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Solutions within.

Analysis of Low Levels of Bisphenol A (BPA) in Canned Broths

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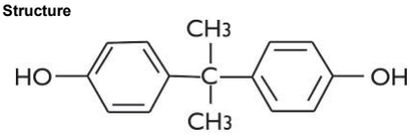
Introduction

Bisphenol A (BPA) is a primary monomer used in the manufacture of many plastics and epoxies (1).

- Used to contain beverages and to line and seal metal food containers
- Unreacted monomer is always present in these materials; as a result, some BPA is present in most foods and beverages stored in such containers (Figure 1).

- BPA exhibits a potential to act as an endocrine disrupter at low concentrations in humans (2).
- Regulations have been enacted regarding BPA concentrations in food and beverages sold for human consumption (3, 4).
- The European Food Safety Authority (EFSA) is currently re-evaluating human risk factors associated with BPA consumption and has temporarily recommended lowering tolerable daily intake standards for BPA for its associated members (5).

Figure 1. Bisphenol A Structure



Bisphenol A

- This work uses molecularly imprinted polymers (MIPs) for low level detection of BPA.
  - A class of highly cross-linked polymer-based molecular recognition elements engineered to bind one target compound or a class of structurally related target compounds with high selectivity.
  - Selectivity is introduced during MIP synthesis in which a template molecule, designed to mimic the analyte, guides the formation of specific cavities or imprints that are sterically and chemically complementary to the target analyte.
- Sample preparation challenges for quantifying low levels of BPA.
  - BPA is ubiquitous in the environment and can contaminate low concentration samples.
  - Careful screening of blank samples, mobile phase and LC system components are recommended when quantifying BPA at low concentrations (1-10 ng/mL).

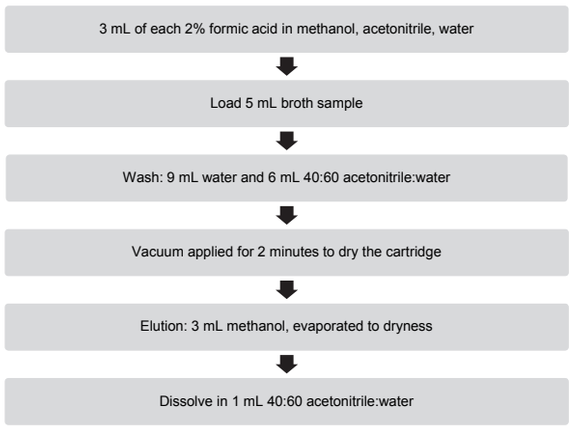
Experimental

Chicken Broth Spike: Canned uncondensed chicken broth was filtered through 2.5 µm filter paper. It was spiked to 60 ng/mL BPA then shaken 5 minutes at medium speed on an orbital shaker.

Beef Broth: filtered through 2.5 µm filter paper and analyzed.

Quantification: Quantification was performed using an external calibration curve from 60 to 900 ng/mL. Non-fortified mobile phase blank samples were used as controls.

MIP SPE Method



Minimizing Potentional BPA Laboratory Background

During early method development, BPA and a closely eluting compound were identified in the laboratory background:

- Vials
- HPLC mobile phase solvents
- Water generated from a lab purifying system.

The following steps were taken to eliminate potential sources of contamination:

1. HPLC grade solvents and lab purified water were replaced with LC-MS water and solvents (Figure 2).
2. As a precaution, glass vials were examined for BPA contribution. A silane-treated amber glass vial showed evidence of BPA contamination. A clear glass vial with a silicone septa showed no contributing BPA or near eluting compounds after >12 hours and was selected for testing.
3. **General precautions implemented to keep low level BPA in the laboratory:**
   - Triple rinsing glassware with LC-MS solvents
   - Swirling (versus shaking) plastic capped reagents
   - LC-MS grade acetic acid
   - All samples were run with accompanying mobile phase blanks

HPLC Method

column:	Ascentis® Express C18, 15 cm x 2.1 mm I.D., 2.7 µm
mobile phase:	(A) 40:60; acetonitrile:water; (B) 100% acetonitrile
gradient:	Hold 100% A for 5.60 min at 0.2 mL/min, change to 100% B and 0.3 mL/min in 0.1 min, hold 100% B for 3 min at 0.3 mL/min, change to 100% A in 0.1 min, Hold 100% A for 7 min at 0.3 mL/min, change to 0.2 mL/min and hold for additional 4 minutes.
flow rate:	variable, see gradient conditions
temp.:	30 °C
det.:	FLD: ex 230 nm, em 315 nm
inj.:	2 µL

Results

Figure 2. Injection of LC-MS Grade Acetonitrile:Water (60:40) into the Same Mobile Phase, Proven to be BPA-free

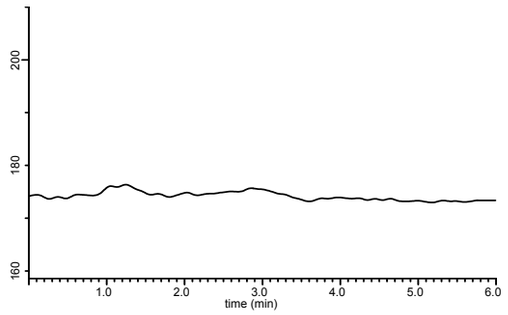
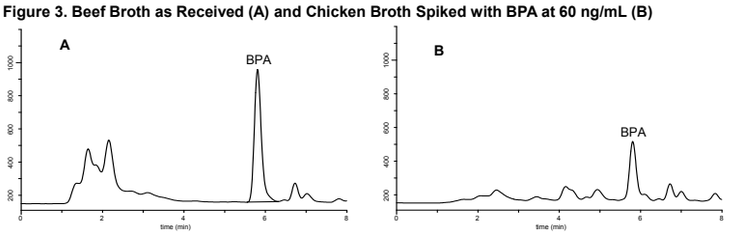


Figure 3. Beef Broth as Received (A) and Chicken Broth Spiked with BPA at 60 ng/mL (B)



BPA Results from Broth Samples

Table 1. BPA Quantification in Broth

Sample	Concentration	Recovery	RSD
Chicken broth, unspiked	8 ng/mL*	-	-
Chicken broth spiked at 60 ng/mL	44 ng/mL[a]	72.5%	0.8%
Beef broth, unspiked	119 ng/mL	-	3.5%

* Estimated, below lowest calibration point
[a] corrected for blank broth value

1. Low levels of BPA were observed in the blank chicken broth sample. The final BPA recovery value in spiked chicken broth was corrected by subtracting the value found in the non-spiked chicken broth.
2. The sample matrix showed some evidence of interfering compounds at the BPA retention time (Figure 3B).
3. Recovery for the chicken broth sample was >70% with very good reproducibility.
4. Non-fortified beef broth contained over 100 ng/mL BPA, the reproducibility of detection was also very good at 3.5%.

Conclusions

- Laboratories and samples may easily become contaminated with environmental BPA including solvents and purified water.
  - Use of LC-MS solvents to prepare mobile phases can eliminate potential contamination.
  - The analyzed LC-MS solvent could be used to triple rinsed all glassware and be used during sample preparation.
  - Glass auto-sample vials should be used as plastic vials can be a BPA contamination source.
  - No major BPA background was found from SupelMIP® SPE tubes.
- The above efforts can result in low laboratory levels of BPA (less than 0.1 ng/mL in final samples).
- The SupelMIP SPE – Bisphenol A SPE cartridge can be used efficiently to concentrate and recover BPA at concentrations of 50 ng/mL or lower in beef and chicken broth samples with recoveries greater than 70% and very low sample to sample variability. It is anticipated that SPE MIP methods can be easily adapted to meet analytical needs in response to increased stringency in BPA regulation.

References

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