



**AFFINIMIP<sup>®</sup> SPE**

**Bisphenols**



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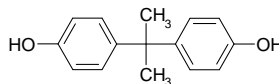
This table reminds the main regulation on the use of Bisphenol A.

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

# Bisphenol A Regulation – USA states

US states	Regulation to ban BPA	Date of votation or application
California	Proposition 65 warning label required for BPA covers packaged foods and drinks	Effective May 2016
USA	in infant formula packaging	Decided July 2013
USA	from use in infant and toddler products such as baby bottles and sippy cups - 21 CFR 177.1580	Decided July 2012
Arizona	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	Effective 01/14
California	in baby bottles and sippy cups	October 11
District of Columbia	bottles, cups or containers designed to be filled with food or liquids	Applied 1/07/11 Effective July 2013
Connecticut	in infant formula containers, baby food cans or jars, reusable food or beverage containers, including baby bottles, spill-proof cups, sports bottles and thermoses	Voted June 09 Applied Oct 11
Connecticut	in thermal receipt paper or cash register receipt paper	Voted June 11 Applied 1/10/13
Delaware	from children's bottles, cups and other food and beverage containers	Effective 01/01/12
Illinois	from children's food and beverage containers	Voted August 12 Applied 2013
Maine	from baby bottles, sippy cups, water bottles and reusable food storage containers	Voted 2011 Applied 2012
Maryland	Ban infant formula containing more than 0.5 ppb Bisphenol A	Voted May 11 Applied July 14
Massachusetts	in children's reusable food or beverage containers	Voted January 11
Minnesota	in sippy cups and baby bottles	Applied 1 January 2010
New Jersey	from children's food and beverage containers	Voted Feb 2013
State of New York	in pacifiers, baby bottles, sippy cups and other unfilled beverage containers for use by children under three years of age after December 1, 2010.	Voted July 10 Applied 1/12/10
Vermont	in reusable food or beverage containers such as baby bottles, spill-proof cups, sports bottles, and thermoses	Voted May 10 Applied 1/12/10
Virginia	In food and beverage containers for children up to 3 years old at the exemption of metal can	Effective July 11
	In sports bottles	Effective July 12
Wisconsin	in pacifiers, baby bottles, sippy cups for children under three years of age after December 1, 2010.	Voted March 2010 Applied June 2010
Washington	in childcare article for children under 3	Effective July 12

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.

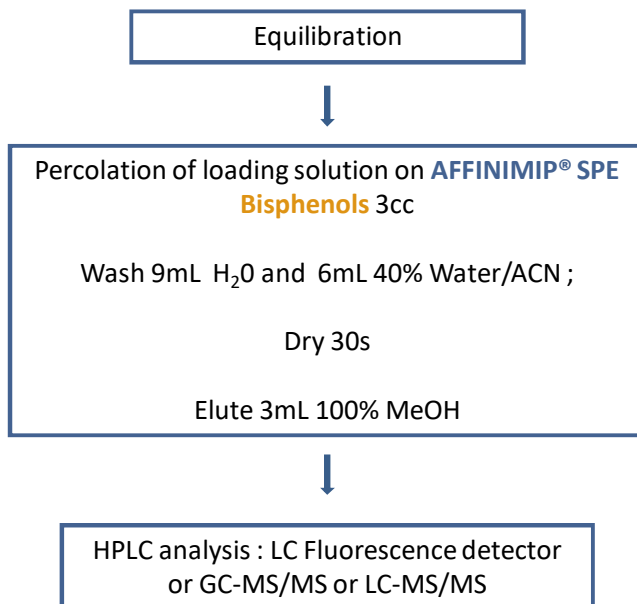
This method is also perfectly suitable for clean-up before GC-MS/MS or LC-MS/MS.

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.



**Performance. Save your time.**

## 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 day

>>> 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 BISPHENOL A IN LIQUID INFANT FORMULA

**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 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

Time (min)	% water	% ACN
0	65	35
2	65	35
12	50	50
20	50	50
20.5	65	35
35	65	35

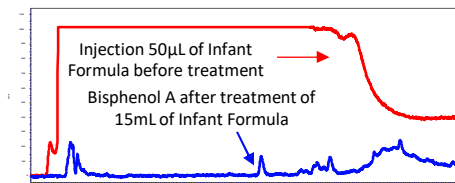
Flow rate: 1mL/min

Fluorescence detection: excitation/emission

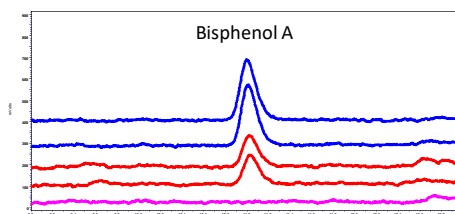
wavelengths: 230 / 315nm

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<sub>r</sub>)

C° (µg/L)	Mean (µg/L)	Recoveries %	% RSD <sub>r</sub>
1.0	0.9	88.4	1.5
2.0	1.7	85.7	2.7

- under **reproducibility** conditions (% RSD<sub>R</sub>)

C° (µg/L)	Mean (µg/L)	Recoveries %	% RSD <sub>R</sub>
1.0	0.8	84.4	7.4
2.0	1.7	85.8	5.3

### 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

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

## 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

Time (min)	% water	% ACN
0	65	35
2	65	35
12	50	50
20	50	50
20.5	65	35
35	65	35

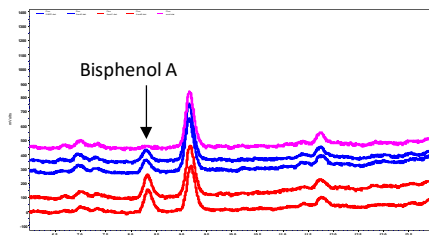
Flow rate: 1mL/min

Fluorescence detection: excitation/emission

wavelengths: 230 / 315nm

Injection volume: 50µL.

## RESULTS



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

Concentration of BPA in reconstituted milk (µg/L)	Mean concentration (µg/L)	Recoveries %	RSD <sub>r</sub> %
2.1	2.3 (n=5)	108	8.7
4.3	4.0 (n=4)	95	3.7

### 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 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

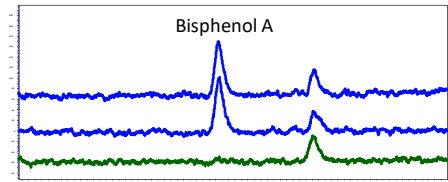
Time (min)	% water	% ACN
0	65	35
2	65	35
12	50	50
20	50	50
20.5	65	35
35	65	35

Flow rate: 1mL/min

Fluorescence detection: excitation/emission wavelengths: 230 / 315nm

Injection volume: 50µL

## RESULTS



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

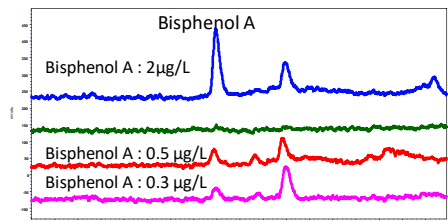
- under reproducibility conditions (n=4).

C° (µg/L)	Mean (µg/L)	Recoveries %	% RSD <sub>R</sub>
1.0	1.05	105.1	5

- under reproducibility conditions (n=4).

C° (µg/L)	Mean (µg/L)	Recoveries %	% RSD <sub>R</sub>
1.0	1.04	104.3	10

## 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:** 1<sup>st</sup> 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

# 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 (4-7 $\mu$ m) and diluted  $\frac{1}{2}$  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

Time (min)	% water	% ACN
0	65	35
2	65	35
12	50	50
20	50	50
20.5	65	35
35	65	35

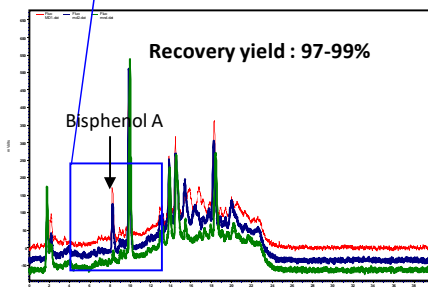
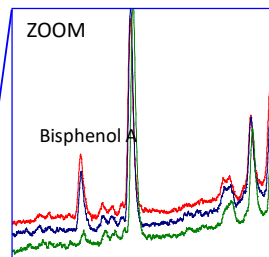
Flow rate: 1mL/min

Fluorescence detection: excitation/emission

wavelengths: 230 / 315nm

Injection volume: 50 $\mu$ L.

## RESULTS



Chromatograms after clean-up with AFFINIMIP® SPE Bisphenols of 20mL loading solution of extract of canned Peas- carrots spiked with Bisphenol A at 2 $\mu$ g/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 2011/8/EU) : Specific migration limit in food from packaging of 0.6mg/kg

## PROTOCOL OF PURIFICATION

Sample preparation

The beer is degassed by sonication for 1 hour.

Purification with a 3mL/100mg **AFFINIMIP® 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

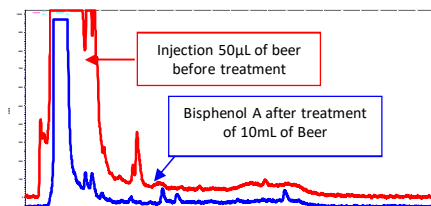
Time (min)	% water	% ACN
0	65	35
2	65	35
12	50	50
12.5	65	35
22	65	35

Flow rate: 1mL/min

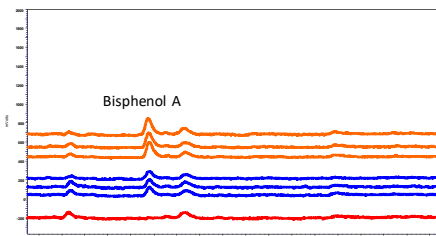
Fluorescence detection: excitation/emission wavelengths: 230 / 315nm

Injection volume: 50µL.

## RESULTS



Chromatograms of beer containing 1µg/L of Bisphenol A before (Red) and after (Blue) **AFFINIMIP® SPE Bisphenols** Clean-up.



Chromatograms obtained after **AFFINIMIP® 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® SPE Bisphenols** clean-up and relative standard deviation calculated from results generated:

- under **repeatability** conditions (n=3, % RSD<sub>r</sub>)

C° (µg/L)	Mean µg/L	Recoveries %	% RSD <sub>r</sub>
1.0	1.1	106.9	1.0
2.0	1.9	93.4	1.0

- under **reproducibility** conditions (% RSD<sub>R</sub>)

C° (µg/L)	Mean µg/L	Recoveries %	% RSD <sub>R</sub>
1.0	1.0	99.3	8.9
2.0	1.8	90.6	6.0

### Catalog number:

**3mL-100mg sorbent in a PP cartridge**

FS106-02 for 25 cartridges

FS106-03 for 50 cartridges

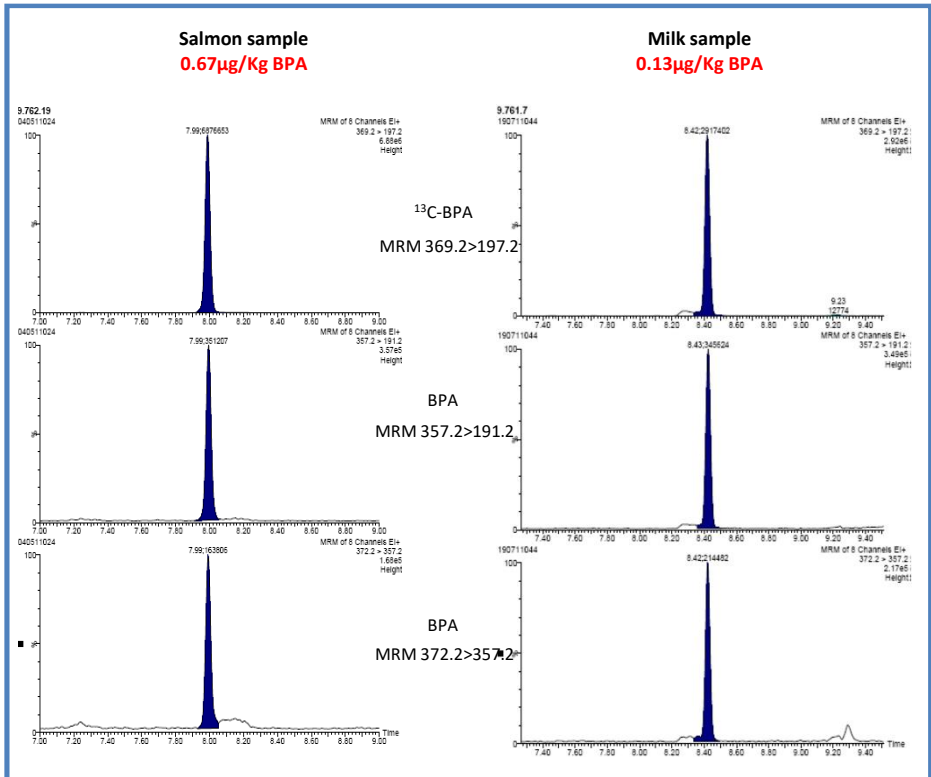
**6mL-100mg sorbent in a glass cartridge**

FS106-02G for 25 cartridges

FS106-03G for 50 cartridges

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



### 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

Time (min)	% water	% ACN
0	65	35
2	65	35
12	50	50
12.5	65	35
22	65	35

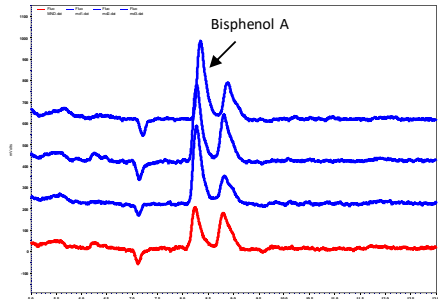
Flow rate: 1mL/min

Fluorescence detection: excitation/emission

wavelengths: 230 / 315nm

Injection volume: 50µL.

## RESULTS



Chromatograms obtained after clean-up with AFFINIMIP® SPE Bisphenols of 10mL of white wine spiked with Bisphenol A at 2µg/kg (tested three times, blue) or not spiked (red). The white wine naturally contained 2µg/kg of BPA

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

Matrice Spiked at 2µg/kg	Mean concentration (µg/kg)	Recoveries %
Red wine 1	1.93 (n=2)	96.6
Red wine 2	2.13 (n=2)	106.5
Red wine 3	1.66 (n=2)	83.0
White wine	1.60 (n=3)	80.0

### 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 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 20 $\mu$ L of  $\beta$ -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

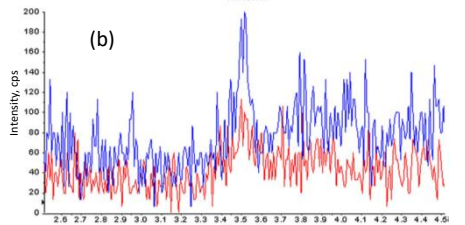
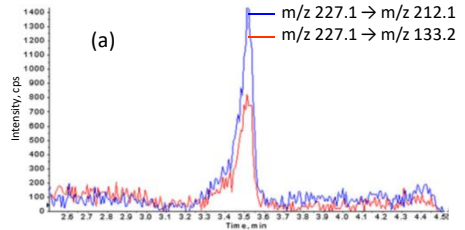
HPLC Column: Kinetex 2.6 $\mu$ m PFP 100mm x 4.6mm

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: 20 $\mu$ L  
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:

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

## RESULTS

### PROTOCOL OF PURIFICATION

Sample preparation

Cola drink is degassed 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

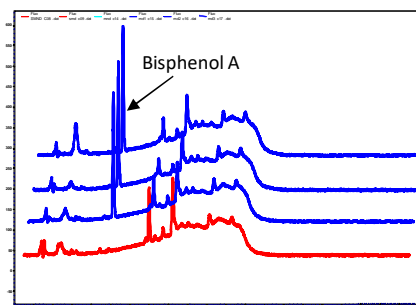
#### Elution (E)

3mL Methanol

The elution fraction was then concentrated and diluted with the mobile phase before HPLC analysis.



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.

### HPLC Method with Fluorescence detection

HPLC Column: Hypersil Gold 150mm x 4.6mm

Mobile phase: gradient profile

Time (min)	% water	% Acetonitrile
0	65	35
2	65	35
12	50	50
20	50	50
20.5	65	35
40	65	35

Flow rate: 1mL/min

Injection volume: 50µL.

Detector: Fluorescence detection ( $\lambda_{exc}$  230nm –  $\lambda_{em}$  315nm)

Matrice Spiked at 5µg/kg	Mean concentration (µg/kg)	Recovery %	RSDr %
Cola	4.54 (n=3)	90.8	1.0

#### Catalog number:

**3mL-100mg sorbent in a PP cartridge**

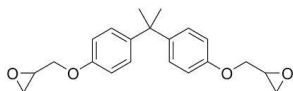
FS106-02 for 25 cartridges

FS106-03 for 50 cartridges

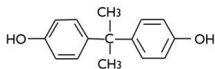
**6mL-100mg sorbent in a PP cartridge**

FS106-02B for 25 cartridges

FS106-03B for 50 cartridges



Bisphenol A Diglycidyl Ether (BADGE)



Bisphenol A

## 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

Time (min)	% water	% ACN
0	65	35
2	65	35
12	50	50
20	20	80
25	20	80
30	65	35
40	65	35

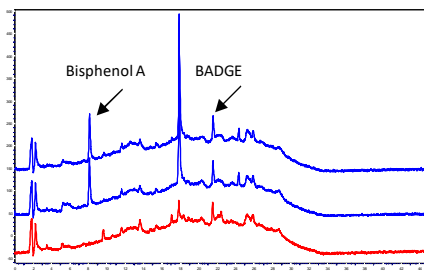
Flow rate: 1mL/min

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.

Matrix Spiked at 10µg/kg	Mean concentration (µg/kg)	Recoveries %
BPA	10.85	108.5
BADGE	7.5	75

#### 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 **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)).

The analytical method has been described by ONIRIS - LABERCA in the article: Development and validation of a specific and sensitive gas chromatography tandem mass spectrometry method for the determination of bisphenol A residues in a large set of food items, Y. Deceuninck, E. Bichon, S. Durand, N. Bemrah, Z. Zendong, M.L. Morvan, P. Marchand, G. Dervilly-Pinel J.P., *J. Chrom. A*, 1362, 241-249 (2014)

Results of the analyses have been published in the article: Assessment of dietary exposure to bisphenol A in the French population with a special focus on risk characterisation for pregnant French women, N. Bemrah, J. Jean, G. Riviere, M. Sanaa, S. Leconte, M. Bachelot, Y. Deceuninck, B. Le Bizec, X. Dauchy, A.-C. Roudot, V. Camel, K. Grob, C. Feidt, N. Picard-Hagen, P.-M. Badot, F. Foures, J. -C. Leblanc, *Food and Chemical Toxicology*, 72, 90–97 (2014)

#### Example of tested food:

Cereals for breakfast, muesli, cornflakes

Bread, toast, brioche, pastries, sweet and salted biscuits, cookies, pasta...

Cereals: rice, wheat...

Cheese: camembert, 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.

#### 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

## RESULTS FOR CANNED FISH

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

#### 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 18 BISPHENOL ANALOGS IN HUMAN BREAST MILK BY GC-MS/MS

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.

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

## Publications

Data extracted from the article

**Determination of bisphenol A and related substitutes/analogues in human breast milk using gas chromatography-tandem mass spectrometry**, Y. Deceuninck, E. Bichon, P. Marchand, C.-Y. Boquien, A. Legrand, C. Boscher, J. P. Antignac, B. Le Bizec, *Anal. and Bioanal. Chem.*, 407 (9), 2485-2497 (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 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 4mL 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

# DETERMINATION OF BPA, BPB, BPF, BADGE AND BFDGE IN CANNED ENERGY DRINKS

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

Time (min)	% water	% Acetonitrile
0	50	50
0.5	50	50
5.5	5	95
8.5	5	95
10.5	50	50

Flow rate: 0.5mL/min

Fluorescence detection: excitation/emission wavelengths: 275 / 305nm

Injection volume: 5µL.

## VALIDATION WITH CANNED ENERGY DRINKS

Analyte	Conc (ng/mL)	Recovery (%) (n=6)	RSD <sub>R</sub> % n=3
BPA	2.0	58	6.0
	10.0	52	8.6
BPB	2.0	93	9.9
	10.0	78	7.7
BPF	2.0	82	6.3
	10.0	89	9.0
BADGE	2.0	88	7.0
	10.0	94	8.1
BFDGE	2.0	87	4.7
	10.0	91	7.0

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

### 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



AFFINISEP proposes the complete set of equipments required to carry out SPE experiments:

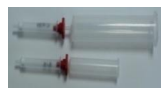
## Manifold



### 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.

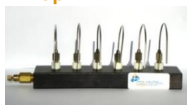
## SPE Adapter & Reservoir kit



### 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

Products	Designation	Definition	Reference	Nber of cartridges
Bisphenol A	AFFINIMIP® SPE Bisphenols	3mL Selective SPE cartridges for Bisphenols (PP)	FS106-02	25
			FS106-03	50
		6mL Selective SPE cartridges for Bisphenols (PP)	FS106-02	25
			FS106-03	50
		6mL Selective SPE cartridges for Bisphenols (Glass)	FS106-02G	25
			FS106-03G	50

## SPE ACCESSORIES – Product list

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



## 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](http://www.affinisep.com).

## ORDERING INFORMATION

For any order, please, choose one of the following ways:

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