

Analysis of Glufosinate and Glyphosate in Water by HPLC With Post-Column Derivatization

Glyphosate is a broad-spectrum herbicide that has been used for several decades to protect a variety of crops. Due to its heavy use in agriculture many weeds have developed resistance to Glyphosate causing increased interest in finding alternative herbicides. Glufosinate also provides broad-spectrum weed control and can be successfully used against many Glyphosate-resistant plants and grasses. Glufosinate has become increasingly popular creating need for analytical methods to monitor food and water to ensure protection of human health and environment.

EPA mandates maximum contamination level (MCL) for Glyphosate in water at 700 ug/L. EPA Method 547 describes Glyphosate analysis in drinking water by direct-injection HPLC with post-column derivatization. Glufosinate contains a primary amino group and is also capable of reacting with o-Phthalaldehyde (OPA) reagent. Utilizing mixed-mode analytical column allowed us to develop a simple method capable of separating Glufosinate and Glyphosate in water with direct injection. This method eliminates complicated and labor-intensive sample pretreatment steps, required by LC/MS analysis. Post-column derivatization with OPA reagent ensures high sensitivity of analysis without matrix interferences or signal suppression.

Method

Calibration

Calibration range for Glyphosate and Glufosinate is from 25 ug/L to 1000 ug/L. A quadratic calibration curve with $R^2=0.9998$ is observed for Glufosinate. A linear calibration curve with $R^2=0.9998$ is observed for Glyphosate.

Sample Preparation

Filter water samples through 0.45 um Nylon filter, inject

Analytical Conditions

Column: Acclaim Mixed-Mode WAX-1, 4.6x150 mm (Thermo Scientific)

Column Temperature: 55 °C

Flow Rate: 1.0 mL/min

Mobile Phase: 85% of K600, 15% of ACN2

Injection Volume: 100 uL

Post-column Conditions

Post-column System: Onyx PCX, Pinnacle PCX or Vector PCX

Heated Reactor Volume: 0.5 mL

Temperature: 36 °C

Ambient Reactor: 0.1 mL

Reagent 1: 90 uL of 5% NaOCl (Bleach) in 950 mL of GA116 Diluent

Reagent 2: 100 mg of OPA and 2 g of Thiofluor in 950 mL of GA104 Diluent

Reagent Flow Rate: 0.3 mL/min each reagent

Detection: λ_{EX} :330 nm, λ_{EM} :465 nm

Conclusion

This simple, fast and sensitive method allows simultaneous detection of Glufosinate and Glyphosate in drinking and surface waters. The method utilizes common HPLC equipment and doesn't require complex extraction and derivatization of the samples prior to injection. Avoiding sample pretreatment steps reduces analysis time and costs as well as minimizes errors. This promising protocol can be easily adopted by laboratories, especially the ones already set up to run Glyphosate analysis according to EPA Method 547.

Pickering Laboratories also plans to expand this protocol to including analysis of Glyphosate and Glufosinate in vegetable samples.

Table 1. Glufosinate and Glyphosate Analysis in water

Analyte	Surface Water		Bottled Water	
	Glufosinate	Glyphosate	Glufosinate	Glyphosate
Spike	100 ug/L	100 ug/L	100 ug/L	100 ug/L
RSD, N=3	3.7%	1.0%	4.2%	0.8%
Recoveries	96%	94%	85%	92%
Spike	300 ug/L	300 ug/L	300 ug/L	300 ug/L
RSD, N=3	2.5%	0.6%	2.1%	0.5%
Recoveries	87%	93%	87%	92%

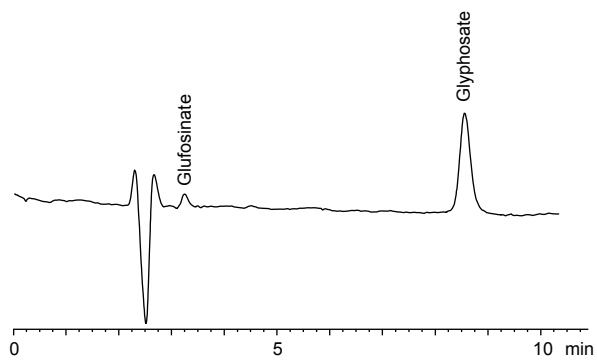


Fig 1. Chromatogram of 50 ug/L calibration standard of Glufosinate and Glyphosate

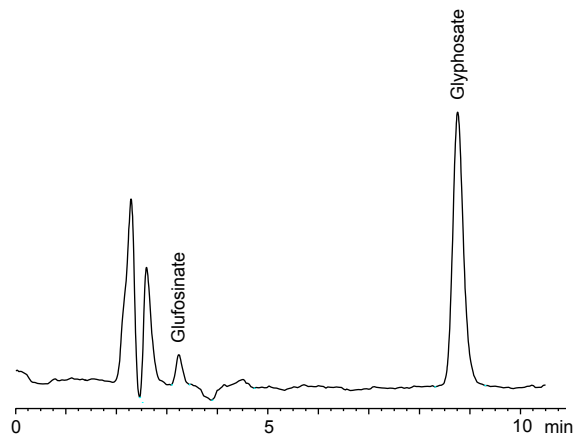


Fig 3. Chromatogram of surface water spiked with Glufosinate and Glyphosate at 100 ug/L

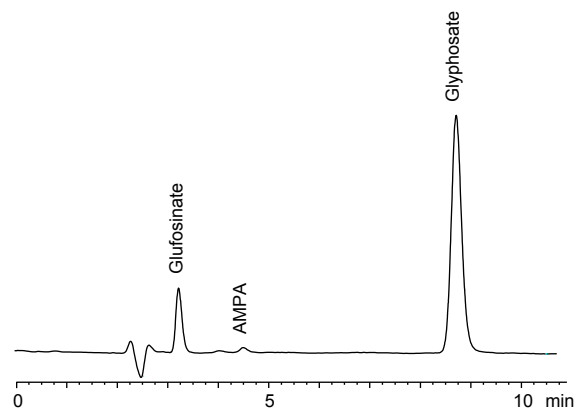


Fig 2. Chromatogram of 500 ug/L calibration standard of Glufosinate, Glyphosate and AMPA

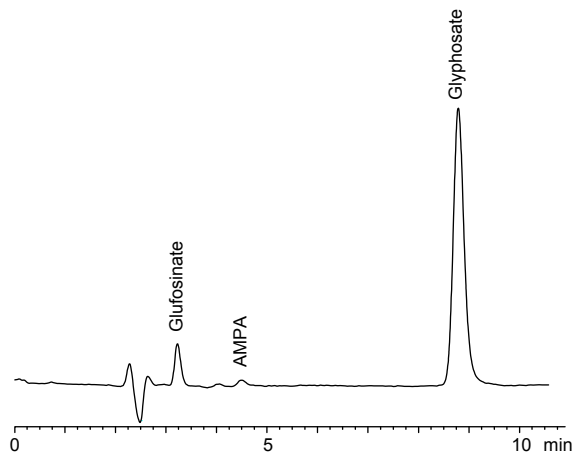


Fig 4. Chromatogram of bottled water spiked with Glufosinate, Glyphosate and AMPA at 300 ug/L