Analysis of Estrogens compounds, a class of Endocrine Disrupting Chemicals Using Solid Phase Extraction based on Molecularly Imprinted Polymer for selective extraction

Kaynoush Naraghi[1], Michel Arotçaréna[1] and Sami Bayoudh[1], Sandrine Rochereau[2], Emmanuelle Bichon[2], Bruno Le Bizec[2]

[1] AFFINISEP - Voie de l'innovation Pharmaparc II 27100 Val de Reuil, FRANCE. www.affinisep.com contact@affinisep.com

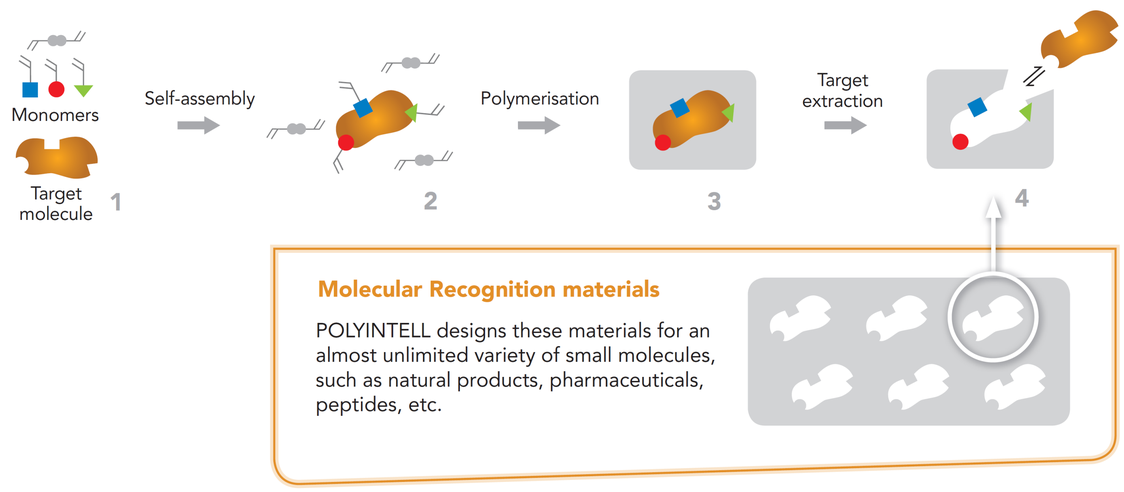
[2] ONIRIS, Laboratoire d'Etude des Résidus et Contaminants dans les Aliments (LABERCA), Nantes, F-44307, France

Estrogens are steroid hormones, a class of **Endocrine Disruptor Compounds** (EDC), suspected to disrupt the endocrine system. More and more reports identify the presence of estrogens in aquatic environment and consequently food and biological media.

To analyses these complex matrices, an efficient clean-up step is crucial to improve the sensitivity and the specificity before analysis. Based on molecularly imprinted polymers, we have developed a powerful method to clean-up and pre-concentrate Estrogens compounds.

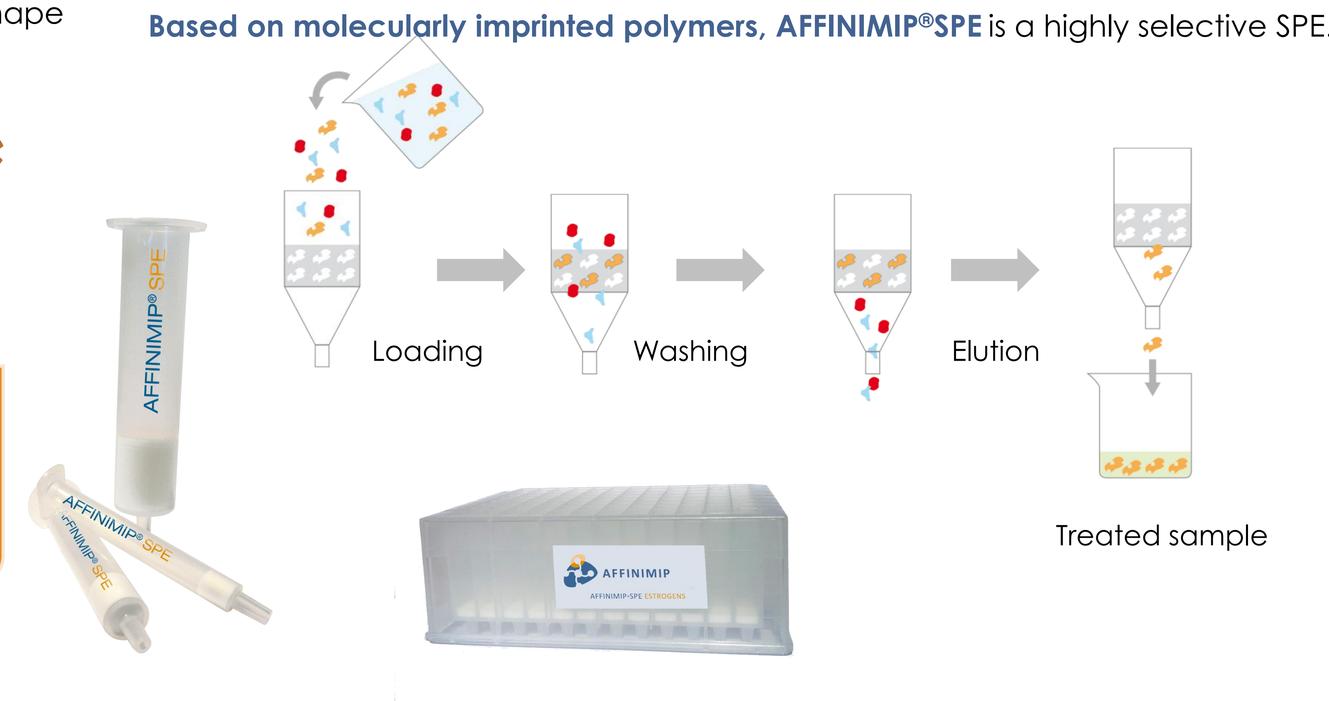
Principle of AFFINIMIP® SPE

Molecularly imprinted polymers is a polymer having a « memory » of the shape and functional group positions of a template molecule.



Application of AFFINIMIP®SPE

Based on molecularly imprinted polymers, AFFINIMIP®SPE is a highly selective SPE.

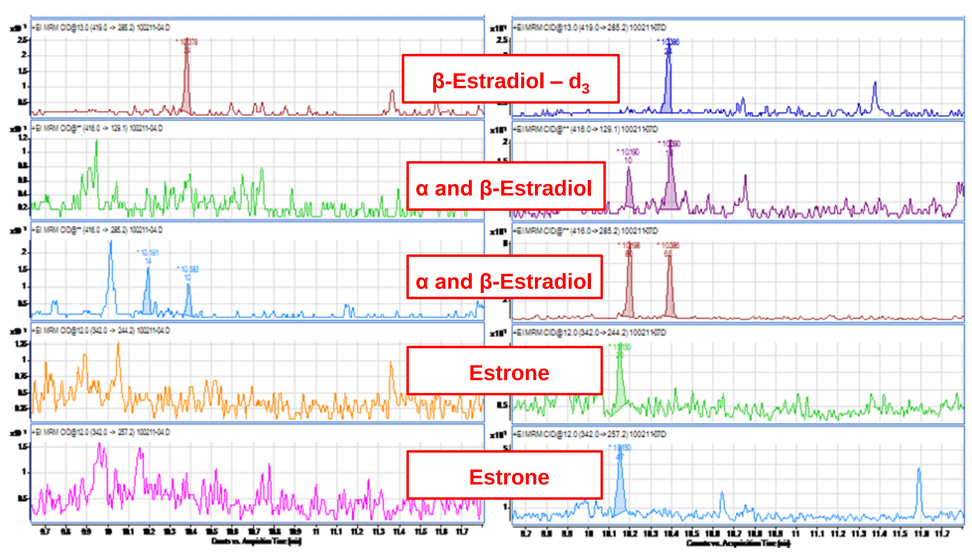


AFFINIMIP® SPE Estrogens : Bovine plasma

Protocol of use

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

Plasma blank — Plasma Spiked at 40 ppt



Results for Bovine plasma

Compounds	Absolute recovery %
17 a-Estradiol	**73**
17 β-Estradiol	**85**

Estrogens measurements by GC-MS/MS

GC column was a 30 m × 0.250 mm × 0.25 µm, Rxi-5HT

Temperature program: 80°C to 320°C (15°C.min^{-1})

- ❑ **Successfully tested on complex matrices**
- ❑ **Works for natural and synthetics estrogens!!!**

AFFINIMIP® SPE Estrogens : River water

Protocol of use

Step	Solvent
Loading	100mL Water
Washing	5mL Water 5mL 60/40 Water/ACN Dry 10 seconds
Elution	3mL Methanol

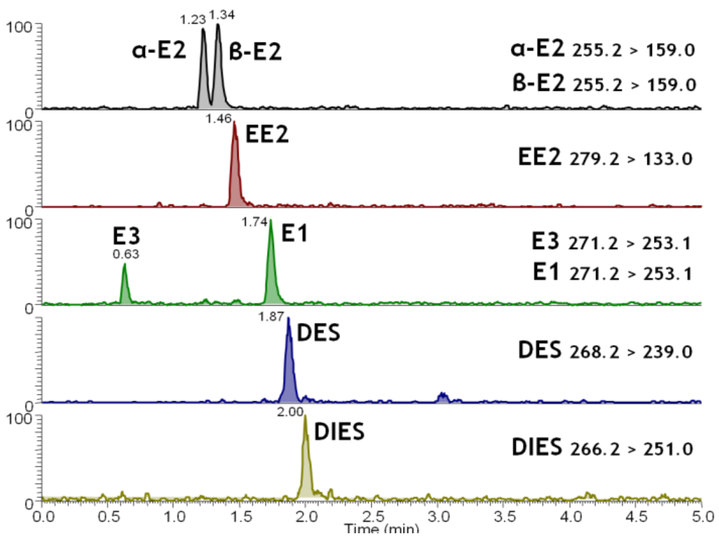
Analysis by UHPLC-MS/MS

Column Phenyl-Hexyl column 150 x 2.1 mm

Solvent :51/44/5 Water /Acetonitrile/Methanol

Flow : 450 µL/min

V_{inj} = 10 µL ; APCI Positive ionisation mode



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

Results for River water

Compounds	Recovery %
17 a-Estradiol (a-E2)	**93**
17 β-Estradiol (β-E2)	**93**
17 a- Ethynilestradiol (EE2)	**99**
Estriol (E3)	**82**
Estrone (E1)	**89**
Diethylstilbestrol (DES)	**54**
Dienestrol (DIES)	**54**

*P. Lucci, O. Nuñez, M.T. Galceran, *J. Chrom. A,* 1218 (30), 4828 (2011)

Conclusion

A new AFFINIMIP® SPE has been developed for extraction of estradiol and others estrogens from aqueous sample and plasma with an efficient clean up. This product has already be tested on various complex matrices : Water, Sediments, Plasma, Urchin, Urine…