AFFINIMIP® SPE

Bisphenols
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</tbody>
</table>
This table reminds the main regulation on the use of Bisphenol A.

<table>
<thead>
<tr>
<th>Countries</th>
<th>Regulation</th>
<th>Date of votation or application</th>
</tr>
</thead>
<tbody>
<tr>
<td>EU</td>
<td>WTO informed of the intention to reduce the Specific migration limit (SML) from 0,6 to 0,05mg/kg</td>
<td>Intended march 17</td>
</tr>
<tr>
<td>EU</td>
<td>Restriction on BPA in thermal paper in the EU</td>
<td>Decided dec 16 Effective 2020</td>
</tr>
<tr>
<td>USA</td>
<td>Ban Bisphenol A in infant formula packaging</td>
<td>Decided July 2013</td>
</tr>
<tr>
<td>France</td>
<td>Ban Bisphenol A in all food containers intended for direct contact with food</td>
<td>Voted 24/12/12 applied 1/01/15</td>
</tr>
<tr>
<td>France</td>
<td>Ban Bisphenol A in food contact materials infants and young children under the age of three</td>
<td>Voted 24/12/12 Applied 01/01/13</td>
</tr>
<tr>
<td>Belgium</td>
<td>Ban Bisphenol A in food contact materials intended for children up to the age of three</td>
<td>Voted Sept 2012 Applied 01/01/13</td>
</tr>
<tr>
<td>Sweden</td>
<td>Ban Bisphenol A in food packaging intended for children under the age of three</td>
<td>Applied 2013</td>
</tr>
<tr>
<td>Austria</td>
<td>Ban Bisphenol A in pacifiers or teethers made with Bisphenol A</td>
<td>1 February 2012</td>
</tr>
<tr>
<td>USA</td>
<td>Ban Bisphenol A from use in infant and toddler products such as baby bottles and sippy cups</td>
<td>Decided July 2012</td>
</tr>
<tr>
<td>Argentina</td>
<td>Ban Bisphenol A in baby bottles</td>
<td>Effective april 12</td>
</tr>
<tr>
<td>Brazil</td>
<td>Ban BPA in baby and infant feeding bottles</td>
<td>Effective january 12</td>
</tr>
<tr>
<td>Ecuador</td>
<td>Ban bisphenol A in bottles</td>
<td>Effective october 11</td>
</tr>
<tr>
<td>China</td>
<td>Ban Bisphenol A in infant feeding bottles</td>
<td>Applied 1/06/11</td>
</tr>
<tr>
<td>Russia</td>
<td>Maximum Permissive Level (MPL) for BPA in water – 10µg/L</td>
<td></td>
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<tr>
<td>EU</td>
<td>No Bisphenol A to be used for the manufacture of polycarbonate infant feeding bottles</td>
<td>Decided 28/01/11 Applied 1/06/11</td>
</tr>
<tr>
<td>EU</td>
<td>Specific migration limit (SML) of Bisphenol A in Food of 0.6mg/Kg</td>
<td>Decided 28/01/11 Applied 01/02/11</td>
</tr>
<tr>
<td>Denmark</td>
<td>Feeding bottles, feeding cups and materials in contact with food for children up to 3 years</td>
<td>Effective July 2010</td>
</tr>
<tr>
<td>Canada</td>
<td>Ban Bisphenol A in baby bottles</td>
<td>Decided August 08</td>
</tr>
</tbody>
</table>
## Bisphenol A Regulation – USA states

<table>
<thead>
<tr>
<th>US states</th>
<th>Regulation to ban BPA</th>
<th>Date of votation or application</th>
</tr>
</thead>
<tbody>
<tr>
<td>California</td>
<td>Proposition 65 warning label required for BPA covers packaged foods and drinks</td>
<td>Effective May 2016</td>
</tr>
<tr>
<td>USA</td>
<td>in infant formula packaging</td>
<td>Decided July 2013</td>
</tr>
<tr>
<td>USA</td>
<td>from use in infant and toddler products such as baby bottles and sippy cups - 21 CFR 177.1580</td>
<td>Decided July 2012</td>
</tr>
<tr>
<td>Arizona</td>
<td>Plastic container, jar or can for baby food or infant formula; Child container, baby bottle or spill proof cup intended for use by children under 5 years old</td>
<td>Effective 01/14</td>
</tr>
<tr>
<td>California</td>
<td>in baby bottles and sippy cups</td>
<td>October 11</td>
</tr>
<tr>
<td>District of Columbia</td>
<td>bottles, cups or containers designed to be filled with food or liquids</td>
<td>Applied 1/07/11 Effective July 2013</td>
</tr>
<tr>
<td>Connecticut</td>
<td>in infant formula containers, baby food cans or jars, reusable food or beverage containers, including baby bottles, spill-proof cups, sports bottles and thermoses</td>
<td>Voted June 09 Applied Oct 11</td>
</tr>
<tr>
<td>Connecticut</td>
<td>in thermal receipt paper or cash register receipt paper</td>
<td>Voted June 11 Applied 1/10/13</td>
</tr>
<tr>
<td>Delaware</td>
<td>from children’s bottles, cups and other food and beverage containers</td>
<td>Effective 01/10/12</td>
</tr>
<tr>
<td>Illinois</td>
<td>from children’s food and beverage containers</td>
<td>Voted August 12 Applied 2013</td>
</tr>
<tr>
<td>Maine</td>
<td>from baby bottles, sippy cups, water bottles and reusable food storage containers</td>
<td>Voted 2011 Applied 2012</td>
</tr>
<tr>
<td>Maryland</td>
<td>Ban infant formula containing more than 0.5 ppb Bisphenol A</td>
<td>Voted May 11 Applied July 14</td>
</tr>
<tr>
<td>Massachusetts</td>
<td>in children’s reusable food or beverage containers</td>
<td>Voted January 11</td>
</tr>
<tr>
<td>Minnesota</td>
<td>in sippy cups and baby bottles</td>
<td>Applied 1 January 2010</td>
</tr>
<tr>
<td>New Jersey</td>
<td>from children’s food and beverage containers</td>
<td>Voted Feb 2013</td>
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<tr>
<td>State of New York</td>
<td>in pacifiers, baby bottles, sippy cups and other unfilled beverage containers for use by children under three years of age after December 1, 2010.</td>
<td>Voted July 10 Applied 1/12/10</td>
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<tr>
<td>Vermont</td>
<td>in reusable food or beverage containers such as baby bottles, spill-proof cups, sports bottles, and thermoses</td>
<td>Voted May 10 Applied 1/12/10</td>
</tr>
<tr>
<td>Virginia</td>
<td>In food and beverage containers for children up to 3 years old at the exemption of metal can</td>
<td>Effective July 11</td>
</tr>
<tr>
<td></td>
<td>In sports bottles</td>
<td>Effective July 12</td>
</tr>
<tr>
<td>Wisconsin</td>
<td>in pacifiers, baby bottles, sippy cups for children under three years of age after December 1, 2010.</td>
<td>Voted March 2010 Applied June 2010</td>
</tr>
<tr>
<td>Washington</td>
<td>in childcare article for children under 3</td>
<td>Effective July 12</td>
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</table>
Bisphenol A (or BPA) is a molecule widely used in industry for the synthesis of polycarbonate plastics and epoxy resins. Polycarbonate plastics are used to make a variety of common products including baby and water bottles. Epoxy resins are used as coatings on the inside of almost all food and beverage cans.

The migration of this endocrine disruptor compound from the packaging to food is the main source of consumers’ exposure to Bisphenol A. Its consumption is critical for babies. So, Bisphenol A is a topical issue with a increasingly restrictive worldwide regulation going to still lower concentrations of Bisphenol A allowed in food. Highly sensitive and reliable detection methods are required for routine analysis of Bisphenol A in food samples, particularly for baby food.

In these application notes, we describe protocols enabling the determination of very low concentration of Bisphenol A in liquid and powdered infant formula, and several other matrices. using AFFINIMIP® SPE Bisphenols cartridge.

These methods show the determination of very low concentration of Bisphenol A with a fluorescence detector. Therefore, the use of AFFINIMIP® SPE Bisphenols enables to eliminate the tedious derivatization step required by gas chromatography. This method is also perfectly suitable for clean-up before GC-MS/MS or LC-MS/MS.

To meet customer specifications, AFFINISEP proposes two different formats of AFFINIMIP® SPE Bisphenols:

- 6mL Glass cartridges with PTFE frits
- 3mL PP plastic cartridges with PE frits (other volume available on demand).

AFFINIMIP® SPE Bisphenols kits contains SPE cartridges as well as an instruction sheet for various complex matrices and certificate of analysis.

To ensure the best quality of its products, the performance is checked by following several QC tests according to each product’s quality control procedure. After passing all these tests, results are gathered in a QC report available on demand for the customer for the purchased batch. Then, products receive a certificate of analysis which proved the compliance with the defined criteria.
Equilibration

Percolation of loading solution on AFFINIMIP® SPE Bisphenols 3cc

Wash 9mL H₂O and 6mL 40% Water/ACN ;

Dry 30s

Elute 3mL 100% MeOH

HPLC analysis : LC Fluorescence detector or GC-MS/MS or LC-MS/MS

Performance. Save your time.
Advantages of using AFFINIMIP® SPE Bisphenols

**Greater Recoveries**
- Minimal sample transfer

**Greater Accuracy**
- No cross contamination

**Save time**
- Faster Protocol
- Fewer steps

**Lower Cost**
- Lower solvent consumption
- Lower reagent consumption
- Less apparatus

**Greater Safety**
- Less exposure to toxic agents

**No Emulsion Problems**
- Less sample handling
- Fewer steps

**No Transporting of Samples to Lab**
- Direct field sampling

**Reduced Harm to Labile Samples**
- Minimal evaporation

**Minimal Glass Breakage**
- Less glassware used, less to wash

**Manual SPE manifold**
10 to 12 SPE could be made in the same time and two series of SPE could be easily made during one days

>>> 20 to 24 samples analyses are easily obtained

**Easy to use with SPE automate**
Format and protocols fully compatible with an use with SPE automate
Application notes

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Determination of TOTAL Bisphenol A in Cola drink 18
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Determination of 7 Bisphenol analogs by LC-MS/MS 21
Determination of 18 Bisphenol analogs in human breast milk by GC-MS/MS 22
Determination of BPA, Nonylphenol and 4-t-Octylphenol in fish feed 23
Determination of BPA, BPB, BPF, BADGE AND BFDGE in canned energy drinks 24
**Regulations for Bisphenol A:**
Europe (directive 2011/8/EU): forbidden in infant feeding bottles

**PROTOCOL OF PURIFICATION**
Sample preparation

**Purification with a 3mL/100mg AFFINIMIP® SPE Bisphenols cartridge**

**Equilibration**
- 3mL Methanol -2% Acetic Acid
- 3mL Acetonitrile
- 3mL Water

**Loading**
Up to 15mL of infant formula

**Washing of interferents**
- 9mL Water
- 6mL Water/Acetonitrile (60/40)

**Drying 30 seconds**

**Elution (E)**
3mL Methanol

The elution fraction was then evaporated and dissolved in the mobile phase before HPLC analysis.

**HPLC Method with Fluorescence detection**
Column: Hypersil Gold C18 column 150mm x 4.6mm
Mobile phase: gradient profile

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>% water</th>
<th>% ACN</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>65</td>
<td>35</td>
</tr>
<tr>
<td>2</td>
<td>65</td>
<td>35</td>
</tr>
<tr>
<td>12</td>
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<td>50</td>
</tr>
<tr>
<td>20</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>20.5</td>
<td>65</td>
<td>35</td>
</tr>
<tr>
<td>35</td>
<td>65</td>
<td>35</td>
</tr>
</tbody>
</table>

Flow rate: 1mL/min
Fluorescence detection: excitation/emission wavelengths: 230 / 315nm
Injection volume: 50µL

**RESULTS**

Chromatograms of Infant Formula containing 1µg/L of Bisphenol A before clean-up (Red) and after clean-up (Blue) with AFFINIMIP® SPE Bisphenols.

Chromatograms obtained after clean-up with AFFINIMIP® SPE Bisphenols of 15mL of Infant Formula spiked with Bisphenol A at 2µg/L (tested twice, blue) or at 1µg/L (tested twice, red) or not spiked (pink).

Recovery of Bisphenol A in 15mL of infant formula after AFFINIMIP® SPE Bisphenols clean-up and relative standard deviation calculated from results generated:
- under **repeatability** conditions (n=3, % RSD_r)

<table>
<thead>
<tr>
<th>C (µg/L)</th>
<th>Mean (µg/L)</th>
<th>Recoveries %</th>
<th>% RSD_r</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>0.9</td>
<td>88.4</td>
<td>1.5</td>
</tr>
<tr>
<td>2.0</td>
<td>1.7</td>
<td>85.7</td>
<td>2.7</td>
</tr>
</tbody>
</table>

- under **reproducibility** conditions (% RSDR).

<table>
<thead>
<tr>
<th>C (µg/L)</th>
<th>Mean (µg/L)</th>
<th>Recoveries %</th>
<th>% RSD_R</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>0.8</td>
<td>84.4</td>
<td>7.4</td>
</tr>
<tr>
<td>2.0</td>
<td>1.7</td>
<td>85.8</td>
<td>5.3</td>
</tr>
</tbody>
</table>

**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
DETERMINATION OF BISPHENOL A IN POWDERED INFANT FORMULA

PROTOCOL OF PURIFICATION

Sample preparation
4.4g powdered infant milk was reconstituted in 30 mL of water and warmed up at ~ 50°C during 20 seconds using microwaves. Then 20 mL of acetonitrile were added to 20 mL of warm milk and centrifuged at 4000 rpm during 10 minutes. The supernatant was collected and filtered on filter paper (4-7µm). This extract was diluted 1:1 with water to form the loading solution.

Purification with a 3mL/100mg AFFINIMIP® SPE Bisphenols cartridge

Equilibration
• 3mL Methanol -2% Acetic Acid
• 3mL Acetonitrile
• 3mL Water

Loading
Up to 40mL of infant formula

Washing of interferences
• 9mL Water
• 6mL Water/Acetonitrile (60/40)

Drying 30 seconds

Elution (E)
3mL Methanol

The elution fraction was then evaporated and dissolved in the mobile phase before HPLC analysis.

HPLC Method with Fluorescence detection

Column: Hypersil Gold C18 column 150mm x 4.6mm
Mobile phase: gradient profile

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>% water</th>
<th>% ACN</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>65</td>
<td>35</td>
</tr>
<tr>
<td>2</td>
<td>65</td>
<td>35</td>
</tr>
<tr>
<td>12</td>
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<td>50</td>
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<tr>
<td>20</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>20.5</td>
<td>65</td>
<td>35</td>
</tr>
<tr>
<td>35</td>
<td>65</td>
<td>35</td>
</tr>
</tbody>
</table>

Flow rate: 1mL/min
Fluorescence detection: excitation/emission wavelengths: 230 / 315nm
Injection volume: 50µL

RESULTS

Concentration of BPA in reconstituted milk (µg/L) | Mean concentration (µg/L) | Recoveries % | RSD, % |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>2.3 (n=5)</td>
<td>108</td>
<td>8.7</td>
</tr>
<tr>
<td>4.3</td>
<td>4.0 (n=4)</td>
<td>95</td>
<td>3.7</td>
</tr>
</tbody>
</table>

Chromatograms obtained after clean-up with AFFINIMIP® SPE Bisphenols of equivalent at 10mL of Infant Formula spiked with Bisphenol A at 4.3µg/L (tested twice, red) or at 2.1µg/L (tested twice, blue) or not spiked (pink).

Recovery of Bisphenol A spiked at different concentrations after 3mL/100mg AFFINIMIP® SPE Bisphenols clean-up of 40mL of loading solution (equivalent to 10mL of reconstituted Infant milk) and relative standard deviation calculated from results generated under repeatability conditions.

Regulations for Bisphenol A:
Europe (directive 2011/8/EU) : forbidden in infant feeding bottles

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

6mL-100mg sorbent in a PP cartridge
FS106-02B for 25 cartridges
FS106-03B for 50 cartridges

6mL-100mg sorbent in a glass cartridge
FS106-02G for 25 cartridges
FS106-03G for 50 cartridges
DETERMINATION OF BISPHENOL A IN CANNED FOOD (Liquid form)

**Regulations for Bisphenol A:**
Europe (directive 2011/8/EU) : Specific migration limit in food from packaging of 0.6mg/kg

**PROTOCOL OF PURIFICATION**
Sample preparation

**Purification with a 3mL/100mg AFFINIMIP® SPE Bisphenols cartridge**

**Equilibration**
- 3mL Methanol -2% Acetic Acid
- 3mL Acetonitrile
- 3mL Water

**Loading**
10mL liquid from canned food after filter paper filtration

**Washing of interferents**
- 9mL Water
- 6mL Water/Acetonitrile (60/40)

**Drying 30 seconds**

**Elution (E)**
3mL Methanol
The elution fraction was then evaporated and dissolved in the mobile phase before HPLC analysis.

**HPLC Method with Fluorescence detection**
Column: Hypersil Gold C18 column 150mm x 4.6mm
Mobile phase: gradient profile

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>% water</th>
<th>% ACN</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>65</td>
<td>35</td>
</tr>
<tr>
<td>2</td>
<td>65</td>
<td>35</td>
</tr>
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<td>12</td>
<td>50</td>
<td>50</td>
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<tr>
<td>20</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>20.5</td>
<td>65</td>
<td>35</td>
</tr>
<tr>
<td>35</td>
<td>65</td>
<td>35</td>
</tr>
</tbody>
</table>

Flow rate: 1mL/min
Fluorescence detection: excitation/emission wavelengths: 230 / 315nm
Injection volume: 50µL

Chromatograms after clean-up with AFFINIMIP® SPE Bisphenols of 10mL liquid form of canned Peas and carrots spiked with Bisphenol A at 1µg/L (tested twice, blue) or not spiked (green).

Recovery of Bisphenol A after AFFINIMIP® SPE Bisphenols clean-up of 10mL of canned peas and carrots (liquid) spiked at 1µg/L and relative standard deviation calculated from results generated
- under reproducibility conditions (n=4).

<table>
<thead>
<tr>
<th>C° (µg/L)</th>
<th>Mean (µg/L)</th>
<th>Recoveries %</th>
<th>% RSD R</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>1.05</td>
<td>105.1</td>
<td>5</td>
</tr>
</tbody>
</table>

- under reproducibility conditions (n=4).

<table>
<thead>
<tr>
<th>C° (µg/L)</th>
<th>Mean (µg/L)</th>
<th>Recoveries %</th>
<th>% RSD R</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>1.04</td>
<td>104.3</td>
<td>10</td>
</tr>
</tbody>
</table>

**EVALUATION OF Bisphenol A IN COMMERCIAL CANNED FOODS**

Chromatograms after clean-up with AFFINIMIP® SPE Bisphenols of 10mL of canned salmon and tuna (liquid form).

Blue: 1st price canned salmon; Green: middle grade canned salmon: no Bisphenol A was detected; Red: premium canned salmon; Pink: canned tuna

**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

www.affinisep.com
DETERMINATION OF BISPHENOL A IN CANNED FOOD (Vegetable)

**Regulations for Bisphenol A:**
Europe (directive 2011/8/EU) : Specific migration limit in food from packaging of 0.6mg/kg

**PROTOCOL OF PURIFICATION**
Sample preparation
150g of drained canned peas - carrots and 200mL of Water /ACN (50/50) are blended during 2 min and centrifuged during 10min at 4000rpm. The supernatant solution is collected, filtered (4-7µm) and diluted ½ with water to give the loading solution

**Purification with a 3mL/100mg AFFINIMIP® SPE Bisphenols cartridge**

**Equilibration**
- 3mL Methanol -2% Acetic Acid
- 3mL Acetonitrile
- 3mL Water

**Loading**
20mL loading solution

**Washing of interferences**
- 9mL Water
- 6mL Water/Acetonitrile (60/40)

**Drying 30 seconds**

**Elution (E)**
3mL Methanol

The elution fraction was then evaporated and dissolved in the mobile phase before HPLC analysis.

**HPLC Method with Fluorescence detection**
Column: Hypersil Gold C18 column 150mm x 4.6mm
Mobile phase: gradient profile

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>% water</th>
<th>% ACN</th>
</tr>
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<tbody>
<tr>
<td>0</td>
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<td>35</td>
</tr>
<tr>
<td>12</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>20</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>20.5</td>
<td>65</td>
<td>35</td>
</tr>
<tr>
<td>35</td>
<td>65</td>
<td>35</td>
</tr>
</tbody>
</table>

Flow rate: 1mL/min
Fluorescence detection: excitation/emission wavelengths: 230 / 315nm
Injection volume: 50µL

**RESULTS**

**Recovery yield : 97-99%**

Chromatograms after clean-up with AFFINIMIP® SPE Bisphenols of 20mL loading solution of extract of canned Peas- carrots spiked with Bisphenol A at 2µg/L (tested twice, blue and red) or not spiked (green).

**Catalog number:**

3mL-100mg sorbent in a PP cartridge
FS106-02 for 25 cartridges
FS106-03 for 50 cartridges
6mL-100mg sorbent in a PP cartridge
FS106-02B for 25 cartridges
FS106-03B for 50 cartridges
6mL-100mg sorbent in a glass cartridge
FS106-02G for 25 cartridges
FS106-03G for 50 cartridges
**REGULATIONS FOR BISPHENOL A: Europe (directive 2009/42/EC): Specific migration limit in food from packaging of 0.6 mg/kg**

**PROTOCOL OF PURIFICATION**

Sample preparation

The beer is degassed by sonication for 1 hour.

**Purification with a 3mL/100mg AFFINIMIP$^\text{®}$ SPE Bisphenols cartridge**

- **Equilibration**
  - 3mL Methanol -2% Acetic Acid
  - 3mL Acetonitrile
  - 3mL Water

- **Loading**
  - 10mL of degassed beer

- **Washing of Interferences**
  - 9mL Water
  - 6mL Water/Acetonitrile (60/40)

- **Drying 30 seconds**

- **Elution (E)**
  - 3mL Methanol

The elution fraction was then evaporated and dissolved in the mobile phase before HPLC analysis.

**HPLC Method with Fluorescence detection**

- **Column:** Hypersil Gold C18 column 150mm x 4.6mm
- **Mobile phase:** gradient profile

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>% water</th>
<th>% ACN</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>65</td>
<td>35</td>
</tr>
<tr>
<td>2</td>
<td>65</td>
<td>35</td>
</tr>
<tr>
<td>12</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>12.5</td>
<td>65</td>
<td>35</td>
</tr>
<tr>
<td>22</td>
<td>65</td>
<td>35</td>
</tr>
</tbody>
</table>

Flow rate: 1mL/min
Fluorescence detection: excitation/emission wavelengths: 230 / 315nm
Injection volume: 50µL.

**RESULTS**

- **Injection:** 50µL of beer before treatment
- **Bisphenol A after treatment of 10mL of Beer**

Chromatograms of beer containing 1µg/L of Bisphenol A before (Red) and after (Blue) AFFINIMIP$^\text{®}$ SPE Bisphenols Clean-up.

Chromatograms obtained after AFFINIMIP$^\text{®}$ SPE Bisphenols Clean-up of 10mL of beer spiked at 2µg/L (tested 3 times, orange) or at 1µg/L (tested 3 times, blue) with Bisphenol A or not spiked (red)

Recovery of Bisphenol A in spiked beer after AFFINIMIP$^\text{®}$ SPE Bisphenols clean-up and relative standard deviation calculated from results generated:

- under **repeatability** conditions ($n=3$, % RSD$_r$)

<table>
<thead>
<tr>
<th>C$^\circ$ (µg/L)</th>
<th>Mean µg/L</th>
<th>Recoveries %</th>
<th>% RSD$_r$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>1.1</td>
<td>106.9</td>
<td>1.0</td>
</tr>
<tr>
<td>2.0</td>
<td>1.9</td>
<td>93.4</td>
<td>1.0</td>
</tr>
</tbody>
</table>

- under **reproducibility** conditions (% RSD$_R$)

<table>
<thead>
<tr>
<th>C$^\circ$ (µg/L)</th>
<th>Mean µg/L</th>
<th>Recoveries %</th>
<th>% RSD$_R$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>1.0</td>
<td>99.3</td>
<td>8.9</td>
</tr>
<tr>
<td>2.0</td>
<td>1.8</td>
<td>90.6</td>
<td>6.0</td>
</tr>
</tbody>
</table>

**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

**www.affinisep.com**
The analysis of BPA (derivatized with TMS) was performed by GC-MS/MS, SRM mode after a clean-up protocol using AFFINIMIP® SPE Bisphenols of various solid and liquid complex food matrices (illustration here for salmon and milk).

**RESULTS**

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>BPA Concentration</th>
<th>MRM Transition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmon</td>
<td>0.67µg/Kg BPA</td>
<td>369.2&gt;197.2</td>
</tr>
<tr>
<td>Milk</td>
<td>0.13µg/Kg BPA</td>
<td>357.2&gt;191.2</td>
</tr>
</tbody>
</table>

**13C-BPA**
MRM 369.2>197.2

**BPA**
MRM 357.2>191.2

**BPA**
MRM 372.2>357.2

**Publications**
Data extracted from the poster *Utilisation de la spectrométrie de masse pour le dosage du Bisphénol A dans les matrices alimentaires*, Emmanuelle Bichon et al. (LABERCA), Poster for SMAP 2011, Avignon (France)

**Catalog number:**
- 3mL-100mg sorbent in a PP cartridge
  - FS106-02 for 25 cartridges
  - FS106-03 for 50 cartridges
- 6mL-100mg sorbent in a PP cartridge
  - FS106-02B for 25 cartridges
  - FS106-03B for 50 cartridges
- 6mL-100mg sorbent in a glass cartridge
  - FS106-02G for 25 cartridges
  - FS106-03G for 50 cartridges
Regulations for Bisphenol A:
Europe (directive 2011/8/EU): Specific migration limit in food from packaging of 0.6mg/kg

PROTOCOL OF PURIFICATION

Purification with a 3mL or 6mL/100mg AFFINIMIP® SPE Bisphenols cartridge

Equilibration
• 3mL Methanol -2% Acetic Acid
• 3mL Acetonitrile
• 3mL Water

Loading
10mL of wine

Washing of interferences
• 9mL Water
• 6mL Water/Acetonitrile (60/40)

Drying 1 minute

Elution (E)
3mL Methanol

The elution fraction was then evaporated and dissolved in the mobile phase before HPLC analysis.

HPLC Method with Fluorescence detection
Column: Hypersil Gold C18 column 150mm x 4.6mm
Mobile phase: gradient profile

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>% water</th>
<th>% ACN</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>65</td>
<td>35</td>
</tr>
<tr>
<td>2</td>
<td>65</td>
<td>35</td>
</tr>
<tr>
<td>12</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>12.5</td>
<td>65</td>
<td>35</td>
</tr>
<tr>
<td>22</td>
<td>65</td>
<td>35</td>
</tr>
</tbody>
</table>

Flow rate: 1mL/min
Fluorescence detection: excitation/emission wavelengths: 230 / 315nm
Injection volume: 50µL.

RESULTS

Recovery of Bisphenol A spiked at 2µg/kg after AFFINIMIP® SPE Bisphenols clean-up of 6mL of red wine or 10mL of white wine.

<table>
<thead>
<tr>
<th>Matrice Spiked at 2µg/kg</th>
<th>Mean concentration (µg/kg)</th>
<th>Recoveries %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red wine 1</td>
<td>1.93 (n=2)</td>
<td>96.6</td>
</tr>
<tr>
<td>Red wine 2</td>
<td>2.13 (n=2)</td>
<td>106.5</td>
</tr>
<tr>
<td>Red wine 3</td>
<td>1.66 (n=2)</td>
<td>83.0</td>
</tr>
<tr>
<td>White wine</td>
<td>1.60 (n=3)</td>
<td>80.0</td>
</tr>
</tbody>
</table>

Catalog number:

3mL-100mg sorbent in a PP cartridge
FS106-02 for 25 cartridges
FS106-03 for 50 cartridges

6mL-100mg sorbent in a PP cartridge
FS106-02B for 25 cartridges
FS106-03B for 50 cartridges

6mL-100mg sorbent in a glass cartridge
FS106-02G for 25 cartridges
FS106-03G for 50 cartridges

www.affinisep.com
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**

- 3mL Methanol -2% Acetic Acid
- 3mL Acetonitrile
- 3mL 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**

- m/z 227.1 → m/z 212.1
- m/z 227.1 → m/z 133.2

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
DETERMINATION OF BISPHENOL A IN COLA DRINK

PROTOCOL OF PURIFICATION
Sample preparation
Cola drink is degazzed during 30min.

Purification with a 3mL/100mg AFFINIMIP® SPE Bisphenols PP cartridge
Equilibration
• 3mL Methanol -2% Acetic Acid
• 3mL Acetonitrile
• 3mL Water
Loading solution
6mL of Cola drink
Washing of interferences
• 9mL Water
• 6mL Water/Acetonitrile (60/40)
Drying 3min
Elution (E)
3mL Methanol
The elution fraction was then concentrated and diluted with the mobile phase before HPLC analysis.

HPLC Method with Fluorescence detection
HPLC Column: Hypersil Gold 150mm x 4.6mm
Mobile phase: gradient profile

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>% water</th>
<th>% Acetonitrile</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>65</td>
<td>35</td>
</tr>
<tr>
<td>2</td>
<td>65</td>
<td>35</td>
</tr>
<tr>
<td>12</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>20</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>20.5</td>
<td>65</td>
<td>35</td>
</tr>
<tr>
<td>40</td>
<td>65</td>
<td>35</td>
</tr>
</tbody>
</table>

Flow rate: 1mL/min
Injection volume: 50µL
Detector: Fluorescence detection ($\lambda_{exc}$ 230nm – $\lambda_{em}$ 315nm)

RESULTS

Comparison of the solution obtained before and after using AFFINIMIP® SPE Bisphenols

Chromatograms obtained after clean-up with AFFINIMIP® SPE Bisphenols of 6mL of cola spiked with Bisphenol A at 5µg/kg (tested three times, blue) or not spiked (red)

Recovery of Bisphenol A spiked at 5µg/kg after AFFINIMIP® SPE Bisphenols clean-up of 6mL of cola.

<table>
<thead>
<tr>
<th>Matrice Spiked at 5µg/kg</th>
<th>Mean concentration (µg/kg)</th>
<th>Recovery %</th>
<th>RSDr %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cola</td>
<td>4.54 (n=3)</td>
<td>90.8</td>
<td>1.0</td>
</tr>
</tbody>
</table>

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

www.affinisep.com
DETERMINATION OF BISPHENOL A AND BADGE IN MILK

PROTOCOL OF PURIFICATION

Purification with a 3mL or 6mL/100mg AFFINIMIP® SPE Bisphenols cartridge

Equilibration
- 3mL Methanol -2% Formic Acid
- 3mL Acetonitrile
- 3mL Water

Loading
9mL of Milk

Washing of interferences
- 9mL Water
- 6mL Water/Acetonitrile (60/40)

Drying 3 minute

Elution (E)
- 3mL Methanol (E1)
- 3mL Acetonitrile (E2)

The elution fractions E1 and E2 were gathered, evaporated and dissolved in the mobile phase before HPLC analysis.

HPLC Method with Fluorescence detection
Column: Hypersil Gold C18 column 150mm x 4.6mm
Mobile phase: gradient profile

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>% water</th>
<th>% ACN</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>65</td>
<td>35</td>
</tr>
<tr>
<td>2</td>
<td>65</td>
<td>35</td>
</tr>
<tr>
<td>12</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>20</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>25</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>30</td>
<td>65</td>
<td>35</td>
</tr>
<tr>
<td>40</td>
<td>65</td>
<td>35</td>
</tr>
</tbody>
</table>

Flow rate: 1mL/min

Fluorescence detection: excitation/emission wavelengths: 230 / 315nm
Injection volume: 50µL.

RESULTS

Fluorescence chromatograms obtained after clean-up with AFFINIMIP® SPE Bisphenols of 9mL of milk spiked with 10µg/kg Bisphenol A and 10µg/kg BADGE (tested twice, blue) or not spiked (red).

Recovery of Bisphenol A and BADGE spiked at 10µg/kg after AFFINIMIP® SPE Bisphenols clean-up of 9mL of milk.

<table>
<thead>
<tr>
<th>Matrice Spiked at 10µg/kg</th>
<th>Mean concentration (µg/kg)</th>
<th>Recoveries %</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPA</td>
<td>10.85</td>
<td>108.5</td>
</tr>
<tr>
<td>BADGE</td>
<td>7.5</td>
<td>75</td>
</tr>
</tbody>
</table>

Catalog number:
- 3mL-100mg sorbent in a PP cartridge
  - FS106-02 for 25 cartridges
  - FS106-03 for 50 cartridges
- 6mL-100mg sorbent in a PP cartridge
  - FS106-02B for 25 cartridges
  - FS106-03B for 50 cartridges
- 6mL-100mg sorbent in a glass cartridge
  - FS106-02G for 25 cartridges
  - FS106-03G for 50 cartridges
A report of the French Health Agency (ANSES) on the assessment of the health risks associated with bisphenol A (BPA) was published on 9 April 2013. Quantitative analysis of Bisphenol A in all liquid or solid food matrices were carried out by using AFFINIMIP® SPE Bisphenols (Analyses carried out by LABERCA and described in Annex 12 of Annexes of the report p132 (in french)).


Results of the analyses have been published in the article:

Example of tested food:
Cereals for breakfast, muesli, cornflakes
Bread, toast, brioche, pastries, sweet and salted biscuits, cookies, pasta...
Cereals: rice, wheat...
Cheese: camenbert, cantal...
Milk (skimmed, concentrated ...), Yoghurt, cream, butter
Oils, eggs
Fish: cooked fish, fried breaded fish, canned atun, steamed and smoked salmon, hake...
Seafood: crustacean, oysters, mussel, shrimp...
Vegetable: salad, tomatoes, radish, onion, soja, carrots, cauliflower, zucchini, peas, spinach....
Cooked food such as paella, couscous
Meat: roasted meat, lamb, pork, duck, beef, sheep, turkey, poultry
Delicatessen: Raw and cooked ham, foie gras, paté, sausage, bacon, chipolatas, merguez...
Fruits and dried fruits: almonds, peach, orange, compote....
Drink water, apple juice, soda...
Coffee, chocolate, cacao...
The analysis of seven bisphenol analogues in beverage and canned food samples was performed by using **AFFINIMIP® SPE Bisphenols** prior LC–MS analysis.

Bisphenol analogs tested: BPS, BPF, BPA, BPB, BPAF, tetrachlorobisphenol A (TCBPA), TBBPA.

Matrices: beverage and canned food (soda, tea drink, juice, red wine, vegetable, fish and meat)

**PROTOCOL OF PURIFICATION**

**Sample preparation for beverage**

10mL beverage is degassed or centrifuged 9000g during 5min.

**Sample preparation for canned food**

1g of canned food is extracted with 5mL acetonitrile with sonication during 20min and centrifugation 9000g for 5min. Fat is removed with 5mL Hexane by LLE. The acetonitrile layer is concentrated to 1mL and diluted with water to 10mL

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

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

**Loading**
- Loading solution

**Washing of interferences**
- 6mL Water
- 3mL Water/Acetonitrile (60/40)

**Drying 30 min**

**Washing of interferences**
- 2mL Acetonitrile
- 2mL Methanol/Acetonitrile (10/90)

**Elution (E)**

4mL Methanol containing 2% Formic Acid

The elution fraction was then evaporated and dissolved in the mobile phase before HPLC analysis.

**RESULTS FOR CANNED FISH**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Conc (ng/mL)</th>
<th>Recovery (%)</th>
<th>LOQ (ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPS</td>
<td>0.1</td>
<td>73</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>82</td>
<td></td>
</tr>
<tr>
<td>BPF</td>
<td>1</td>
<td>78</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>73</td>
<td></td>
</tr>
<tr>
<td>BPA</td>
<td>0.5</td>
<td>81</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>89</td>
<td></td>
</tr>
<tr>
<td>BPB</td>
<td>1</td>
<td>79</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>82</td>
<td></td>
</tr>
<tr>
<td>BPAF</td>
<td>0.1</td>
<td>81</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>79</td>
<td></td>
</tr>
<tr>
<td>TCBPA</td>
<td>0.5</td>
<td>72</td>
<td>0.28</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>78</td>
<td></td>
</tr>
<tr>
<td>TBBPA</td>
<td>1</td>
<td>57</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>61</td>
<td></td>
</tr>
</tbody>
</table>

**Catalog number:**

- **3mL-100mg sorbent in a PP cartridge**
  - FS106-02 for 25 cartridges
  - FS106-03 for 50 cartridges

- **6mL-100mg sorbent in a PP cartridge**
  - FS106-02B for 25 cartridges
  - FS106-03B for 50 cartridges

- **6mL-100mg sorbent in a glass cartridge**
  - FS106-02G for 25 cartridges
  - FS106-03G for 50 cartridges

**Publications**

Data extracted from the article Molecularly imprinted solid phase extraction for the selective extraction of bisphenol analogues in beverages and canned food, Y. Yang et al., *J. Agric. Food Chem.*, 2014, 62 (46), pp 11130–11137
ONIRIS – LABERCA describes an accurate and sensitive method of determination of 18 Bisphenol analogues in human breast milk by GC-MS/MS. By using AFFINIMIP® SPE Bisphenols in the sample preparation protocol, LABERCA analyzes FREE and TOTAL bisphenol analogues with recovery yields higher than 90% for all analogues.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Recovery (%) Spiked at 0.1ng</th>
<th>Recovery (%) Spiked at 1ng</th>
<th>Recovery (%) Spiked at 10ng</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bisphenol A</td>
<td>97</td>
<td>94</td>
<td>105</td>
</tr>
<tr>
<td>Bisphenol B</td>
<td>96</td>
<td>99</td>
<td>102</td>
</tr>
<tr>
<td>Bisphenol AP</td>
<td>100</td>
<td>90</td>
<td>92</td>
</tr>
<tr>
<td>Bisphenol AF</td>
<td>100</td>
<td>96</td>
<td>90</td>
</tr>
<tr>
<td>Bisphenol BP</td>
<td>108</td>
<td>109</td>
<td>99</td>
</tr>
<tr>
<td>Bisphenol C</td>
<td>92</td>
<td>94</td>
<td>97</td>
</tr>
<tr>
<td>Bisphenol CI2</td>
<td>102</td>
<td>101</td>
<td>93</td>
</tr>
<tr>
<td>Bisphenol E</td>
<td>96</td>
<td>94</td>
<td>102</td>
</tr>
<tr>
<td>Bisphenol PH</td>
<td>94</td>
<td>93</td>
<td>102</td>
</tr>
<tr>
<td>Bisphenol S</td>
<td>100</td>
<td>99</td>
<td>93</td>
</tr>
<tr>
<td>Bisphenol F</td>
<td>103</td>
<td>109</td>
<td>104</td>
</tr>
<tr>
<td>DHDPE</td>
<td>104</td>
<td>92</td>
<td>100</td>
</tr>
<tr>
<td>Bisphenol FL</td>
<td>103</td>
<td>100</td>
<td>96</td>
</tr>
<tr>
<td>Bisphenol Z</td>
<td>100</td>
<td>97</td>
<td>103</td>
</tr>
<tr>
<td>Biphenyl-4,4’-diol</td>
<td>109</td>
<td>103</td>
<td>104</td>
</tr>
<tr>
<td>Bisphenol M</td>
<td>96</td>
<td>96</td>
<td>94</td>
</tr>
<tr>
<td>Bisphenol P</td>
<td>97</td>
<td>92</td>
<td>99</td>
</tr>
<tr>
<td>Bis-2(hydroxyphenyl)methane</td>
<td>108</td>
<td>103</td>
<td>109</td>
</tr>
</tbody>
</table>

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

**Publications**
Data extracted from the article
DETERMINATION OF BPA, NONYLPHENOL AND 4-t-OCTYLPHENOL IN FISH FEED

The metabolic effects induced by feed contaminated with a lower or a higher Concentration of nonylphenol (NP), 4-tert-octylphenol (t-OP) or bisphenol A (BPA), three environmental endocrine disruptors, were assessed in juvenile sea bream liver.

The extraction of NP, t-OP and BPA in water and feed was performed by using AFFINIMIP® SPE Bisphenols prior LC/ESI-QTRAP-MS/MS analysis.

PROTOCOL OF PURIFICATION

Sample preparation for feed
1g of homogenized feed and 5mL water/Acetonitrile 50/50 were shaken for 10min then centrifuged at 1267g for 10min. The supernatant was collected and the extraction on feed was repeated. Then 2mL supernatant and 50µL solution NaCL 20% were mixed with 4mL ethyl acetate, vortexed and centrifuged at 1267g for 5 min. The upper layer was evaporated under nitrogen and diluted with 2mL Water/Acetonitrile (50/50) and 6mL water to form the loading solution.

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

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

Loading
- Loading solution

Washing of interferences
- 10mL Water
- 6mL Water/Acetonitrile (60/40)

Elution (E)
- 3mL Methanol

The elution fraction was then evaporated and dissolved in the mobile phase before HPLC analysis.

Publications

Data extracted from the article Xenobiotic-contaminated diets affect hepatic lipid metabolism: implications for liver steatosis in Sparus aurata juveniles, F. Maradonna, V. Nozzi, S. Santangeli, I. Traversi, P. Gallo, E. Fattore, D.G. Mita, A. Mandich, O. Carnevali, Aquatic Toxicology, 257–264 (167), 2015

Catalog number:
3mL-100mg sorbent in a PP cartridge
FS106-02 for 25 cartridges
FS106-03 for 50 cartridges
6mL-100mg sorbent in a PP cartridge
FS106-02B for 25 cartridges
FS106-03B for 50 cartridges
6mL-100mg sorbent in a glass cartridge
FS106-02G for 25 cartridges
FS106-03G for 50 cartridges
The analysis of 5 bisphenol analogues in canned energy drinks was performed by using **AFFINIMIP® SPE Bisphenols** prior UPLC - Fluorescence analysis.

Bisphenol analogs tested: BPF, BPA, BPB, BADGE, BFDGE.

**PROTOCOL OF PURIFICATION**

Sample preparation for beverage
20mL of energy drinks is degassed for 60min in an ultrasonic bath. Then 5mL of solution plus 1mL 0.2M aqueous ammonium acetate were vortexed for 30s. Adjust pH at 4 to form the loading solution.

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

**Equilibration**
- 3mL Methanol - 2% Acetic Acid
- 3mL Acetonitrile
- 3mL Water

**Loading**
Loading solution

**Washing of interferences**
- 9mL Water
- 6mL Water/Acetonitrile (60/40)

**Drying 30 s**

**Elution (E)**
- 3mL Methanol
- 3mL Acetonitrile

The elution fractions were gathered, evaporated and dissolved in methanol before UPLC-FLD analysis.

**UPLC Method with Fluorescence detection**

Column: Ascensis Express RP-Amide 75mm x 4.6mm
Mobile phase: gradient profile

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>% water</th>
<th>% Acetonitrile</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>0.5</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>5.5</td>
<td>5</td>
<td>95</td>
</tr>
<tr>
<td>8.5</td>
<td>5</td>
<td>95</td>
</tr>
<tr>
<td>10.5</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

Flow rate: 0.5mL/min
Fluorescence detection: excitation/emission wavelengths: 275 / 305nm
Injection volume: 5μL.

**VALIDATION WITH CANNED ENERGY DRINKS**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Conc (ng/mL)</th>
<th>Recovery (%) (n=6)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPA</td>
<td>2.0</td>
<td>58</td>
<td>6.0</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>52</td>
<td>8.6</td>
</tr>
<tr>
<td>BPB</td>
<td>2.0</td>
<td>93</td>
<td>9.9</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>78</td>
<td>7.7</td>
</tr>
<tr>
<td>BPF</td>
<td>2.0</td>
<td>82</td>
<td>6.3</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>89</td>
<td>9.0</td>
</tr>
<tr>
<td>BADGE</td>
<td>2.0</td>
<td>88</td>
<td>7.0</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>94</td>
<td>8.1</td>
</tr>
<tr>
<td>BFDGE</td>
<td>2.0</td>
<td>87</td>
<td>4.7</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>91</td>
<td>7.0</td>
</tr>
</tbody>
</table>

LOQ = 0.50 ng/mL
LOD = 0.15 ng/mL (n=3)

**Publications**

Data extracted from the article **Determination of BPA, BPB, BPF, BADGE and BFDGE in canned energy drinks by molecularly imprinted polymer cleaning up and UPLC with fluorescence detection**, P. Gallo *et al.*, *Food Chemistry* 220 (2017) 406–412
AFFINISEP proposes the complete set of equipments required to carry out SPE experiments:

**Manifold**  
ACC-MAN1  
Like all chromatography techniques, Use of SPE cartridges needs a precise control of flow rate for maintaining reproducible extractions. Solid Phase extraction Vacuum Manifold allows you to control the flow and to process up to 12 (12-port version) or 24 (24-port version) AFFINIMIP® SPE samples simultaneously, to gain significantly time during sample preparation steps.

**SPE Adapter & Reservoir kit**  
ACC-AR1  
Tube adapters serve to pile one SPE tube on top of another to provide different selectivities. A larger empty syringe barrel can be stacked on top of a smaller SPE tube to act as a larger load reservoir. Or, they can serve as an adapter for positive pressure methods (e.g. from a syringe or air/ N2 line).

**Mini-Vap**  
ACC-VAP1  
The 6-Port Mini-Vap concentrator/evaporator processes six vials at one time. The Mini-Vap includes a needle valve for fine metering of air or nitrogen drying gas.

**Mini PUMP**  
ACC-PUMP  
Mini diaphragm vacuum pump for solid phase extraction experiments  
Portable  
➢ 5.5L/min  
➢ ~120 torr vacuum  
➢ Oil-free  
➢ portable

**Vacuum pump trap**  
ACC-TRAP  
SPE Vacuum pump trap kit  
Installed between the manifold and the vacuum pump, it collects all liquids that are aspirated preventing contamination of the vacuum pump with a capacity of 1L.
### AFFINIMIP SPE and Reactive – Product list

<table>
<thead>
<tr>
<th>Products</th>
<th>Designation</th>
<th>Definition</th>
<th>Reference</th>
<th>Nber of cartridges</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bisphenol A</td>
<td>AFFINIMIP® SPE Bisphenols</td>
<td>3mL Selective SPE cartridges for Bisphenols (PP)</td>
<td>FS106-02</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6mL Selective SPE cartridges for Bisphenols (PP)</td>
<td>FS106-02</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6mL Selective SPE cartridges for Bisphenols (Glass)</td>
<td>FS106-02G</td>
<td>25</td>
</tr>
</tbody>
</table>

### SPE ACCESSORIES – Product list

<table>
<thead>
<tr>
<th>SPE Accessories</th>
<th>Designation</th>
<th>Definition</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manifold</td>
<td>SPE Vacuum Manifold</td>
<td>12-port model</td>
<td>ACC-MAN1</td>
</tr>
<tr>
<td>SPE Adapter &amp; Reservoir kit</td>
<td>SPE Adapter &amp; Reservoir kit</td>
<td>Kit of 12 reservoirs 60ml and adapters for use with 1,3 &amp; 6 mL cartridges</td>
<td>ACC-AR1</td>
</tr>
<tr>
<td>Mini-Vap</td>
<td>Mini Evaporator/Concentrator</td>
<td>6 port Mini-Vap Evaporator/Concentrator for use with 1 to 250mL containers</td>
<td>ACC-VAP1</td>
</tr>
<tr>
<td>Mini PUMP</td>
<td>Mini vacuum pump</td>
<td>Laboport diaphragm vacuum mini pump, 5.5L/min</td>
<td>ACC-PUMP</td>
</tr>
<tr>
<td>Vacuum pump trap</td>
<td>SPE Vacuum pump trap kit</td>
<td>1L trap kit</td>
<td>ACC-TRAP</td>
</tr>
</tbody>
</table>
About AFFINISEP

AFFINISEP is a worldwide expert in purification and sample preparation applications as well as for the design and the development of intelligent polymers with Molecularly Imprinted Polymers (MIP).

AFFINISEP is dedicated to the development of analytical applications in various fields such as water, biological fluids, food and feed analysis with a complete set of products and services for sample preparation.

Our mission is to develop and market innovative products of high value to customers by a practical contribution to their work. By offering you a most comprehensive range of solid phase extraction products:

- AFFINIMIP® SPE products based on molecularly imprinted polymers,
- AttractSPE™ a range of polymeric phases
- SilactSPE™ Silica based products, associated reagents,
- QuEChERS small equipment,

the analytical chemists can find any solution for sample preparation, selective extraction and sample clean-up needs in various sectors: food and feed safety and quality, pharmaceutical R&D and quality control, clinical diagnosis, environment and doping.

Furthermore, by exploiting our library of innovative polymers and our know-how in chromatography and solid phase extraction, we have a strong capacity to adapt these polymers to meet any specific requirements and to solve unsatisfied purification and extraction needs.

Numerous documents related to our products (Application notebooks, publication references, posters, catalog for different applications…) can be found on our website www.affinisep.com.

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