

Application Note



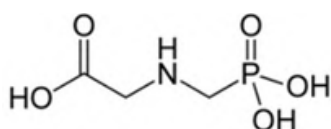
**Analysis of Glyphosate/AMPA/Glufosinate by LC-MS/MS
without derivatization in cereals with AFFINIMIP® SPE
GLYPHOSATE**

Food testing

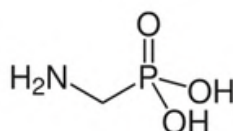
This application note shows an efficient Solid Phase Extraction (SPE) cleanup of glyphosate, AMPA, and glufosinate in cereal extracts using **AFFINIMIP® SPE GLYPHOSATE**. The quantitation of these molecules is shown WITHOUT DERIVATIZATION prior to LC/MS-MS analysis.



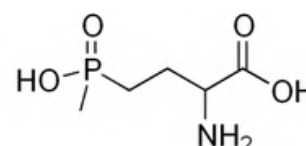
Glyphosate is one of the most vastly used herbicides in the world for cultivation, especially cereals. Due to its important use, it can be detected at relatively high concentrations. With Glufosinate, another commonly used herbicide, they are closely structured herbicides referred to as phospho-herbicides. Glyphosate undergoes rapid microbial degradation in plants, soil and water to the metabolite aminomethylphosphonic acid (AMPA). These three molecules are often analyzed simultaneously. Codex alimentarius has defined a MRL (maximum residue limit) for Glyphosate of 30mg/Kg in cereals.



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

Extraction of the three analytes was tested on cereals (barley) at concentrations of 92 µg/Kg with a clean up with **AFFINIMIP® SPE GLYPHOSATE** cartridges (6 mL format with more sorbent).

Loading solution: Mix 9g of crushed cereals + 75mL ultrapure water with 1% formic acid. Sonicate 30 min, centrifuge 10 min. The supernatant is put to pH = 7 with ammonia solution and filtered to form the loading solution.

CONDITIONING

9 mL ultrapure water

LOADING

9mL of loading solution (~1mL/min)

WASHING

24 mL ultrapure water

ELUTION

8 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-Na2.

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



AttractSPE™ Positive Pressure Manifold

The analytes were simultaneously analyzed by LC-MS/MS without derivatization. The results obtained are presented in the table below. The analytical method is described at the end of the application note.

Analyte	Concentration measured (µg/Kg)		Recovery for spiked sample	RSDr (n = 3)
	Not spiked	Spiked		
Glyphosate	<5	93.4	101%	3%
AMPA	<5	90.6	98%	2%
Glufosinate	<5	86.0	93%	3%

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				
			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.

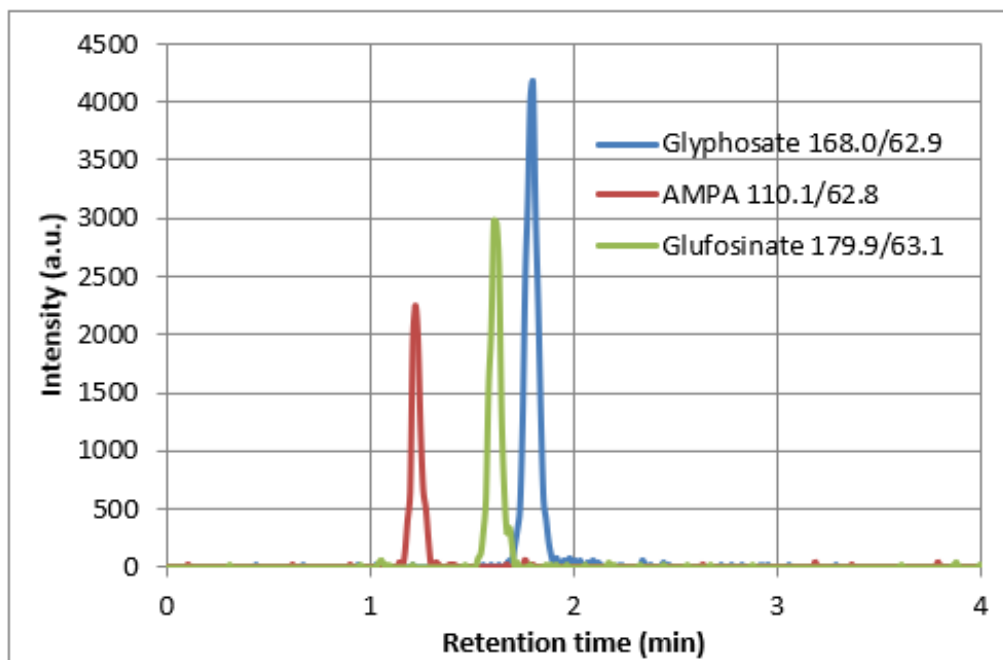


Figure 2. Typical LC-MS/MS chromatogram obtained for the three main ion transitions of glyphosate, AMPA, and glufosinate from a sample purified using **AFFINIMIP® SPE Glyphosate**.

Conclusion

AFFINIMIP® SPE GLYPHOSATE has been successfully used for the enrichment and cleanup of Glyphosate, AMPA, and Glufosinate in cereals at lower concentrations than MRLs defined by Codex Alimentarius. The method has shown excellent performances with recoveries from 93% to 101%. In addition, the protocol is very simple and fast to proceed.

Product reference

- **AFFINIMIP® SPE Glyphosate**

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

- **AttractSPE™ Positive Pressure unit**

Catalog number: **MPP-1** for 1 unit

- **15 position, 6 mL**

Catalog number: **MPP-6ML-KIT** for 1 unit

