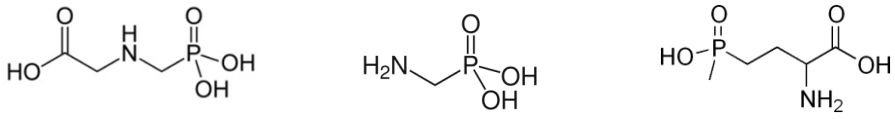
LC-MS/MS detection of trace levels of glyphosate, AMPA, glufosinate in large volume of sea water without prior derivatization after AFFINIMIP® SPE Glyphosate cleanup

How to overcome Glyphosate and derivatives analysis

This application note describes the purification of glyphosate, AMPA, and glufosinate in large sample volumes of sea water using AFFINIMIP® SPE Glyphosate. The method is suitable for trace analysis by LC-MS/MS WITHOUT THE NEED FOR PRIOR DERIVATIZATION. The automated system Gilson 274 ASPEC Large Volume System was used to realize SPE protocol.

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 aminomethylphosphonic acid (AMPA). Given these ties, the three molecules are often analyzed 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 time-consuming and introduces uncertainties in the analysis, especially with complex matrices. On the other hand, for water analysis, very low concentrations can make their detection difficult. A concentration of the sample is then necessary.

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, to name a few[1]. This application note discusses an efficient SPE cleanup and concentration process for the three molecules from sea water.

The quantitation of these molecules is shown WITHOUT DERIVATIZATION with a clean-up with AFFINIMIP® SPE Glyphosate prior to LC/MS-MS analysis.

1 – Glyphosate, AMPA, Glufosinate in sea water

1.1 - SPE sample processing

It is preferable to avoid glass because of potential adsorption of glyphosate on glass surfaces. PEHD containers were used as samples containers. Polypropylene tubes were used for collection of the elutions and evaporation. Polypropylene vials were used for the analysis.

For this study, sea water was gathered in the city of Le Havre (France) in a PEHD bottle, and used within a week. pH and salts concentration have been measured and are presented in the table below.

Measured pH	**8.42***
Measured concentration of salts	**37.8 g/L**

*It is recommended to get a pH above 5 to avoid loss of AMPA.

The automated system Gilson 274 ASPEC Large Volume System was used to realize SPE protocol. 6mL cartridges of AFFINIMIP® SPE Glyphosate with improved capacity were used.

Step	SPE Protocol
Sample preparation	Water is filtered through 1.2µm membrane filter. 500 mL samples are then spiked at 200 ng/L with each analytes. (One sample not spiked for blank determination)
Conditioning/ Equilibration	9 mL ultrapure water
Loading	500 mL of loading solution (~4mL/min)
Washing	12 mL ultrapure water
Elution	**2 x 4 mL HCl 0.2M in water**
Analysis*	Elutions are collected in polypropylene vials, evaporated with rotative vacuum evaporator at 60°C for 2 hours, and dissolved in 1 mL of mobile phase containing **0.8mM of EDTA-Na_2**. (800µL of 0.1M EDTA-Na2 aqueous solution mixed with 99.2 mL of mobile phase)

Figure 2. Gilson ASPEC Large Volume System

***EDTA is important for full recovery of underivatized glyphosate during LC-MS/MS analysis. Although EDTA-Na_2 was used, other EDTA salts may be used (EDTA-Na_4 also tested).**

1.2 - RESULTS

After the AFFINIMIP® SPE Glyphosate procedure, the molecules were simultaneously analyzed by LC-MS/MS (see method Table 2) without derivatization. A sample of sea water without added glyphosate, AMPA, glufosinate, was tested as blank control to determine eventual initial presence the analytes. Three sea water samples spiked at 200ng/L were tested to show repeatability. The results obtained are presented in Table 1.

Compound	Presence in blank	Recovery	RSDr (n=3)	Observed matrix effect
Glyphosate	ND	**102%**	3%	-4%
AMPA	146 ng/L	**157%** **(91% with blank subtracted)**	3%	-17%
Glufosinate	ND	**61%**	5%	-6%

Table 1. Recovery for glyphosate, AMPA, glufosinate in **500 mL** of unspiked sea water and spiked sea water (200 ng/L) after purification with AFFINIMIP® SPE Glyphosate. The recovery values already take into account the matrix effects. (ND = Not detected)

Recoveries observed were **102%** for glyphosate and **61%** for glufosinate. The presence of AMPA was detected in sea water before spike at a concentration of **146ng/L** leading to higher recoveries. The subtraction of the blank allowed calculating a recovery yield of **91%** for AMPA. The method showed excellent repeatability with relative standard deviation from **3%** to **5%**. Few to no matrix effects were observed with a maximum of 17% signal supression for AMPA, demonstrating the success of the purification method using AFFINIMIP® SPE Glyphosate.

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							

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

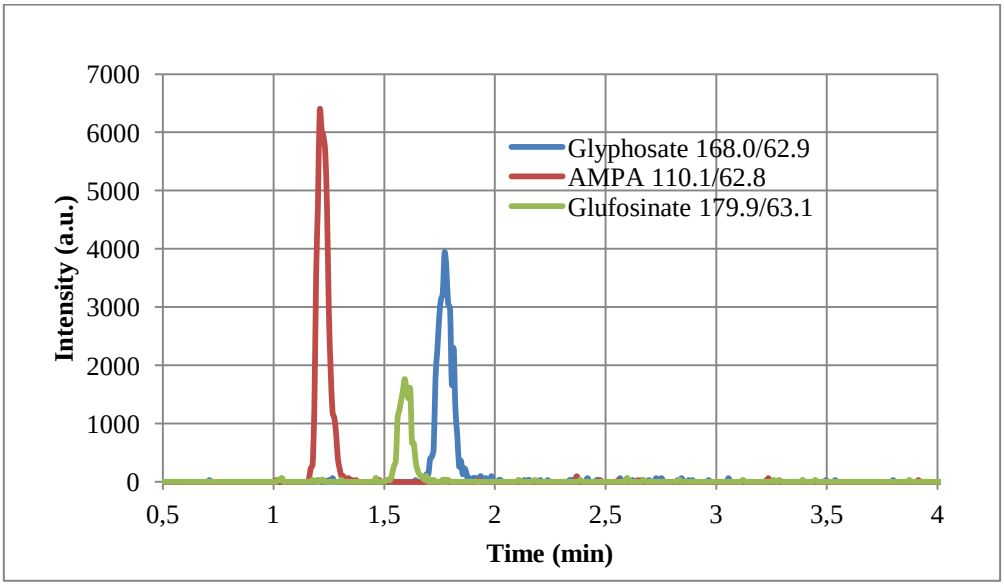


Figure 3. LC-MS/MS chromatogram obtained for the three main ion transitions for glyphosate, AMPA, and glufosinate from a sea water sample spiked at 200ng/L and purified using **AFFINIMIP® SPE Glyphosate**.

CONCLUSION

AFFINIMIP® SPE Glyphosate has been successfully used for the enrichment and cleanup of trace levels of glyphosate, AMPA, and glufosinate from large volumes of sea water samples (500mL). **AFFINIMIP® SPE Glyphosate** demonstrated a high selectivity for the three molecules (Figure 3), producing excellent recoveries for glyphosate and AMPA **(<90%),** and good recoveries for glufosinate **(61%).** Moreover, the protocol showed excellent repeatability with relative standard deviation from **3% to 5%**.

The method made it possible to **concentrate 500 times** the sample, making the analysis possible with low sensitivity devices.

Finally, the protocol is very simple, does not use organic solvents, and was easily automated with **Gilson 274 ASPEC Large Volume System.**

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

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