

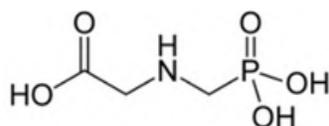
# Application Note



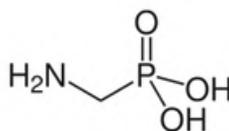
**Analysis of Glyphosate/AMPA/Glufosinate by LC-MS/MS  
without derivatization in mineral water with  
AFFINIMIP® SPE GLYPHOSATE**

This application note describes the purification of glyphosate, AMPA, and glufosinate in large sample volumes of mineral water using AFFINIMIP® SPE Glyphosate. The method is suitable for trace analysis by LC-MS/MS WITHOUT THE NEED FOR PRIOR DERIVATIZATION.

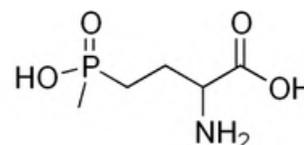
Glyphosate is one of the most widely used herbicides for agriculture. Due to its widespread use, it can be detected at relatively high concentrations. Both glyphosate and glufosinate, another commonly used herbicide, have similar chemical structures and are referred to as phospho-herbicides. In plants, soil, and water, microbes rapidly degrade glyphosate to the metabolite aminomethylphosphonic acid (AMPA). Given these ties, the three molecules are often analyzed simultaneously.



Glyphosate



AMPA



Glufosinate

**Figure 1.** Chemical structures of glyphosate, AMPA, and glufosinate.

The very polar nature of these three molecules makes them difficult to analyze, and usually requires a derivatization step with fluorenylmethyloxycarbonyl chloride (FMOC-Cl) for study with many analytical methods. This derivatization method is time-consuming and introduces uncertainties in the analysis, especially with complex matrices. On the other hand, for water analysis, very low concentrations can make their detection difficult. A concentration of the sample is then necessary.

AFFINIMIP® SPE Glyphosate was proven to be highly effective for the rapid purification and concentration of glyphosate, AMPA, and glufosinate from various matrices, such as large volumes of water, cereals, and honey, to name a few[1]. This application note discusses an efficient SPE cleanup and concentration process for the three molecules from mineral water.

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

## SPE sample processing

For this experiment, 1L of mineral water was spiked with very low concentration of each analyte for trace analysis of the solution and loaded on a 12 mL **AFFINIMIP® 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 60°C for 2 hours and dissolved in 1mL of mobile phase containing 0.8mM of EDTA-Na<sub>2</sub>. The solution is then analyzed.

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	

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

**\*EDTA is important for full recovery of underivatized glyphosate during LC-MS/MS analysis. Although EDTA-Na<sub>2</sub> was used, other EDTA salts may be used (EDTA-Na<sub>4</sub> also tested).**

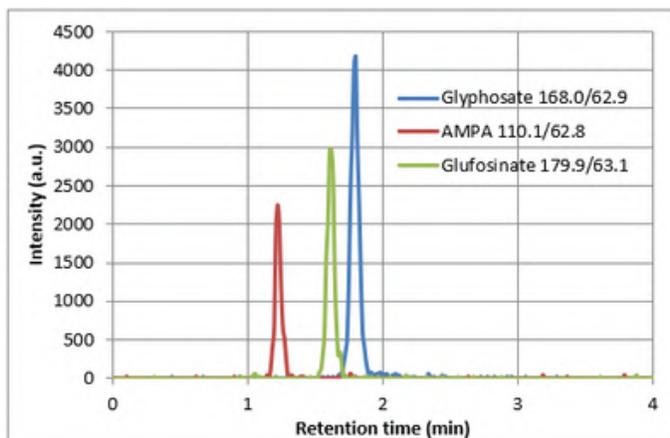
The analytes were simultaneously analyzed by LC-MS/MS. A blank matrix was also performed. The results obtained are presented in the table below. The analytical method is described at the end of the application note.

Analyte	Concentration measured (ng/L)		Recovery for spiked sample (blank deduced)
	Not spiked	Spiked	
Glyphosate	9.7	118.3	108 %
AMPA	4.4	82.5	78%
Glufosinate	0	81.3	81%

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

LC Conditions			MS/MS Conditions				
LC Dionex U3000			Sciex Qtrap 4000 ESI- MS/MS				
Column : Acclaim Trinity Q1 100 mm x 3 mm ID (3 µm) + prefilter			Curtain gas: 30				
Injection volume : 20µL			CAD: High				
T° sampler : 10°C			IS: -4500V				
Flow rate : 0.5mL/min			Temperature: 700°C				
			GSI/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	62.9	-32
3	100%	0%			168.0	78.9	-50
3.2	0%	100%	AMPA	1.2	110.1	62.8	-24
6	0%	100%			110.1	78.8	-34
6.2	100%	0%	Glufosinate	1.6	179.9	63.1	-58
10.2	100%	0%			179.9	95.0	-24
<b>Solvent A</b> : 50mM Ammonium formate, pH 2.9 (adjusted with formic acid) <b>Solvent B</b> : Acetonitrile							

**Table 2.** LC-MS/MS conditions for tested analytes



**Figure 2.** Typical LC-MS/MS chromatogram obtained for the three main ion transitions for glyphosate, AMPA, and glufosinate from a sample purified using **AFFINIMIP® SPE Glyphosate**.

## Conclusion

**AFFINIMIP® SPE GLYPHOSATE** has been successfully used for the enrichment and cleanup of Glyphosate, AMPA, and Glufosinate in mineral water. These experiments show that the analytes strongly interact with the sorbent making possible to load high volume and thus allowing a concentration of the analytes more than a thousand times. The method has shown excellent performances with recoveries around 78% to 109%. In addition, the protocol is very simple.

[1] Application notebook for glyphosate including tests in various matrices available at: <https://www.affinisep.com/support-services/documentations/>

## Product reference

- **AFFINIMIP® SPE Glyphosate**

Catalog number: **FS113-03B** for 50 cartridges 6mL

- **AFFINIMIP® SPE Glyphosate**

Catalog number: **FS113-03C** for 50 cartridges 12mL



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