affinisep

The art of making sample preparation easier

BISPHENOLS ANALYSIS

by Solid Phase Extraction & Passive Sampling

Bisphenol A Regulation - Countries

This table gathers some main regulation on the use of Bisphenol A.

Countries	Regulation	Date of votation or application
EU	Ban Bisphenol A in all food contact materials	2024
USA	Ban Bisphenol A in infant formula packaging	2023
Austria	Ban Bisphenol A in pacifiers or teethers made with Bisphenol A	2012
Argentina, Australia, Brazil, China, EU, Japan, Malaysia, New Zealand, USA, South Africa and others...	Ban Bisphenol A in baby bottles and infant feeding bottles for children up to 3 years	Since around 2011 for most countries
EU	Migration limit of BPA permitted to leach is 0,04mg/L for toys for children up to three years of age and toys meant for placement in a child's mouth	2009
EU	Ban Bisphenol A in cosmetics	2009
EU, South Korea, Switzerlands, some US states	Ban or restriction of Bisphenol A in thermal paper	

INTRODUCTION

Bisphenol A (or BPA) is a molecule widely used in industry for the synthesis of polycarbonate plastics and epoxy resins, used for bottles or food and bevevage cans.

The migration of this endocrine disruptor compound from the packaging to food is the main source of consumers' exposure to Bisphenol A.

At the request of the French Health Agency, LABERCA- Oniris, a French National Reference Lab, has assessed dietary exposure to Bisphenol A in the French population by carrying out a quantitative analysis of this compound in all types of liquid and solid food matrices (publication 2014).
Nowadays, Bisphenol A remains a topical issue with an increasingly restrictive worldwide regulation. In 2024, EU has prohibited bisphenol A in all food contact materials.
As an alternative, a lot of diverse Bisphenols exist and can be used as substitute such as Bisphenol S.

ANALYTICAL METHODS

Highly sensitive and reliable analytical methods are required for routine analysis of Bisphenol A in food samples, particularly in baby food.
This booklet gathers several application notes or references of scientific articles that describe for the determination of very low concentrations of Bisphenol A in several food matrices using **AFFINIMIP® SPE Bisphenols** cartridges before detection by fluorescence, GC-MS/MS or LC-MS/MS.

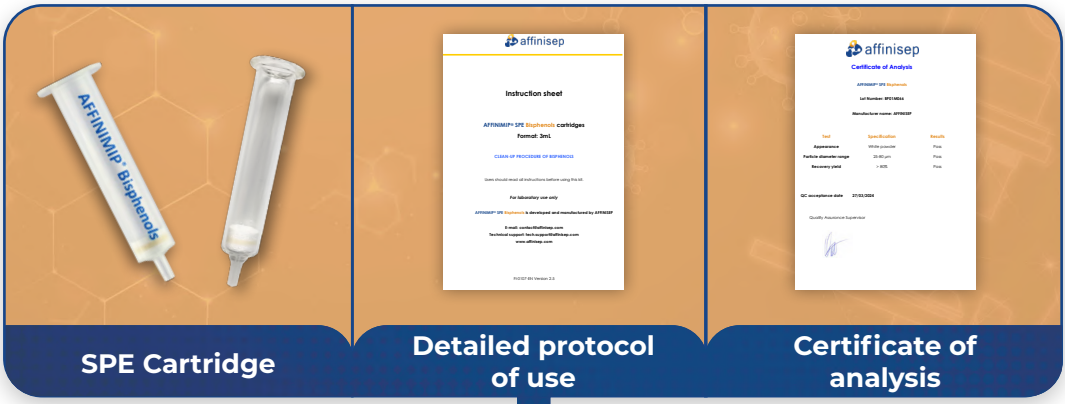
AFFINIMIP® SPE Bisphenols are suitable not only for Bisphenol A but for all Bisphenols. For instance, in 2015, LABERCA-Oniris published a peer-reviewed article for the determination of **18 Bisphenols** in human breast milk.

Formats of AFFINIMIP® SPE Bisphenols

To meet customer specifications, AFFINISEP proposes several different formats of **AFFINIMIP® SPE Bisphenols** :
•6mL Glass cartridges with PTFE frits
•6mL/ 3mL or 1mL PP plastic cartridges with PE frits
Please contact us for any other formats for high throughput analysis or for SPE automation.

BISPHENOLS KITS

- Ready-to-use kit
- Efficient for a wide variety of bisphenols
- Simple and fast process
- High capacity cartridges
- Tested on a large variety of matrices (Fish, meat, milk, canned food , fruits, beverages, ...)
- Suitable for GC-MS/MS, LC-MS/MS, LC-Fluorescence



SPE Cartridge | Detailed protocol of use | Certificate of analysis

A Ready-to-use AFFINIMIP® SPE Bisphenols kit

GENERAL PROTOCOL

EQUILIBRATION
1. 3 mL 2% acetic acid in methanol
2. 3 mL acetonitrile
3. 3 mL water

↓

Sample loading on **AFFINIMIP®SPE Bisphenols**

↓

WASHING
1. 6mL water
2. 3mL 40% acetonitrile in water
3. 3 min dry (optionnal)

↓

ELUTION: 3mL 0,2% acetic acid in methanol

↓

ANALYSIS GC-MS/MS, LC-MS/MS, LC Fluo

✓ **Simple, efficient protocol**

⏳ **Save your time**

affinisep

WHY USING A DELAY COLUMN FOR BISPHENOLS ANALYSIS?

For trace analyses (LC-MS/MS), some bisphenols are likely to be found in mobile phase or LC parts and tubing. They concentrate at the front of the LC column during runs, leading to contamination. To change LC parts and tubing and control solvents purity is very expensive, time consuming, and sometimes impossible. The other solution is the use of a delay column.

Principle of delay column

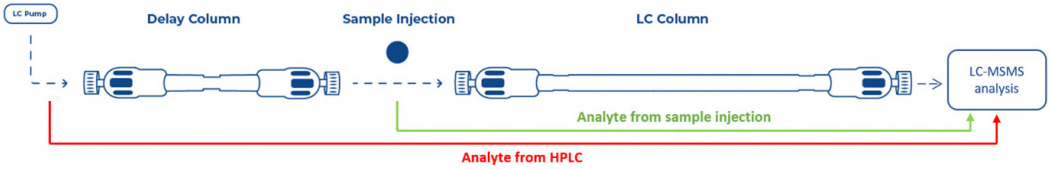


Figure 1: Installation of Delay column for LC analysis

A delay column is put between LC pumps and sample injection (Figure 1). It allows the contaminant coming from the LC device to concentrate at the front of the delay column instead of LC column, while the analyte from sample injection will concentrate directly on the LC column. This will lead to a longer retention time for the contaminant from LC device (See figure 2 for example). This solution is very easy to put in place and is cost effective.

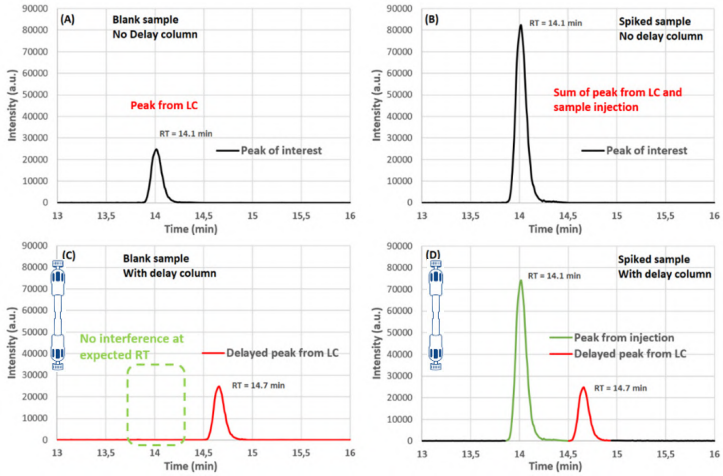


Figure 2: Effects of a delay column on samples. A: Blank sample without delay column. B: Spiked sample without delay column. C: Blank sample with delay column. D: Spiked sample with delay column.

Catalog number

- **SilactHPLC DELAY -BPA - 50x2,1mm (5µm) Delay column - 1pc** — **DELAY-BPA-50.2.1**

DETERMINATION OF 4 BISPHENOLS IN COLA DRINKS

Protocol of purification

Sample preparation

The cola drink is degassed in an ultrasonic bath for 30 minutes. The solution was tested non spiked (blank) and spiked at 50ng/Kg with the 4 bisphenols.

Purification with a 1mL AFFINIMIP® SPE Bisphenols cartridge.

EQUILIBRATION
1. 1mL 2% Acetic acid (in methanol)
2. 1mL Acetonitrile
3. 1mL Ultrapure water

LOADING
8 mL loading solution at a rate of 1mL/min

WASHING
1. 2mL Ultrapure water
2. 1mL 40% (v/v) acetonitrile (in water)

DRYING 3 minute

ELUTION
1mL 0.2% acetic acid (in methanol)
The elution fraction is evaporated to dryness and dissolved in 200µL of 25% Acetonitrile (in water) prior to analysis.

See analysis method 1 described page 27

Results

Recovery of 4 bisphenols in cola drink (n = 2). (ND = Not detected).

Matrice Spiked at 50ng/kg	C° in blank [ng/Kg]	Recoveries %
BPS	ND	77
BPF	ND	75
BPE	2.9	89
BPA	7.1	87

Catalog number

- 1mL format - 50/pk — FS106-03A
- 3mL format - 50/pk — FS106-03
- 6mL format - 50/pk (glass cartridges) — FS106-03G

affinisep

DETERMINATION OF BISPHENOL A IN BEER

Protocol of purification

Sample preparation

Degas beer using sonication for 1 hour. Adjust pH to 5–6 prior to SPE.

Purification with a 3mL AFFINIMIP® SPE Bisphenols cartridge.

EQUILIBRATION
1. 3mL Methanol -2% Acetic Acid
2. 3mL Acetonitrile
3. 3mL Water

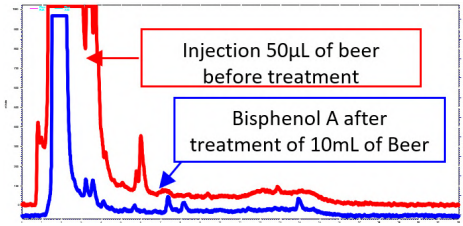
LOADING
10mL of degassed beer

WASHING
1. 9mL Water
2. 6mL Water/Acetonitrile (60/40)

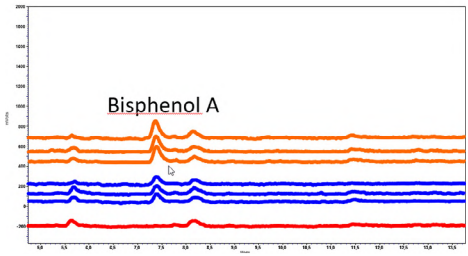
DRYING 30 seconds

ELUTION
3mL Methanol
Evaporation of elution and dissolution in mobile phase prior to analysis.

See analysis method 2 described page 27



Chromatograms containing 1µg/L of Bisphenol A before (Red) and after (Blue) Clean-up.



Chromatograms after clean-up of 10mL of beer spiked at 2µg/L(n=3, orange) or at 1 µg/L (n=3, blue) with Bisphenol A or not spiked (red)

Results

Recovery of Bisphenol A in spiked beer after clean-up and relative standard deviation:

- under repeatability conditions (n=3, % RSD_r)

C° (µg/L)	Mean µg/L	Recoveries %	% RSD_r
1.0	1.1	107	1.0
2.0	1.9	93	1.0

- under reproducibility conditions (% RSD_R)

C° (µg/L)	Mean µg/L	Recoveries %	% RSD_R
1.0	1.1	99	8.9
2.0	1.8	91	6.0

Catalog number

3mL format - 50/pk (PP cartridges)	FS106-03
6mL format - 50/pk (glass cartridges)	FS106-03G

affinisep

DETERMINATION OF BISPHENOL A IN WINE

Protocol of purification

Sample preparation

Adjust wine pH to 5–6 prior to SPE.

Purification with a 3mL AFFINIMIP® SPE Bisphenols cartridge.

EQUILIBRATION
1. 3mL Methanol -2% Acetic Acid
2. 3mL Acetonitrile
3. 3mL Water

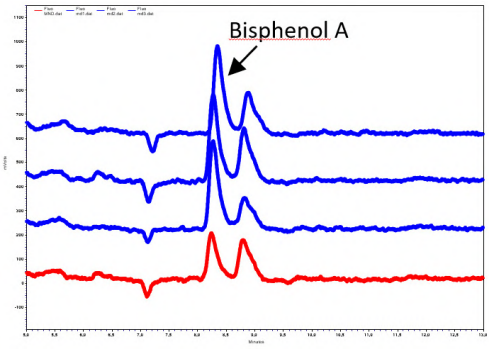
LOADING
10mL of wine

WASHING
1. 9mL Water
2. 6mL Water/Acetonitrile (60/40)

DRYING 1 minute

ELUTION
3mL Methanol
Evaporation of elution and dissolution in mobile phase prior to analysis.

See analysis method 2 described page 27



Chromatograms obtained after clean-up of 10mL of white wine spiked with Bisphenol A at 2µg/kg (n=3, blue) or not spiked (red). The white wine naturally contained 2µg/kg of BPA

Results

Recovery of Bisphenol A after 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	1.9 (n=2)	97
Red wine 2	2.1 (n=2)	106
Red wine 3	1.7 (n=2)	83
White wine	1.6 (n=3)	80

Catalog number

3mL format - 50/pk (PP cartridges)	FS106-03
6mL format - 50/pk (PP cartridges)	FS106-03B
6mL format - 50/pk (glass cartridges)	FS106-03G

affinisep

DETERMINATION OF BISPHENOL A AND BADGE IN MILK

Protocol of purification

Sample preparation

Adjust milk pH to 5–6 prior to SPE.

Purification with a 3mL or 6mL AFFINIMIP® SPE Bisphenols cartridge.

EQUILIBRATION
1. 3mL Methanol -2% Formic Acid
2. 3mL Acetonitrile
3. 3mL Water

LOADING
9mL of Milk

WASHING
1. 9mL Water
2. 6mL Water/Acetonitrile (60/40)

DRYING 30 seconds

ELUTION
1. 3mL Methanol
2. 3mL Acetonitrile

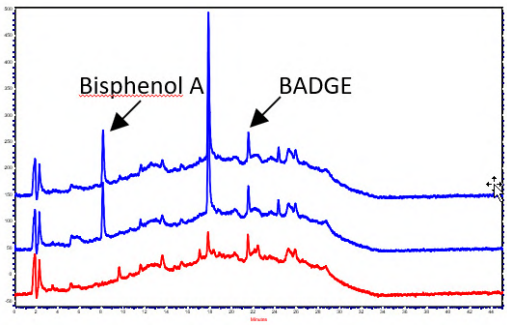
Evaporation of elutions and dissolution in mobile phase prior to analysis.

See analysis method 3 described page 27

Catalog number

3mL format - 50/pk (PP cartridges)	FS106-03
6mL format - 50/pk (PP cartridges)	FS106-03B
6mL format - 50/pk (glass cartridges)	FS106-03G

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 (n=2, 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.

Matrice Spiked at 10µg/kg	Mean concentration (µg/kg)	Recoveries %
BPA	10.8	108
BADGE	7.5	75

affinisep

DETERMINATION OF BISPHENOL A IN LIQUID INFANT FORMULA

Protocol of purification

Sample preparation

Purification with a 3mL AFFINIMIP® SPE Bisphenols cartridge.

EQUILIBRATION
1. 3mL Methanol -2% Acetic Acid
2. 3mL Acetonitrile
3. 3mL Water

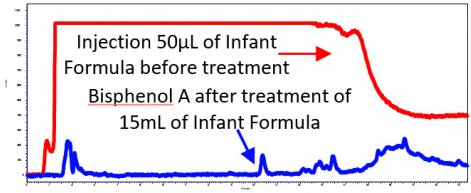
LOADING
Up to 15mL of infant formula

WASHING
1. 9mL Water
2. 6mL Water/Acetonitrile (60/40)

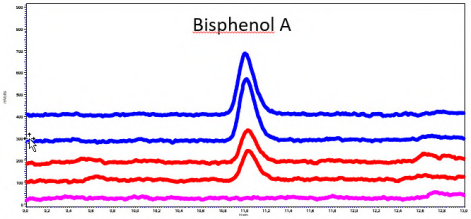
DRYING 30 seconds

ELUTION
3mL Methanol
Evaporation of elution and dissolution in mobile phase prior to analysis.

See analysis method 4 described page 28



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



Chromatograms obtained after clean-up of 15mL of Infant Formula spiked with Bisphenol A at 2µg/L (n=2, blue) or at 1µg/L (n=2, red) or not spiked (pink).

Results

Recovery of BPA in 15mL of infant formula after clean-up and relative standard deviation:

- under repeatability conditions (n=3, % RSD_r)

C° (µg/L)	Mean µg/L	Recoveries %	% RSD_r
1.0	0.9	88	1.5
2.0	1.7	86	2.7

- under reproducibility conditions (% RSD_R)

C° (µg/L)	Mean µg/L	Recoveries %	% RSD_R
1.0	0.8	84	7.4
2.0	1.7	86	5.3

Catalog number

3mL format - 50/pk (PP cartridges)	FS106-03
6mL format - 50/pk (glass cartridges)	FS106-03G

affinisep

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 20mL 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 AFFINIMIP® SPE Bisphenols cartridge.

EQUILIBRATION
1. 3mL Methanol -2% Acetic Acid
2. 3mL Acetonitrile
3. 3mL Water

LOADING
Up to 40mL of infant formula

WASHING
1. 9mL Water
2. 6mL Water/Acetonitrile (60/40)

DRYING 30 seconds

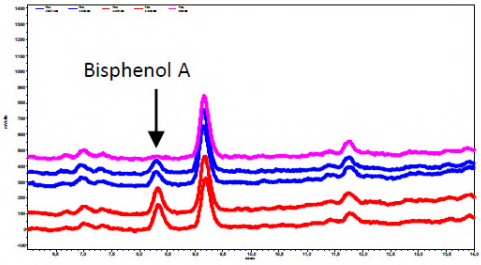
ELUTION
3mL Methanol
Evaporation of elution and dissolution in mobile phase prior to analysis.

See analysis method 4 described page 28

Catalog number

3mL format - 50/pk (PP cartridges)	FS106-03
6mL format - 50/pk (PP cartridges)	FS106-03B
6mL format - 50/pk (glass cartridges)	FS106-03G

Results



Chromatograms obtained after clean-up of equivalent at 10mL of Infant Formula spiked with Bisphenol A at 4.3µg/L (n=2, red) or at 2.1µg/L (n=2, blue) or not spiked (pink).

Recovery of Bisphenol A spiked at different concentrations after clean-up of 40mL of loading solution (equivalent to 10mL of reconstituted Infant milk) and relative standard deviation.

C° (µg/L)	Mean µg/L	Recoveries %	% RSD_r
2.1	2.3 (n=5)	108	8.7
4.3	4.0 (n=4)	95	3.7

affinisep

DETERMINATION OF BISPHENOL A IN LIQUID CANNED FOOD

Protocol of purification

Sample preparation

The liquid from canned food was filtered on filter paper to form the loading solution.

Purification with a 3mL AFFINIMIP® SPE Bisphenols cartridge.

EQUILIBRATION
1. 3mL Methanol -2% Acetic Acid
2. 3mL Acetonitrile
3. 3mL Water

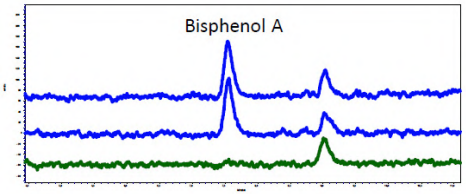
LOADING
10mL liquid from canned food

WASHING
1. 9mL Water
2. 6mL Water/Acetonitrile (60/40)

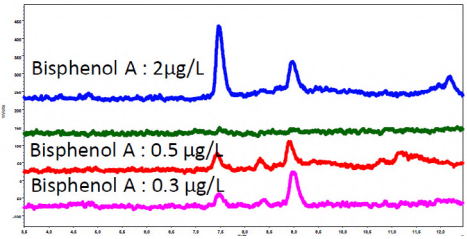
DRYING 30 seconds

ELUTION
3mL Methanol
Evaporation of elution and dissolution in mobile phase prior to analysis.

See analysis method 4 described page 28



Chromatograms after clean-up of 10mL of liquid of canned Peas and carrots spiked with BPA at 1µg/L (n=2, blue) or not spiked (green).



Chromatograms after clean-up of 10mL of canned salmon and tuna. **Blue:** 1st price canned salmon; **Green:** middle grade canned salmon: no Bisphenol A was detected; **Red:** premium canned salmon; **Pink:** canned tuna

Results

Recovery of Bisphenol A after clean-up of 10mL of canned peas and carrots (liquid) spiked at 1µg/L and relative standard deviation:

- under repeatability conditions (n = 3)

C° (µg/L)	Mean µg/L	Recoveries %	% RSD_r
1.0	1.05	105	5

- under reproducibility conditions (n = 4)

C° (µg/L)	Mean µg/L	Recoveries %	% RSD_R
1.0	1.04	104	10

Catalog number

3mL format - 50/pk (PP cartridges)	FS106-03
6mL format - 50/pk (glass cartridges)	FS106-03G

affinisep

DETERMINATION OF BISPHENOL A IN VEGETABLE CANNED FOOD

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 form the loading solution.

Purification with a 3mL AFFINIMIP® SPE Bisphenols cartridge.

EQUILIBRATION
1. 3mL Methanol -2% Acetic Acid
2. 3mL Acetonitrile
3. 3mL Water

LOADING
20mL of loading solution

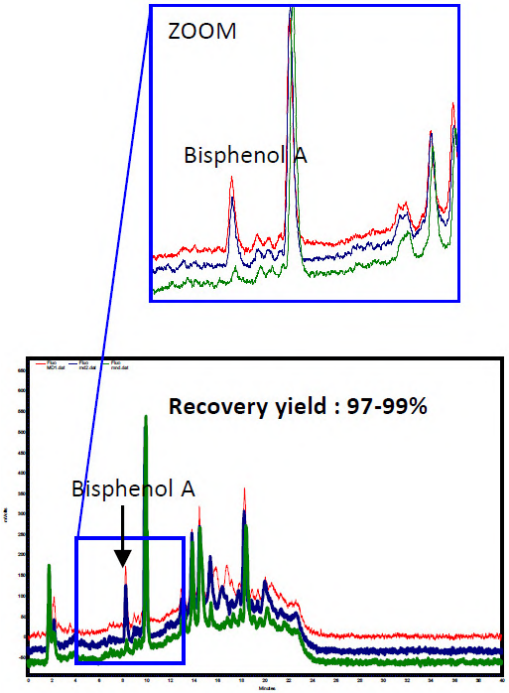
WASHING
1. 9mL Water
2. 6mL Water/Acetonitrile (60/40)

DRYING 30 seconds

ELUTION
3mL Methanol
Evaporation of elution and dissolution in mobile phase prior to analysis.

See analysis method 4 described page 28

Results



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 (n=2, blue and red) or not spiked (green).

Catalog number

3mL format - 50/pk (PP cartridges)	FS106-03
6mL format - 50/pk (PP cartridges)	FS106-03B
6mL format - 50/pk (glass cartridges)	FS106-03G

affinisep

DETERMINATION OF 5 BISPHENOLS IN CANNED ENERGY DRINKS

Data extracted from publication:
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

Protocol of purification

Sample preparation

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 AFFINIMIP® SPE Bisphenols cartridge.

EQUILIBRATION
1. 3mL Methanol -2% Acetic Acid
2. 3mL Acetonitrile
3. 3mL Water

LOADING
Loading solution

WASHING
1. 9mL Water
2. 6mL Water/Acetonitrile (60/40)

DRYING 30 seconds

ELUTION
1. 3mL Methanol
2. 3mL Acetonitrile

Evaporation of elutions and dissolution in mobile phase prior to analysis.

Catalog number

6mL format - 50/pk (PP cartridges)	FS106-03B
6mL format - 50/pk (glass cartridges)	FS106-03G

Results

Analyte	Conc (ng/mL)	Recovery (%) (n=6)	RSD_R % 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=0.50ng/mL LOD=0.15ng/mL

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 (ex/em): 275 / 305nm
Injection volume: 5µL.

affinisep

FRENCH HEALTH AGENCY REPORT ON BISPHENOL A IN ALL LIQUID AND SOLID FOOD

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:

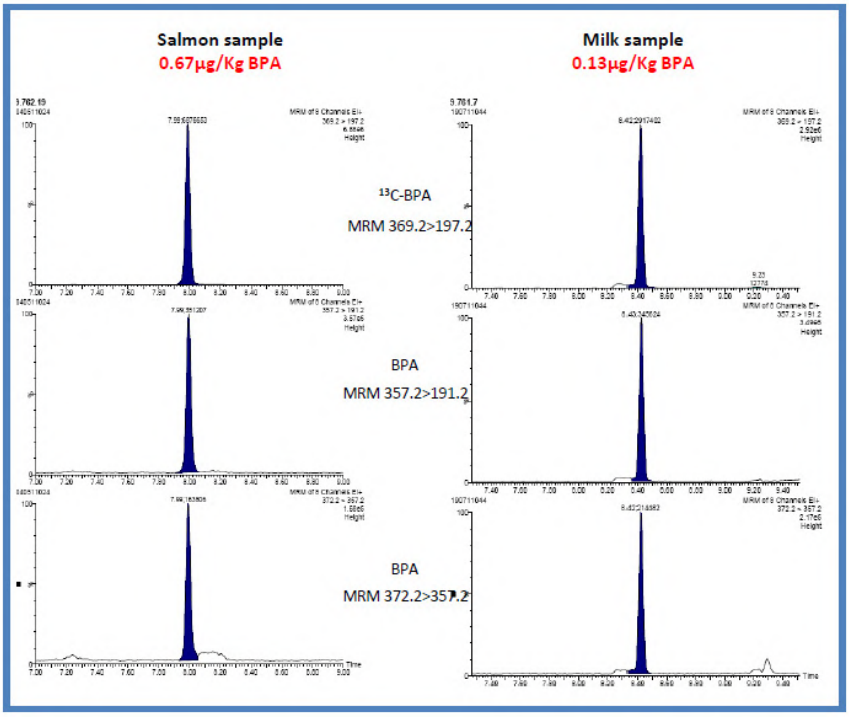
- **Cereals for breakfast, muesli, cornflakes**
- **Bread, toast, brioche, pastries, sweet and salted biscuits, cookies, pasta…**
- **Cereals: rice, wheat…**
- **Cheese: camenbert, cantal…**
- **Milk (skimmed, concentrated …), Yoghurt, cream, butter, Oils, eggs**
- **Fish: cooked fish, fried breaded fish, canned atun, steamed and smoked salmon, hake…**
- **Seafood: crustacean, oysters,mussel, shrimp…**
- **Vegetable: salad, tomatoes, radish, onion, soja, carrots, cauliflower, zucchini, peas, spinash….**
- **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…**

DETERMINATION OF BISPHENOL A BY GC-MS/MS

Data extracted from 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)

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



Catalog number

- **3mL format - 50/pk (PP cartridges)** — **FS106-03**
- **6mL format - 50/pk (PP cartridges)** — **FS106-03B**
- **6mL format - 50/pk (glass cartridges)** — **FS106-03G**

DETERMINATION OF 7 BISPHENOLS ANALOGS BY LC-MS/MS

Data extracted from publication:
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

The analysis of seven bisphenol analogues in beverage and canned food samples was performed by using **AFFINIMIP® SPE Bisphenols** prior LC–MS analysis.
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
Add 5mL of acetonitrile to 1g of canned food and sonicate for 20min then centrifuge at 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 AFFINIMIP® SPE Bisphenols cartridge.

EQUILIBRATION
1. 5mL Methanol -2% Acetic Acid
2. 5mL Acetonitrile
3. 5mL Water

LOADING
Loading solution

WASHING
1. 6mL Water
2. 3mL Water/Acetonitrile (60/40)

DRYING 30 min

WASHING
1. 2mL Acetonitrile
2. 2mL Methanol/Acetonitrile (10/90)

ELUTION
4mL 2% formic acid in methanol
Evaporation of elution and dissolution in mobile phase prior to analysis.

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

6mL format - 50/pk (PP cartridges)	FS106-03B
6mL format - 50/pk (glass cartridges)	FS106-03G

DETERMINATION OF 18 BISPHENOLS ANALOGS IN HUMAN BREAST MILK BY GC-MS/MS

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.

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
Bisohenol Cl2	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
Bisohenol 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

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.

Catalog number

3mL format - 50/pk (PP cartridge)	FS106-03
6mL format - 50/pk (PP cartridge)	FS106-03B
6mL format - 50/pk (glass cartridge)	FS106-03G

affinisep

DETERMINATION OF TOTAL BISPHENOL A IN HUMAN URINE

Data extracted from 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.

By courtesy of Nadia Diano, Dept. of Experimental Medicine, Second University of Naples (Italy)

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 AFFINIMIP® SPE Bisphenols glass cartridge.

EQUILIBRATION
1. 3mL Methanol -2% Acetic Acid
2. 3mL Acetonitrile
3. 3mL Water

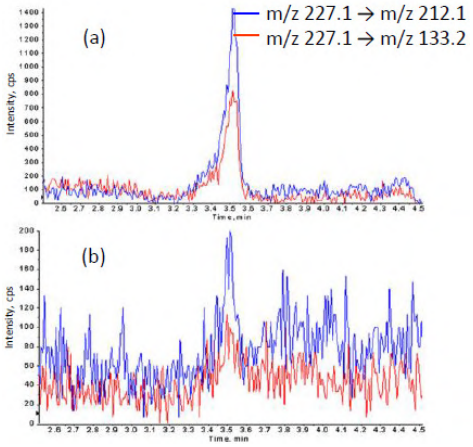
LOADING
Up to 12mL of loading solution (Equivalent to around 9mL of urine)

WASHING
1. 4mL Water
2. 4mL Water/Acetonitrile (60/40)

ELUTION
3mL Methanol
The elution was then concentrated and diluted to 1mL prior to analysis.

LC-MS/MS method

- LC Column: Kinetex 2.6µm PFP 100mm x 4.6mm.
- Mobile phase: 0-1min: 70/30 H2O/MeOH; 2min: 5/95 H2O/MeOH; 2-5min: 5/95 H2O/MeOH; 6min: 70/30 H2O/MeOH; 6-9min: 70/30 H2O/MeOH.
- Flow rate: 0.5mL/min
- Injection volume: 20µL.
- Detector: ESI-MS/MS



LC-MS/MS Chromatograms obtained after clean-up of (a) children urine at 0.38ng/mL BPA, signal to noise (S/N = 13.9) (b) blank sample (neither urine nor BPA), (S/N = 1.9)

Results

Mean recoveries of BPA spiked at different concentrations in 3mL of urine

C° (ng/mL)	1	10	100
Recoveries %	103	95	98

Catalog number

3mL format - 50/pk (PP cartridges)	FS106-03
6mL format - 50/pk (glass cartridges)	FS106-03G

affinisep

BISPHENOL A IN RIVER AND SOURCE WATER WITH ATTRACTSPE® DISKS - HLB

Data extracted from application note :
Multiresidue analysis of Pesticides and pharmaceuticals at trace levels in river water and source water using AttractSPE® Disks - HLB. Application note AN-0020-01 from Affinisep.

AttractSPE® Disks are thin, dense, soft, and uniform extraction SPE membranes allowing excellent interactions with analytes even with high flow rate without any channeling. They are available with a broad variety of sorbents such as anion or cation exchanges, SDB-XC to meet the needs of main applications requiring an enrichment of water or air samples.
AttractSPE® Disks are particularly suitable for processing large volumes in a short space of time.

Protocol of purification

Sample preparation

1000mL of river water (Le Cailly river, Le Houlme) and source water (bottled water) were spiked with a list of analytes including bisphenol A at 25ng/L.

Purification with a 47mm AttractSPE® Disks – HLB membrane

EQUILIBRATION
1. 50mL methanol
2. 25mL water

LOADING
1000mL of sample in 20 minutes

WASHING
1. 20mL water
2. Apply vacuum for 30s to dry the disk

ELUTION
1. 20mL acetonitrile
2. 20mL 1% formic acid in acetonitrile

The elution was evaporated with N2 and dissolved in mobile phase prior to analysis

See analysis method 5 described page 28

Results

Recovery of Bisphenol A after **AttractSPE® Disks - HLB** clean-up of river and source water spiked at 25ng/L.

	River water	Source water
Yield (%)	109%	96%
RSD_r (n=5)	15%	7%

Catalog number

- **AttractSPE® Disks – HLB 47mm 20/pk** — **SPE-Disks-HLB-47.T1.20**

affinisep

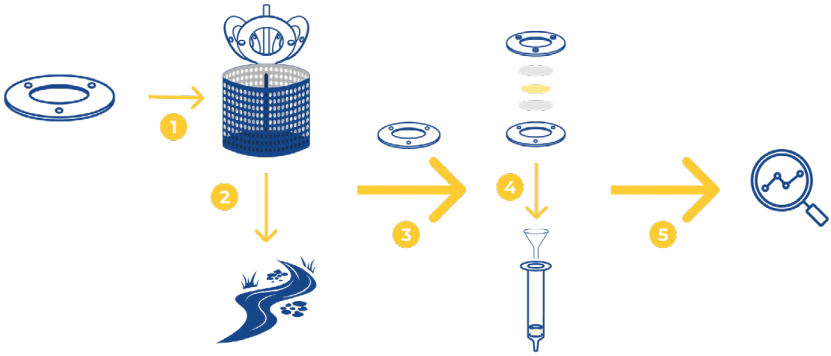
HOW TO USE POCIS?

Polar Organic Chemical Integrative Sampler (POCIS) are very common and useful passive samplers for monitoring polar organic chemicals in several aquatic environments such as freshwater, groundwater, seawater, etc...

This Integrative sampling method can adsorb an extensive range of contaminants such as endocrine disruptors, plasticizers (including bisphenol A), pharmaceuticals and personal care products (PPCPs), pesticides, fragrances, fire retardants, perfluorinated compounds, and their degradation products depending on the choice of the POCIS.

This sampler consists of a solid powder sorbent between two filtration membranes.

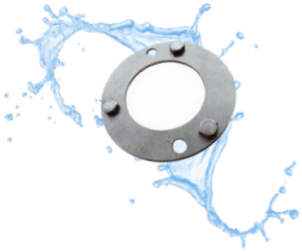
1. **POCIS is mounted on a holder inside a canister to protect from any floating object.**
2. **During the exposure period, the organic pollutants pass through the membranes and accumulate on the sorbent integratively for one or more weeks.**
3. **The POCIS is then washed and sent to the laboratory to extract the sorbent.**
4. **AFFINISEP provides empty cartridges and frits for powder transfer.**
5. **Once the sorbent is in the SPE column, contaminants can be eluted prior to an analysis by chromatography.**



Several formats are available to adapt sampling to field constraints.
The most popular product is the round POCIS (90mm diameter). However, two other narrow formats (5 cm width) are also available for groundwater monitoring.

Passive sampling of Bisphenol A in river water using AttractSPE® POCIS – EDC+

Data extracted from application note :
Passive sampling for the analysis of 12 endocrine disruptors in river water using AttractSPE® POCIS – EDC+. Application note AN-0018-04 from Affinisep.



Passive Sampling with POCIS

Polar Organic Chemical Integrative Sampler (POCIS) is a passive sampler designed to provide the time weighted average (TWA) concentration of chemicals during a sampling period of several weeks.

AttractSPE® POCIS – EDC+ enables the passive sampling of many analytes including bisphenol A

Experimental conditions:
In a 50L tank, river water (Le cailly, Le Houlme) was spiked at 5µg/L with analytes including bisphenol A and the concentrations kept constant during the experiment. The water was kept agitated with a propeller at 200 RPM.
AttractSPE® POCIS –EDC+ were then used for passive sampling using with a sampling duration from 1 to 7 days prior to extraction.

Extraction of the analyte from POCIS

WASHING
1. 5mL water
2. Apply vacuum for 1min to dry the cartridge

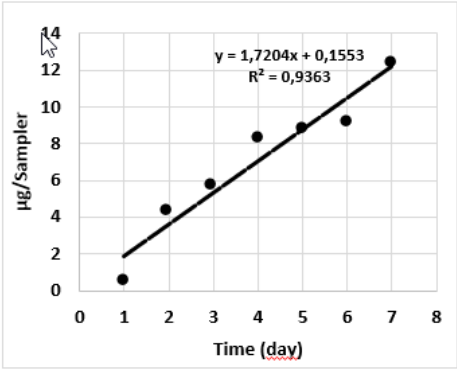
ELUTION
1. 5mL acetonitrile
2. 5mL 1% formic acid in acetonitrile

The elution was then diluted with water/ acetonitrile (90/10) prior to analysis.

See analysis method 5 described page 28

Results

Uptake curve of bisphenol A during the experiment with **AttractSPE® POCIS – EDC+.**



Laboratory sampling rate (Rs) estimation of Bisphenol A in river water

	River water
Sampling rate (L/Day)	0.486
Standard deviation (n=5)	0.087

Catalog number

- **AttractSPE® POCIS – EDC+ 10/pk** — **POCIS.EDC+.90.55.kit.10**

affinisep

Passive sampling of Bisphenol A in river water using AttractSPE® Disks Passive sampler– EDC

Data extracted from application note :
Disk based passive samplers for the analysis of 12 endocrine disruptors (EDC) in river water with AttractSPE® Disks Passive Sampler – EDC. Application note AN-0045-01 from Affinisep.

AttractSPE® Disks are thin and uniform extraction SPE membranes made of more than 90% of SPE sorbent allowing strong interactions with analytes to obtain excellent recoveries. AttractSPE® Disks, used as passive samplers, makes extraction and processing easier after the sampling step.
AttractSPE® Disks Passive Sampler – EDC enables the passive sampling of many analytes including bisphenol A.

Results

Experimental conditions:
In a 50L tank, river water (Le cailly, Le Houlme) was spiked at 5µg/L with analytes including bisphenol A and the concentrations kept constant during the experiment. The water was kept agitated with a propeller at 200 RPM. **AttractSPE® Disks Passive Sampler – EDC** were then used for passive sampling using with a sampling duration up to 7 days prior to extraction.

Extraction of the analyte from disk

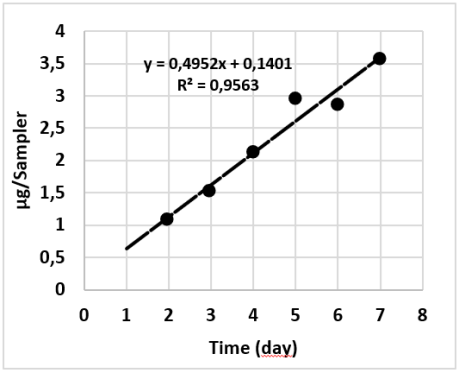
WASHING
1. 20mL water
2. Apply vacuum for 1min to dry the disk

ELUTION
1. 20mL acetonitrile
2. 20mL 1% formic acid in acetonitrile

The elution was then diluted with water/ acetonitrile (90/10) prior to analysis.

See analysis method 5 described page 28

Uptake curve of bisphenol A during the experiment with **AttractSPE® Disks Passive Sampler – EDC**.



Laboratory sampling rate (Rs) estimation of Bisphenol A in river water using **AttractSPE® Disks passive sampler – EDC**.

	River water
Sampling rate (L/Day)	0.141
Standard deviation (n=5)	0.023

Catalog number

- **AttractSPE® Disks Passive Sampler – EDC 10/pk** — **DBPS.EDC.90.40.kit.10**

ANALYTICAL CONDITIONS FOR ANALYSIS

Method 1 (LC-MS/MS) :

LC Conditions	MS/MS Conditions
LC Dionex U3000	Qtrap 4000 ESI- MS/MS
Column : Hypersil Gold 50*2.1mm at 30°C Delay column : SilactHPLC Delay-BPA 50*2.1mm	Curtain gas: 30
	CAD: High
Injection volume : 20µL	IS: -4500V
T° sampler : 10°C	Temperature: 600°C
Flow rate : 0.25mL/min	GS1/GS2: 30/30

Time (min)	Water	Acetonitrile	Analyte	Retention time (min)	Q1	Q3	CE (V)
0	75%	25%	BPS	1.6	248.9	107.9	-38
1	75%	25%			248.9	92.0	-46
3	0%	100%	BPF	3.3	199.0	92.8	-30
8	0%	100%			199.0	104.9	-32
8.5	75%	25%	BPE	4.1	212.9	197.8	-24
13	75%	25%			212.9	197.0	-38
			BPA	4.3	227.2	211.9	-26
					227.2	132.9	-36

Method 2 (LC-Fluo) :

HPLC	ThermoFinnigan Spectra System	HPLC gradient		
Flow rate	1mL/min	Time (min)	% water	% Acetonitrile
Column	Hypersil Gold C18 150 x 4.6 mm (3 µm)	0	65	35
Injection volume	50 µL	2	65	35
Detector	Jasco FP-2020 with Fluorescence detector	12	50	50
Wavelength	230 nm/315 nm (ex/em)	12.5	65	35
		22	65	35

Method 3 (LC-Fluo) :

HPLC	ThermoFinnigan Spectra System	HPLC gradient		
Flow rate	1mL/min	Time (min)	% water	% Acetonitrile
Column	Hypersil Gold C18 150 x 4.6 mm (3 µm)	0	65	35
Injection volume	50 µL	2	65	35
Detector	Jasco FP-2020 with Fluorescence detector	12	50	50
Wavelength	230 nm/315 nm (ex/em)	20	20	80
		25	20	80
		30	65	35
		40	65	35

ANALYTICAL CONDITIONS FOR ANALYSIS

Method 4 (LC-Fluo) :

		HPLC gradient		
HPLC	ThermoFinnigan Spectra System			
Flow rate	1mL/min	**Time (min)**	**% water**	**% Acetonitrile**
Column	Hypersil Gold C18 150 x 4.6 mm (3 µm)	0	65	35
Injection volume	50 µL	2	65	35
Detector	Jasco FP-2020 with Fluorescence detector	12	50	50
Wavelength	230 nm/315 nm (ex/em)	20	50	50
		20.5	65	35
		35	65	35

Method 5 (LC-MS/MS) :

LC Conditions				MS/MS Conditions
LC Dionex U3000	Gradient			Qtrap 4000 ESI- MS/MS
Column : Hypersil Gold 150*2.1mm at 30°C	**Time (min)**	**H2O + 0.05%NH3**	**Methanol + 0.05% NH3**	Curtain gas: 20
Delay column : Hypersil Gold 50*2.1mm	0	90%	10%	CAD: High
Injection volume : 20µL	1	90%	10%	IS: -4500V
T° sampler : 10°C	10	10%	90%	Temperature: 650°C
Flow rate : 0.5mL/min	15	10%	90%	GS1/GS2: 50/50
	16	90%	10%	
	21	90%	10%	

Analyte	Retention time (min)	Q1(m/z)	Q3(m/z)	CE(V)
Bisphenol A	12.4	227.2	211.9	-26
		227.2	132.9	-36

PUBLICATIONS AND POSTERS

Bisphenol A and Bisphenol S Induce Endocrine and Chromosomal Alterations in Brown Trout, Frenzilli G, Martorell-Ribera J, Bernardeschi M, Scarcelli V, Jönsson E, Diano N, Moggio M, Guidi P, Sturve J and Asker N; *Front. Endocrinol*. 12:645519. (2021) ; **OPEN ACCESS**

A rapid analysis of bisphenol A using MISPE coupled with HPLC-FLD in tissues of food-producing animals, Cerkvenik-Flajs V., Sturm S., *MethodsX* 8, 101351 (2021). **OPEN ACCESS.**

A New LC-MS/MS Method for Simultaneous and Quantitative Detection of Bisphenol-A and Steroids in Target Tissues: A Power Tool to Characterize the Interference of Bisphenol-A Exposure on Steroid Levels. Errico S., Chioccarelli T., Moggio M., Diano N., Cobellis G., *Molecules 25*, 48 (2020). **OPEN ACCESS.**

A novel experimental approach for liver analysis in rats exposed to Bisphenol A by means of LC-mass spectrometry and infrared spectroscopy, , Errico S., Portaccio M., Nicolucci C., Meccariello R., Chianese R., Scafuro M., Lepore M., Diano N., *J. Pharm. Biomed. Anal*. 165, 207 (2019)

Determination of free and total bisphenol A in the urine and feces of orally and subcutaneously dosed sheep by high-performance liquid chromatography with fluorescence detection, Šturm S., Škibin A., Pogačnik M., Cerkvenik-Flajs V., J. *Environ. Sci. Health B 55(7)*, 655 (2020).

Analysis and Occurrence of Bisphenol A in Mediterranean Mussels (Mytilus galloprovincialis) Sampled from the Slovenian Coastal Waters of the North Adriatic Sea, Cerkvenik-Flajs V., Fonda I. & Gombač M, *Bull Environ Contam Toxicol* 101, 439 (2018).

Analysis and occurrence of some phenol endocrine disruptors in two marine sites of the northern coast of Sicily (Italy), Errico S., Nicolucci C., Migliaccio M., Micale V., Mita D. G., Diano N., *Marine Pollution*, 120 (1-2), 68 (2017).

PUBLICATIONS AND POSTERS

Determination of BPA, BPB, BPF, BADGE and BFDGE in canned energy drinks by molecularly imprinted polymer cleaning up and UPLC with fluorescence detection, Gallo P., Di Marco Pisciottano I., Esposito F., Fasano E., Scognamiglio G., Damiano Mita G., Cirillo T., *Food Chemistry*, 220, 406, (2017).

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

Determination of bisphenol A and related substitutes/analogues in human breast milk using gas chromatography-tandem mass spectrometry, Deceuninck Y., Bichon E., Marchand P., Boquien C.-Y., Legrand A., Boscher C., Antignac J. P., Le Bizec B., *Anal. and Bioanal. Chem*., 407 (9), 2485 (2015).

Molecularly imprinted solid-phase extraction for the selective extraction of bisphenol analogues in beverages and canned food, Yang Y., Yu J., Yin J., Shao B., Zhang J., *J. Agric. Food Chem*., 62 (46), 11130 (2014).

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, Deceuninck Y., Bichon E., Durand S., Bemrah N., Zendong Z., Morvan M.L., Marchand P., Dervilly-Pinel G., Antignac J.P., Leblanc J.C., Le Bizec B., *J. Chrom. A*, 1362, 241 (2014).

Assessment of dietary exposure to bisphenol A in the French population with a special focus on risk characterisation for pregnant French women, Bemrah N., Jean J., Rivière G., Sanaa M., Leconte S., Bachelot M., Deceuninck Y., Le Bizec B., Dauchy X., Roudot A-C., Camel V., Grob K., Feidt C., Picard-Hagen N., Badot P-M., Foures F., Leblanc J-C., *Food Chem. Toxicol*. 72, 90 (2014).

A high selective and sensitive liquid chromatography-tandem mass spectrometry method for quantization of BPA urinary levels in children, Nicolucci C., Rossi S., Menale C., Giudice E., Miraglia del Giudice P., Perrone L., Gallo P., Mita D., Diano N., *Anal. Bioanal. Chem.*, 1618 (2013) (use of glass cartridges).

SPE – Product list

Designation	Definition	Reference	Nber of units
SPE columns			
AFFINIMIP® SPE Bisphenols	1mL SPE cartridge for bisphenols analysis	FS106-03A	50
	3mL SPE cartridge for bisphenols analysis	FS106-03	50
	6mL SPE cartridge for bisphenols analysis	FS106-03B	50
	6mL SPE glass cartridge for bisphenols analysis	FS106-03G	50
SPE disks and passive samplers			
AttractSPE® Disks - HLB	47mm SPE disk for analysis of bisphenols	SPE-Disks-HLB-47.T1.20	20
AttractSPE® POCIS – EDC+	Passive sampler based on POCIS for the uptake of Bisphenols	POCIS.EDC+.90.55.kit.10	10
AttractSPE® Disks Passive Sampler – EDC	Passive sampler based on SPE disks for the uptake of Bisphenols	DBPS.EDC.90.40.kit.10	10

SPE ACCESSORIES – Product list

Designation	Definition	Reference	Nber of units
SPE columns			
Manifold	SPE Vacuum manifold 12 port model for SPE cartridges	ACC-MAN2	1
SPE Adapter & Reservoir kit	Kit of 12 reservoirs 60mL and adapters for use with 1, 3 & 6 mL cartridges	ACC-AR1	1
Trap 1L	1l trap - 1 unit	ACC-TRAP-1L	1
AttractSPE® N2 Heat Evaporator 12	Nitrogen Evaporator concentrator with dry heater - 1 unit 12 needles - require a block for test tubes (not supplied) Heat : +5°C - 160°C Voltage: 220V - 50/60Hz	ACC-EVN2H-12.0	1
SPE disks			
SPE Disk manifold	SPE disks manifold - 3 stations - for 47 mm SPE disks - glass support with PTFE grid	ACC-DISKSPE-G47-3	1
Trap 4L	4L trap kit + hose (2x1m)	ACC-TRAP-4L	1
AttractSPE® N2 Heat Evaporator 4	Nitrogen Evaporator concentrator with dry heater - 1 unit 4 needles - supplied with a block for test tubes - 28mm Heat : +5°C - 160°C Voltage: 220V - 50/60Hz	ACC-EVN2H-4.BK28	1

affinisep

ABOUT AFFINISEP

Affinisep develops and manufactures in France (Normandy) various kits for passive sampling and sample preparation dedicated to the development of analytical applications in various fields such as water monitoring, food safety and quality control, proteomics, metabolomics and genomics.

Brands	Applications	Matrices
AFFINIMIP® AttractSPE® SilactSPE BioSPE®	Sample Preparation Passive Sampling Solid Phase Extraction Filtration Microextraction	Food, Feed, Soil, Oil, Water, Biological fluids, Proteolytic digest

www.affinisep.com