

Application Note



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

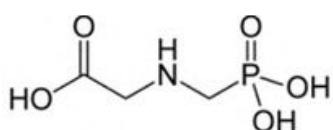


Introduction

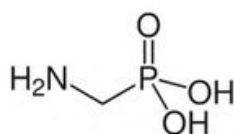
This application note shows an efficient Solid Phase Extraction (SPE) cleanup of glyphosate, AMPA, and glufosinate in black tea (earl grey) extracts using **AFFINIMIP® SPE GLYPHOSATE**. The quantitation of these molecules is shown **WITHOUT DERIVATIZATION** prior to LC/MS-MS analysis.



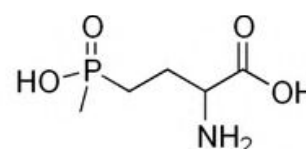
Glyphosate is one of the most vastly used herbicides in the world for cultivation. Due to its important use, it can be detected at relatively high concentrations. With Glufosinate, another commonly used herbicide, they are closely structured herbicides referred to as phospho-herbicides. Glyphosate undergoes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



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 dry black tea (Earl grey) at concentrations of 1.67 mg/Kg with a clean up with **AFFINIMIP® SPE GLYPHOSATE** 6mL cartridges.

Loading solution: Mix 3g of black tea powder + 50mL 1% Formic acid in ultrapure water. Sonicate 30 min, centrifuge 10 min. The supernatant is filtered and put to pH = 7 with ammonia solution to form the loading solution.

CONDITIONING

6 mL ultrapure water

LOADING

3mL of loading solution (~1mL/min)

WASHING

12 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 3mL 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.



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 (mg/Kg)		Recovery for spiked sample	RSDr (n = 3)
	Not spiked	Spiked		
Glyphosate	Not detected	1.72	103%	10%
AMPA	Not detected	1.35	81%	12%
Glufosinate	Not detected	1.37	82%	6%

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.

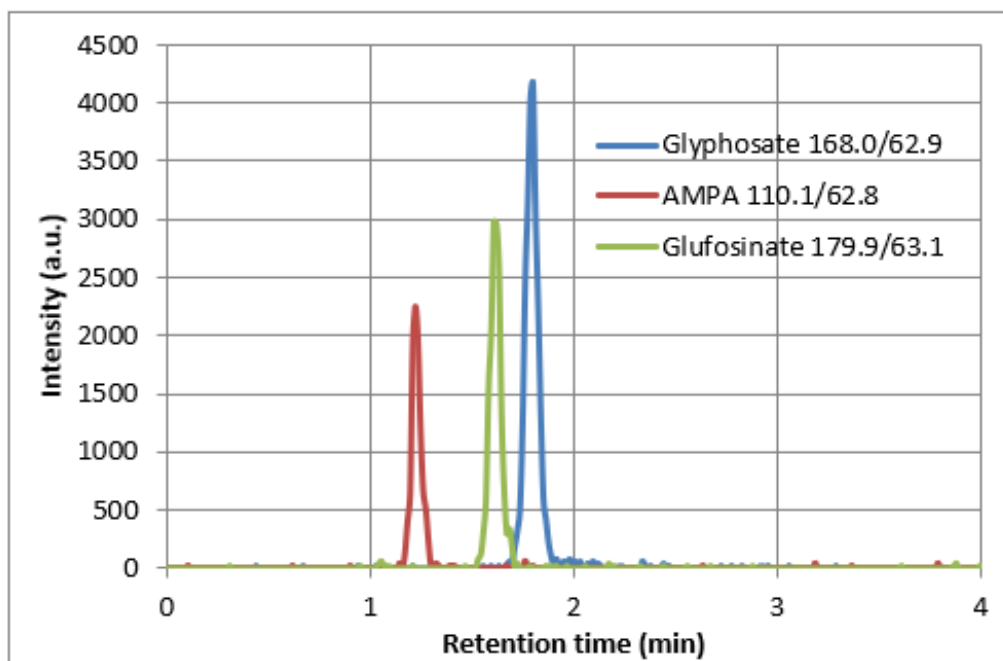


Figure 2. Typical LC-MS/MS chromatogram obtained for the three main ion transitions of glyphosate, AMPA, and glufosinate from a sample purified using **AFFINIMIP® SPE Glyphosate**.

Conclusion

AFFINIMIP® SPE GLYPHOSATE has been successfully used for the cleanup of Glyphosate, AMPA, and Glufosinate in honey. The method has shown excellent performances with recoveries from 81% to 103%. In addition, the protocol is very simple and fast to proceed.

Product reference

- **AFFINIMIP® SPE Glyphosate**

Catalog number: **FS113-03B** for 50 cartridges 6mL

- **AFFINIMIP® SPE Glyphosate**

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

