DETERMINATION OF TOTAL BISPHENOL A IN HUMAN URINE

PROTOCOL OF PURIFICATION
Sample preparation
3mL urine sample, 1mL of sodium acetate buffer 0.1M at pH 5.0 and 20μL of β-glucuronidase/sulfatase Helix pomatia enzyme solution at 1.0mg/mL in the same buffer were mixed thoroughly by vortex. The enzymatic reaction was carried out for 2h at 37°C to obtain the loading solution.

Purification with a 6mL/100mg AFFINIMIP® SPE Bisphenols glass cartridge

Equilibration
- 5mL Methanol -2% Acetic Acid
- 5mL Acetonitrile
- 5mL Water

Loading solution
Up to 12mL of loading solution (Equivalent to around 9mL of urine)

Washing of interferences
- 4mL Water
- 4mL Water/Acetonitrile (60/40)

Elution (E)
3mL Methanol
The elution fraction was then concentrated and diluted to 1mL before HPLC analysis.

HPLC Method with LC-MS/MS
HPLC Column: Kinetex 2.6µm PFP 100mm x 4.6mm
Mobile phase: gradient profile

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>% water</th>
<th>% Methanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>70</td>
<td>30</td>
</tr>
<tr>
<td>1</td>
<td>70</td>
<td>30</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>95</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>95</td>
</tr>
<tr>
<td>6</td>
<td>70</td>
<td>30</td>
</tr>
<tr>
<td>9</td>
<td>70</td>
<td>30</td>
</tr>
</tbody>
</table>

Flow rate: 0.5mL/min
Injection volume: 20μL.
Detector: ESI-MS/MS

RESULTS
LC-MS/MS Chromatograms obtained after clean-up with AFFINIMIP® SPE Bisphenol A
(a) of children urine at 0.38ng/mL BPA, signal to noise (S/N) 13.9
(b) for the blank sample (neither urine nor BPA), S/N=1.9

Mean percentage recoveries of Bisphenol A spiked at different concentrations in 3mL of urine after AFFINIMIP® SPE Bisphenols clean-up:

<table>
<thead>
<tr>
<th>C° (ng/mL)</th>
<th>1</th>
<th>10</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recoveries %</td>
<td>102.6</td>
<td>94.7</td>
<td>97.6</td>
</tr>
</tbody>
</table>

By courtesy of Nadia Diano, Dept. of Experimental Medicine, Second University of Naples (Italy)
More details in the following article

Catalog number:
3mL-100mg sorbent in a PP cartridge
FS106-02 for 25 cartridges
FS106-03 for 50 cartridges
6mL-100mg sorbent in a glass cartridge
FS106-02G for 25 cartridges
FS106-03G for 50 cartridges

More applications notes with AFFINIMIP® SPE Bisphenols