Introduction
Bisphenol A (BPA), shown in Figure 1, has made headlines over the past few years due to its potential effects on human health. The headlines began with concerns over BPA in baby bottles and spread to include other types of contact materials such as re-useable plastic Tupperware, can linings, thermal paper, cosmetics, and plastic toys. BPA is a chemical byproduct used in the production of plastics for its optical clarity and heat resistance properties. It has been shown to exhibit hormone-like properties, causing concerns over possible health risks. Since 2008, several governments have supported on-going studies to investigate its safety and to establish safe levels of exposure. In the U.S., the Food and Drug Administration (FDA) set the safe level to 5 mg/kg (by body weight) per day, while, in Europe, the European Food Safety Authority (EFSA) recently lowered its safe level to 4 µg/kg (by body weight) per day.1, 2

Analysis of Bisphenol A in Toys by HPLC with Fluorescence Detection
As a result of the health concerns over human exposure to BPA, this compound is now closely monitored in specific products, including children’s goods. This application focuses on the extraction and HPLC analysis of BPA in three store-bought children’s toys. Method conditions and performance data, including linearity and repeatability, are presented.

![Structure of Bisphenol A (BPA)](image)

**Experimental**

**Hardware/Software**

For all chromatographic separations, a PerkinElmer Altus™ HPLC System was used, including the Altus A-10 solvent/sample module, integrated vacuum degasser, A-10 column module and Altus A-30 fluorescence (FL) detector. All instrument control, analysis and data processing was performed using the Waters® Empower® 3 CDS platform.

**Method Parameters**

The HPLC method parameters are shown in Table 1.

<table>
<thead>
<tr>
<th>Table 1. HPLC Method Parameters.</th>
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<tr>
<td><strong>HPLC Conditions</strong></td>
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<tr>
<td>Column: PerkinElmer Brownlee™ HRes Biphenyl 1.9 µm, 50 x 2.1 mm (Part No. N9303912)</td>
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<tr>
<td>Mobile Phase: Solvent A: water, Solvent B: Acetonitrile, Solvent program (isocratic):</td>
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<td>Time (min)</td>
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<td>Initial</td>
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<td>Analysis Time: 4.0 min.</td>
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<tr>
<td>Flow Rate: 0.5 mL/min. (5500 psi; 367 bar)</td>
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<td>Oven Temp.: 40 ºC</td>
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<td>Detection: Altus A-30 FL detector, Excitation: 275 nm; Emission: 313 nm</td>
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<tr>
<td>Injection Volume: 3 µL</td>
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<tr>
<td>Sampling (Data) Rate: 5 pts/sec</td>
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**Solvents, Standards and Samples**

All solvents and diluents used were HPLC grade and filtered via 0.45 µm filters.

The BPA standard was obtained from Sigma-Aldrich, Inc® (Allentown, PA). A stock 100-ppm BPA standard was made using purified water as diluent. The lower level standards were then prepared from this stock solution.

The children’s toy samples were purchased at a local store. These included a toy car, pony and keychain (Figure 2). The extraction procedure used was intended to simulate the contact route through which children are likely to encounter BPA. The toy samples were immersed in 800 mL of purified water, at 40 ºC for 24 hours. Following the extraction, all water extracts were filtered and then chromatographed using the conditions listed in Table 1.

Prior to injection, all calibrants and samples were filtered through 0.45-µm filters to remove any small particles.

**Results and Discussion**

Figure 3 shows the chromatogram of the 1-ppm BPA Standard, using the optimized conditions described above. BPA eluted at 0.95 min. A second peak, eluting at 2.57 min, was also detected in the standard. As this second peak was also observed in subsequent blanks (see Figure 6), this peak was not further pursued.

![Children’s toy samples analyzed for BPA in this application: A) car, B) pony, C) keychain.](image)
Figure 3. Chromatogram of the 1-ppm BPA Standard.

Figure 4 shows the overlay of ten replicate injections of the 1-ppm BPA Standard, demonstrating exceptional reproducibility. The retention time %RSD was 0.04%.

Figure 4. Overlay of 10 replicates of the 1-ppm BPA Standard.

Figure 5 shows the calibration result for the BPA Standard over a concentration range of 0.1 to 1.0 ppm. BPA followed a linear (1st order) fit and had an $R^2$ coefficient of 0.99821 ($n = 3$ at each level).

Figure 5. Results of 5-level calibration set for BPA.
Figure 6 shows the overlay of a 0.1-ppm standard and the following water blank, confirming that the system was free of any BPA contamination or carry over.

Using the same chromatographic conditions, the three toy sample water-extracts were then analyzed. As shown in Figure 7, the chromatograms for both the car and keychain water-extracts showed no quantifiable level of BPA. However, as shown in Figure 8, the chromatogram of the water extract from the pony showed a significant level of BPA. Based on the calibration curve, the amount of water-extractable BPA in the pony sample was calculated to be 0.274 mg. As an example, if one considers the average weight of a 4-year old child to be 15 kg, the amount of BPA extractable from the pony sample was within the FDA’s safe maximum level of 5 mg/kg (by body weight) per day. However, using the above example, this amount exceeded the EFSA’s safe maximum level of 4 µg/kg (by body weight) per day.
Conclusion
This work has demonstrated the effective chromatographic separation of BPA using a PerkinElmer Altus HPLC System with FL detection. The results show very good retention time repeatability as well as excellent linearity over the tested concentration range. Considering the lower calibration levels that were run, the sensitivity levels were well below the maximum safe levels stipulated by both the FDA and EFSA regulatory agencies.

This work also focused on the BPA extraction and analysis of three store bought toys, comparing them both chromatographically and quantitatively. It effectively identified one of the toys as containing a significant amount of water-extractable BPA. This is especially relevant in today's environmentally-sensitive climate, as the uncertainties surrounding potential health effects of BPA exposure, especially in plastics, have raised widespread concern.

References

Figure 8. Chromatographic overlay of water-extracts from the pony sample (red) and 1-ppm BPA Standard (black).