

# UHPLC-MS/MS Analysis of Polar Pesticides on the Supel<sup>™</sup> Carbon LC Column

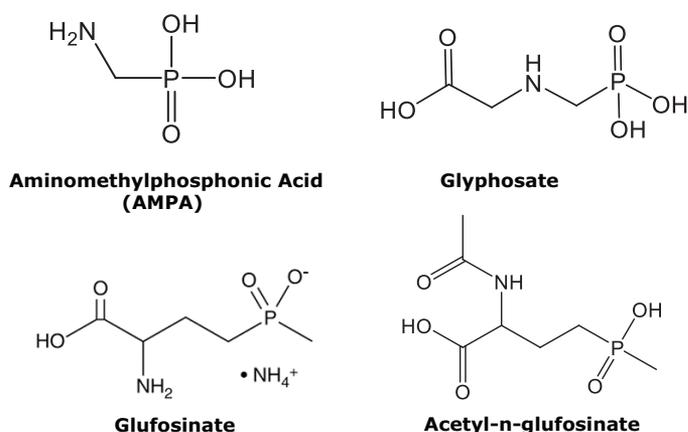
## Introduction

Glyphosate is a broad-spectrum herbicide, widely used to prevent the growth of broadleaf weeds and grasses. Although glyphosate was adopted for use as a premier herbicide in 1970; the intervening years saw many studies being conducted that indicated the harmful effects of glyphosate on humans and wildlife. Currently, glyphosate is monitored closely for its presence in trace amounts in agricultural produce and environmental samples of river, water, and soil.

The hydrophilic nature of glyphosate, its metabolites, and related compounds causes their poor retention onto reversed-phase columns, thereby making the analysis of these compounds by high-performance liquid chromatography (HPLC) difficult. In addition, the methods based on hydrophilic interaction liquid chromatography (HILIC) face the challenge of solvating these polar compounds in a non-polar diluent. But, porous graphitic carbon (PGC) columns like the Supel<sup>™</sup> Carbon LC column, with its unique, mixed-mode retention mechanism, has shown promising results in the analysis of these compounds. This application note describes a series of experiments using the Supel<sup>™</sup> Carbon LC column for resolving these hydrophilic compounds with excellent peak shapes and resolution.

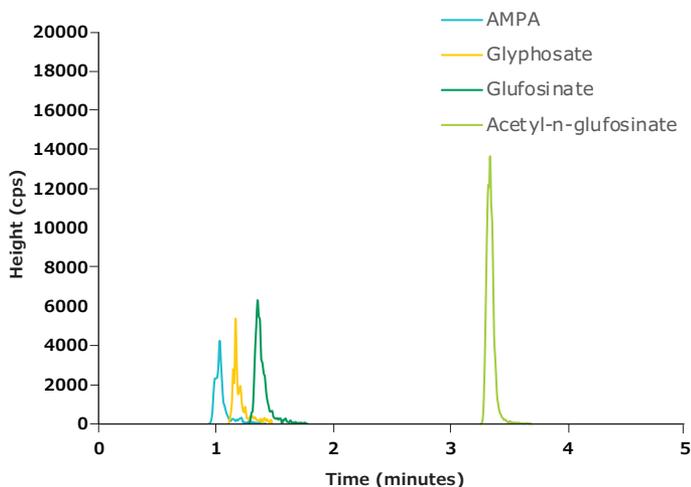
## Glyphosate and its Structural Analogs

The structural similarities among many of the metabolites and breakdown products of glyphosate can offer challenges in the resolution of these compounds by HPLC-mass spectrometry (LC-MS). Also, to avoid regulatory oversight, some pesticide manufacturers have developed compounds structurally similar to glyphosate for use as herbicides that are also toxic to humans and wildlife. **Figure 1** displays the chemical structures of some of these compounds.



**Figure 1:** Chemical structures of glyphosate and related compounds.

Figure 2 displays the MS/MS spectral results of the analysis, while Table 1 displays the MS conditions for the analysis and Table 2 displays the multiple reaction monitoring (MRM) conditions. One of the major challenges with many of these highly polar pesticides is their poor retention on typical reversed-phase columns. Other difficulties include their general need for exotic sample preparation or mobile phase conditions and the necessary derivatization needed to achieve acceptable retention times. However, PGC is not as handicapped as generally used reversed-phase columns for the analysis of these compounds. Reasonable retention of all four pesticides is achieved during their separation using a simple gradient with an aqueous buffer and organic solvent.



**Figure 2:** Separation of glyphosate and related compounds on Supel™ Carbon LC. Condition: Column: Supel™ Carbon LC, 5 cm x 3.0 mm I.D., 2.7 μm; Mobile Phase: [A] 20 mM Ammonium bicarbonate in water, pH 9.0 with ammonium hydroxide; [B] Acetonitrile; Gradient: Hold at 0% B for 1 min; 0% B to 40% B in 3 min; 40% B to 100% B in 3.5 min; hold at 100% B for 2.5 min; Flow rate: 0.3 mL/min; Column Temp.: 40 °C; Detector: MSD, ESI (-); Injection: 6.0 μL; Sample: Glyphosate and related compounds, varied concentration, 20 mM ammonium bicarbonate in water, pH 9.0 with ammonium hydroxide

**Table 1: MS Parameters**

Ion Source Type	Turbo Spray
Curtain Gas	25
Ion Spray Voltage	4000 V
Temperature	325 °C
Ion Source Gas 1	20
Ion Source Gas 2	30
Interface Heater	On

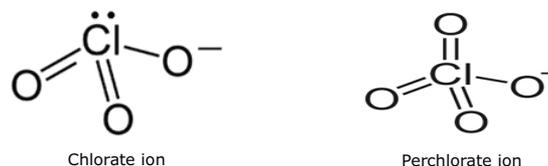
**Table 2: Optimized MRM Parameters**

Q1	Q3	Name	DP	EP	CEP	CE	CXP
110.0	81	AMPA	-31.0	-5.8	-15.0	-21.0	-8.2
110.0	79	AMPA	-31.1	-10.6	-4.6	-29.0	-10.25
110.0	63	AMPA	-32.0	-10.0	-4.6	-29.0	-11.7
168.1	63	Glyphosate	-26.1	-5.9	-48.2	-33.1	-21.8
168.1	124	Glyphosate	-33.0	-9.4	-14.7	-18.7	-3.8
168.1	81	Glyphosate	-22.1	-7.9	-39.0	-19.1	-12.5
168.1	150	Glyphosate	-21.9	-9.9	-11.9	-16.9	-4.7
180.1	136	Glufosinate	-32.2	-5.0	-4.0	-18.9	-4.2
180.1	95	Glufosinate	-32.7	-5.0	-4.0	-19.1	-6.7
180.1	85	Glufosinate	-33.0	-5.0	-4.5	-18.2	-6.9
180.1	63	Glufosinate	-32.5	-5.0	-5.3	-58.1	-7.2
222.2	136	Acetyl-n-glufosinate	-37.1	-3.0	-6.0	-28.1	-2.3
222.2	63	Acetyl-n-glufosinate	-35.0	-6.4	-6.0	-29.2	-2.5

\*Note: Highlighted rows correspond to transitions used to create Figure 2.

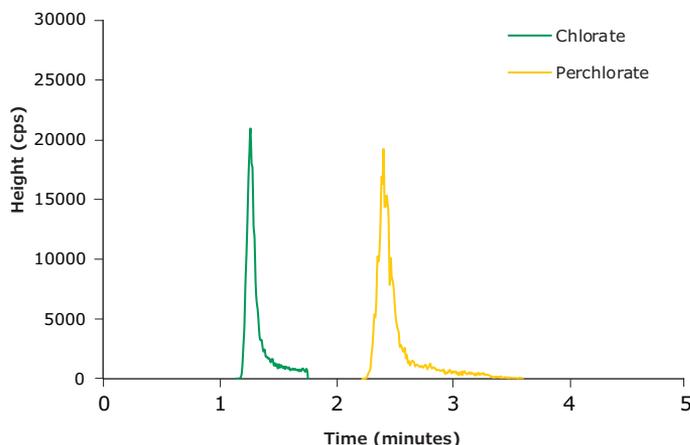
## Analysis of Chlorate and Perchlorate on Supel™ Carbon LC Column

Chlorate and perchlorate are two polar compounds, abundantly used as propellants in rocket fuel and highway flares and as anti-static agents in processed food packaging. They also have been used in the past for the treatment of hyperthyroidism. Both chlorate and perchlorate are toxic in nature and have been detected in groundwater, soil, and plant tissues. The highly polar nature of these compounds (Figure 3) have made their chromatographic analysis difficult.



**Figure 3:** Molecular structures of chlorate and perchlorate ions.

Figure 4 displays the MS/MS results for the analysis of chlorate and perchlorate on the Supel™ Carbon LC column while Tables 3 and 4 display the MS conditions and optimized MRM parameters, respectively. The long-established approach for the analysis of these two compounds has been ion chromatography. However, the major downside of using ion chromatography for the analysis is the need for specialized equipment. Other approaches utilize HILIC or mixed-mode HPLC columns. This application illustrates the use of PGC for the separation of these difficult compounds. Using isocratic conditions with a simple aqueous buffer and acetonitrile, Supel™ Carbon LC column can separate both the compounds with a short run time and reasonable peak shape.



**Figure 4:** LC-MS/MS analysis of chlorate and perchlorate on Supel™ Carbon LC. Conditions: Column: Supel™ Carbon LC, 5 cm x 3.0 mm I.D., 2.7 μm; Mobile Phase: [A] 20 mM Ammonium bicarbonate in water, pH 9.0 with ammonium hydroxide; [B] Acetonitrile (90:10 A:B); Flow Rate: 0.3 mL/min; Column Temp.: 40 °C; Detector: MSD, ESI (-); Injection: 8.0 μL; Sample: Chlorate/Perchlorate mixture, varied concentration, 20 mM Ammonium bicarbonate in water, pH 9.0 with ammonium hydroxide

**Table 3: MS Parameters**

Ion Source Type	Turbo Spray
Curtain Gas	25
Ion Spray Voltage	4000 V
Temperature	325 °C
Ion Source Gas 1	20
Ion Source Gas 2	30
Interface Heater	On

**Table 4: Optimized MRM Parameters**

Q1	Q3	Name	DP	EP	CEP	CE	CXP
99	83	Perchlorate	-46.1	-9.9	-38.2	-37	-1.9
101	85	Perchlorate	-47.7	-9	-38.6	-40	-1.9
83	67	Chlorate	-20.2	-8.9	-7	-27	-9.3
85	69	Chlorate	-21	-7	-6.8	-29.3	-9.1

\*Note: Highlighted rows correspond to transitions used to generate Figure 4.

## Analysis of Fosetyl-Al and Metabolites on Supel™ Carbon LC Column

Fosetyl-Al (fosetyl-aluminum) is an organophosphorus compound used as a fungicide. Upon absorption by plants, it metabolizes into two predominant forms: hydroxyethylphosphonic acid (HEPA) and 3-(Hydroxymethylphosphinyl)propionic acid. The structures for these compounds are depicted in Figure 5. Many regulatory authorities monitor for the metabolites of fosetyl-Al, as well as the parent compound. The polar nature of all three compounds have led to the hypothesis that these compounds could be retained on the Supel™ Carbon LC column.

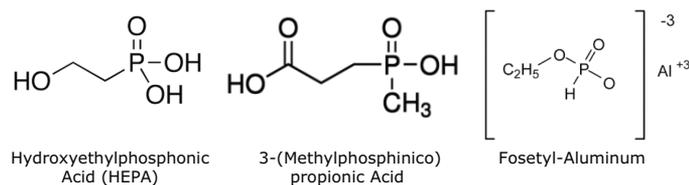


Figure 5: Molecular structures of fosetyl-Al and related metabolites.

Figure 6 displays the LC-MS/MS results of the analysis of fosetyl-Al and its metabolites, while Tables 5 and 6 display the MS Parameters and Optimized MRM Parameters, respectively. As can be seen from Figure 6, excellent peak shape and resolution of the three compounds are achieved with the Supel™ Carbon LC column. This, in turn, will enable the accurate quantitation of the different metabolites and/or of fosetyl-Al itself.

**Table 5: MS Parameters**

Ion Source Type	Turbo Spray
Curtain Gas	25
Ion Spray Voltage	4000 V
Temperature	325 °C
Ion Source Gas 1	20
Ion Source Gas 2	30
Interface Heater	On

**Table 6: Optimized MRM Parameters**

Q1	Q3	Dwell/RT	Name	DP	EP	CEP	CE	CXP
125.1	95	5	HEPA	-28.1	-7.0	-10.3	-20.0	-3.0
125.1	79	5	HEPA	-35.0	-6.1	-10.2	-31.0	-3.0
151.0	133	5	MPPA	-24.3	-4.9	-18.9	-21.8	-1.0
151.0	107	5	MPPA	-24.0	-10.1	-15.2	-21.2	-2.1
151.0	63	5	MPPA	-19.2	-9.9	-15.2	-44.0	-1.4
109.0	81	5	Fosetyl-aluminum	-21.9	-10.0	-6.0	-15.0	-2.1
109.0	63	5	Fosetyl-aluminum	-33.1	-7.0	-20.0	-27.9	-3.0

\*Note: Highlighted rows correspond to transitions used to generate Figure 6.

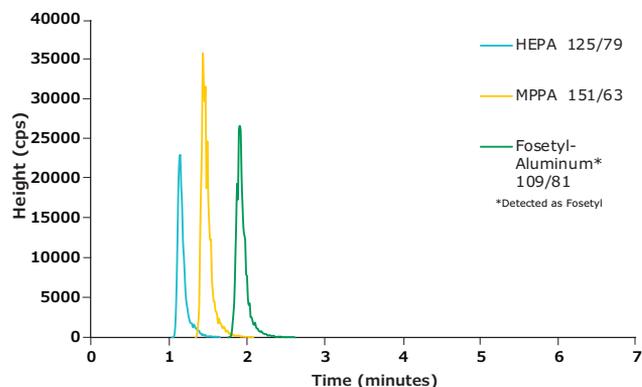


Figure 6: LC-MS/MS analysis of fosetyl-Al and its related metabolites. Conditions: Column: Supel™ Carbon LC, 5 cm x 3.0 mm I.D., 2.7 μm; Mobile Phase: [A] 20 mM Ammonium bicarbonate in water, pH 9.0 with ammonium hydroxide; [B] Acetonitrile; Gradient: Hold at 0% B for 1 min; 0% B to 40% B in 4 min; 40% B to 100% B in 2 min; hold at 100% B for 3 min; Flow Rate: 0.3 mL/min; Column Temp.: 40 °C; Detector: MSD, ESI(-); Injection: 5.0 μL; Sample: Fosetyl-Al mixture, varied concentration, 20 mM Ammonium bicarbonate in water, pH 9.0 with ammonium hydroxide

## Analysis of Bialaphos, Ethephon, and Phosphonic Acid on Supel™ Carbon LC Column

Bialaphos is a natural herbicide produced by certain strains of bacteria. This pesticide is a protoxin and is nontoxic until taken up by a plant. Ethephon is an organophosphorus pesticide that regulates plant growth and is widely used in the European Union. Phosphonic acid is a common byproduct of the metabolism of these pesticides by plants. Regulators monitor for the presence of all three of these compounds, to ensure compliance with local regulations. The polar nature of these compounds (Figure 7), and their resulting poor retention on traditional reversed-phase columns, asserted for the use of Supel™ Carbon LC column for their analysis.

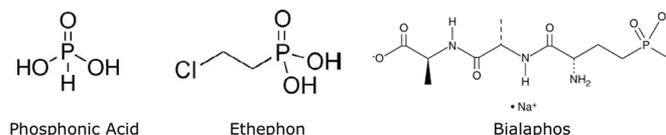
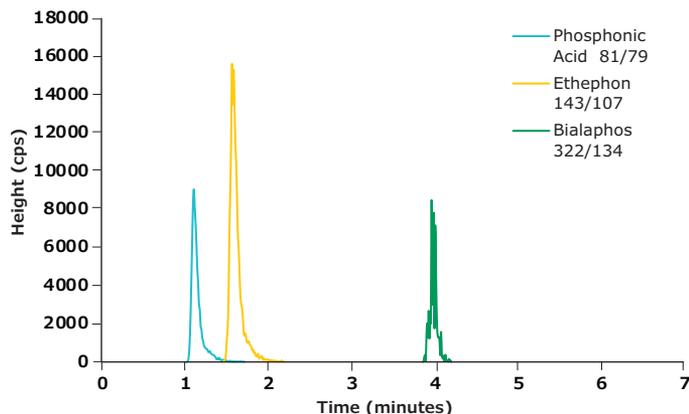


Figure 7: Molecular structures of phosphonic acid, ethephon, and bialaphos.

Figure 8 displays the LC-MS/MS results of the analysis, while Tables 7 and 8 display the MS parameters and optimized MRM parameters. As can be seen in Figure 8, excellent resolution is achieved among all three of the compounds.



**Figure 8:** Analysis of polar pesticides on Supel™ Carbon LC. Conditions: Column: Supel™ Carbon LC, 5 cm 3.0 mm I.D., 2.7 μm; Mobile Phase: [A] 20 mM Ammonium bicarbonate in water, pH 9.0 with ammonium hydroxide; [B] Acetonitrile; Gradient: Hold at 0% B for 1.5 min; 0% B to 40% B in 3.5 min; 40% B to 100% B in 2 min; hold at 100% B for 3 min; Flow Rate: 0.3 mL/min; Column Temp.: 40 °C; Detector: MSD, ESI(-); Injection: 5.0 μL; Sample: Pesticide mix, 5 μg/mL, 20 mM Ammonium bicarbonate, pH 9.0 with ammonium hydroxide

**Table 7: MS Parameters**

Ion Source Type	Turbo Spray
Curtain Gas	25
Ion Spray Voltage	4000 V
Temperature	325 °C
Ion Source Gas 1	20
Ion Source Gas 2	30
Interface Heater	On

**Table 8: Optimized MRM Parameters**

Q1	Q3	Dwell/ RT	Name	DP	EP	CEP	CE	CXP
81.0	79	5	Phosphonic acid	-33.9	-8.0	-35.0	-26.2	-7.0
81.0	63	5	Phosphonic acid	-33.3	-8.0	-35.0	-37.0	-6.0
143.0	107	5	Ethephon	-13.0	-7.3	-17.0	-11.2	-3.8
143.0	79	5	Ethephon	-12.0	-7.0	-15.0	-22.0	-3.8
145.0	107	5	Ethephon	-14.1	-7.1	-16.1	-15.0	-3.8
322.1	216	5	Bialaphos	-35.0	-7.9	-7.2	-25.0	-3.0
322.1	134	5	Bialaphos	-33.8	-3.2	-11	-34.4	-3.0
322.1	94	5	Bialaphos	-34.4	-8.8	-11.0	-34.1	-3.0
322.1	88	5	Bialaphos	-36.1	-10	-11.0	-44.0	-3.0

\*Note: Highlighted rows correspond to transitions used to generate Figure 8.

## Conclusion

These applications illustrated the use of the Supel™ Carbon LC column in retaining and resolving multiple series of polar pesticides and their metabolites. Many of these results were achieved using a combination of simple mobile phases and gradients, without needing special modifications. The Supel™ Carbon LC column is a unique column and will prove to be a beneficial addition to the chromatographer's toolbox.

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