

Application Note



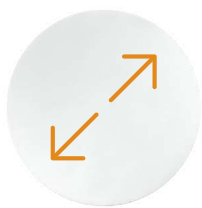
**Analysis of Pharmaceuticals and Personal Care Products in
Water with SPE Disks **AttractSPE®Disks - HLB****



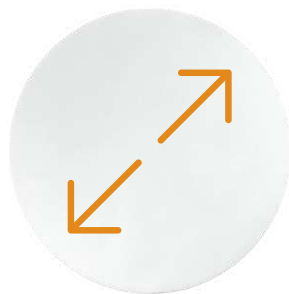
AttractSPE®Disks are thin, dense, soft and uniform extraction SPE membranes allowing the best interactions with analytes even with high flow rate without any **channeling**. AttractSPE®Disks make possible the loading of large water volume thanks to a fast flow rate and a high surface area of exchange and reduce the time of sample clean-up for 1L from 45min to 10min for each extraction. AttractSPE®Disks is also the perfect membrane to use for the passive sampler thanks to a very good hold and ease to use.

In this application note, **AttractSPE®Disks – HLB 47mm** have been tested for the analysis of several pharmaceutical compounds.

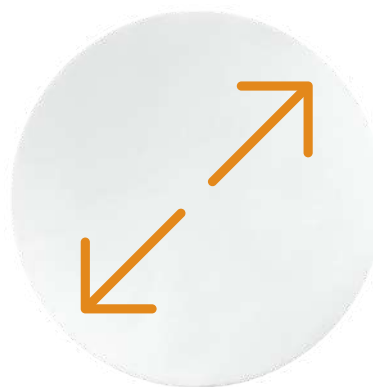
8 compounds from group 1 (according to EPA 1694) were spiked in water at a concentration of 16 to 80ng/L. 1L of the solution was loaded with a high flow rate.



25mm



47mm



90mm

An alternative method for the analysis of this group 1 drugs and 7 tetracyclines is also described.

LOADING SOLUTION:

One liter of water was adjusted to pH 2-2.5 with HCl 37%. Add 80mg of sodium thiosulfate and 500 mg of EDTA-Na₄ – 2 H₂O. Solution is then spiked with analytes of interest.

Important: *For each conditioning and elution step, apply a fast vacuum to soak the disk and wait 1 minute before starting elution.*

CONDITIONNING OF **AttractSPE®** Disks – HLB - 47mm

1. 20 mL Acetone
2. 50 mL Methanol
3. 20 mL of ultrapure water

LOADING

1. 1 L of loading solution in 15 minutes

WASHING

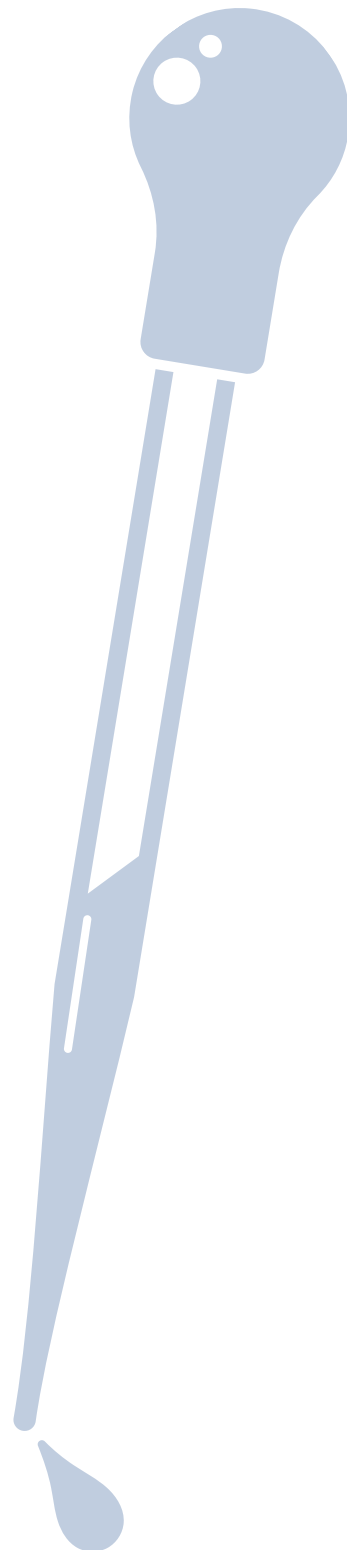
1. 20 mL ultrapure water
2. Apply vacuum for 30 s to dry the disk

ELUTION

1. 20 mL Methanol

ANALYSIS

1. Evaporation under N₂ and dissolution in the mobile phase.



Results

Group 1 compounds were analyzed by LC-MS/MS. The complete method parameters are described in table 3.

Table 1: Recoveries obtained for tested analytes, and corresponding concentrations

Group 1	Analyte	Blank	Spiked Recovery (%)	Concentration (ng/L)
	Penicillin V	ND	105	160
Flucloxacillin	ND	105	80	
Sulfathiazole	ND	92	16	
Sulfadimethoxine	ND	84	16	
Sulfamethazine	ND	88	80	
Sulfadiazine	ND	95	32	
Caffeine	ND	106	80	
Carbamazepine	ND	98	16	

AttractSPE®Disks HLB were successfully tested in experimental conditions close to **EPA 1694** and showed recovery yields higher than 80% for tested analytes.

Alternative conditions :

An alternative method, also based on the use of AttractSPE®Disks HLB with similar and simple operating conditions, was demonstrated for the 8 previous compounds in addition of 7 tetracyclines. Main changes are the nature of the loading and elution steps.

Alternative method for group 1 compounds and tetracyclines

An alternative method is also developed for the analysis of group 1 and group 2 compounds with the use of AttractSPE® Disks HLB 47mm with excellent recoveries. Main changes are the nature of the loading and elution steps.

LOADING SOLUTION : Mix 22.195g of disodium hydrogen phosphate heptahydrate, 11.257g of citric acid, and 500mg of ETDA-Na₄ - 2H₂O to one liter of water until complete dissolution (pH should be around 4,2). The solution was then spiked with the analytes for demonstration.

CONDITIONNING OF AttractSPE® Disks – HLB - 47mm

1. 20 mL Acetone
2. 50 mL Methanol
3. 20 mL of ultrapure water

LOADING

1. 1 L of loading solution in 15 minutes

WASHING

1. 20 mL ultrapure water

Apply vacuum for 30 s to dry the disk

ELUTION

1. 20 mL Methanol + 3% Formic acid

ANALYSIS

1. Evaporation under N₂ and dissolution in the mobile phase.

Table 2: Recoveries obtained for tested analytes, and corresponding concentrations

	Analyte	Blank	Spiked Recovery (%)	Concentration (ng/L)
Group 1	Penicillin V	ND	102	80
	Flucloxacillin	ND	102	40
	Sulfathiazole	ND	62	8
	Sulfadimethoxine	ND	82	8
	Sulfamethazine	ND	80	40
	Sulfadiazine	ND	69	16
	Caffeine	ND	113	40
Group 2	Carbamazepine	ND	90	8
	4-epitetracycline	ND	70	69
	4-epioxytetracycline	ND	97	37
	Oxytetracycline	ND	87	97
	Tetracycline	ND	75	71
	4-epichlorotetracycline	ND	105	60
	Chlortetracycline	ND	75	67
Doxycycline	ND	93	67	

The use of McIlvaine Buffer (set at pH = 4.2) as the loading solution allows good recoveries for Tetracyclines and most other molecules.



Table 3 : Analytical conditions for molecules of group 1

LC Conditions <ul style="list-style-type: none"> • LC Dionex U3000 • Column : Hypersil Gold 150*2.1mm (3µm) + guard Hypersil Gold 1cm at 30°C • Injection volume : 20µL • T° sampler : 10°C • Flow rate : 0.2mL/min 			MS Conditions <ul style="list-style-type: none"> • Qtrap 4000 ESI + MS/MS • Curtain gas : 20 • CAD : Medium • IS : 5500V • Temperature : 350°C • GS1/GS2 : 50/50 				
Time (min)	Solvent A	Solvent B	Analyte	Retention time (min)	Q1	Q3	CE (V)
0	100%	0	Penicillin V	19,3 +20,2	383,1	160.00	23
					383,1	114.1	53
4	100%	0	Flucloxacillin	20,8	486,00	160.1	25
					486,00	196.00	55
22	10%	90	Sulfathiazole	13,1	256.00	156.0	23
					256.00	92.1	37
24,5	10%	90	Sulfadimethoxine	17,6	311.00	156.00	31
					311.00	108.00	43
25	100%	0	Sulfamethazine	14,6	279.1	186.00	25
					279.1	92.1	45
31	100%	0	Sulfadiazine	12,3	251.0	156.00	23
					251.0	92.1	41
Solvent A : Ammonium Formate 0.1% + 0.1% Formic Acid Solvent B : Methanol/Acetonitrile 50/50			Caffeine	13,4	195.1	138.1	27
					195.1	110.1	33
			Carbamazepine	19,5	237.1	194.2	27
					237.1	165.2	61

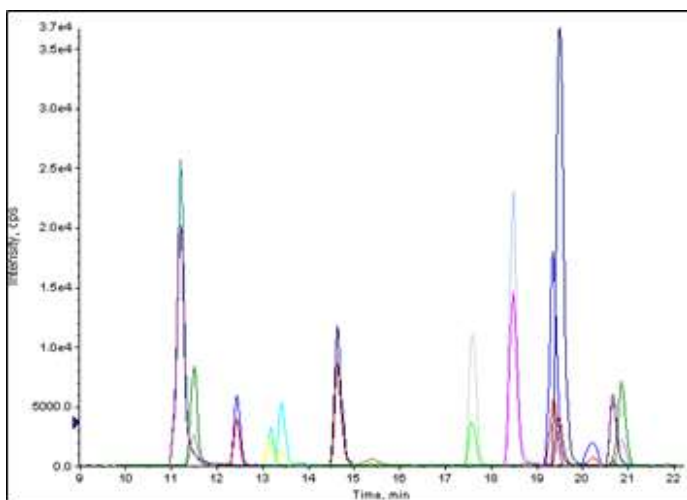


Figure 1: Typical chromatogram obtained for the analysis of group 1 compounds.

Table 4: Conditions of analysis for group 2 molecules (tetracyclines)

LC Conditions			MS Conditions				
<ul style="list-style-type: none"> LC Dionex U3000 Column : Hypersil Gold 150*2.1mm (3µm) + guard Hypersil Gold 1cm at 30°C Injection volume : 20µL T° sampler : 10°C Flow rate : 0.2mL/min 			<ul style="list-style-type: none"> Qtrap 4000 ESI+ MS/MS Curtain gas : 30 CAD : Medium IS : 5500V Temperature : 600°C GS1/GS2 : 50/50 				
Time (min)	H2O 5mM Oxalic Acid	MeOH/ACN 50/50 5mM Oxalic Acid	Analyte	Retention time (min)	Q1	Q3	CE (V)
0	90%	10%	4-epitetracycline	10,1	445.1	410.1	27
					445.1	427.1	19
1	90%	10%	4-epioxytetracycline	10,5	461.1	426.1	29
					461.1	443.1	19
13	69%	31%	Oxytetracycline	11,1	461.1	426.1	29
					461.1	443.1	19
20	10%	90%	Tetracycline	12,1	445.1	410.1	27
					445.1	427.1	19
20,5	10%	90%	4-epichlorotetracycline	15,3	479.1	444.2	31
					479.1	462.2	27
20,8	90%	10%	Chlorotetracycline	17,2	479.1	444.2	31
					479.1	462.2	27
24,8	90%	10%	Doxycycline	18,9	445.1	428.2	27
					445.1	410.1	27

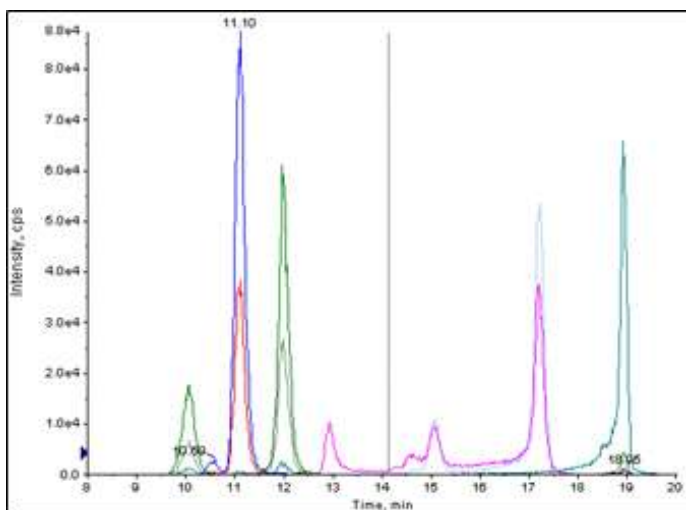


Figure 2: Typical chromatogram obtained for the analysis of group 2 compounds (tetracyclines)

AttractSPE® Disks HLB have been used for the enrichment of 15 pharmaceutical compounds according to EPA method 1694. They have shown an excellent hold giving a great ease to handle. In addition, excellent performances were obtained at a high flow rate as recoveries are higher than 80% for most analytes.

AFFINISEP supplies a wide variety of sorbents such as HLB, C18, SDB-RPS, SDB-XC, Anion and cation exchanges (disks, cartridges and other formats available).

Used in the application note

AFFINIMIP® SPE Disks HLB - 47mm - 20/pk

- SPE-Disks-HLB-47.T1.20

Related products

SPE Disks Manifold - 3 stations - 47mm

- ACC-DISKSPE-G47-3

AFFINIMIP® SPE Disks HLB - 25mm - 20/pk

- SPE-Disks-HLB-25.T1.40

AFFINIMIP® SPE Disks HLB - 90mm - 20/pk

- SPE-Disks-HLB-90.T1.10

AttractSPE® Prefilter Glassfiber - 1µm - 47mm - 50/pk

- PF-GF-50.T1.47.1

AttractSPE® Prefilter Glassfiber - 3µm - 47mm - 50/pk

- PF-GF-50.T1.47.3