

## 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.

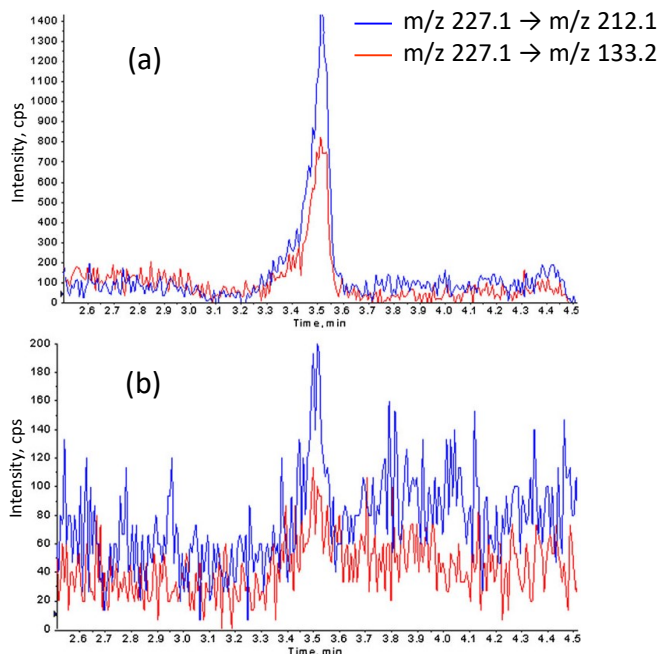
### HPLC Method with LC-MS/MS

HPLC Column: Kinetex 2.6µm PFP 100mm x 4.6mm  
Mobile phase: gradient profile

| Time (min) | % water | % Methanol |
|------------|---------|------------|
| 0          | 70      | 30         |
| 1          | 70      | 30         |
| 2          | 5       | 95         |
| 5          | 5       | 95         |
| 6          | 70      | 30         |
| 9          | 70      | 30         |

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

### RESULTS



LC-MS/MS Chromatograms obtained after clean-up with AFFINIMIP® SPE Bisphenols 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:

| C° (ng/mL)   | 1     | 10   | 100  |
|--------------|-------|------|------|
| Recoveries % | 102.6 | 94.7 | 97.6 |

By courtesy of Nadia Diano, Dept. of Experimental Medicine, Second University of Naples (Italy)  
More details in the following article  
C. Nicolucci, S. Rossi, C. Menale, E. Giudice, P. Miraglia del Giudice, L. Perrone, P. Gallo, D. Mita, N. Diano, *Analytical and Bioanalytical Chemistry*, 1618-2642, 2013.

#### 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

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