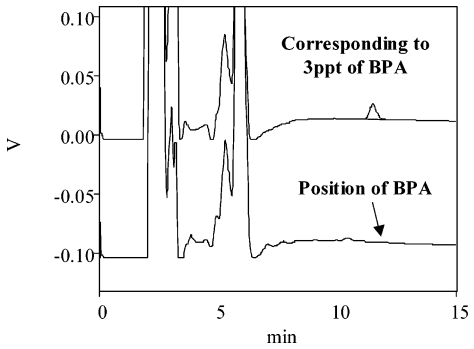

Figure 6. Comparative chromatograms of water and filtered water. Water was obtained from a Milli-Q water purification system. HPLC conditions were the same as Figure 3.

and that of filtered water. By filtration with the Empore disk, BPA contamination was suppressed below the detection limit.

On the basis of these results, the water purification system seems to generate small but not negligible BPA contamination, as we confirmed from direct analysis of tap water that had no significant peaks recognized as BPA. One of latest water purification systems guarantees that the BPA contamination will be below 5 ppt, whereas not negligible for ultralow determination, such as a 1 ppt level.

CONCLUSION

BPA determination in analytical samples at ultralow concentrations, such as below 10 ppt, cannot be accomplished without suppressing or reducing the inherent background levels of BPA contamination introduced by the method or sample preparation. Manual syringes with both cemented and removable needles generate BPA contamination. Considering the degree of contamination and assuming that the acceptable contamination revel is 1%, the former procedure or method mentioned above can be used when the BPA concentration is >500 ppb, and the latter method for sample analysis at the 10 ppb level based on our investigation. It is also very important to note that glassware used for sample preparation requires heat treatment at 400 °C or more to evaporate possible BPA contamination. The water purified with the commercially available water purification system cannot be used as it is for preparing standard BPA solutions for analysis at the ultralow

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concentration range. It must be filtered with a hydrophobic membrane; only then is the BPA contamination level sufficiently reduced below the detection limit of the ECD providing more reliable results. The most suitable HPLC method, as demonstrated in this extensive study, is the on-line HPLC column-switching system and method. BPA below 1 ppt can be accurately and reproducibly detected using this HPLC system. Hopefully, this methodology and HPLC system should provides a useful tool for more careful, accurate investigation and determination of BPA levels in the full spectrum of samples ranging from environmental to food products and, consequently, research on endocrine disruptors in biological matrixes.

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