LC-MS/MS detection of glyphosate/AMPA/glufosinate in red wine without prior derivatization after AFFINIMIP® SPE Glyphosate cleanup

APPLICATION NOTE AN1028

APPLICATION BENEFITS

This application note describes the rapid purification 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.

SOLUTIONS

AFFINIMIP® SPE Glyphosate is 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 (from 70% to 96%). In a simple, fast, and easily automated with the use of Gilson's GX-241 liquid handler protocol.

INTRODUCTION

Glyphosate is one of the most widely used herbicides for agriculture and can be detected at relatively high concentrations in agricultural areas. 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.

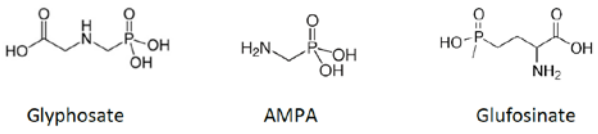

Figure 1
Chemical structures of glyphosate, AMPA, and glufosinate.

The very polar nature of these three molecules makes them difficult to analyze, and usually requires a derivatization step with fluorenylmethyloxycarbonyl chloride (FMOC-CI) for study with many analytical methods. This derivatization method is time-consuming and introduces uncertainties in this 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, and honey[1], to name a few. This application note discusses an efficient SPE cleanup and concentration preocess for the three molecules from red wine.

For this study, an organic red wine (Bordeaux) was chosen in order to avoid any presence of glyphosate.

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.

AFFINISEP

GILSON®

CONDITIONING

1. 9 mL ultrapure water

LOADING

1. 24 mL of loading solution at 1.5 mL/min

WASHING

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

ELUTION

1. 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. The solution is then analyzed by LC-MS/MS (Table 2).

NOTE We recommend the use of plastic labware to avoid potential adsorption of the analytes on glassware.

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

RESULTS

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 1.

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)

Analyte	Concentratration 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%

Recoveries ranging from 70% to 96% for the three molecules were observed, demonstrating the success of the purification method using **AFFINIMIP® SPE Glyphosate**.

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

LC Conditions	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
	CAD: High
Injection volume: 20 µL	IS: -4500V
T° sampler: 10°C	Temperature: 700°C
Flow rate: 0.5 mL/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	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

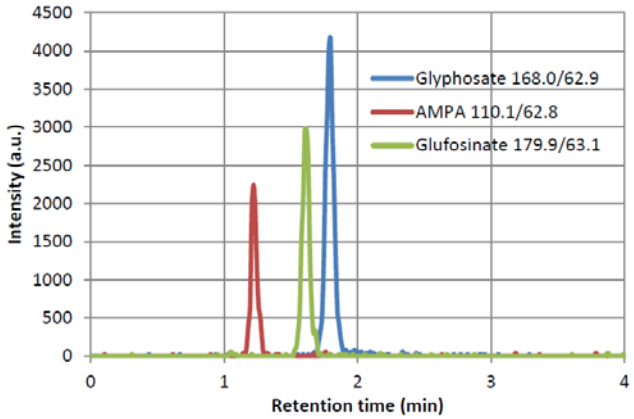

Figure 2
LC-MS/MS chromatogram obtained for the three main ion transitions for glyphosate, AMPA, and glufosinate from a red wine sample purified using **AFFINIMIP® SPE Glyphosate**.

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 (Figure 2), producing excellent recoveries of the three compounds (from 70% to 96%). In addition, the protocol is simple, fast, and easily automated with the use of Gilson's GX-241 liquid handler.

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**.

REFERENCES

[1]Application notebook for glyphosate including tests in various matrices available at:

https://www.affinisep.com/spe-kits-applications/spe-kit-for-sample-preparation/affinimip-spe---selectives-mip-spe-cartridges/affinimip-spe-glyphosate---ampa/

Part number of products used in this application note

Product	Quantity	Part number
AFFINIMIP® SPE Glyphosate - 6 mL cartridges with improved capacity	50/PK	FS113-15-03B