Chromatography
Separation Techniques

Migliaccio et al., J Chromatogr Sep Tech 2015, 6:6
DOI: 10.4172/2157-7064.1000295

Short Communication Open Access

BPA Free Waters Essential to Perform Laboratory Studies

Migliaccio M[1], Errico S[1], Diano N[1]*, Nicolucci C[1,2]

[1]Department of Experimental Medicine, Second University of Naples, Via S. M. di Costantinopoli, 16, 80138 Naples, Italy
[2]Department of Experimental Medicine, Università degli Studi di Messina, AOU Policlinico G. Martino - Viale Gazzi - 98122 Messina, Italy

Abstract

The present study aims at assessing Bisphenol A (BPA) level in water used in laboratories. A total of five types of water commonly used in laboratory (tap, softened, distilled, double distilled, commercial LC-MS pure water), were analyzed and BPA was quantified by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Only the samples of ultrapure water showed BPA levels above detection limits (>0.004 ng/mL). The mean BPA level ranged from 0.008 to 0.473 ng/mL. A higher mean BPA level was found in water obtained from ultrafilter process compared to commercial sources. Activated carbon filtration is necessary to achieve a BPA free level in ultrapure water.

Keywords: Bisphenol A; Uncontrolled contamination; Laboratory water; LC-MS/MS

Introduction

Bisphenol A (BPA) is an endocrine-disrupting chemical (EDC) used in the production of plastic food and beverage containers, leading to ubiquitous low-dose human exposure. It has been suggested that this exposure to low doses of BPA may be associated with increased susceptibility to reproductive and neurobehavioral disorders, heart disease, type 2 diabetes, prostate and breast cancer, and many other diseases [1-5]. These potential adverse health effects have been also assessed by several studies performed in rodent models for the purpose of extrapolating data to humans [6,7].

Part of scientific community asserts that the studies in rodents have provided inconsistent data regarding its estrogenic activity and toxicity, due to the fact that the BPA is found in much equipment used during the experiments in the laboratory, contributing to unwanted exposure [8-12]. The low levels of exposure used in the studies increase the possibility that contamination can obscure true exposures and negatively influence the assessment of potential adverse health effects. So, the critical importance is to consider all possible sources of contamination of BPA or other potential EDC. In studies involving mice or rats, many authors have focused their attention on the diets and on the release of BPA from cages and water bottles [13-14], but few have analyzed the water, main route of administration, and main solvent for instrumental and biological analyses. Aim of this paper is to provide data to minimize accidental BPA exposure by analyzing the water commonly used in laboratories (tap, softened, distilled, double distilled, commercial LC-MS pure water). To have previously developed and optimized a method for the extraction, purification and analysis of BPA has allowed a rapid screening of samples from different purification system and repeatedly sampled in time.

Materials and Methods

Materials

BPA (purity: 98%), d_6-BPA, used as internal standard (IS), Granular Activated Carbon and reagents were purchased from Sigma Aldrich (Sigma-Aldrich, Milano, Italy). HPLC grade reagents, including methanol (MeOH) and acetonitrile (ACN) were purchased from Romil (ROMIL Ltd, UK). The MIP cartridges, purchased from Polyntell (Polyntell SA, Paris, France), were Glass AFFINIMIP® SPE Bisphenol A. To avoid contamination, no plastics were allowed to be used in the experiment; also the SPE cartridges were in glass tube.

Different sources of laboratory water were tested for BPA: municipal tap water, softned water, distilled water and ultrapure water, the latter generated by three typical laboratory-based water purification systems. The occurrence of the target compound was also determined in commercial LC-MS grade water provided in glass bottles, produced by two different brands (for privacy, the names of the manufacturers was not declared). In particular, the analyses were performed in triplicate on water sampled four times at regular interval or from four production lots.

Extraction method and LC-MS/MS analysis

The water samples were enriched and analyzed according to the procedures described in Nicolucci et al. [14]. An aliquot of 25-mL of water sample were added with 100 µL IS solution at 1 µg/mL, then loaded onto solid extraction AFFINIMIP® Bisphenol A cartridges, previously conditioned according to the manufacturer instructions. After extraction in MeOH and concentrating up to a final volume of 1 mL, the samples were analyzed on a Phenomenex Kinetex PFP reversed phase column (100 × 4.6 mm, 2.6 µm), by using a Dionex UltiMate 3000 HPLC system (Thermo Fisher Scientific Inc, Italy), coupled to a triple quadrupole mass spectrometer by an electrospray ion source (API 2000; AB Sciex, Germany). Analyses were performed in the multiple reaction monitoring (MRM) mode and negative ionization. The product ions at 133.2 *m/z* and 212.1 *m/z* for BPA and at 138.2 *m/z* and 215.0 *m/z* for d_6-BPA (IS) were monitored to assess unambiguous identification. The linearity of the detector response was verified over the concentration range 0.100-200 ng/mL. The detection limit (LOD) and the limit of quantification (LOQ) were calculated with S/N 3 and 10, respectively. Using the enrichment method, BPA was concentrated to a level of 25 times the initial solution. The resultant LOD and LOQ were 0.0012 and 0.004 ng/mL, respectively. Standard and spiked samples at three different BPA concentration (1, 10, 100 ng/mL) were extracted

***Corresponding author:** Nadia Diano, Department of Experimental Medicine, Second University of Naples, Via S. Maria di Costantinopoli 16, 80138 Naples, Italy, Tel: +39 081 5665822; Fax: +39 081 5665822; E-mail: nadia.diano@unina2.it

Received September 09, 2015; **Accepted** September 28, 2015; **Published** October 10, 2015

Citation: Migliaccio M, Errico S, Diano N, Nicolucci C (2015) BPA Free Waters Essential to Perform Laboratory Studies. J Chromatogr Sep Tech 6: 295. doi:10.4172/2157-7064.1000295

in the same manner as all the water samples. The average recovery, precision and accuracy data for the analytical procedure are shown in Table 1. At each working session, method blank was prepared in methanol to capture possible environmental contamination of analyte released from material used for sample preparation (including tubes, pipette tips and vials). When we detected trace levels of BPA in tested laboratory blank samples, background subtraction was used to correct for this minor contamination.

Activated carbon filtration

First, 25 mL pure water sample were added with 2 mL of granular active carbon (GAC) in glass tube. The solution was stirred (40 min) at room temperature and then centrifuged (10 min at 3500 rpm) at room temperature. The supernatant was collected and filtered first on Whatman No 1 paper then by 0.2 µm MCE (Cellulose Mixed Esters) syringe filter (Macherey-Nagel GmbH and Co. KG, Germany). In each treated water, BPA was concentrated and analyzed using the SPE-LC-MS technique, previously described.

Results and Discussion

The chemical characteristics of analyzed waters, in order of increasing water quality, are summarized in Table 2. Specific values have been provided from the municipal company and from the manufacturers of laboratory purification systems and bottled LC-MS grade water. As previously reported, for privacy, the names of the companies were not reported. No detectable BPA was found in municipal tap, softened and distilled water. The results are different for the ultrapure water samples. By analyzing triplicate samples for each water, BPA was detected in all of the samples, with mean concentrations ranging from 0.008 ± 0.002 ng/mL to 0.473 ± 0.050 ng/mL. The softening and distillation water systems, typically fed by tap water, do not release BPA. Contrary, ultrapure water systems, based on processes of filtration and reverse osmosis, can leach BPA in water. By comparing the water generated by three different laboratory-based water purification systems, there are relevant differences in BPA concentration. Consequently, each system should be characterized in terms of yield in BPA purification. Furthermore, also bottled commercial water samples from different brands have different BPA level, while different production lots have nearly the same BPA content. BPA contents in serum, urine and any other relevant tissues should be measured when assessing the effects of BPA in rodent or human studies. Typical levels of BPA are in the range of 0.05-3.0 ng/mL [1-7]. Our data has shown that similar level of BPA is into purified water. Thus, unexpected high background or fluctuation is possible in biological analyses when ultrapure water is used as extraction or sampling media. In the same manner, in 60% of the studies using rats or mice, animals were exposed to BPA orally (in feed or water). The BPA presence in pure water contributing to unwanted exposure may have the potential to complicate the understanding of some toxicological data and challenge the validity of investigational results [8-12]. Thus, it is imperative to be aware of the type of water used. BPA has now become a ubiquitous contaminant not only in the human environment, but also in research laboratories. On the basis of these experimental results, we performed the following consecutive step to fully extract BPA from ultrapure water.

Correlation coefficient (R^2)[a]	0.997
LOD (ng/mL)	0.0012
LOQ (ng/mL)	0.004
Recovery[b]	95 ± 3%
Accuracy[b]	98 ± 5%
Precision (%RSD)[b]	3.8 ± 1.2%

[a]The linearity was verified over the concentration range 0.100-200 ng/mL.
[b]Water samples spiked at three different BPA concentration were analyzed in triplicate.

Table 1: Method validation parameters.

Figure 1 shows BPA concentration in ultrapure water, generated by system 2 and system 3, before and after the treatment with GAC. The water samples most representatives have been chosen. It is clear that activated carbon in the form of GAC column can provide for the effective removal of BPA. The exact design of the GAC unit (e.g., bed depth, column diameter, process time etc.) depends on the initial concentration of target compound and the removal efficiency required. It is well documented in the literature that inorganic constituents of water, such as Ca, may reduce the adsorption capacity of activated carbon filters. But the processing of water already at low hardness and salt concentration makes the additional step of adsorption on GAC, the most effective and economically feasible process for achieve a BPA free level in ultrapure water.

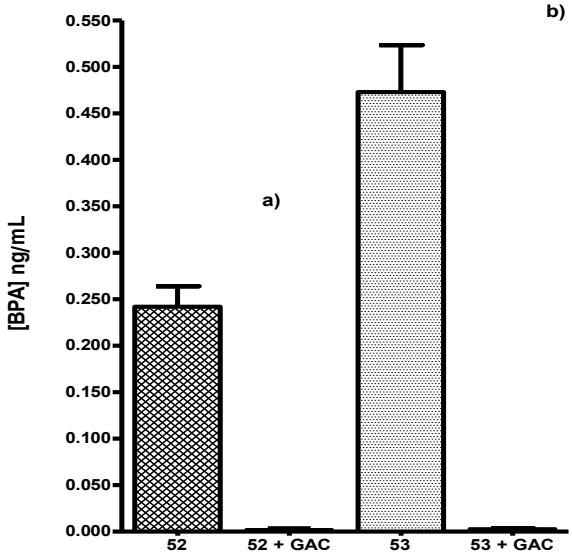

Figure 1: Effect of GAC filtration on pure water generated by System 2 (a) and by System 3 (b). The values are given as mean ± standard error of three independent determinations for each sample.

Contaminant	Parameter	Tap water	Soft water	Distilled water	Pure Water				
					system 1	system 2	system 3	Brand 1	Brand 2
Ions	Resistivity at 25°C [MΩ·cm]	0.001	-	0.2	18	18	18	18	18
	Conductivity at 25°C [µS·cm^{-1}]	1186	290	5.6	-	-	-	-	-
Acidity/Alkalinity	pH at 25°C	7.0-7.5	5.5-6.0	4.5-5.0	4.6-5.0	4.5-5.0	4.6-5.0	4.4-5.0	5.5-8.0
Organics	Total Organic Carbon/p.p.b. (µg/L)	3500	-	-	-	-	-	≤ 5	<2
Impurities	Residue (%)	-	-	-	-	-	-	<0.0001	<0.00005
Estrogenic activity	BPA [ng/mL]	<LOQ	<LOQ	<LOQ	0.014 ± 0.003	0.242 ± 0.022	0.473 ± 0.050	0.027 ± 0.006	0.008 ± 0.002

Table 2: Chemical characteristics of different types of water.

Conclusion

Our investigation confirmed that in laboratories there are numerous potential sources of BPA contamination. BPA is also in ultrapure water, probably resulted from leaching from filters installed in purification system. The types and the sources of analyzed waters were just representative samples of different types of water and not all-encompassing in water types or brands. This study was not intended to advise or warn against any particular brand or water purification method. Since BPA content in ultrapure water is resulted to vary depending on the water purification method, it is necessary to limit contamination by attempting to regularly characterize the purification systems. These data want to highlight the importance of critically considering all nonspecific sources of estrogenic compounds when performing studies where the primary goal is to evaluate the effects of EDC such as BPA, especially at low levels of exposure. The value of biological analyses and of animal studies hinges on the ability to carry out experiments in a tightly controlled environment. Chemicals, solvents, laboratory equipments, as well as diet, water and housing materials of animals should be tested for BPA to help characterize the background levels to the tested compound.

Acknowledgements

We thank for the scientific and technical support Prof. Gilda Cobellis and Dr. Teresa Chioccarelli of Department of Experimental Medicine - Second University of Naples. We thank Mr Sergio Rossi of the Institute of Genetics and Biophysics "ABT" - CNR for its active collaboration in performing experiments.

References

1. Keri RA, Ho SM, Hunt PA, Knudsen KE, Soto AM, et al. (2007) An evaluation of evidence for the carcinogenic activity of bisphenol A. Reprod Toxicol 24: 240-252.
2. Lang IA, Galloway TS, Scarlett A, Henley WE, Depledge M, et al. (2008) Association of urinary bisphenol A concentration with medical disorders and laboratory abnormalities in adults. JAMA 300: 1303-1310.
3. Maffini MV, Rubin BS, Sonnenschein C, Soto AM (2006) Endocrine disruptors and reproductive health: the case of bisphenol-A. Mol Cell Endocrinol 254-255: 179-86.
4. Newbold RR, Padilla-Banks E, Jefferson WN, Heindel JJ (2008) Effects of endocrine disruptors on obesity. Int J Androl 31: 201-208.
5. Trasande L, Attina TM, Blustein J (2012) Association between urinary bisphenol A concentration and obesity prevalence in children and adolescents. JAMA 308: 1113-1121.
6. Hengstler JG, Foth H, Gebel T, Kramer PJ, Lilienblum W, et al. (2011) Critical evaluation of key evidence on the human health hazards of exposure to bisphenol A. Crit Rev Toxicol 41: 263-291.
7. Richter CA, Birnbaum LS, Farabollini F, Newbold RR, Rubin BS, et al. (2007) In vivo effects of bisphenol A in laboratory rodent studies. Reprod Toxicol 24: 199-224.
8. Gray LE, Ryan B, Hotchkiss AK, Crofton KM (2010) Rebuttal of "Flawed Experimental Design Reveals the Need for Guidelines Requiring Appropriate Positive Controls in Endocrine Disruption Research" by vom Saal. Toxicol Sci 115: 614-620.
9. Vandenberg LN, Maffini MV, Sonnenschein C, Rubin BS, Soto AM (2009) Bisphenol-A and the great divide: a review of controversies in the field of endocrine disruption. Endocr Rev 30: 75-95.
10. vom Saal FS, Akingbemi BT, Belcher SM, Crain DA, Crews D, et al. (2010) Flawed experimental design reveals the need for guidelines requiring appropriate positive controls in endocrine disruption research. Toxicol Sci 115: 612-613.
11. vom Saal FS, Myers JP (2010) Good laboratory practices are not synonymous with good scientific practices, accurate reporting, or valid data. Environ Health Perspect 118: A60.
12. Vom Saal FS, Prins GS, Welshons WV (2012) Report of very low real-world exposure to bisphenol A is unwarranted based on a lack of data and flawed assumptions. Toxicol Sci 125: 318-320.
13. Howdeshell KL, Peterman PH, Judy BM, Taylor JA, Orazio CE, et al. (2003) Bisphenol A is released from used polycarbonate animal cages into water at room temperature. Environ Health Perspect 111: 1180-1187.
14. Thigpen JE, Setchell KD, Kissling GE, Locklear J, Caviness GF, et al. (2013) The estrogenic content of rodent diets, bedding, cages, and water bottles and its effect on bisphenol A studies. J Am Assoc Lab Anim Sci 52: 130-141.