



**AFFINIMIP<sup>®</sup> SPE**

**Estrogens**

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Natural and synthetic estrogens are known to be growth promoters for livestock. So, Diethylstilbestrol (DES), a synthetic estrogens was used at a large scale before being prohibited on USA at the end of the 70's.

17 $\beta$ -Estradiol, a natural estrogen is naturally present in cattle plasma at very low concentration. Calves' plasma contains ca. 1-2 pg/mL and plasma of adult animals approximately 1-5 pg/mL. Only in plasma of pregnant cows, concentrations ranging from 100 to 1000 pg/mL are found.

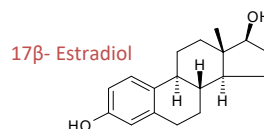
In 1989, the European Community issued a ban on all meat from animals treated with steroid growth hormones (including Estradiol). Several directives were edicted and amended to obtain a permanent prohibition of 17 $\beta$ -Estradiol and ester-like derivatives as growth promoter use and to regulate their use for therapeutic or zootechnical purposes. (Directive 96/22/EC, Directive 2003/74/EC and Directive 2008/97/EC).

In order to protect public health, maximum residue limits (MRLs) of veterinary medical products in foodstuffs of animal origin (liver, milk, egg, kidney, muscle, fat, etc.) have been established according to European Union regulation (2377/90/CEE).

In the EU currently a MRL of 40 pg/mL of Estradiol is valid for biological fluids such as blood, urine and serum.

Estrogens are a group of compounds which play an important role in the estrous cycle. They are either natural (Estrone, Estriol, 17 $\alpha$ - and 17 $\beta$ -Estradiol) or synthetic compounds (17 $\alpha$ -EthinylEstradiol, Dienestrol, Diethylstilbestrol).

Estrogens play a key role in developmental and reproductive functions. They also affect a diversity of biological processes involved in coronary artery disease, immunocompetence and cancer susceptibility. When they are present in wastewater, these endocrine disrupting chemicals (EDC) have adverse effects on endocrine systems of human beings and animals.



In addition, because of their anabolic effects, estrogens have been used in animal fattening. Steroid hormones are used in animal fattening because of their capacity to increase weight gain and to reduce the feed conversion ratio which is the average feed intake in relation to the weight gain. For several years now, the use of anabolic steroids in animal fattening is prohibited in the European Community because of their possible toxic effects on public health (96/22/EC). Nevertheless, they are still offered on the 'black' market for animal fattening purposes.

AFFINIMIP® SPE Estrogens are selective solid phase extraction cartridges that selectively clean and concentrate natural and synthetic Estrogens compounds from complex matrices such as Water, Plasma or Serum prior to analysis by HPLC.

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.



**Catalog number:**

PH100-02 for 25 cartridges, 3mL

PH100-03 for 50 cartridges, 3mL

PH100-1.96W for 1 96-well plate

- Extraction of a broad family of natural and synthetic estrogens by direct percolation of diluted plasma or water
- Fast and simple protocol

## **Lower Cost**

- Lower solvent consumption
- Lower reagent consumption
- Less apparatus

## **Faster Protocol**

- Fewer steps

## **Greater Safety**

- Less exposure to toxic agents

## **Greater Accuracy**

- No cross contamination

## **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**

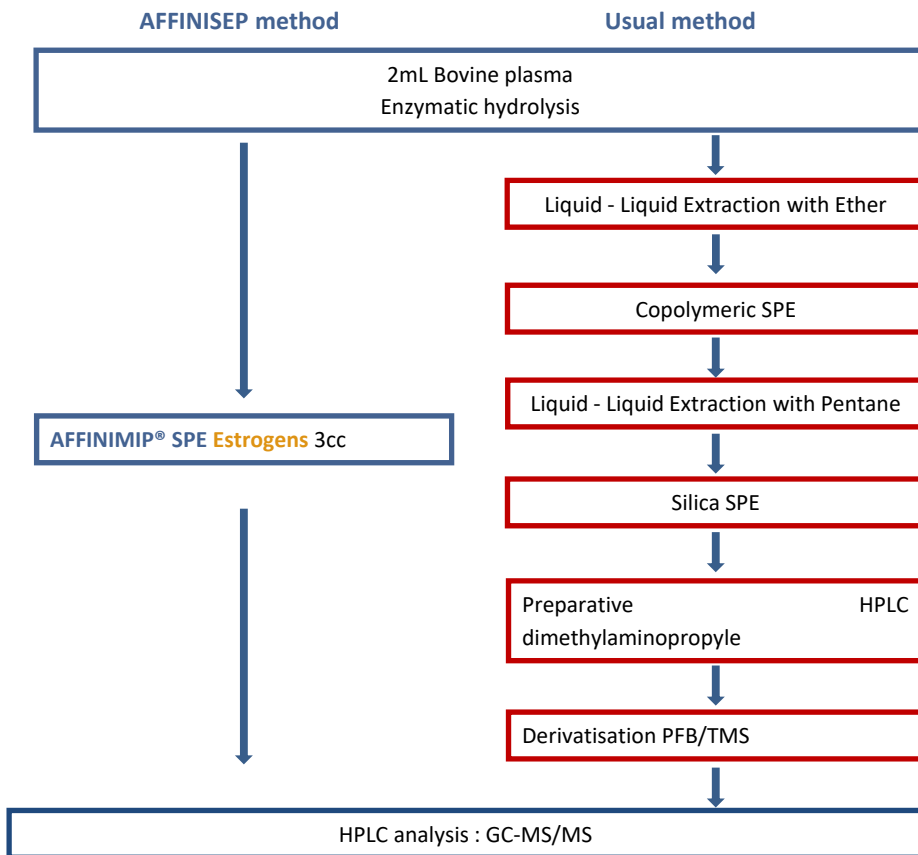
- Less glassware used, less to wash

## **Easy to use with SPE automate**

Compatible with the SPE automate

## **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



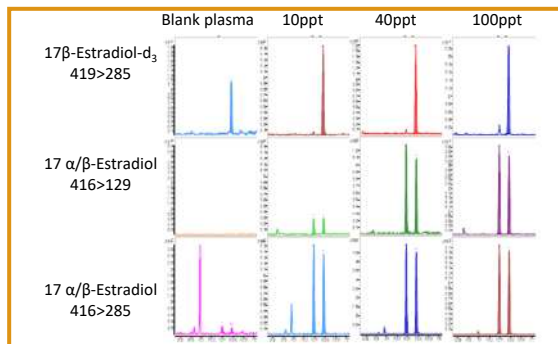
**Performance. Save your time.**

\*Data extracted from Quantification of estrogens at ppt levels in bovine plasma by Molecularly Imprinted Solid Phase Extraction and GC-MS/MS analysis, Emmanuelle Bichon *et al.* (LABERCA) Poster session, HTSP-2 and HTC 2012

## Application notes

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## RESULTS



MRM chromatograms from GC-MS/MS analysis of fortified calves' plasma samples at 0, 10, 40 and 100  $\mu\text{g}\cdot\text{mL}^{-1}$  with 17 $\alpha$ -estradiol, 17 $\beta$ -estradiol and estrone. Chromatograms obtained after a clean-up with AFFINIMIP® SPE Estrogens (Courtesy of Emmanuelle Bichon - LABERCA)

## PROTOCOL OF PURIFICATION

### Sample preparation

2mL serum samples spiked with 40pg 17 $\beta$ -Estradiol-d<sub>3</sub>. Then 2mL of Acetate buffer (0.8M, pH 6.8) and 100 $\mu\text{L}$   $\beta$ -glucuronidase were added. Hydrolysis performed overnight at 37°C and samples centrifuged at 4000 rpm for 10min. Upper layer was used as loading solution.

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

### Equilibration

- 4mL Methanol
- 4mL Acetonitrile
- 4mL Water

### Loading solution from sample preparation

### Washing of interferences

- 5mL Water
- 5mL Water/Acetonitrile (60/40)

### Elution (E)

3mL Methanol

The elution fraction was then evaporated and estrogens were derivatised 40min at 60°C with BSTFA before GC-MS/MS analysis.

### GC-MS/MS Analysis

Column: RTX-1614 Resteck 15m x 0.25mm x 0.10 $\mu\text{m}$   
 Gradient temperature: 80 to 320°C (15°C/min)

Data extracted from **Quantification of estrogens at ppt levels in bovine plasma by Molecularly Imprinted Solid Phase Extraction and GC-MS/MS analysis**, Emmanuelle Bichon *et al.* (LABERCA) Poster session, HTSP-2 and HTC 2012



## PROTOCOL OF PURIFICATION

Sample preparation  
100mL of river water were filtered through 0.45µm cellulose filter to obtain the loading solution.

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

### Equilibration

- 5mL Acetonitrile
- 5mL Water

### Loading solution from sample preparation

#### Washing of interferents

- 4mL Water/Acetonitrile (80/20)
- 2mL Water

### Drying under vacuum during 5min

#### Washing of interferents

- 2mL Acetonitrile
- 2mL Methanol/Acetonitrile (5/95)

### Elution (E)

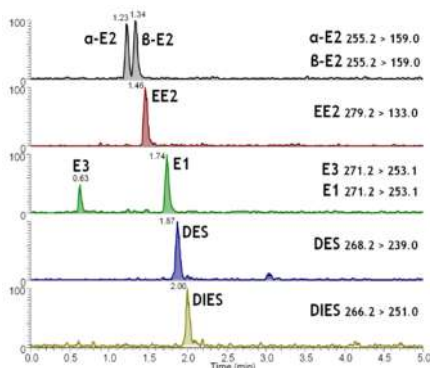
- 3mL Methanol

The elution fraction was then evaporated and reconstituted in 500µL of UHPLC.

## LC-MS Analysis

Column: Ascentis Express Phenyl-Hexyl  
150mmx2.1mm, 2.7µm  
Column Temperature: 35°C  
Mobile phase: Water/Acetonitrile/Methanol (51/44/5) at 450µL/min

## RESULTS



SRM Chromatograms of Estrogens extracted from 100 mL river water spiked at 100 ng L<sup>-1</sup> (Courtesy of P. Lucci, University of Barcelona, SPAIN)

## Recovery yield in river water

Matrix	Recoveries %
Estrone (E1)	89
17α-Estradiol (α-E2)	101
17β-Estradiol (β-E2)	93
Estriol (E3)	82
17α- Ethynilestradiol (EE2)	100

### Publications

Data extracted from Solid-phase extraction using molecularly imprinted polymer for selective extraction of natural and synthetic estrogens from aqueous samples, Paolo Lucci, Oscar Núñez, M.T. Galceran, *Journal of Chromatography A*, 1218(30), 4828-4833, 2011

# DETERMINATION OF ESTROGENS IN SEA URCHIN BODY FLUID SAMPLE

**Molecules analyzed : Estradiol, estrone, estriol, 17 $\alpha$ -Estradiol ( $\alpha$ -E2)**

## PROTOCOL OF PURIFICATION

### Sample preparation

40 mL of coelomic fluid were spiked with the internal standard estradiol-d2 to the final concentration of 10 ng ml<sup>-1</sup> and centrifuged to obtain the loading solution.

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

### Equilibration

- 3mL Acetonitrile
- 3mL Water

### Loading solution from sample preparation

#### Washing of interferents

- 3mL water
- 3mL Water/Acetonitrile (60/40)

### Elution (E)

3mL Methanol

The elution fraction was evaporated until dryness under nitrogen before derivatization with 100  $\mu$ l of dansyl chloride (1mg ml<sup>-1</sup> in acetone) and 100  $\mu$ l of 0,1 M sodium bicarbonate in water, heated at 60°C for 3 minutes. The derivatized extract was reconstituted in 1mL of methanol:water (70:30 v/v).

## LC-MS/MS Analysis

Column: Synergi Hydro RP (150mmx2.0 mm, 4 $\mu$ m)

Column Temperature: 30°C

Injection volume: 10 $\mu$ L

Flow rate: 0.3mL/min

Detection : LC-MS/MS ESI+

Mobile phase: gradient

Time (min)	% Methanol	% Water-0.1% acid formic
0	70	30
1	70	30
10	95	5
12	5	95
14	70	30
20	70	30

## Publications

Data extracted from

**Unraveling estradiol metabolism and involvement in the reproductive cycle of non-vertebrate animals: the sea urchin model.** S. Mercurio, P. Tremolada, M. Nobile, D. Fernandes, C. Porte, M. Sugni, Steroids, vol. 104, 25-36, 2015

## PROTOCOL OF PURIFICATION

### Sample preparation

100mL of tap water spiked with  $17\beta$ -E<sub>2</sub>-d<sub>3</sub> to a final concentration of 75ng/L was the loading solution.

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

**SPE Estrogens cartridge**

### Equilibration

- 3mL Acetonitrile
- 3mL Water

### Loading solution from sample preparation

### Washing of interferences

- 3mL water
- 3mL Water/Acetonitrile (60/40)

### Elution (E)

3mL Methanol

The elution fraction was then evaporated to dryness under a stream of nitrogen. Residues was treated with 10  $\mu$ L of a mixture containing BSTFA +1 % TMCS and 8  $\mu$ L of pyridine (dried with solid KOH). After a vortex stirring, derivatisation was performed for 30 min at 55 °C. The derivatives were cooled to room temperature, 2- $\mu$ L aliquots of the recovery standard (pyrene-d10) were added to each vial and the samples were subjected to GC-MS analysis.

## GC-MS/MS Analysis

Column: Rtx-5 fused silica capillary columns (30 m, 0.25-mm ID, 0.25- $\mu$ m film thickness)

Gas carrier: Helium at a flow 1.2mL/min

Injection temperature: 50 to 300 °C at 100 °C/min, held at 300 °C for 10 min

GC-MS transfer line temperature: 280°C

Temperature program: 100°C during 2min; 10°C/min to 265°C; 265°C during 2min ; 10°C/min to 300°C; 300°C during 3 min ; 20°C/min to 310°C; 310°C during 3min

Injection volume: 5 $\mu$ L

Detector : GC-MS/MS EI<sup>+</sup> mode

Detection mode: Selected reaction monitoring (SRM)

## RESULTS

Method validation for  $17\beta$ -E<sub>2</sub> and  $17\alpha$ -EE<sub>2</sub> by GC-MS/MS

	$17\beta$ -E <sub>2</sub>	$17\alpha$ -EE <sub>2</sub>
Linearity range, ng/ L	0.08-80.0	0.08-80.0
Linearity (R <sup>2</sup> )	0.995	0.9998
m-LOQ, ng/L	0.08	0.08
Spiking level ng/L (n=5)	4	4
Recovery %	111	104
Precision (n=5)	6.2	6.8
Spiking level ng/L (n=5)	20	20
Recovery %	108	110
Precision (n=5)	9.7	15.3

### Publications

Data extracted from **Determination of steroidal estrogens in tap water samples using solid-phase extraction on a molecularly imprinted polymer sorbent and quantification with gas chromatography-mass spectrometry (GC-MS)**, D. Zacs, I. Perkons, V. Bartkevics, *Environ Monit Assess* 188, 433, 2016.

## PROTOCOL OF PURIFICATION

### Sample preparation

100mL of tap water spiked with  $17\beta$ -E<sub>2</sub>-d<sub>3</sub> to a final concentration of 75ng/L was the loading solution.

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

### Equilibration

- 3mL Acetonitrile
- 3mL Water

### Loading solution from sample preparation

### Washing of interferences

- 3mL water
- 3mL Water/Acetonitrile (60/40)

### Elution (E)

3mL Methanol

The elution fraction was then evaporated to dryness under a stream of nitrogen. Residues was treated with 10  $\mu$ L of a mixture containing BSTFA +1 % TMCS and 8  $\mu$ L of pyridine (dried with solid KOH). After a vortex stirring, derivatisation was performed for 30 min at 55 °C. The derivatives were cooled to room temperature, 2- $\mu$ L aliquots of the recovery standard (pyrene-d10) were added to each vial and the samples were subjected to GC-MS analysis.

## GC-HRMS Analysis

Column: Rtx-5 fused silica capillary columns (30 m, 0.25-mm ID, 0.25- $\mu$ m film thickness)

Gas carrier: Helium at a flow 1.2mL/min

Injection temperature: 260°C

GC-MS transfer line temperature: 280°C

Temperature program: 100°C during 2min; 10°C/min to 265°C; 265°C during 2min ; 10°C/min to 300°C; 300°C during 3 min ; 20°C/min to 310°C; 310°C during 3min

Injection volume: 1 $\mu$ L

Detector : GC-MS/MS EI<sup>+</sup> mode

Detection mode: Selected Ion Recording (SIR)

## RESULTS

Method validation for  $17\beta$ -E<sub>2</sub> and  $17\alpha$ -EE<sub>2</sub> by GC-MS/MS

	$17\beta$ -E <sub>2</sub>	$17\alpha$ -EE <sub>2</sub>
Linearity range, ng/ L	0.08-80.0	0.08-80.0
Linearity (R <sup>2</sup> )	0.9990	0.9990
m-LOQ, ng/L	0.08	0.08
Spiking level ng/L (n=5)	4	4
Recovery %	113	111
Precision (n=5)	4.6	5.4
Spiking level ng/L (n=5)	20	20
Recovery %	99	106
Precision (n=5)	4.3	14.3

### Publications

Data extracted from **Determination of steroidal oestrogens in tap water samples using solid-phase extraction on a molecularly imprinted polymer sorbent and quantification with gas chromatography-mass spectrometry (GC-MS)**, D. Zacs, I. Perkons, V. Bartkevics, *Environ Monit Assess* 188, 433, 2016.

# 13 ESTROGENIC EDCs FROM BIOLOGICAL FLUIDS BY UHPLC-MS/MS WITH AFFINIMIP®SPE PHENOLICS



The analysis of 13 analytes: estrone (E1), 17 $\alpha$ -estradiol ( $\alpha$ -E2), 17 $\beta$ -estradiol ( $\beta$ -E2), estriol (E3), 17 $\alpha$ -ethinyloestradiol (EE2), diethylstilbestrol (DES), bisphenol A (BPA), bisphenol S (BPS), 4-n-octylphenol (OP), 4-n-nonylphenol (NP), coumestrol (COU), genistein (GEN), and enterolactone (ENT) was performed by using **AFFINIMIP® SPE PHENOLICS** prior UHPLC–MS /MS analysis.

## PROTOCOL OF PURIFICATION

### Sample preparation

100mL of tap water spiked with 17 $\beta$ -E2-d<sub>3</sub> to a final concentration of 75ng/L was the loading solution.

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

### Equilibration

- 3mL Methanol – Formic acid (98/2)
- 3mL Acetonitrile
- 3mL Water

### Loading solution from sample preparation

### Washing of interferents

- 4mL water
- 5mL Water/Acetonitrile (80/20)

### Elution (E)

- 10mL Methanol
- 10mL Methanol – Acetic acid (98/2)

Elution solution was evaporated to dryness, reconstituted in 50  $\mu$ L of dansyl chloride solution and held at 30 °C for 30 min. After further evaporation, a liquid/liquid extraction with 1.5 mL hexane and 1.5 mL H<sub>2</sub>O was performed. The organic solvent containing dansyl derivatives was collected and evaporated to dryness; the final residue was reconstituted in 50  $\mu$ L ACN/ H<sub>2</sub>O (50:50, v/v).

### Analysis by UPLC-MS/MS Analysis

Column: Accucore phenyl-hexyl (100mmx2.1 mm, 2.6 $\mu$ m)

Column Temperature: 50°C

Injection volume: 5 $\mu$ L

Flow rate: 0.5mL/min

Detection : LC-MS/MS ESI+ mode

Detection mode: Selected reaction monitoring (SRM)

Mobile phase: gradient

Time (min)	% Water-0.1% acid acetic	% Acetonitrile-0.1% acid acetic
0	70	30
1	70	30
4	40	60
5	40	60
7	0	100
8	0	100

## RESULTS

Validation parameters in ultra-pure water: recoveries, reproducibility, reproducibility and quantification limits

Analyte	Recovery (%) (n=2)	RSD <sub>R</sub> % n=10 $\mu$ g/L	LOQ (ng/g)
E1	107.6	10.8	0.07
$\alpha$ -E2	124.2	7.9	0.30
E3	121.6	60.2	2.7
$\beta$ -E2	107.4	7.3	0.20
EE2	106.7	6.6	0.10
OP	51.8	Nd	0.07
NP	45.1	Nd	0.15
BPA	104.6	9.8	0.04
BPS	129.6	36.2	0.07
COU	235.7	15.9	0.10
ENT	253.1	73.1	0.10
GEN	208.4	47.7	0.25
DES	109.5	38.7	0.04

### Publications

Data extracted from **Development of a molecular recognition based approach for multi-residue extraction of estrogenic endocrine disruptors from biological fluids coupled to liquid chromatography-tandem mass spectrometry measurement**, R. Bousoumah, J. P. Antignac, V. Camel, M. Grimaldi, P. Balaguer, F. Courant, E. Bichon, M.-L. Morvan, B. Le Bizec, *Anal Bioanal Chem*, 407: 8713, 2015.

- Determination of steroidal oestrogens in tap water samples using solid-phase extraction on a molecularly imprinted polymer sorbent and quantification with gas chromatography-mass spectrometry (GC-MS), D. Zacs, I. Perkons, V. Bartkevics, *Environ Monit Assess* 188, 433, 2016. doi:10.1007/s10661-016-5435-8
- Unraveling estradiol metabolism and involvement in the reproductive cycle of non-vertebrate animals: the sea urchin model. S. Mercurio, P. Tremolada, M. Nobile, D. Fernandes, C. Porte, M. Sugni, *Steroids*, vol. 104, 25-36, 2015.
- On-line molecularly imprinted solid-phase extraction coupled to liquid chromatography-tandem mass spectrometry for the determination of hormones in water and sediment samples, D. Matějček, J. Vlček, A. Burešová, P. Pelcová, *J. Sep. Sci.*, 36 (9-10), 1509-1515, 2013.
- The use of molecularly imprinted polymers for the multicomponent determination of endocrine-disrupting compounds in water and sediment, D. Matějček, A. Grycová, J. Vlček, *J. Sep. Sci.*, 36(6), 1097-1103, 2013.
- Molecularly imprinted polymer applied to the selective isolation of urinary steroid hormones: An efficient tool in the control of natural steroid hormones abuse in cattle, M. Doué, E. Bichon, G. Dervilly-Pinel, V. Pichon, F. Chapuis-Hugon, E. Lesellier, C. West, F. Monteau, B. Le Bizec, *J. Chrom A*, 1270, 51-56, 2012.
- Solid-phase extraction using molecularly imprinted polymer for selective extraction of natural and synthetic estrogens from aqueous samples, P. Lucci, O. Núñez, M.T. Galceran, *J. Chrom. A*, 1218,(30), 4828-4833, 2011.  
Posters et conférences
- Quantification of estrogens at ppt levels in bovine plasma by AFFINIMIP® SPE and GC-MS/MS analysis, S. Rochereau, E. Bichon, F. Courant, F. Monteau, S. Prévost, F. Hanganu, N. Cesbron, G. Dervilly-Pinel, B. Le Bizec. This poster was presented at Euroresidues VIIIth conference, Egmond aan Zee, The Netherlands, 14th-16th May 2012 by the French reference laboratory on residues and contaminants in food (LABERCA - ONIRIS). Original method for analysis of Estrogens and Bisphenol A, Endocrine Disrupting Chemicals using solid phase extraction based on molecularly imprinted polymer, D.Derrien, M. Mulet, B. Chevalier, F. Alix, C.Pérollier, O.Lépine, K.Naraghi\* , Johann Travers, S. Bayouhd, présenté à ISEAC-37 at Antwerp, Belgium, May 22nd, 2012.
- High efficiency of semi-preparative Supercritical Fluid Chromatography with Molecularly Imprinted Polymer as stationary phase (SFC-MIP). Application on urinary steroids purification for IRMS analysis, M. DOUE, E. BICHON, F. MONTEAU, B. LE BIZEC.  
Poster presented at the 2nd International Symposium on HTSP , Brugge, Belgium, 31st January - 3rd February 2012 by the French reference laboratory on residues and contaminants in food (LABERCA - ONIRIS).
- How to improve analytical strategies to monitor growth promoting agents misuse in cattle, E. Bichon, S. Rochereau, L. Sérée, S. Prevost, F. Monteau, B. Le Bizec.  
This conference was presented at 5th international Symposium on Recent Advances in Food Analysis (RAFA), Prague, Czech Republic, 1-4 November 2011 by the French reference laboratory on residues and contaminants in food (LABERCA - ONIRIS).
- New method for extraction of Estrogens On Molecularly Imprinted Polymer, D. Derrien, C. Pérollier, O. Lépine, S. Bayouhd, K. Naraghi presented at PITTCO2011, Atlanta, Georgia, March 13 - 18, 2011.

## AFFINIMIP SPE and Reactive – Product list

Products	Designation	Definition	Reference	Nber of cartridges
Estrogens	AFFINIMIP® SPE Estrogens	Selective SPE cartridges for Estrogens, 3mL	FS104-02	25
			FS104-03	50
		Selective SPE cartridges for Estrogens, 1mL	FS104-02A	25
			FS104-03A	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).