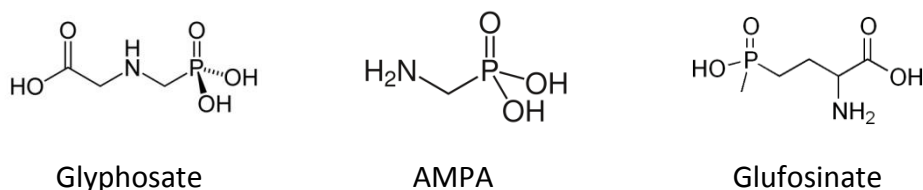




This application note gathers several experiments with AFFINIMIP<sup>®</sup> SPE - GLYPHOSATE to clean-up efficiently Glyphosate, Glufosinate and AMPA. Trace analysis conditions (high volume of mineral water at very low concentration) and high capacity (high concentration of analytes) were tested in water. Analysis of these compounds in cereals are also shown.

Glyphosate is one of the most vastly used herbicides in the world for cultivation, especially cereals. Due to its important use, it can be detected at relatively high concentrations. With Glufosinate, another commonly used herbicide, they are closely structured herbicides referred to as phospho-herbicides. Glyphosate undergoes rapid microbial degradation in plants, soil and water to the metabolite aminomethylphosphonic acid (AMPA). These three molecules are often analyzed simultaneously. Codex alimentarius has defined a MRL (maximum residue limit) for Glyphosate of 30mg/Kg in cereals and for Glufosinate, 2mg/kg of soybean.



**Figure 1.** Chemical structures of Glyphosate, AMPA and Glufosinate

The very polar nature of these molecules makes them difficult to analyse. For instance, main analytical methods require a derivatization step with fluorenylmethyloxycarbonyl chloride (FMOC-Cl). This method is time consuming and introduces uncertainties in the analysis. On the other hand, for water analysis, very low concentrations can make their detection difficult. A concentration of the sample is then necessary.

This application note shows an efficient Solid Phase Extraction (SPE) cleanup with both simple and complex matrices. It describes the quantitation of the three molecules at trace levels in a large volume of distilled water and mineral water, and in cereals (Barley), a more complex matrix.

**The quantitation of these molecules is shown WITHOUT DERIVATIZATION with a clean-up with AFFINIMIP<sup>®</sup> SPE GLYPHOSATE prior to LC/MS-MS analysis.**

## 1 – Quantitation of Glyphosate/AMPA/Glufosinate in water

For this experiment, 100 mL of water was spiked with a high concentration of each analyte and loaded on a 6 mL **AFFINIMIP<sup>®</sup> SPE GLYPHOSATE** cartridges to demonstrate the high capacity of these cartridges.

**Loading solution:** 100 mL of water are spiked in a plastic bottle with a solution of Glyphosate, AMPA and Glufosinate at 3 µg/L each.

### CONDITIONNING

6 mL ultrapure water

### LOADING

100 mL of loading solution in 15 minutes

### WASHING

6 mL ultrapure water

### ELUTION

8 mL ultrapure water with HCl 0.1M

### ANALYSIS

Elutions are evaporated under vacuum at 60°C for 2 hours and dissolved in 3mL of mobile phase containing 0.8mM of EDTA-Na<sub>2</sub>. The solution is then analyzed.

*Note: It is advised to use plastic labware to avoid potential adsorption of the analytes on glassware.*



## Results:

The analytes were simultaneously analyzed by LC-MS/MS. A blank matrix was also performed. The results obtained are presented in the table below. The analytical method is described at the end of the application note.

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

Analyte	Concentration measured (µg/L)		Recovery for spiked sample	RSDr (n = 3)
	Not spiked	Spiked		
Glyphosate	0	2.73	91%	2%
AMPA	0	2.10	70%	1%
Glufosinate	0	2.40	80%	6%

**AFFINIMIP<sup>®</sup> SPE GLYPHOSATE** was successfully tested for the quantitation of Glyphosate, AMPA, and Glufosinate in water with a high concentration of each compound. Thanks to the high capacity of the cartridge, very good recovery yields and repeatability.

## 2 – Quantitation of Glyphosate/AMPA/Glufosinate in mineral water

For this experiment, 1L of water was spiked with very low concentration of each analyte for trace analysis of the solution and loaded on a 12 mL **AFFINIMIP<sup>®</sup> SPE GLYPHOSATE** cartridges.

**Loading solution:** One liter of mineral water is spiked in a plastic bottle with a solution of Glyphosate, AMPA and Glufosinate at 100 ng/L each.

### CONDITIONNING

12 mL ultrapure water

### LOADING

1 L of loading solution in 150 minutes

### WASHING

12 mL ultrapure water

### ELUTION

12 mL ultrapure water with HCl 0.1M

### ANALYSIS

Elutions are evaporated under vacuum at 80°C for 1.5 hours and dissolved in 1mL of mobile phase containing 0.8mM of EDTA-Na<sub>2</sub>. The solution is then analyzed.

*Note: It is advised to use plastic labware to avoid potential adsorption of the analytes on glassware.*

Characteristic composition of water	
pH = 7.2	
Ca <sup>2+</sup> 104mg/L	Cl <sup>-</sup> <0.05mg/L
Na <sup>+</sup> 6mg/L	SO <sub>4</sub> <sup>2-</sup> 25mg/L
Mg <sup>2+</sup> 17mg/L	NO <sub>3</sub> <sup>-</sup> <1mg/L
K <sup>+</sup> 1mg/L	

## Results:

The analytes were simultaneously analyzed by LC-MS/MS. A blank matrix was also performed. The results obtained are presented in the table below. The analytical method is described at the end of the application note.

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

Analyte	Concentration measured (ng/L)		Recovery for spiked sample (blank deduced)
	Not spiked	Spiked	
<b>Glyphosate</b>	9.7	118.3	<b>108.5 %</b>
<b>AMPA</b>	4.4	82.5	<b>78.1 %</b>
<b>Glufosinate</b>	0	81.3	<b>81.3 %</b>

**AFFINIMIP<sup>®</sup> SPE GLYPHOSATE** was successfully tested for the quantitation of Glyphosate, AMPA, and Glufosinate at trace concentrations in water with a very high volume of water. Analytes are retained on the cartridges with very strong interactions and are not washed out during the loading in spite of the high volume. Very good recovery yields were obtained. The method allows concentrating the analytes more than a thousand times, so the detector sensitivity should not be a limiting factor.

### 3 – Quantitation of Glyphosate/AMPA/Glufosinate in cereals

Extraction of the three analytes was tested on cereals (barley) at concentrations of 92 µg/Kg with a clean up with **AFFINIMIP<sup>®</sup> SPE GLYPHOSATE** cartridges (6 mL format with more sorbent). For this experiment, the AttractSPE<sup>™</sup> positive pressure manifold was used for each SPE step.

**Loading solution:**

Mix 9g of crushed cereals + 75mL ultrapure water with 1% formic acid. Sonicate 30 min, centrifuge 10 min. The supernatant is put to pH = 7 with ammonia solution and filtered to form the loading solution.

**CONDITIONNING**

9 mL ultrapure water

**LOADING**

9mL of loading solution

**WASHING**

24 mL ultrapure water

**ELUTION**

8 mL ultrapure water with HCl 0.1M

**ANALYSIS**

Elutions are evaporated under vacuum at 80°C for 1.5 hours and dissolved in 1mL of mobile phase containing 0.8mM of EDTA-Na<sub>2</sub>. The solution is then analyzed.

*Note: It is advised to use plastic labware to avoid potential adsorption of the analytes on glassware.*



**AttractSPE<sup>™</sup> Positive Pressure Manifold**

**Results:**

The analytes were simultaneously analyzed by LC-MS/MS. The results obtained are presented in the table below. The analytical method is described at the end of the application note.

*Table 3: Recoveries obtained for tested analytes, and corresponding concentrations*

Analyte	Concentration measured (µg/Kg)		Recovery for spiked sample	RSDr (n = 3)
	Not spiked	Spiked		
<b>Glyphosate</b>	<5	93.4	<b>101%</b>	3%
<b>AMPA</b>	<5	90.6	<b>98%</b>	2%
<b>Glufosinate</b>	<5	86.0	<b>93%</b>	3%

**AFFINIMIP<sup>®</sup> SPE GLYPHOSATE** was successfully tested for the quantitation of Glyphosate, AMPA, and Glufosinate in cereals with very good recovery yields and a good repeatability. The concentration measured is lower than defined MRLs.

*Table 4: Analytical conditions tested analytes*

LC Conditions			MS Conditions				
LC Dionex U3000			Qtrap 4000 ESI- MS/MS				
Column : Acclaim Trinity Q1 100mm x 3mm ID (3µm) + prefilter			Curtain gas : 30				
			CAD : High				
Injection volume : 20µL			IS : -4500V				
T° sampler : 10°C			Temperature : 700°C				
Flow rate : 0.5mL/min			GS1/GS2 : 50/50				
Time (min)	Solvent A	Solvent B	Analyte	Retention time (min)	Q1	Q3	CE (V)
0	100%	0%	Glyphosate	1.8	168.0	149.8	-16
3	100%	0%			168.0	62.9	-32
3.2	0%	100%			168.0	78.9	-50
6	0%	100%	AMPA	1.2	110.1	62.8	-24
6.2	100%	0%			110.1	78.8	-34
10.2	100%	0%	Glufosinate	1.6	179.9	63.1	-58
<b>Solvent A</b> : Ammonium Formate 50mM pH = 2.9 (adjusted with Formic Acid) <b>Solvent B</b> : Acetonitrile					179.9	95.0	-24

## Conclusion

**AFFINIMIP<sup>®</sup> SPE GLYPHOSATE** has been successfully used for the enrichment and cleanup of Glyphosate, AMPA, and Glufosinate in several matrices. These experiments show that the analytes strongly interact with the sorbent making possible to load high volume of samples. In addition, **AFFINIMIP<sup>®</sup> SPE GLYPHOSATE** show high capacity of retention. The method has shown excellent performances with recoveries around **80% to 100%**. In addition, the protocol is very simple and fast to proceed.

### Part number of products used in this application note:

<u>Product:</u>	<u>Quantity:</u>	<u>Part number:</u>
<b>AFFINIMIP<sup>®</sup> SPE Glyphosate</b> – 6 mL cartridges	50/pk	FS113-03B
<b>AFFINIMIP<sup>®</sup> SPE Glyphosate</b> – 6 mL cartridges used with more sorbent for cereals	50/pk	FS113-15-03B
<b>AFFINIMIP<sup>®</sup> SPE Glyphosate</b> – 12 mL cartridges	50/pk	FS113-03C
AttractSPE™ Positive Pressure unit	1 unit	MPP-1
15 position, 6 mL	1 unit	MPP-6ML-KIT