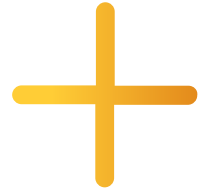




# affinisep

Glyphosate

## Application Note



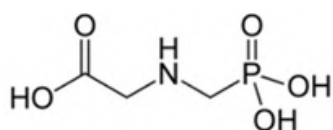
**Analysis of glyphosate/AMPA/glufosinate by LC-MS/MS  
without derivatization in beers with  
AFFINIMIP® SPE Glyphosate**

Food testing

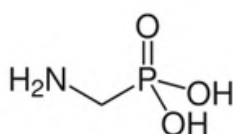
## Introduction

This application note describes the fast purification of glyphosate, aminomethylphosphonic acid (AMPA), and glufosinate from blond beer and dark beer using **AFFINIMIP®SPE Glyphosate**. The purified samples are ready for subsequent LC-MS/MS analysis **WITHOUT THE NEED FOR PRIOR DERIVATIZATION**

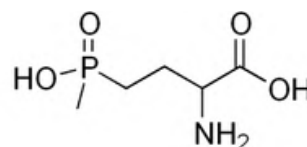
Glyphosate is one of the most widely used herbicides for agriculture. Due to its widespread use, it can be detected at relatively high concentrations. Both glyphosate and glufosinate, another commonly used herbicide, have similar chemical structures and are referred to as phospho-herbicides. In plants, soil, and water, microbes rapidly degrade glyphosate to the metabolite AMPA. Given these ties, the three molecules are often analysed 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 tricky and time-consuming and introduces uncertainties in the analysis, especially with complex matrices.

**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, honey, and wine to name a few[1]. In this application note, an efficient SPE cleanup and concentration process is shown for the three molecules from blond and dark beer.

A 12 mL AFFINIMIP® SPE Glyphosate cartridge was used for this study. An organic blond beer (Alcohol 4.5%) and a dark beer (alcohol 7%) were tested.

**Loading solution:** The beer was firstly degassed by sonication for 30 minutes. Then, 10 mL of beer 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 at 1.56 µg/L and with AMPA and glufosinate at 3.12 µg/L each.

### CONDITIONING

9 mL ultrapure water

### LOADING

16 mL of loading solution at 1.5 mL/min

### WASHING

- 8 mL methanol/water (80/20 v/v)
- 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, and dissolved in 1 mL of mobile phase containing **0.8mM of EDTA-Na<sub>2</sub>**.



*Note: We recommend the use of plastic labware to avoid potential adsorption of the analytes on glassware.*

After the **AFFINIMIP® SPE Glyphosate** process, molecules were simultaneously analyzed by LC-MS/MS (analytical conditions described Table 2) without derivatization. Beers without added glyphosate, AMPA, or glufosinate were also checked as a blank control. The results obtained are presented in Table 1.

		Glyphosate	AMPA	Glufosinate
Spike concentration in non-diluted beer		15.6 µg/L	31.2 µg/L	31.2 µg/L
Blond beer	Mean % recovery	104%	60%	80%
	RSDr (n = 4)	4%	9%	10%
Dark beer	Mean % recovery	99%	58%	77%
	RSDr (n = 3)	6%	9%	10%
Average % recovery		102%	59%	79%
RSDR (n = 7)		5%	8%	9%

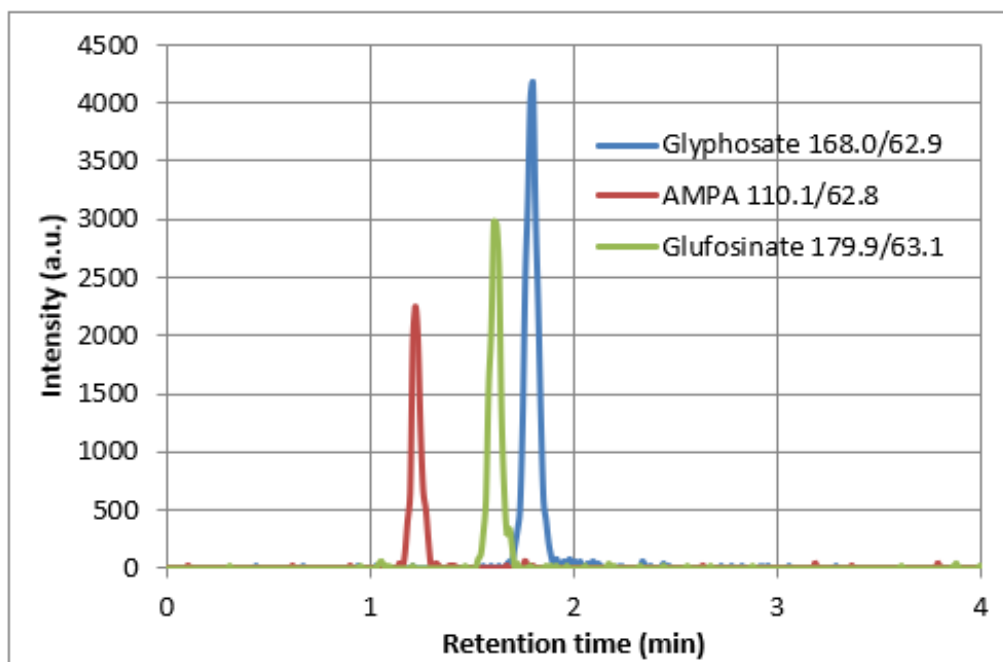
**Table 1.** Recovery of glyphosate, AMPA, and glufosinate in blond and dark beer after purification with **AFFINIMIP® SPE Glyphosate**.

Recoveries ranging from 59% to 102% for the three molecules were observed, demonstrating the success of the purification method using **AFFINIMIP® SPE Glyphosate**.

After the **AFFINIMIP® SPE Glyphosate** process, molecules were simultaneously analyzed by LC-MS/MS (analytical conditions described Table 2) without derivatization. Beers without added glyphosate, AMPA, or glufosinate were also checked as a blank control. The results obtained are presented in Table 1.

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
<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 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 blond beer and dark beer. **AFFINIMIP® SPE Glyphosate** demonstrated a high selectivity for the three molecules, giving good recoveries. In addition, the protocol has shown a good reproducibility with a relative standard deviation between 5% and 9% over 7 samples.

## Product reference

- **AFFINIMIP® SPE Glyphosate**

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

- **AFFINIMIP® SPE Glyphosate**

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



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