Comprehensive LC-MS/MS Analysis of 15 Bisphenols in 8 Minutes

- Excellent peak shape and separation for bisphenol A and common analogues.
- Simple, no-additives mobile phases and gradient program.
- Fast, 8-minute total cycle time.

Bisphenol A (BPA), widely used in the production of polycarbonate plastic and epoxy resins, is an endocrine disruptor that imitates naturally occurring hormones or acts as an antagonist, both of which can cause harmful effects on hormone biosynthesis, metabolism, distribution, and mode of action. Hormone disruption can result in detrimental effects to health, growth, and reproduction. Children and unborn babies are most susceptible to hormonal and neurological development problems, and the prevalent use of BPA in many consumer products, including food and beverage packaging, adhesives, and toys, has caused several governments to investigate its safety. Due to the wide range of exposure routes and vulnerability of children, negative public perception has driven many products to advertise as "BPA-free," instead opting to use BPA analogues that have similar physicochemical properties. However, these alternate bisphenols are understudied and may also have harmful toxicological profiles. Some research has shown that these compounds, notably BPF, BPS, BPAF, BPZ, BPE, and BPB, are estrogenic endocrine disruptors and may cause health effects similar to BPA [1,2].

Establishing accurate methods for the analysis of bisphenols that include a broad suite of compounds, rather than just BPA, is imperative for both investigating toxicology and monitoring human exposure. The simple LC-MS/MS gradient method presented here was developed on a Raptor Biphenyl column because it provides excellent chromatographic peak shape and baseline separation of 15 bisphenols, including BPA and its most prevalent analogues. The 1.8 µm, 2.1 x 50 column format takes advantage of the inherent speed gains of small particle size technology, resulting in a fast, 8-minute analysis that is ideal for high-throughput testing.
<table>
<thead>
<tr>
<th>Peaks</th>
<th>ts (min)</th>
<th>Conc. (ng/mL)</th>
<th>Precursor Ion</th>
<th>Product Ion</th>
<th>Product Ion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Bisphenol S</td>
<td>0.84</td>
<td>5.00</td>
<td>244.2</td>
<td>108.1</td>
<td>92.1</td>
</tr>
<tr>
<td>2. Bisphenol F</td>
<td>1.62</td>
<td>350</td>
<td>199.3</td>
<td>93.1</td>
<td>103.1</td>
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<tr>
<td>3. Bisphenol E</td>
<td>2.06</td>
<td>100</td>
<td>223.3</td>
<td>198.3</td>
<td>197.4</td>
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<tr>
<td>4. Bisphenol A</td>
<td>2.50</td>
<td>100</td>
<td>227.3</td>
<td>222.3</td>
<td>133.1</td>
</tr>
<tr>
<td>5. Bisphenol AF</td>
<td>2.71</td>
<td>2.00</td>
<td>335.2</td>
<td>265.3</td>
<td>177.3</td>
</tr>
<tr>
<td>6. Bisphenol B</td>
<td>3.13</td>
<td>100</td>
<td>241.3</td>
<td>232.4</td>
<td>211.3</td>
</tr>
<tr>
<td>7. Bisphenol C</td>
<td>3.43</td>
<td>350</td>
<td>255.3</td>
<td>240.4</td>
<td>147.3</td>
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<tr>
<td>8. Bisphenol AP</td>
<td>3.98</td>
<td>25.0</td>
<td>289.3</td>
<td>274.3</td>
<td>273.1</td>
</tr>
<tr>
<td>9. Bisphenol Z</td>
<td>4.25</td>
<td>250</td>
<td>267.2</td>
<td>173.4</td>
<td>145.2</td>
</tr>
<tr>
<td>10. Bisphenol G</td>
<td>4.72</td>
<td>250</td>
<td>311.2</td>
<td>295.4</td>
<td>296.6</td>
</tr>
<tr>
<td>11. Bisphenol FL</td>
<td>4.90</td>
<td>50.0</td>
<td>348.8</td>
<td>256.2</td>
<td>-</td>
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<td>12. Bisphenol BP</td>
<td>5.14</td>
<td>50.0</td>
<td>351.2</td>
<td>273.3</td>
<td>274.3</td>
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<td>13. Bisphenol M</td>
<td>5.39</td>
<td>15.0</td>
<td>345.2</td>
<td>330.3</td>
<td>253.4</td>
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<tr>
<td>14. Bisphenol P</td>
<td>5.67</td>
<td>50.0</td>
<td>345.2</td>
<td>330.4</td>
<td>315.3</td>
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<tr>
<td>15. Bisphenol PH</td>
<td>6.11</td>
<td>350</td>
<td>379.2</td>
<td>209.4</td>
<td>364.4</td>
</tr>
</tbody>
</table>

**Column**: Raptor Biphenyl (cat.# 9309252)
**Dimensions**: 50 mm x 2.1 mm ID
**Particle Size**: 1.8 µm
**Pore Size**: 90 Å
**Temp.**: 25 °C
**Sample Diluent**: 75:25 Water:methanol
**Conc.**: 2.00-350 ng/mL
**Inj. Vol.**: 2 µL
**Mobile Phase**
- A: Water
- B: Methanol

**Time (min)** | **Flow (mL/min)** | **%A** | **%B**
---|---|---|---
0.00 | 0.45 | 50 | 50
6.50 | 0.45 | 50 | 50
6.51 | 0.45 | 50 | 50
8.00 | 0.45 | 50 | 50

**Detector**: MS/MS
**Ion Mode**: ESI-
**Mode**: MRM
**Instrument**: UHPLC
The innovative Biphenyl is Restek’s most popular LC stationary phase because it is particularly adept at separating compounds that are hard to resolve or that elute early on C18 and other phenyl chemistries. As a result, the rugged Raptor Biphenyl column is extremely useful for fast separations in bioanalytical testing applications like drug and metabolite analyses, especially those that require a mass spectrometer (MS). Increasing retention of early-eluting compounds can limit ionization suppression, and the heightened selectivity helps eliminate the need for complex mobile phases that are not well suited for MS detection.

**Raptor Biphenyl LC Columns (USP L11)**

**Chromatographic Properties**

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**USLC Column Interaction Profile**

**Column Characteristics:**
- Stationary Phase Category: Phenyl (L11)
- Ligand Type: Biphenyl
- Particle: 1.8 µm, 2.7 µm, or 5 µm superficially porous silica (SPP or “core-shell”)
- Pore Size: 90 Å
- Carbon Load: 7% (1.8 µm), 7% (2.7 µm), 5% (5 µm)
- End-Cap: yes
- Surface Area: 125 m²/g (1.8 µm), 130 m²/g (2.7 µm), or 100 m²/g (5 µm)
- Recommended Usage:
  - pH Range: 2.0 to 8.0
  - Maximum Temperature: 80 °C
  - Maximum Pressure: 1,034 bar/15,000 psi* (1.8 µm), 600 bar/8,700 psi (2.7 µm), 400 bar/5,800 psi (5 µm)
- * For maximum lifetime, recommended maximum pressure for 1.8 µm particles is 830 bar/12,000 psi.

**Properties:**
- Increased retention for dipolar, unsaturated, or conjugated solutes.
- Enhanced selectivity when used with methanolic mobile phase.
- Ideal for increasing sensitivity and selectivity in LC-MS analyses.
- Switch to a Biphenyl when:
  - Limited selectivity is observed on a C18.
  - You need to increase retention of hydrophilic aromatics.

**References**

