AUTOMATED EXTRACTION OF GLYPHOSATE/AMPA/GLUFOSINATE IN RED WINE PRIOR TO LC-MS/MS ANALYSIS WITHOUT DERIVATIZATION

OVERVIEW

Since 1970, glyphosate has been used as a systemic, non-selective herbicide and crop desiccant on farms, in fields, and in public and residential areas. It is one of the most widely used herbicides worldwide.

Microbial action rapidly decomposes glyphosate into aminomethyl phosphonic acid (AMPA).

Glufosinate, another common phospho-herbicide, is structurally and functionally similar to AMPA and glyphosate, and the three molecules are often analyzed simultaneously.

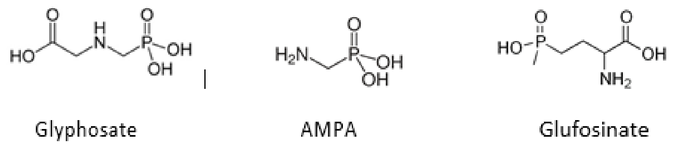

Figure 1
Chemical structures of glyphosate, AMPA, and glufosinate

Due to its relative stability in soils and sediments, and its high solubility in water, glyphosate and its principal decomposition product, AMPA, are present at low concentrations in environmental matrices and across the food chain.

The high polarity of these three molecules makes analysis difficult and typically requires a derivatization step with fluorenylmethyloxycarbonyl chloride (FMOC-Cl) to enable detection and quantitation.

Experimental

AFFINIMIP® SPE Glyphosate is a selective molecularly imprinted polymer phase (MIP) that is highly efficient for the rapid purification and concentration of glyphosate, AMPA, and glufosinate from various matrices, such as large volumes of water, cereals, and honey.

In this study, we have investigated the automation of the **AFFINIMIP SPE Glyphosate** protocol for red wine using the **Gilson ASPEC® 241 Liquid Handler**, a compact liquid handler dedicated to Solid Phase Extraction (SPE) using positive pressure elution.

METHOD

Analysis Method

Elutions are collected in polypropylene vials to avoid potential adsorption of analytes on glassware, evaporated under vacuum at 60°C for 2 hours, and dissolved in 3 mL of mobile phase containing 0.8 mM of EDTA-Na_2. After the **AFFINIMIP SPE Glyphosate** procedure, the molecules were simultaneously analyzed by LC-MS/MS without derivatization (Table 1).

Table 1
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 : -4500 V
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: 50 mM ammonium formate, pH 2.9 (adjusted with formic acid)
Solvent B: Acetonitrile

RESULTS AND CONCLUSION

Nonspiked wine serves as a blank control.

Spiked solution preparation:

- 10 mL of red wine is diluted with 90 mL of ultrapure water
- pH is adjusted to 6–8 with 35% aqueous ammonia solution
- Solution is then spiked with glyphosate, AMPA, and glufosinate at 12.5 µg/L each

Table 2
SPE protocol

Step	SPE protocol on AFFINIMIP® SPE Glyphosate
Condition	9 mL ultrapure water
Load	24 mL loading solution at 1.5 mL/min
Wash	8 mL 80% methanol (in water)
Wash	4 mL ultrapure water
Elution	8 mL HCl 0.2M (in water)

The complete SPE protocol is carried out using the **Gilson ASPEC 241 Liquid Handler** controlled by **TRILUTION® LH** Software.

Figure 2
TRILUTION® LH method sequence

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**.

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%

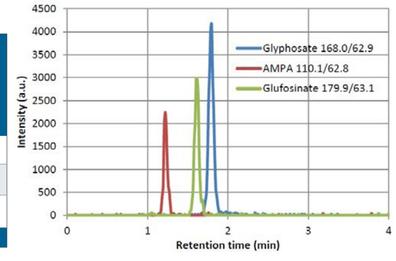

Figure 3
Recovery of glyphosate, AMPA, and glufosinate in diluted red wine spiked at 12.5 µg/L after purification with **AFFINIMIP SPE Glyphosate**
(ND = Not detected)

The **AFFINIMIP SPE Glyphosate** protocol has been automated on the **Gilson ASPEC 241 Liquid Handler** for the enrichment and cleanup of glyphosate, AMPA, and glufosinate from red wine. LC-MS/MS analysis demonstrate high selectivity and excellent recoveries of the three compounds (from 70% to 96%).

This automated solution does not require time-consuming derivatization which introduced variability, especially in complex matrices.

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