COGENT 2.o
Bidentate C18™

BPA, BPF and BPS in Water Transferred from Baby Bottles

An HPLC Analysis

Extended Application Note

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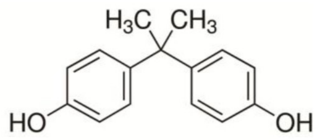
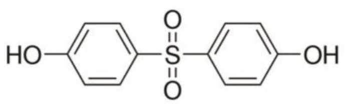


BPA



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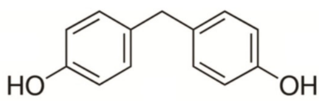

BPF

Mobile Phase: Isocratic reversed phase solvent mixture (acetonitrile/DI water/ formic acid or methanol/ H_2O water/ formic acid)
Flow Rate: 0.3 mL/min
Injection Volume: 5µL

Introduction

Bisphenol A (BPA), a compound used as a plasticizer in the production of plastic and epoxy resins is known to have endocrine-disrupting effects. Studies suggest that it may present a health hazard to infants through consumption of food and beverage products in BPA containing bottles or cans. After scrutinizing these findings the FDA has since banned the use of BPA in baby bottle containers.

As a result of the ban, many companies that manufacture containers used for these purposes have switched to ostensibly safer alternative compounds. Touting the new materials as "BPA free," manufacturers may in fact be misleading the consumer; often, the compounds used in place of BPA are not disclosed by the manufacturer and in many cases other bisphenol compounds such as BPS or BPF. Rather than being innocuous alternatives, BPF and BPS have been shown by some research to be just as dangerous, if not more so, as BPA itself. Hence, these manufacturers may be simply circumventing the BPA ban by replacing one toxic substance for another, while under the guise of safety compliance.

The bisphenol compounds can be leached from the wall of the container and into the liquid or food which is then consumed. The extraction is more rapid at elevated temperatures, such as in typical kitchen microwaving usage. Therefore LC-MS and HPLC-UV studies were conducted on microwaved water that had been in contact with various baby bottles of different brands. The aim was to determine the concentrations of bisphenol compounds that ended up in the water samples. The findings can be applicable in representing the amount of bisphenol compounds ingested by the consumer due to contact with these containers.

Experimental

Materials
"BPA free" Evenflo SimplyMilk 5-oz. Breast Milk Storage Bottles and "BPA free" Parent's Choice Slow Flow 5-oz bottles were purchased. Formic acid LC-MS ultra-grade and the three bisphenol reference standards were from Sigma–Aldrich (St. Louis, MO, USA). Undenatured Ethanol waa from Spectrum (New Brunswick, NJ, USA). Burdick & Jackson (Morristown, NJ, USA). HPLC grade acetonitrile was obtained from OmniSolv (McLean, VA, USA).

Instrumentation
An Agilent 6224/6230 LC-MS TOF system, equipped with an APCI ion source, was used. The analytical column was a Bidentate C18 2.o™ stationary phase, which had dimensions 2.1 x 50 mm, a particle diameter of 2.2 µm, and a pore size of 120Å. a Hewlett–Packard 1090 HPLC system was also used.

Sample Preparation
Four 25.0 mL samples were prepared for analysis. Two samples were prepared by microwaving (75 sec.) 125 mL HPLC grade water inside bottles of each brand, and then cooling to room temperature before collecting. The remaining two samples were prepared by microwaving (75 sec.) 125 mL of water in glass beakers, transferring to baby bottles of each brand, and allowing samples to reach room temperature before collecting. 3M (Saint Paul, MN, USA) C18 extraction disks were conditioned with 3 mL of methanol followed by 5 mL of water. Using vacuum suction, 25 mL water samples were loaded onto pre-conditioned sorbent disks and then eluted with 1.0 mL methanol. Eluents were collected and transferred into HPLC vials and analyzed.

Results and Discussion

Each of the four samples was designated A–D. The details of each sample (see Sample Preparation for more information) are provided in the following table:

Sample	Contents
A	EvenFlo. Heated out of bottle
B	EvenFlo. Microwaved in Bottle
C	Parent's Choice. Microwaved in Bottle
D	Parent's Choice. Heated out of Bottle

The results from Sample A confirmed that, in accordance with the label, no BPA was detected. BPS was also not detected. However, a peak with the same m/z as BPF was observed in this sample (**Figure 1**). Since its retention time differed from BPF in the reference standard, it was believed to be a different compound.

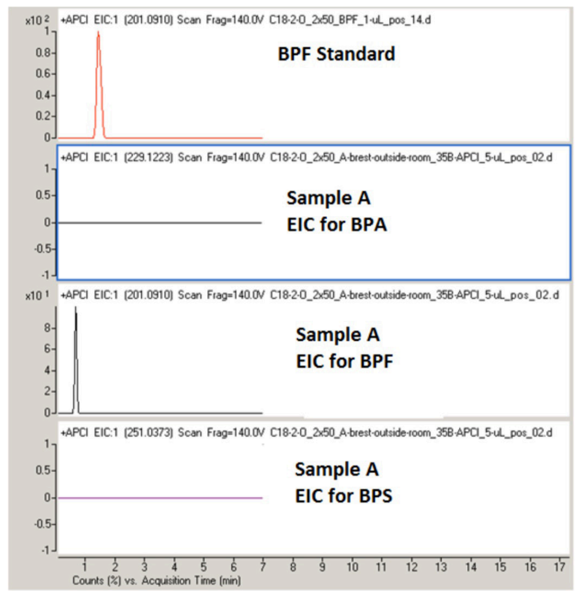

Figure 1

This was not the case in Sample C, where BPF was observed and its retention time was comparable to that of the standard (**Figure 2**). BPS was also observed in the sample as well, but not detected unless an APCI ion source and a methanol-based mobile phase were used. **Figure 3** illustrates this behavior.

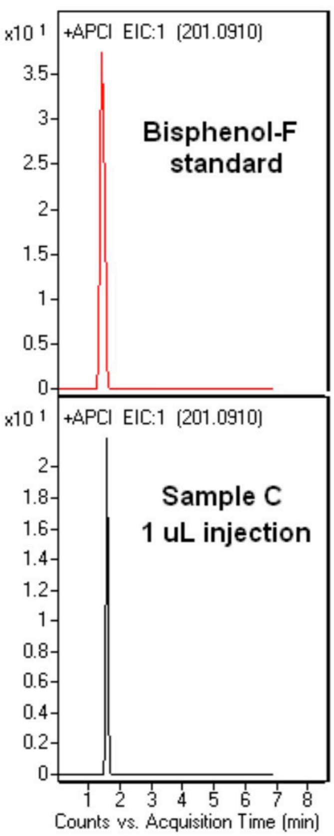

Figure 2

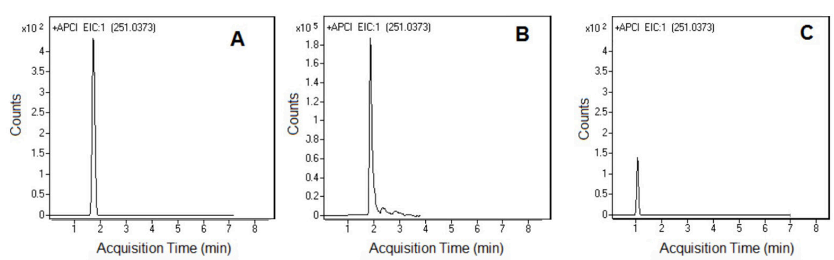

Figure 3

A - Sample C EIC for BPS
B - BPS Standard (0.1 mg/mL)
Mobile phase: 65% DI H_2O/ 35% methanol/ 0.1% formic acid
C - BPS Standard (0.1 mg/mL)
Mobile phase: 65% DI H_2O/ 35% methanol/ 0.1% formic acid

In **Figure. 3C**, the signal of the standard is very low, indicating that the use of acetonitrile is unsuitable in this case compared to methanol.

UV detection was also studied on the samples as well. In this case, BPF was detected in three of the four samples. There was a peak at 7.5 min in sample B, but its retention time was different than BPF in the other samples (Fig. 4).

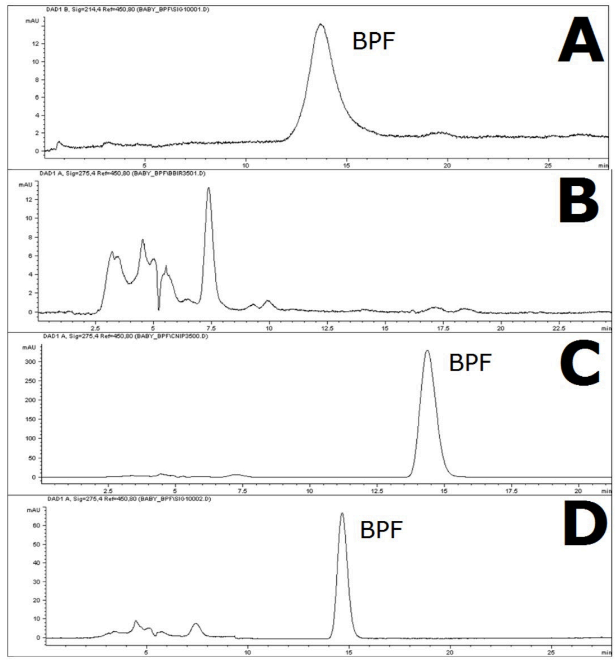

Figure 4

The data for Samples A and B suggests that microwaving directly in the bottle compared to heating outside of the bottle and then adding may make a difference in the extraction of BPF in the case of the EvenFLo™ product. However, a BPF peak was observed in either case with the Parent's Choice™ brand Fig. 4C, 4D). Further studies with replicate samples could help determine how reproducible the extraction is in the case of Samples A and B.

In any case, the chromatograms illustrate that although the products are free of BPA, they contain other harmful bisphenol compounds that can be leached into the liquid stored inside. Upon consumption of the liquid, the bisphenol compounds can cause health issues due to their endocrine-disrupting effects.

Conclusion

The Bidentate C18 2.o™ column is a valuable tool in the analysis of bisphenol compounds in consumable liquids that have been in contact with "BPA-free" bottles. The data demonstrates how bisphenol compounds do in fact leach from the bottle container to the liquid under elevated temperature conditions. As for the "BPA free" labeling, no BPA was observed in any of the samples. However, BPF was found instead, which is believed to have the same deleterious endocrine-disrupting properties as BPA.

Further studies could investigate room temperature liquids to see if BPF is still observed in the samples and, by comparing to the current data from this research, to what extent the elevated temperatures increase the leaching of these compounds.