



affinisep

Application Note



Multiresidue analysis of Pesticides and Pharmaceuticals at trace levels in river water and source water using AttractSPE® Disks



Introduction

This application note describes the rapid and quantitative multiresidue determination of 12 pesticides and pharmaceuticals (see table 1) at trace levels from environmental waters. These molecules were concentrated from large volumes of water with a very high flowrate thanks to the use of **AttractSPE® Disks – HLB**, a SPE disk designed for wide variety of compounds prior to their detection by LC-MSMS.

Nowadays, a broad range of anthropogenic contaminants such as pesticides, pharmaceuticals and personal care products (PPCPs) are present in environmental waters. Their monitoring is a major challenge due to the large number of contaminants and their diversities. Grab sampling remains one of the main methods of monitoring to comply with regulations. However, it requires the concentration of large amounts of water to detect trace levels of these pollutants. Affinisep has developed an **exhaustive portfolio of SPE disks** for this purpose. These **AttractSPE® Disks enable the processing of large volumes of water with a very high flow rate and an outstanding capacity.**



AttractSPE® Disks are thin, dense, soft, and uniform extraction SPE membranes allowing the best interactions with analytes even with high flow rate without any channeling. **AttractSPE® Disks** 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**. They can be used for environmental monitoring of contaminants by concentrating the sample in the lab or as passive sampler sorbents for Disk Based Passive Sampler. This application note describes the rapid and quantitative multiresidue determination of 12 pesticides and pharmaceuticals (see table 1) at trace levels from environmental waters.

Table 1 - List of tested compounds

Compound	CAS Number	Category
Carbendazim	10605-21-7	Pesticide (fungicide)
Atrazine	1912-24-9	Pesticide (herbicide)
Bisphenol A	80-05-7	Plasticizer
Carbamazepine	298-46-4	Drug
Diclofenac	15307-86-5	Drug
Cortisol	50-23-7	Hormone
Cortisone	53-06-5	Hormone
Testosterone	58-22-0	Hormone
Progesterone	57-83-0	Hormone
Ethynil Estradiol	57-63-6	Hormone
17- β -estradiol	50-28-2	Hormone
Estrone	53-16-7	Hormone

1 Proceeding of the experiment and recoveries

For each type of water, five spiked samples were processed. A non-spiked water sample was also processed as a blank to check the eventual initial presence of the molecules of interest. The loading solution is 1 L of water spiked with 12 analytes at a concentration of 25ng/L.

Place the **AttractSPE® Disks – HLB** onto the SPE disk manifold.
Note: A glassfiber prefilter (1 μ m and 3 μ m) can be added on top of the disk to prevent clogging from suspended particles.

CONDITIONNING

1. 50 mL Methanol
2. 25 mL of ultrapure water

LOADING

1 L of loading solution in 20 minutes

WASHING

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

ELUTION

1. 20 mL Acetonitrile
2. 20 mL Acetonitrile +1% Formic Acid

ANALYSIS

Evaporation under N₂ and dissolution in the mobile phase.



Figure 1. SPE disk manifold for AttractSPE® Disks

Two separate methods (described in paragraph 2) were used for the LC-MS/MS analysis of the molecules. One in positive ionization mode (see table 3), and one in negative ionization mode (see table 4). In both methods, a delay column was used in addition to HPLC column to remove contamination from HPLC tubing and solvents (description in paragraph 2).

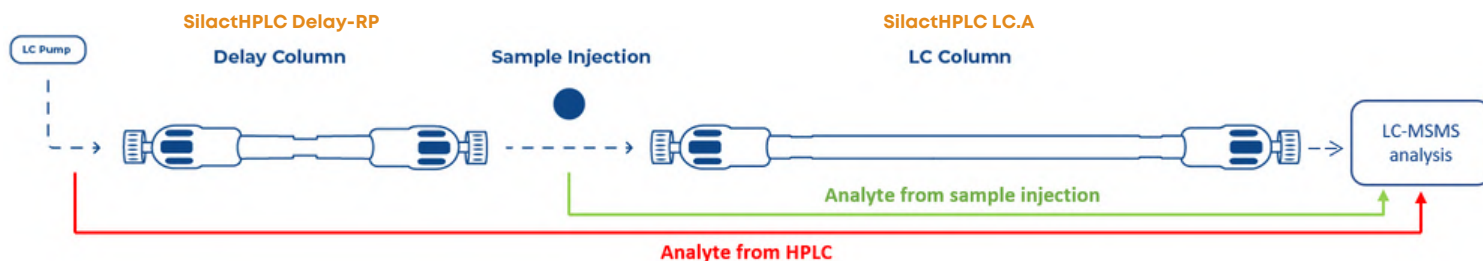
Mode	Analyte	Source water			River water		
		Blank (ng/L)	Spiked recovery %	RSDr (n = 5)	Blank (ng/L)	Spiked recovery %	RSDr (n = 5)
Esi+	Carbendazim	11.5	96%	4%	11.0	93%*	11%*
	Cortisol	N.D	98%	5%	N.D	103%	3%
	Cortisone	N.D	102%	5%	N.D	113%	5%
	Carbamazepine	N.D	100%	3%	8.5	103%	2%
	Atrazine	N.D	84%	4%	10.0	99%	2%
	Testosterone	N.D	91%	4%	N.D	99%	3%
	Diclofenac	N.D	100%	6%	N.D	106%	8%
	Progesterone	N.D	101%	4%	N.D	107%	14%
Esi-	Bisphenol A	N.D	96%	7%	N.D	109%	15%
	Ethynil Estradiol	N.D	105%	8%	N.D	118%	4%
	17- β -estradiol	N.D	89%	2%	N.D	111%	3%
	Estrone	N.D	91%	4%	N.D	106%	2%

Table 2- Recovery yields obtained for tested analytes (N.D = Not Detected). *For carbendazim in river water, recovery and RSDr were obtained from n = 4, as the fifth value was considered as an outlier (%Recovery is 103% and RSDr = 24% including fifth value).

2 Analytical conditions

During analyses, some analytes of interest are likely to be found in mobile phase or LC parts and tubing. These analytes concentrate at the front of the LC column during each sample run, leading to analysis interference due to contamination. To change LC parts and tubing and control solvents purity is very expensive and time consuming. Moreover, some contaminant may never be totally removed. The other solution is the use of a delay column.

Figure 2. Installation of **SilactHPLC Delay-RP** Delay column for LC analysis



The delay column **SilactHPLC Delay-RP** is put between LC pumps and sample injection (Figure 1). It allows the analyte of interest coming from the LC device to concentrate at the front of the delay column instead of LC column, while the analyte coming from sample injection will concentrate directly on the LC column. This will lead to a longer retention time for the analyte from LC device because it must pass through the delay column in addition to the LC column whereas the analyte from sample injection must only pass through LC column (See figure 2 for example). This solution is very easy to install and is cost effective.

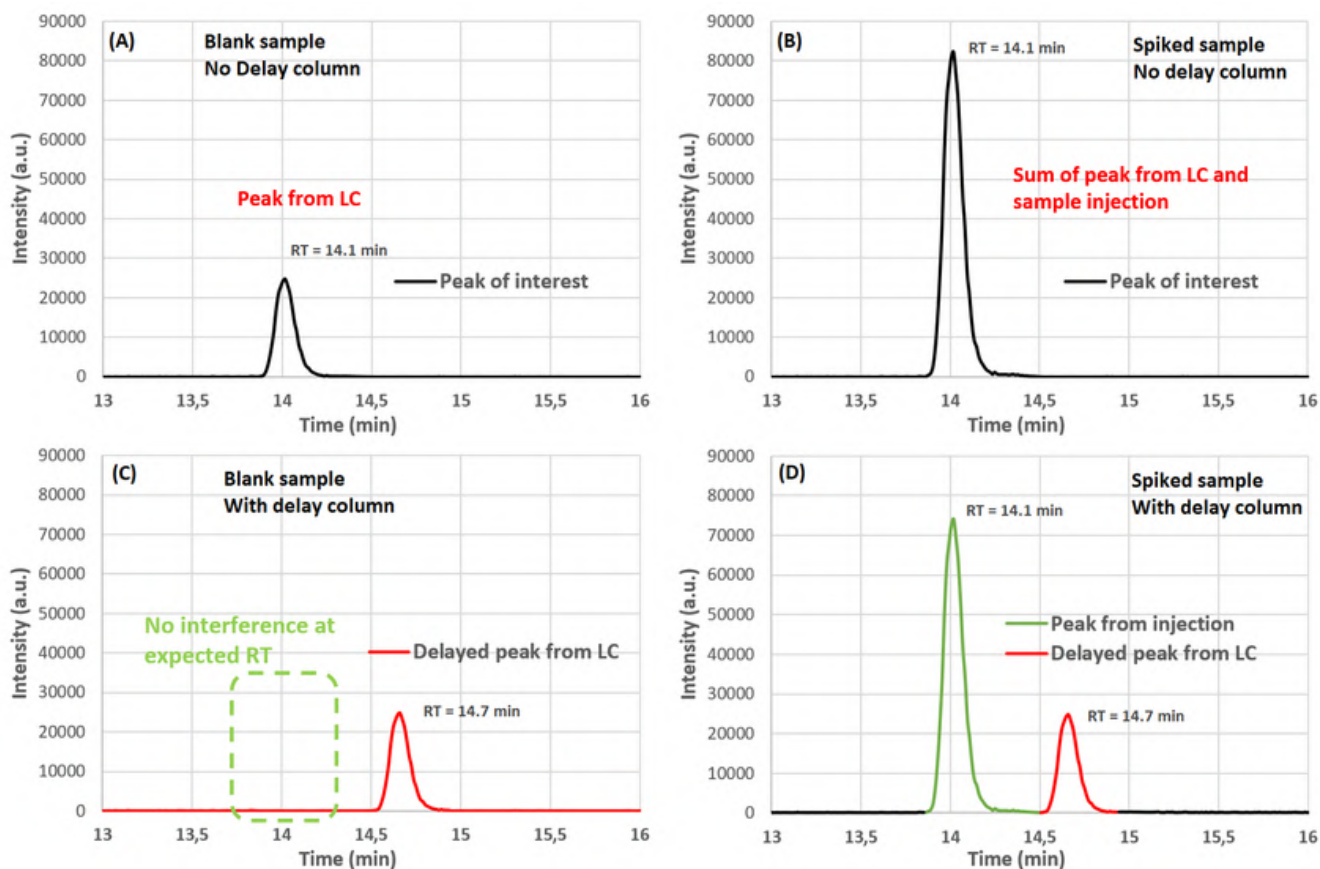


Figure 3. Effects of the delay column **SilactHPLC Delay-RP** on samples. A: Blank sample without delay column. B: Spiked sample without delay column. C: Blank sample with delay column. D: Spiked sample with delay column.

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

LC Conditions			MS/MS Conditions				
LC Dionex U3000			Qtrap 4000 ESI+ MS/MS				
Column : SilactHPLC – LC.A 150*2.1mm at 30°C			Curtain gas : 20				
Delay column : SilactHPLC Delay-RP 50*2.1mm			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%	Testosterone	12.6	289.2	109.1	86
					289.2	97.1	86
Solvent A : H2O + 0.01% formic acid Solvent B : Acetonitrile			Diclofenac	13.1	296.0	214.1	45
					296.0	250.1	19
			Progesterone	14.7	315.2	109.1	86
					315.2	97.1	86

Table 4- LC-MS/MS analysis method in negative 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			Curtain gas : 20				
Delay column : Hypersil Gold 50*2.1mm (3µm)			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%	Bisphenol A	13.0	227.2	211.9	-26
					227.2	132.9	-36
10	10%	90%	Ethinil Estradiol	13.8	295.2	144.9	-56
					295.2	183.1	-56
16	90%	10%	17-β-estradiol	13.9	271.2	145.2	-58
					271.2	182.9	-54
21	90%	10%	Estrone	14.0	269.0	144.9	-54
					269.0	143.0	-76
Solvent A : H2O + 0.05%NH3 Solvent B : Methanol + 0.05% NH3							

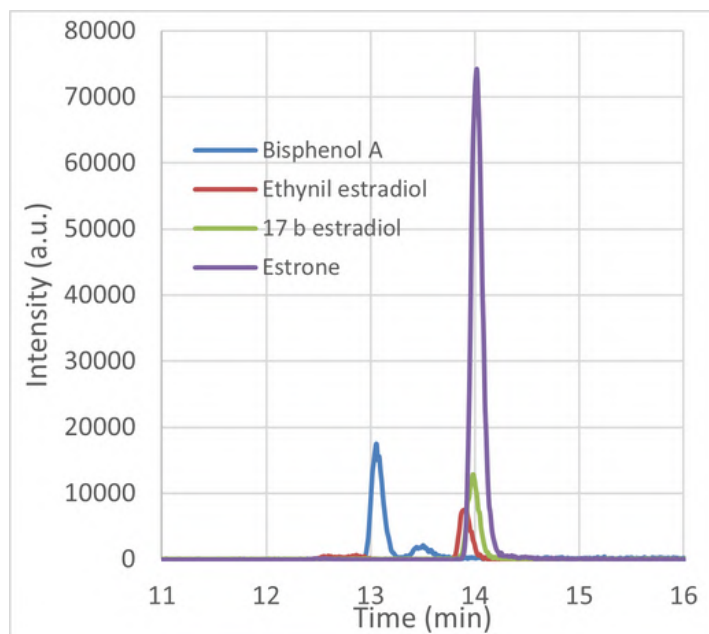
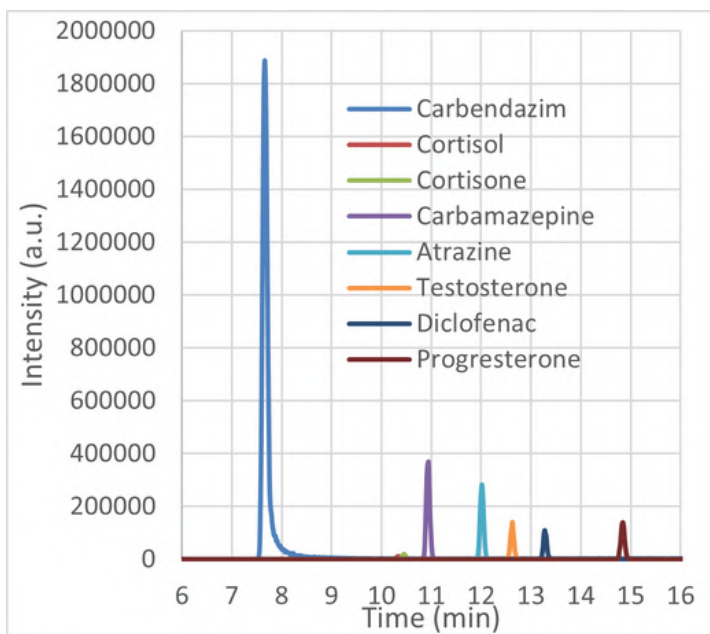


Figure 4. LC-MSMS chromatograms in positive mode (left) and negative mode (right)

Conclusion

AttractSPE® Disks - HLB have shown excellent performances to concentrate 12 common and very diverse organic contaminants (pesticides and PPCPs) from source water and river water with excellent recovery yields (>84%) and a good repeatability. **AttractSPE® Disks** format allowed a fast treatment of large sample volumes (~20-25 min). This method makes possible a 1000 times concentration of samples.

Part number of products used in this application note:		
Product:	Quantity:	Part number:
AttractSPE® Disks - HLB for environmental applications- 47mm diameter	20/pk	SPE-Disks-HLB-47.T1.20
AttractSPE® Disks - HLB for environmental applications- 90mm diameter	10/pk	SPE-Disks-HLB-90.T1.10
SilactHPLC LC.A 150*2.1mm (3µm)	1 unit	LC.A-150.2.1
SilactHPLC Delay.RP 50*2.1mm (5µm)	1 unit	DELAY-RP-50.2.1
SPE Disks manifold 47mm – 1 station	1 unit	ACC-DISKSPE-G47-1
SPE Disks manifold 47mm – 3 station	1 unit	ACC-DISKSPE-G47-3
SPE Disks manifold 47mm – 6 station	1 unit	ACC-DISKSPE-G47-6
AttractSPE® Prefilter Glassfiber for 47mm disks (1µm)	50/pk	PF-GF-50.T1.47.1
AttractSPE® Prefilter Glassfiber for 47mm disks (3µm)	50/pk	PF-GF-50.T1.47.3