Passive sampling for the analysis of 12 endocrine disruptors in river water using AttractSPE® POCIS – EDC+

This application note describes an in-vitro experiment over a period of 7 days for the passive sampling and analysis of 12 endocrine disruptors (see table 1) in river water. The method relies on AttractSPE® POCIS – EDC+ to catch endocrine disruptors in water prior to their analysis by LC-MSMS. The desorption kinetic of DIA-D5 as a Performance and Reference Compounds (PRC) was also studied.

Endocrine disruptor (EDC) are chemicals that can interfere with the hormonal system and produce harmful effects in both humans and wildlife. These chemicals are linked to developmental, reproductive, brain, immune and other problems. A wide range of chemicals, both natural and man-made, may cause endocrine disruption. Some of the major chemicals that are known as water contaminants are endocrine disrupting chemicals such as bisphenols, pesticides or drugs.

Passive sampling allows the monitoring of contaminants in surface or groundwater for several weeks with no energy, maintenance or control required. An average of the concentration of contaminants is then determined. Agitation and temperature conditions being variable in natural environments, Performances and Reference Compounds (PRC) can be used for the quality control. The desorption kinetics of the PRC can also allow the correction of the sampling rates (Rs).

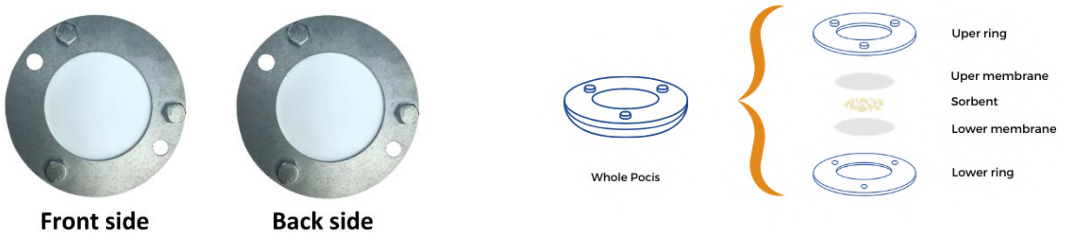

Figure 1. Description of POCIS

AttractSPE® POCIS – EDC+ (Polar Organic Chemical Integrative Sampler) are passive samplers for a wide range of organic compounds such as endocrine disruptors, pharmaceuticals, pesticides, perfluorinated compounds, etc.… The sorbent as a powder is trapped by two membranes held between two stainless steel rings (Figure 1). Several formats and chemistries are available to best suit each application. For this test, sorbent was spiked with DIA-D5 as PRC.

Compound	CAS Number	Category
Carbendazim	10605-21-7	Pesticide (fungicide)
Cortisol	50-23-7	Hormone
Cortisone	53-06-5	Hormone
Carbamazepine	298-46-4	Drug
Atrazine	1912-24-9	Pesticide (herbicide)
3,4-dichloroaniline	95-76-1	Herbicide preparation related
Diclofenac	15307-86-5	Drug
Ibuprofen	15687-27-1	Drug
Bisphenol A	80-05-7	Plasticizer
Ethynil Estradiol	57-63-6	Hormone
17-β-estradiol	50-28-2	Hormone
Estrone	53-16-7	Hormone
Desisopropyl atrazine D5 (PRC)	1189961-78-1	PRC

Table 1. List of tested compounds

1 - Description of the assembly (figure 2):

Two sets of experiments were carried out during a period of 7 days in a glass fish tank (40 x 40 x 40 cm). They differ in their speed of agitation, respectively 50 RPM and 200 RPM.

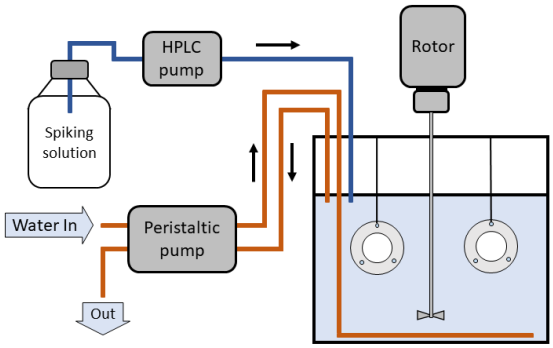

Figure 2. Assembly description

50 liters of river water (La Rouvre, France) spiked at 5µg/L with the analytes were first put into the tank. During the whole experiment, the tank water was drained and replaced with unspiked mineral water using a peristaltic pump at a flowrate of around 20 L per day.

A spiking solution (12 endocrine disruptors in ultrapure water) was constantly added into the tank with an HPLC pump with a flowrate of around 0.2mL/min (288mL/day). Therefore, analytes concentrations in the tank were kept constant at 5 µg/L.

The spiking solution was kept in an amber glass bottle at ambient temperature and magnetically stirred at moderate speed.

The temperature during the experiments was between 20°C and 22°C.

The tank water was constantly agitated using a steel propeller stirrer to simulate a flow with respectively 50 RPM and 200 RPM for each experiment.

Figure 3. Picture of the experimental setup

2 - Proceeding of the experiment:

AttractSPE® POCIS – EDC+ spiked with DIA-D5 at 2µg/g were used for this study. For each set of experiment, the sampling capacity of the POCIS was tested from 1 to 7 days with one passive sampler for each day, for a total of 7 passive samplers. The POCIS were immersed without prior conditioning. At any time, 4 samplers were simultaneously immersed in the tank following the immersion schedule (Figure 4). For example, after 2 days, the "2 days sampler" was removed from the tank and replaced by the "4 days sampler".

	Day							
	0	1	2	3	4	5	6	7
1 day sampler								
2 days sampler								
3 days sampler								
4 days sampler								
5 days sampler								
6 days sampler								
7 days sampler								

Figure 4. Immersion schedule. (Blue = Immersed; White = Not immersed)

3 - Extraction and analysis:

Each time a passive sampler was took out of the tank, it was rinsed with ultrapure water and stored in the fridge in an aluminum bag until the extraction procedure.

The procedure for processing each POCIS is as follows:

1- Sorbent recovery: A 6mL empty SPE cartridge with one frit was weighted (to determine the weight of dry sorbent at the end of the experiment) and put on a vacuum manifold with a funnel to collect the sorbent. POCIS is opened. The two PES membranes are carefully separated and the sorbent is poured thanks to ultrapure water into the funnel and the SPE column. A secund frit (previously weighted) is pushed into the cartridge to trap the sorbent.
2- Washing/drying: The cartridge is washed with 5 mL of ultrapure water then dried by applying full vacuum for 1 minute.
3- Elution: The molecules are eluted from the cartridge with 5 mL acetonitrile, followed by 5 mL 1% formic acid in acetonitrile.
4- Analysis: The eluates are then agitated and diluted (ratio 1:10 or 1:200 for the most concentrated ones) with a mixture of 90:10 (v/v) water/acetonitrile prior to analysis. Each cartridge is dried, and the sorbent weighted.

Two separate methods were used for the LC-MS/MS analysis of the molecules. One in positive ionization mode (see table 2), and the second in negative ionization mode (see table 3). In both methods, a delay column was used in addition to HPLC column to remove contamination from HPLC tubing and solvents. The use of a delay column is described in the application note AN-0009-01 about perfluorinated compounds (see reference [1])

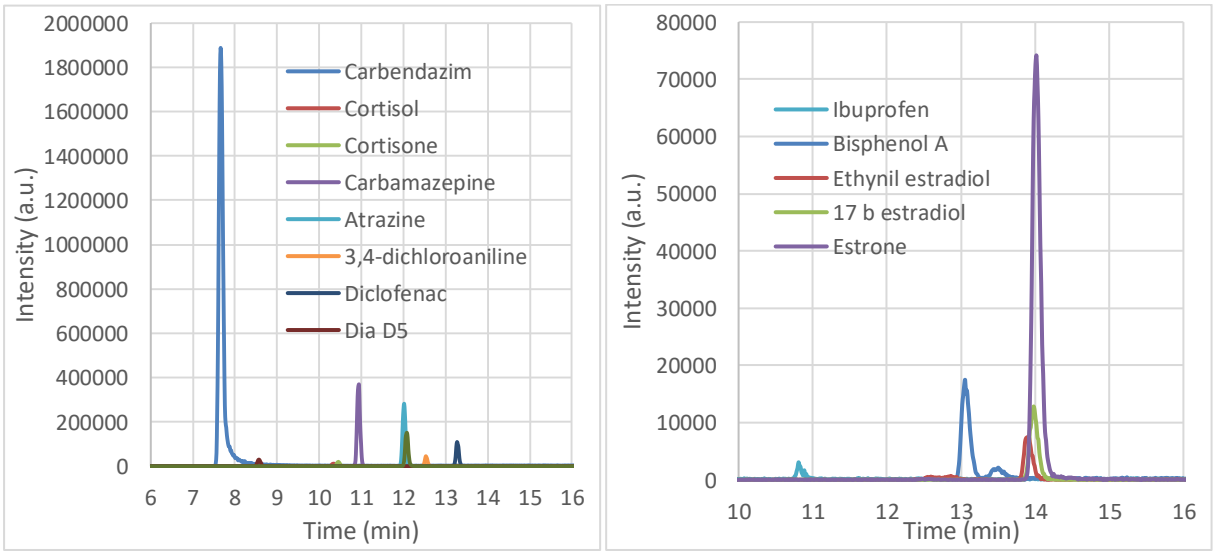

Figure 5. LC-MSMS chromatograms in positive mode (left) and negative mode (right) at 5µg/L.

LC Conditions	MS/MS Conditions
LC Dionex U3000	Qtrap 4000 ESI+ MS/MS
Column : SilactHPLC – LC.A 150*2.1mm at 30°C Delay column : SilactHPLC Delay-EDC 50*2.1mm	Curtain gas : 20
	CAD : High
Injection volume : 10µL	IS : 5500V
T° sampler : 10°C	Temperature : 650°C
Flow rate : 0.2mL/min	GS1/GS2 : 50/50

Time (min)	Solvent A	Solvent B	Analyte	Retention time (min)	Q1 (m/z)	Q3 (m/z)	CE (V)
0	90%	10%	Carbendazim	7.6	192.1	160.1	27
					192.1	132.1	41
1	90%	10%	Cortisol	10.3	363.2	121.1	35
					363.2	91.2	91
10	10%	90%	Cortisone	10.4	361.2	163.2	33
					361.2	91.1	97
15	10%	90%	Carbamazepine	10.9	237.1	194.2	27
					237.1	165.2	61
16	90%	10%	Atrazine	11.9	216.2	174.0	25
					216.2	104.0	41
21	90%	10%	3,4-dichloroaniline	12.4	162.0	127.1	35
					162.0	109.0	45
Solvent A : H_2O + 0.01% formic acid Solvent B : Acetonitrile			Diclofenac	13.1	296.0	214.1	45
					296.0	250.1	19
			DIA-D5	8.6	179.2	101.1	29
					179.2	69.1	43

Table 2. LC-MS/MS analysis method in positive mode.

LC Conditions	MS/MS Conditions
LC Dionex U3000	Qtrap 4000 ESI- MS/MS
Column : Hypersil Gold 150*2.1mm (3µm) + guard Hypersil Gold 1cm at 30°C Delay column : Hypersil Gold 50*2.1mm (3µm)	Curtain gas : 20
	CAD : High
Injection volume : 10µL	IS : -4500V
T° sampler : 10°C	Temperature : 650°C
Flow rate : 0.2mL/min	GS1/GS2 : 50/50

Time (min)	Solvent A	Solvent B	Analyte	Retention time (min)	Q1 (m/z)	Q3 (m/z)	CE (V)
0	90%	10%	Ibuprofen	10.8	205.0	161.0	-12
1	90%	10%			205.0	158.8	-10
10	10%	90%	Bisphenol A	12.4	227.2	211.9	-26
15	10%	90%			227.2	132.9	-36
16	90%	10%	Ethynil Estradiol	13.2	295.2	144.9	-56
21	90%	10%			295.2	183.1	-56
Solvent A : H_2O + 0.05%NH_3 Solvent B : Methanol + 0.05% NH_3			17-β-estradiol	13.3	271.2	145.2	-58
					271.2	182.9	-54
			Estrone	13.3	269.0	144.9	-54
					269.0	143.0	-76

Table 3. LC-MS/MS analysis method in negative mode.

4 - Results

The curve of the adsorbed mass on the sorbent versus the sampling duration was drawn for each molecule (see examples figure 6). The uptake was observed as linear over the 7 days of each experiment for the 12 molecules.

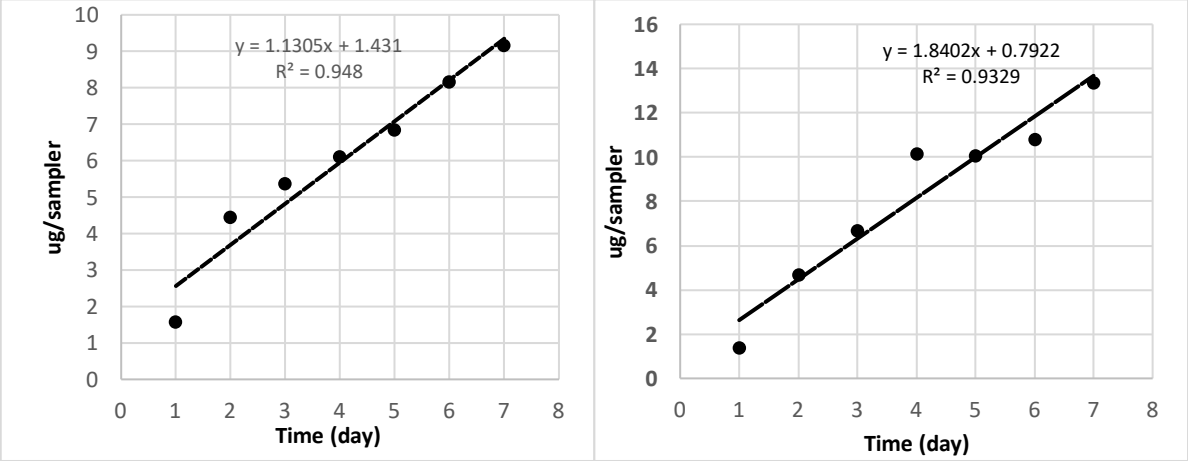

Figure 6. Uptake curve for Cortisol (left) and Estrone (right) measured at 200 RPM during the experiment.

The sampling Rate (Rs) was calculated for each molecule using the following formula:

$$Rs\ (L/days) = \frac{Csampler\ (\mu g/g)\ \times Msampler\ (g)}{C\ tank\ (\mu g/L)\ \times\ time\ (days)}$$

5 values from day 3 were used to determine the mean calculated Rs and a standard deviation value. The results obtained are presented in Table 4.

		Stirring 50 RPM		Stirring 200 RPM	
Mode	**Analyte**	**Rs (L/Day)**	**SD (n = 5)**	**Rs (L/Day)**	**SD (n = 5)**
Esi+	**Carbendazim**	**0.168**	0.063	**0.275**	0.075
	Cortisol	**0.167**	0.061	**0.294**	0.039
	Cortisone	**0.130**	0.053	**0.240**	0.048
	Carbamazepine	**0.209**	0.084	**0.380**	0.082
	Atrazine	**0.198**	0.078	**0.345**	0.046
	3,4-dichloroaniline	**0.114**	0.059	**0.162**	0.017
	Diclofenac	**0.198**	0.046	**0.562**	0.118
Esi-	**Ibuprofen**	**0.164**	0.052	**0.289**	0.037
	Bisphenol A	**0.154**	0.038	**0.486**	0.087
	Ethynil Estradiol	**0.149**	0.033	**0.470**	0.075
	17-β-estradiol	**0.103**	0.025	**0.539**	0.091
	Estrone	**0.269**	0.062	**0.557**	0.084

Table 4. Sampling rates and standard deviation (SD) obtained for the 12 endocrine disruptors in river water using **AttractSPE® POCIS – EDC+**.

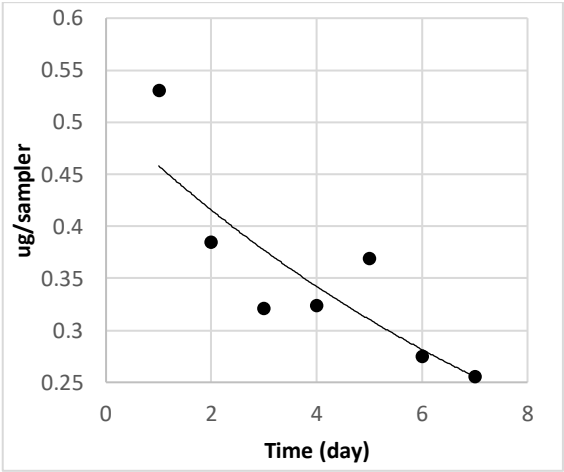

Figure 7. Measured desorption curve for Dia-D5 measured at 200 RPM during the experiment.

As expected, the desorption of DIA-D5 was observed. Over a period of 7 days, about 50% of the initial amount was desorbed.

CONCLUSION

AttractSPE® POCIS – EDC+ was successfully used as a passive sampler for the analysis of 12 endocrine disruptors over a period of 7 days in river water. Two set of experiments were carried out with slow and fast agitation speeds (50 and 200 RPM) leading to the sampling rate determination of each molecule. DIA-D5 was used as PRC and partially desorbed during that period as expected.

Part number of products used in this application note:

Product:	Quantity:	Part number:
SIlactHPLC LC.A 150*2.1mm (3µm)	1 unit	LC.A-150.2.1
SilactHPLC Delay-EDC 50*2.1mm (5µm)	1 unit	DELAY-EDC-50.2.1
Canister for 3 POCIS + 1 holder for 3 POCIS	1 unit	CH-3P.A.1
Canister for 6 POCIS + 2 holders for 3 POCIS each	1 unit	CH-6P.A.1
AttractSPE® POCIS – EDC+ POCIS + empty cartridges	10/pk	POCIS.EDC+.90.55.kit.10
AttractSPE® POCIS – EDC+ with PRC POCIS + empty cartridges + 3 reference cartridges	10/pk	POCIS.EDC+.90.55.kit.10.DIA

[1] How to overcome challenges in PFC analysis: Application note (AN-0009-01)
https://www.affinisep.com/applications/pops-pahs-perfluorinated-compounds/pfcs/