Application Note: 20029

LC/MS of Glyphosate and AMPA

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Key Words

- Glyphosate and AMPA
- Herbicide and metabolite analysis
- Hypercarb
- LC/MS
- Polar retention

Introduction

Glyphosate [N-(phosphonomethyl) glycine] is a broad spectrum, non-selective herbicide which acts by inhibiting the shikimic acid pathway in plants. Glyphosate has been considered a benign herbicide with low toxicity, but recent studies have raised global health and environmental concerns about its use.[1] Glyphosate readily breaks down into aminomethyl phosphonic acid (AMPA) in the environment; hence both compounds require accurate measurement to protect drinking water supplies.

However, both compounds present an analytical challenge as they are highly polar (Figure 1). Typical silica-based reversed phase C18 columns experience difficulty with retention of polar compounds, and may result in non-resolved co-eluting peaks, often with polar analytes eluting in the void volume. Traditional analytical methods requires complex eluents and time consuming derivatization steps to achieve retention on a reversed phase support.

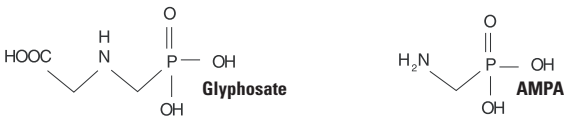

Figure 1: Structure of glyphosate and AMPA

Hypercarb™ is a 100% porous graphitic carbon (PGC) stationary phase with unique capacity for retention of very polar molecules. Through its primary retention mechanisms (dispersive and dipole induced interactions), it shows greater retention and resolution for underivatized glyphosate and AMPA than silica based C18 without requiring an ion pair reagent in the mobile phase.

The method developed on Hypercarb uses a mobile phase containing formic acid, which promotes protonation of the amino/NH group and therefore a fast and sensitive +ESI detection method is achieved.

Experimental

The analyses of glyphosate and AMPA (50 ng) were carried out using a Thermo Scientific Finnigan™ LC/MS system. The separations were performed using 50 x 2.1 mm columns; 5 µm Thermo Scientific Hypersil™ BDS C18 (part number 28105-052130) and 5 µm Hypercarb (part number 35005-052130) with the following conditions:

Eluent:	A: H_2O + 0.1% formic acid B: ACN + 0.1% formic acid
Gradient:	5 to 100% B in 10 mins
Flow Rate:	0.3 mL/min
Detection:	MS +ESI 450 °C, 4.5 kV, 20 V SIM $[M+H]^+$ (m/z 112, 170)

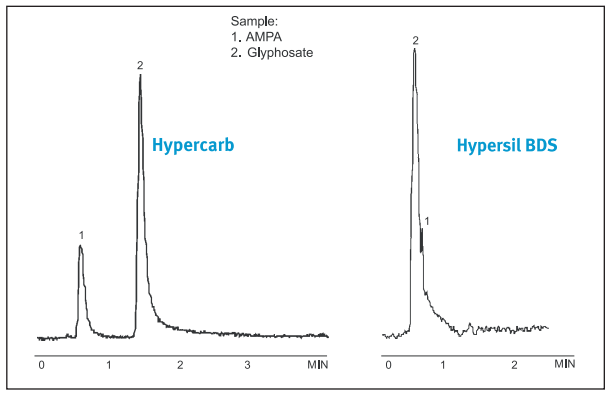

Figure 2: Analysis of underivatized glyphoste and AMPA on Hypercarb and Hypersil BDS C18

Results and Discussion

Figure 2 compares the separation of glyphosate and AMPA on silica-based C18 and Hypercarb.

The silica C18 column (Hypersil™ BDS C18) shows typical behavior for polar compounds, with co-elution of glyphosate and AMPA near the void volume (0.48 and 0.51 minutes respectively).

Hypercarb retains and resolves both AMPA and glyphosate ensuring that a robust quantitative method can be developed. The capacity factor for glyphosate is 2.2. The baseline resolution between the two compounds allows for maximum sensitivity when quantifying them together.

Hypercarb solid phase extraction formats (HyperSep™ Hypercarb) have proven successful for trace enrichment of polar pesticides and metabolites[2], and may provide a solution for effective extraction of glyphosate and AMPA from environmental matrices.

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Conclusion

The combination of retention, ease of method development and increased sample throughput makes Hypercarb ideal for accurate routine measurement of glyphosate and AMPA.

References

1. Cox, C., Herbicides Fact Sheet. Glyphosate (Roundup). *Journal of Pesticide Reform*, 1989. 18, 3 – 17.
2. M.C. Hennion, *J. of Chromatogr*. A, 885 (2000) 73-95.

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AN20029_E 12/06M

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