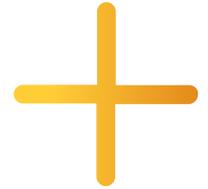




affinisep

Glyphosate

Application Note



**Analysis of glyphosate/AMPA/glufosinate by LC-MS/MS
without derivatization in red wine with AFFINIMIP®
SPE Glyphosate**

Food testing

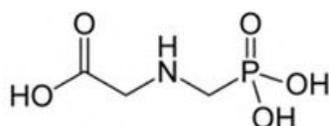


Introduction

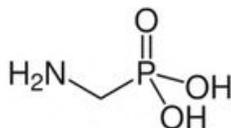
This application note describes the Solid Phase Extraction (SPE) cleanup of glyphosate, aminomethylphosphonic acid (AMPA), and glufosinate from red wine using **AFFINIMIP® SPE Glyphosate**. The purified samples are ready for subsequent LC-MS/MS analysis **WITHOUT THE NEED FOR PRIOR DERIVATIZATION**. The automated system Gilson GX-241 liquid handler was used to realize the SPE protocol.



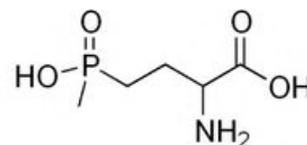
Glyphosate is one of the most widely used herbicides for agriculture. Due to its widespread use, it can be detected at relatively high concentrations. Both glyphosate and glufosinate, another commonly used herbicide, have similar chemical structures and are referred to as phospho-herbicides. In plants, soil, and water, microbes rapidly degrade glyphosate to the metabolite 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 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 some matrices, very low concentrations can make their detection difficult. A concentration of the sample is then necessary.

Proceeding of the experiment and recoveries

For this study, an organic red wine (Bordeaux) was chosen to avoid the presence of glyphosate. A 6 mL **AFFINIMIP® SPE Glyphosate cartridge with improved capacity** was used for this study.

Loading solution: 10 mL of red wine is diluted with 90 mL of ultrapure water. The pH is adjusted to 6–8 with 35% ammonia solution. The solution is then spiked with glyphosate, AMPA, and glufosinate at 12.5 µg/L each. The following SPE protocol is carried out from conditioning to elution using GX-241 autosampler.

CONDITIONING

9 mL ultrapure water

LOADING

24 mL of loading solution at 1.5 mL/min

WASHING

1. 8 mL 80% methanol (in water)
2. 4 mL ultrapure water

ELUTION

8 mL HCl 0.2M (in water)

ANALYSIS

Elutions are collected in polypropylene vials, evaporated under vacuum at 60°C for 2 hours, and dissolved in 3 mL of mobile phase containing 0.8mM of EDTA-Na₂.



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



SPE protocol was carried out using the Gilson GX-241 liquid handler.

After the **AFFINIMIP® SPE Glyphosate** procedure, the molecules were simultaneously analyzed by LC-MS/MS (Table 2) without derivatization. Wine without added glyphosate, AMPA, or glufosinate was also tested as a blank control. The results obtained are presented in Table 2.

Analyte	Concentration in blank control (µg/L)	Spike level (µg/L) (diluted wine)	Recovery from spiked sample
Glyphosate	ND	12.5	96%
AMPA	ND	12.5	81%
Glufosinate	ND	12.5	70%

Table 1. Recovery of glyphosate, AMPA, and glufosinate in diluted red wine spiked at 12.5 µg/L after purification with **AFFINIMIP® SPE Glyphosate**. (ND = Not detected)

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				
			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	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
Solvent A : 50mM Ammonium formate, pH 2.9 (adjusted with formic acid) Solvent B : Acetonitrile							

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

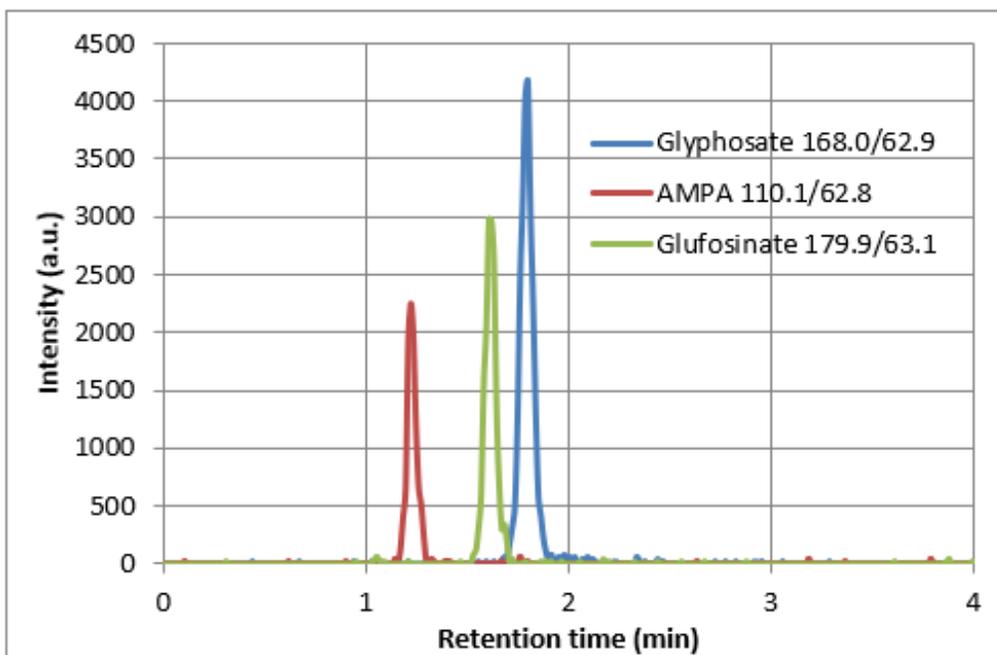


Figure 2. Typical LC-MS/MS chromatogram obtained for the three main ion transitions of glyphosate, AMPA, and glufosinate from a sample purified using **AFFINIMIP® SPE Glyphosate**.

Conclusion

AFFINIMIP® SPE Glyphosate has been successfully used for the enrichment and cleanup of glyphosate, AMPA, and glufosinate from red wine. **AFFINIMIP® SPE Glyphosate** demonstrated a high selectivity for the three molecules, producing excellent recoveries of the three compounds (above 70%). In addition, the protocol is very simple, fast, and easily automated with the use of the GX-241 liquid handler from Gilson.

Product reference

- **AFFINIMIP® SPE Glyphosate**

Catalog number: **FS113-15-03B** for 50 cartridges 6mL with improved capacity



Figure 3. 24 mL of the loading solution in a 50 mL falcon (left), and 24 mL of loading solution concentrated using **AFFINIMIP® SPE Glyphosate** (right).