

## Introduction

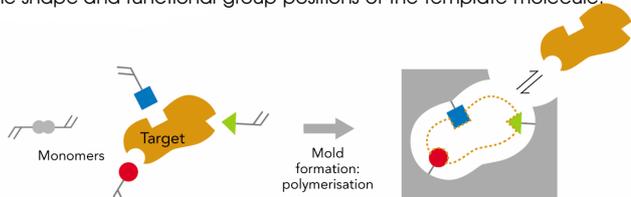
**Estrogens** are steroid hormones suspected to disrupt the endocrine system in wildlife and humans. More and more reports identify the presence of estrogens in aquatic environment.

17 $\beta$ -Estradiol is the most active naturally secreted estrogen steroid hormone. It plays a key role in developmental and reproductive functions. It also affects a diversity of biological processes involved in coronary artery disease, immunocompetence and cancer susceptibility. When it is present in wastewater, this endocrine disrupting chemical (EDC) has adverse effects on endocrine systems of man and animals.

We have developed a new class of intelligent polymers based on molecularly imprinted polymers and this phase has proved to be a powerful technique for clean-up and preconcentration of estrogens.

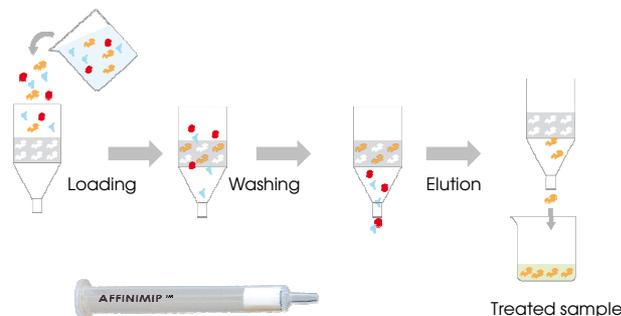
## Principle of AFFINIMIP®

MIP is a process that creates a three-dimensional network that has a « memory » of the shape and functional group positions of the template molecule.



This is a process where functional and cross-linking monomers are copolymerized in the presence of the target analyte (the imprint molecule). The functional monomers form a complex with the imprint molecule, and following copolymerization, their functional groups are held in position by the highly crosslinked polymeric structure. Subsequently thanks to the removal of the imprint molecule, it reveals binding sites that are complementary in size and shape to the analyte.

## Application of AFFINIMIP® SPE

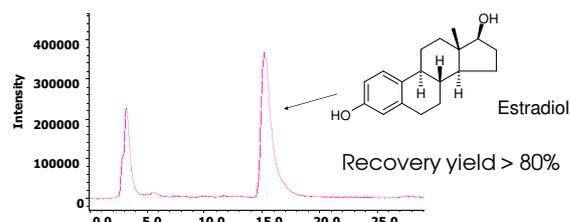


## AFFINIMIP® Estrogens : Protocol

Step	Solvent
Loading	10mL of diluted plasma or serum (dilution by 5 with water)
Washing	5mL Water 5mL 60/40 Water/Acetonitrile Dry 30 seconds
Elution	3mL Methanol

## Extraction from Plasma

Fluorescence profile of 10mL diluted plasma in water (1 :5). The plasma is spiked with 25ppb of 17 $\beta$ -Estradiol

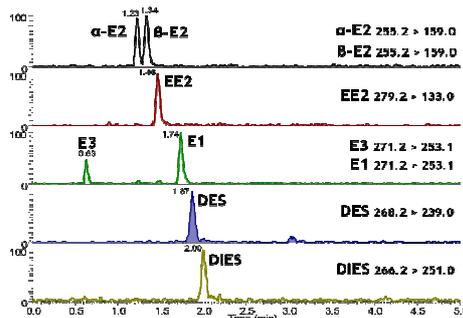


Analysis by LC-Fluo on Hypersil Gold C18 column 150 x 2.1 mm, 3 $\mu$ m  
65/35 Water -0.085% phosphoric acid/Acetonitrile  
0.2 mL/min, Vinj = 20  $\mu$ L, I<sub>ex</sub>=280 nm - I<sub>em</sub>=310 nm

## Extraction from river water

SRM Chromatograms of estrogens extracted from 100 mL river water spiked at 100ppt \*

Analysis by UHPLC-MS/MS  
Ascentis Express Phenyl-Hexyl column 150 x 2.1 mm  
51/44/5 Water /Acetonitrile/Methanol,  
Flow :450  $\mu$ L/min  
Vinj = 10  $\mu$ L  
APCI Positive ionisation mode



\*P. Lucci, O. Nuñez, M.T. Galceran, J. Chrom. A (2011)

Compounds	Recovery %
17 $\alpha$ -Estradiol ( $\alpha$ -E2)	93
17 $\beta$ -Estradiol ( $\beta$ -E2)	93
17 $\alpha$ - ethynilestradiol (EE2)	99
Estril (E3)	82
Estrone (E1)	89
Diethylstilbestrol (DES)	54
Dienestrol (DIES)	54

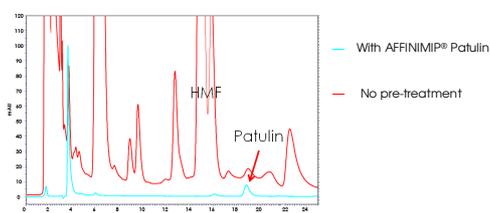
## Conclusion

The detection of estrogens is a real interest to ensure wildlife and humans health. A new AFFINIMIP® SPE has been developed for extraction of Estradiol and others estrogens from aqueous sample. More than 80 % recovery were obtained with an efficient clean up. AFFINIMIP® SPE can also recognize structurally related compounds like the synthetic estrogens diethylstilbestrol and dienestrol.

## Other related AFFINIMIP® SPE

### AFFINIMIP® Patulin: SPE from apple product

Perfect clean-up method with AFFINIMIP® Patulin

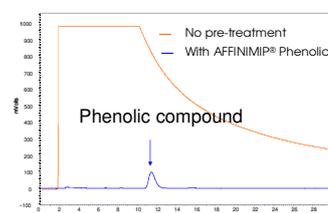


Chromatogram obtained after a purification with AFFINIMIP® Patulin (50 $\mu$ g/kg) of an apple juice sample



### AFFINIMIP® Phenolic : SPE from complex matrix

Perfect clean-up method with AFFINIMIP® Phenolic (class specific)



Phenolic compounds:

- Bisphenol A
- Paraben
- Hydroxylated HAP
- Hydroquinone
- Resorcinols compounds
- Catechols compounds

Chromatogram obtained after a purification with AFFINIMIP® Phenolic (100ng/mL) of a complex matrix