# Supel™ Carbon LC Column

## APPLICATION GUIDE

- Supel™ Carbon LC Column: Porous Graphitic Carbon (PGC) particle packed column for separating polar compounds in Reversed Phase.
- UHPLC Analysis of Vitamin D2 & D3 Metabolites and Epimers
- LC-MS/MS Analysis of 20 Underivatized Amino Acids
- UHPLC-MS/MS Analysis of Polar Pesticides

The Life Science business of Merck operates as MilliporeSigma in the U.S. and Canada.

Supelco®
Analytical Products

# Supel™ Carbon LC Column

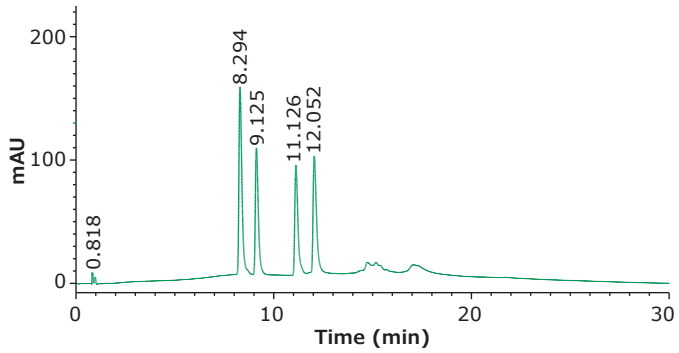
## Porous Graphitic Carbon (PGC) particle packed column for separating polar compounds with reversed phase mobile phase conditions.

Supel™ Carbon LC particles offer a unique retention mechanism that can retain polar compounds under reversed-phase conditions. The same retention mechanism can also enable resolution of geometric isomers of compounds.

### Supel™ Carbon LC Specifications

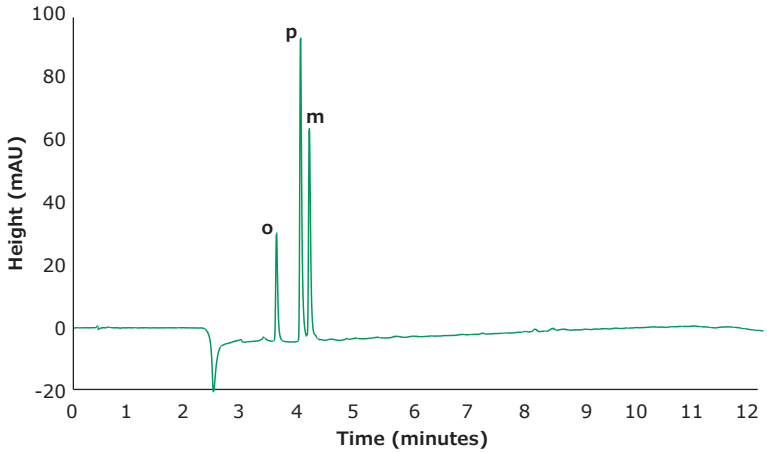
| | |
|---|---|
| **Particle Platform** | Porous Graphitic Carbon (PGC) |
| **Particle Size** | 2.7 µm |
| **Pore Size** | 200 Å |
| **Surface Area** | 155 $m^2/g$ |
| **pH Range** | 1 - 14 |
| **Maximum Temperature** | 250 °C |

| Peaks | Compound | Ret. Time (min) |
|---|---|---|
| 1 | 3-epi-25-hydroxyvitamin D3 (50 µg/mL) | 8.294 |
| 2 | 25-hydroxyvitamin D3 (25 µg/mL) | 9.125 |
| 3 | 3-epi-25-hydroxyvitamin D2 (50 µg/mL) | 11.126 |
| 4 | 25-hydroxyvitamin D2 (100 µg/mL) | 12.052 |




Chromatographic separation of Vitamin D2 and D3 metabolites on Supel™ Carbon LC. Conditions: Column: Supel Carbon™ LC, 10 cm x 2.1 mm I.D., 2.7 µm; Mobile Phase: [A] 2-Propanol; [B] Tetrahydrofuran; Gradient: 0% B to 70% B in 15 min; hold at 70% B for 5 min; Flow Rate: 0.3 mL/min; Column Temp.: 25 °C; Detector: UV, 275 nm; Injection: 2.0 µL; Sample: Vitamin D2 and D3 metabolites mix, varied concentration, ethanol

## Separation of Toluic Acid Isomers on Supel™ Carbon LC




| | |
|---|---|
| **Column** | Supel™ Carbon LC;<br>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 100% B in 10 min; Hold at 100% B for 1 min |
| **Flow Rate** | 0.6 mL/min |
| **Column Temp** | 50 °C |
| **Detector** | UV, 230 nm |
| **Sampling Rate** | 20 Hz |
| **Injection** | 0.750 µL |
| **Sample** | m/o/p-toluic acid, 67 µg/mL in water |

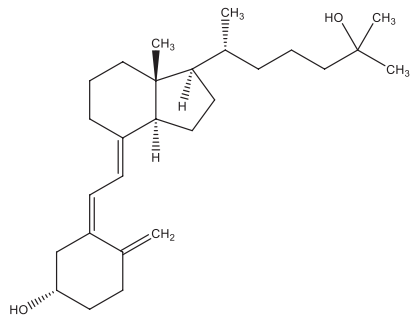
| Product | I.D. (mm) | Length (cm) | Content | Cat. No. |
|---|---|---|---|---|
| Supel™ Carbon LC column | 2.1 | 10 | 1 U/HPLC Column | **59986-U** |
| Supel™ Carbon LC column | 3.0 | 10 | 1 U/HPLC Column | **59993-U** |
| Supel™ Carbon LC column | 4.6 | 10 | 1 U/HPLC Column | **59998-U** |
| Supel™ Carbon LC column | 2.1 | 15 | 1 U/HPLC Column | **59987-U** |
| Supel™ Carbon LC column | 3.0 | 15 | 1 U/HPLC Column | **59994-U** |
| Supel™ Carbon LC column | 2.1 | 5.0 | 1 U/HPLC Column | **59984-U** |
| Supel™ Carbon LC column | 3.0 | 5.0 | 1 U/HPLC Column | **59991-U** |
| Supel™ Carbon LC column | 4.6 | 5.0 | 1 U/HPLC Column | **59997-U** |
| Supel™ Carbon LC column Guard Cartridge Kit | 2.1 | 2.0 | 1 Guard Cartridge + 1 Guard Cartridge Holder | **59982-U** |
| Supel™ Carbon LC column Guard Cartridge Kit | 3.0 | 2.0 | 1 Guard Cartridge + 1 Guard Cartridge Holder | **59989-U** |
| Supel™ Carbon LC column Guard Cartridge Kit | 4.0 | 2.0 | 1 Guard Cartridge + 1 Guard Cartridge Holder | **59996-U** |
| Supel™ Carbon LC Guard Cartridge | 2.1 | 2.0 | 3 Guard Cartridges | **59981-U** |
| Supel™ Carbon LC Guard Cartridge | 3.0 | 2.0 | 3 Guard Cartridges | **59988-U** |
| Supel™ Carbon LC Guard Cartridge | 4.0 | 2.0 | 3 Guard Cartridges | **59995-U** |
| Guard Cartridge Holder for Supel™ Carbon LC Guard Cartridges | N/A | N/A | 1 Guard Cartridge Holder | **59999-U** |

Visit: **SigmaAldrich.com/carbonLC**

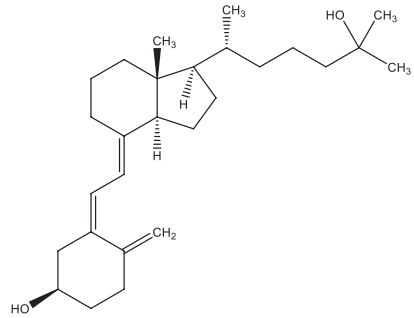
# UHPLC Analysis of Vitamin D2 & D3 Metabolites and Epimers on Supel™ Carbon LC Column

## Introduction

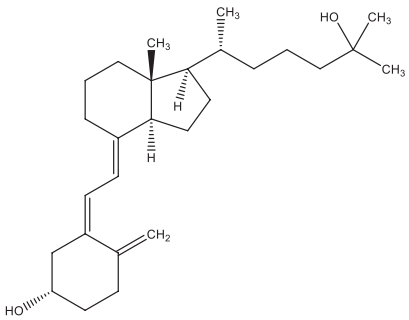
Analysis of vitamin D metabolites has continued to be a topic of interest in recent publications, primarily as biomarkers for possible disease states and vitamin deficiency. While vitamin D is present in two forms, vitamin D3 and vitamin D2, current ELISA methods demonstrate different cross-reactivities and cannot distinguish between D2 and D3 forms of the vitamin metabolites resulting in erroneous reporting of total 25-hydroxyvitamin D concentrations (**Figure 1**). Further, there is interest in an analytical means to differentiate the D2 and D3 forms from the D2 and D3 epimers because of their different degrees of bioactivity. This application demonstrates the use of the Supel™ Carbon LC UHPLC column, with its ability to resolve structural isomers, to baseline separate all four analytes.



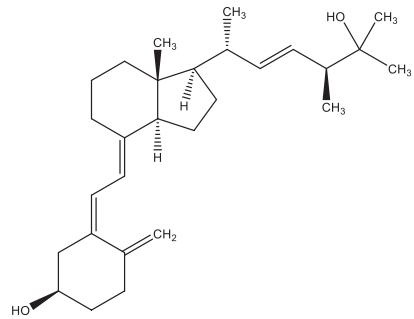

25-hydroxyvitamin D3




3-epi-25-hydroxyvitamin D3




25-hydroxyvitamin D2

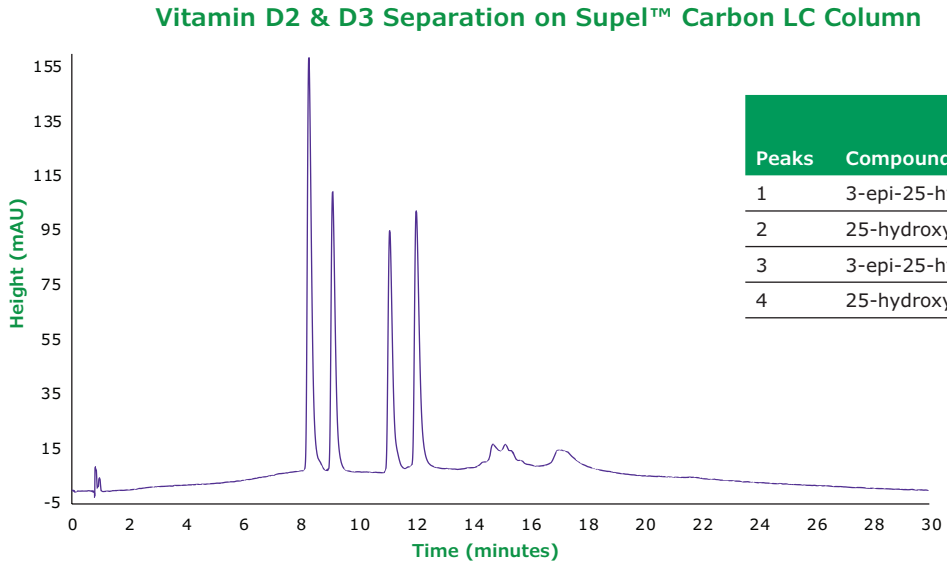



3-epi-25-hydroxyvitamin D2

**Figure 1**: Structures of Vitamin D2 and D3 metabolites.

## Results/Conclusion

**Figure 2** shows the results of the separation of the Vitamin D2 and D3 metabolites. Note the sharp peak shape and baseline resolution between all four analytes.




| Peaks | Compound | Retention Time (min) |
|---|---|---|
| 1 | 3-epi-25-hydroxyvitamin D3 (50 µg/mL) | 8.294 |
| 2 | 25-hydroxyvitamin D3 (25 µg/mL) | 9.125 |
| 3 | 3-epi-25-hydroxyvitamin D2 (50 µg/mL) | 11.126 |
| 4 | 25-hydroxyvitamin D2 (100 µg/mL) | 12.052 |

**Figure 2**: Chromatographic separation of Vitamin D2 and D3 metabolites on Supel™ Carbon LC. Conditions: Column: Supel™ Carbon LC, 10 cm x 2.1 mm I.D., 2.7 µm; Mobile Phase: [A] 2-Propanol; [B] Tetrahydrofuran; Gradient: 0% B to 70% B in 15 min; hold at 70% B for 5 min; Flow Rate: 0.3 mL/min; Column Temp.: 25 °C; Detector: UV, 275 nm; Injection: 2.0 µL; Sample: Vitamin D2 and D3 metabolites mix, varied concentration, ethanol

This application demonstrated the use of the Supel™ Carbon LC column to resolve vitamins D2 and D3 metabolites and their epimers. Baseline separation of all four analytes was achieved with excellent peak shape and sensitivity.

| Product list | Cat. No. |
|---|---|
| Supel™ Carbon LC Column, 10 cm x 2.1mm I.D., 2.7 µm | **59986-U** |
| 2-Propanol for HPLC, 99.5% | **439207** |
| Tetrahydrofuran for HPLC, > 99.9%, Inhibitor-free | **439215** |
| 25-hydroxyvitamin D3 solution, 100 µg/mL in ethanol | **H-083** |
| 25-hydroxyvitamin D2 solution, 50 µg/mL in ethanol | **H-073** |
| 3-epi-25-hydroxyvitamin D3, 50 µg/mL in ethanol | **E-086** |
| 3-epi-25-hydroxyvitamin D2, 98% | **753149** |

# LC-MS/MS Analysis of 20 Underivatized Amino Acids on Supel™ Carbon LC Column

## Introduction

Amino acids are the building blocks of proteins and peptides in biological systems. Amino acids have also been used as supplements to support health and as indicators for certain diseases. The challenge with analyzing amino acids is due to the wide-ranging polarities spanning across all 20 amino acids (**Figure 3**). Due to this complexity, methods in the past have relied on derivatization of the amino acids; however, derivatization can lead to further complexity due to the presence of derivatized/underivatized amino acids and interferences from the derivatizing reagent itself. This application outlines an LC-MS/MS method for analyzing all 20 amino acids, without derivatization, utilizing the Supel™ Carbon LC column. All 20 amino acids are retained on the column, with good peak shape.

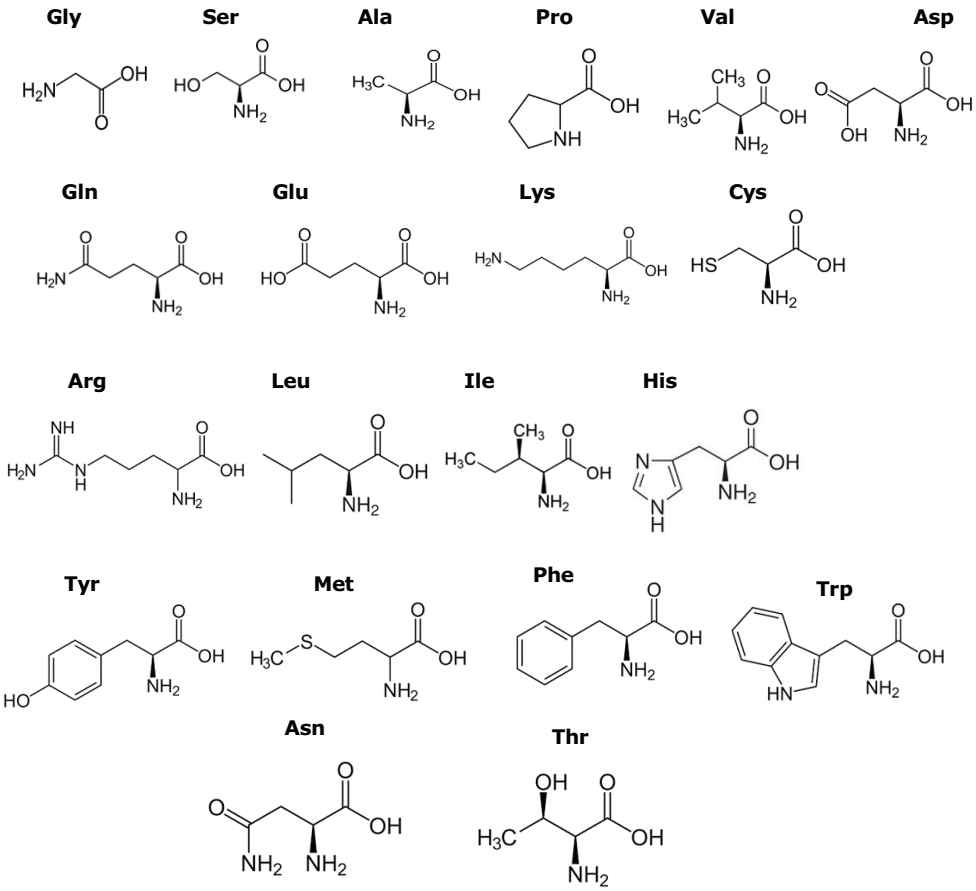



**Figure 3**: Structures and three letter codes of amino acids.

## Results/Conclusion

**Figure 4** displays the MS spectral results for the analysis of 20 underivatized amino acids while **Table 1** displays the optimized MS conditions for the separation and **Table 2** displays the fragment ions and fragmentation parameters for the amino acids.

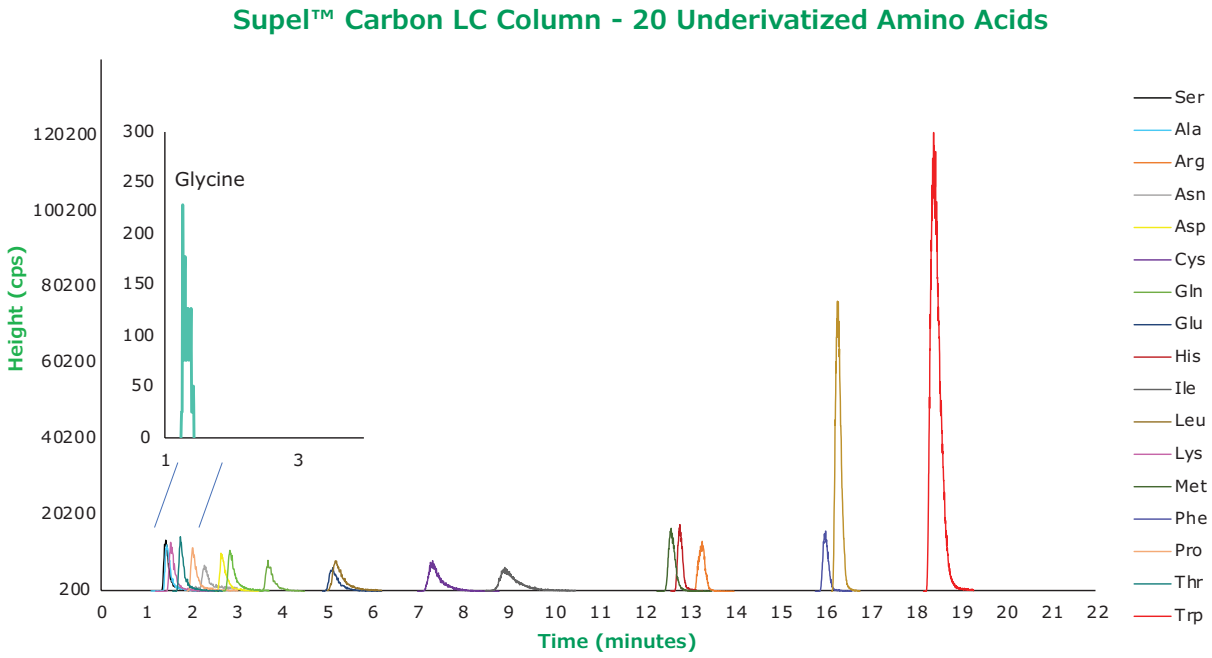



**Figure 4**: Separation of 20 underivatized amino acids by LC-MS/MS. Conditions: Column: Supel™ Carbon LC, 10 cm x 2.1 mm I.D., 2.7 µm; Mobile Phase: [A] Water (0.1% (v/v) DFA); [B] Acetonitrile (0.1% (v/v) DFA); Gradient: Hold at 0% B for 7 min; 0% B to 5% B in 5 min; 5% B to 100% B in 10 min; Flow Rate: 0.2 mL/min; Column temp.: 12 °C; Detector: MSD; Injection: 1.0 µL; Sample: Amino Acid Mix, varied concentration, water (0.1% (v/v) DFA)

| Elution Order | Compound | Retention Time (min) |
|---|---|---|
| 1 | Glycine | 1.27 |
| 2 | Serine | 1.43 |
| 3 | Alanine | 1.45 |
| 4 | Lysine | 1.54 |
| 5 | Threonine | 1.75 |
| 6 | Proline | 2.03 |
| 7 | Asparagine | 2.29 |
| 8 | Aspartic Acid | 2.65 |
| 9 | Valine | 2.85 |
| 10 | Glutamine | 3.69 |

| Elution Order | Compound | Retention Time (min) |
|---|---|---|
| 11 | Glutamic Acid | 5.13 |
| 12 | Leucine | 5.18 |
| 13 | Cystine | 7.34 |
| 14 | Isoleucine | 8.93 |
| 15 | Methionine | 12.61 |
| 16 | Histidine | 12.81 |
| 17 | Arginine | 13.30 |
| 18 | Phenylalanine | 16.03 |
| 19 | Tyrosine | 16.29 |
| 20 | Tryptophan | 18.42 |

**Table 1: MS Conditions for the Separation of 20 Amino Acids**

| Ion Source Type | Turbo Spray |
|---|---|
| Curtain Gas | 25 |
| Ion Spray Voltage | 4000 V |
| Temperature | 300 °C |
| Ion Source Gas 1 | 20 |
| Ion Source Gas 2 | 30 |
| Interface Heater | On |

**Table 2: MRM Fragmentation Parameters and Ions for the 20 Amino Acids**

| Name | Q1 | Q3 | DP | EP | CEP | CE | CXP |
|---|---|---|---|---|---|---|---|
| Lysine | 147.1 | 84.0 | 21.7 | 7.0 | 5.3 | 21.0 | 3.0 |
| Proline | 116.1 | 70.1 | 22.2 | 7.9 | 6.2 | 19.1 | 4.1 |
| Aspartic Acid | 134.1 | 74.0 | 17.0 | 8.3 | 12.8 | 17.0 | 3.7 |
| Serine | 106.1 | 60.0 | 13.0 | 4.0 | 8.8 | 15.0 | 3.1 |
| Glycine | 76.1 | 30.0 | 7.0 | 7.0 | 4.9 | 17.0 | 5.5 |
| Alanine | 90.1 | 44.0 | 16.0 | 6.0 | 4.9 | 16.9 | 5.6 |
| Threonine | 120.1 | 56.0 | 19.0 | 5.3 | 7.0 | 22.0 | 3.3 |
| Asparagine | 133.1 | 87.0 | 17.0 | 3.3 | 8.2 | 14.0 | 2.6 |
| Valine | 118.1 | 72.0 | 14.5 | 6.0 | 7.5 | 14.0 | 3.2 |
| Glutamine | 147.1 | 84.0 | 12.0 | 6.8 | 6.1 | 23.0 | 3.5 |
| Leucine | 132.1 | 86.0 | 16.0 | 7.4 | 10.2 | 14.0 | 3.2 |
| Glutamic Acid | 148.1 | 84.0 | 24.0 | 6.1 | 9.0 | 20.0 | 3.0 |
| Isoleucine | 132.1 | 86.0 | 16.0 | 7.4 | 10.2 | 14.0 | 3.2 |
| Methionine | 150.2 | 104.0 | 16.0 | 5.5 | 11.8 | 13.6 | 3.9 |
| Histidine | 156.1 | 110.0 | 24.0 | 3.5 | 11.0 | 19.0 | 3.7 |
| Arginine | 175.2 | 70.0 | 25.2 | 6.7 | 10.0 | 37.7 | 3.0 |
| Phenylalanine | 166.2 | 120.2 | 18.0 | 7.0 | 9.1 | 16.8 | 3.2 |
| Tryptophan | 205.2 | 146.2 | 19.4 | 4.8 | 17.7 | 23.0 | 4.0 |
| Tyrosine | 182.2 | 136.2 | 19.2 | 7.7 | 6.4 | 18.04 | 4.0 |
| Cystine | 241.2 | 120.0 | 26.0 | 7.8 | 9.8 | 26.0 | 4.1 |

This application demonstrated the effectiveness of the Supel™ Carbon LC column in resolving 20 amino acids without the need for a derivatization reagent. Analysis of amino acids is typically done with speciality, silica-based columns, ion exchange columns, or normal phase columns. However, by utilizing tandem MS/MS detection, analyses of amino acids can be accomplished on Supel™ Carbon LC column with fast run times compared to most commercial approaches. Due to porous graphitic carbon's unique ability to discriminate three-dimensional differences between compounds, Leucine and Isoleucine can be resolved with exceptional resolution.

# UHPLC-MS/MS Analysis of Polar Pesticides on the Supel™ 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™ 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™ 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 5** displays the chemical structures of some of these compounds.

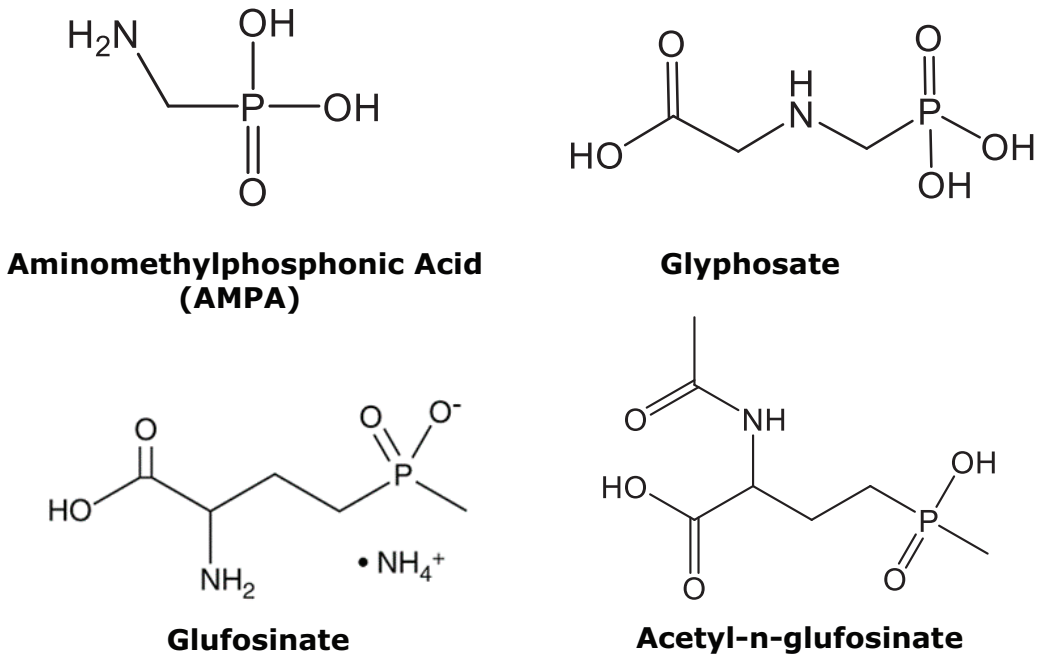



**Figure 5**: Chemical structures of glyphosate and related compounds.

**Figure 6** displays the MS/MS spectral results of the analysis, while **Table 3** displays the MS conditions for the analysis and **Table 4** 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.

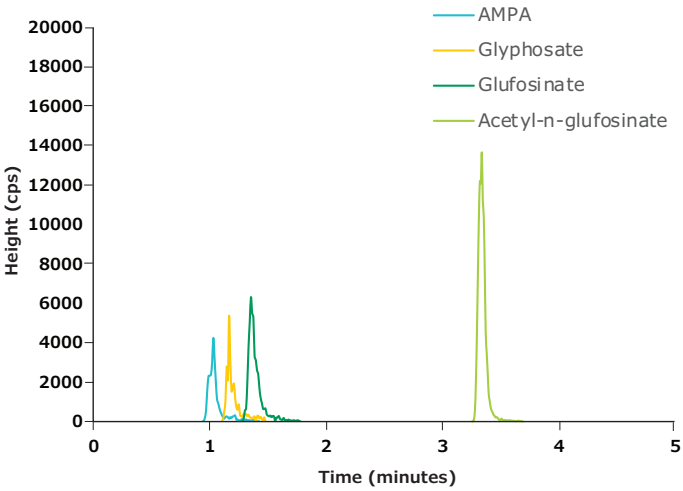



**Figure 6**: Separation of glyphosate and related compounds on Supel™ Carbon LC. Condition: Column: Supel™ Carbon LC, 5 cm x 3.0 mm I.D., 2.7 µm; Mobile Phase: [A] 20 mM Ammonium bicarbonate in water, pH 9.0 with ammonium hydroxide; [B] Acetonitrile; Gradient: Hold at 0% B for 1 min; 0% B to 40% B in 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 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 |
|---|---|---|---|---|---|---|---|
| 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 6**.

## 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 7**) have made their chromatographic analysis difficult.


**Figure 7**: Molecular structures of chlorate and perchlorate ions.

**Figure 8** displays the MS/MS results for the analysis of chlorate and perchlorate on the Supel™ Carbon LC column while **Tables 5** and **6** 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.

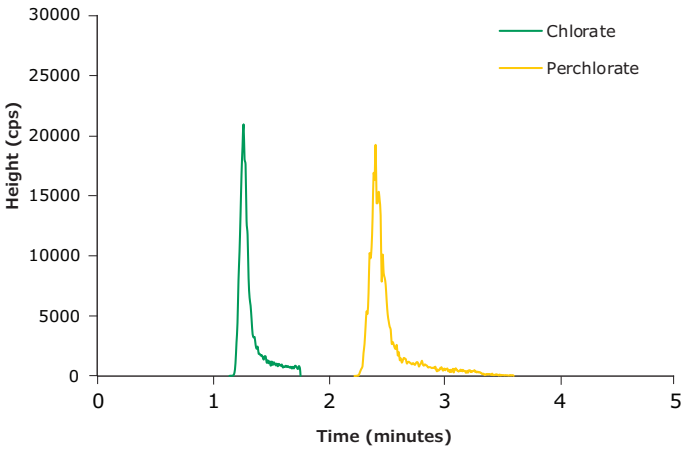



**Figure 8**: LC-MS/MS analysis of chlorate and perchlorate on Supel™ Carbon LC. Conditions: Column: Supel™ Carbon LC, 5 cm x 3.0 mm I.D., 2.7 µm; Mobile Phase: [A] 20 mM Ammonium bicarbonate in water, pH 9.0 with ammonium hydroxide; [B] Acetonitrile (90:10 A:B); Flow Rate: 0.3 mL/min; Column Temp.: 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 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 |

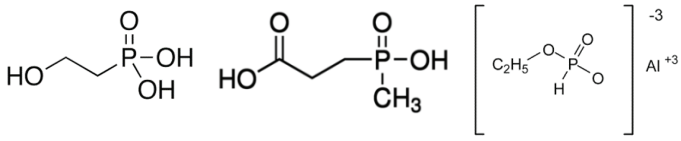
### Table 6: Optimized MRM Parameters

| Q1 | Q3 | 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 8**.

## 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 9**. 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.




Hydroxyethylphosphonic Acid (HEPA) | 3-(Methylphosphinico) propionic Acid | Fosetyl-Aluminum

**Figure 9**: Molecular structures of fosetyl-Al and related metabolites.

**Figure 10** displays the LC-MS/MS results of the analysis of fosetyl-Al and its metabolites, while **Tables 7** and **8** display the MS Parameters and Optimized MRM Parameters, respectively. As can be seen from **Figure 10**, 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 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 |
|---|---|---|---|---|---|---|---|---|
| 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 10**.

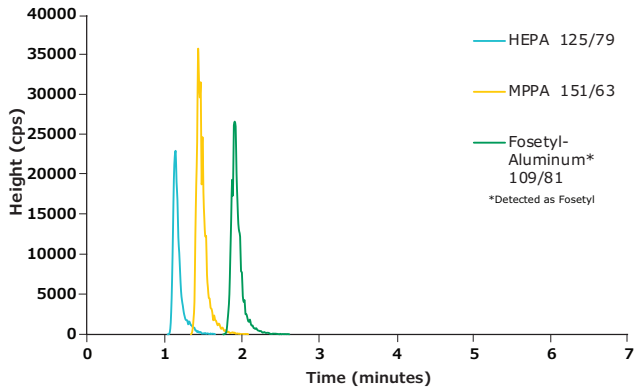
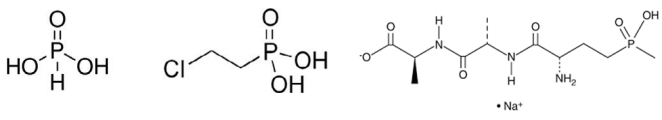



**Figure 10**: LC-MS/MS analysis of fosetyl-Al and its related metabolites. Conditions: Column: Supel™ Carbon LC, 5 cm x 3.0 mm I.D., 2.7 µm; Mobile Phase: [A] 20 mM Ammonium bicarbonate in water, pH 9.0 with ammonium hydroxide; [B] Acetonitrile; Gradient: Hold at 0% B for 1 min; 0% B to 40% B in 4 min; 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 11**), and their resulting poor retention on traditional reversed-phase columns, asserted for the use of Supel™ Carbon LC column for their analysis.




Phosphonic Acid | Ethephon | Bialaphos

**Figure 11**: Molecular structures of phosphonic acid, ethephon, and bialaphos.

**Figure 12** displays the LC-MS/MS results of the analysis, while **Tables 9** and **10** display the MS parameters and optimized MRM parameters. As can be seen in **Figure 12**, excellent resolution is achieved among all three of the compounds.

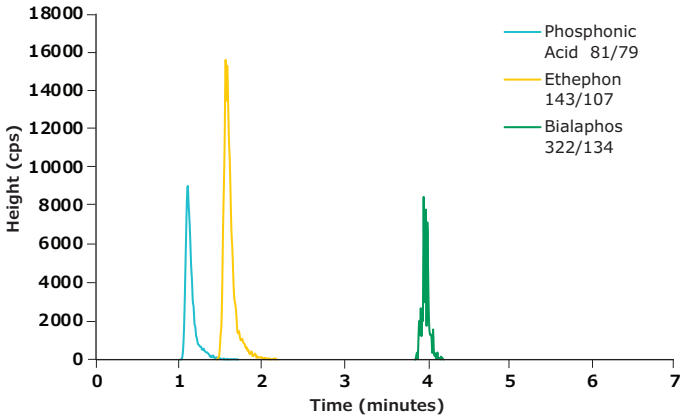



**Figure 12**: Analysis of polar pesticides on Supel™ Carbon LC. Conditions: Column: Supel™ Carbon LC, 5 cm 3.0 mm I.D., 2.7 µm; Mobile Phase: [A] 20 mM Ammonium bicarbonate in water, pH 9.0 with ammonium hydroxide; [B] Acetonitrile; Gradient: Hold at 0% B for 1.5 min; 0% B to 40% B in 3.5 min; 40% B 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 9:** 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 |

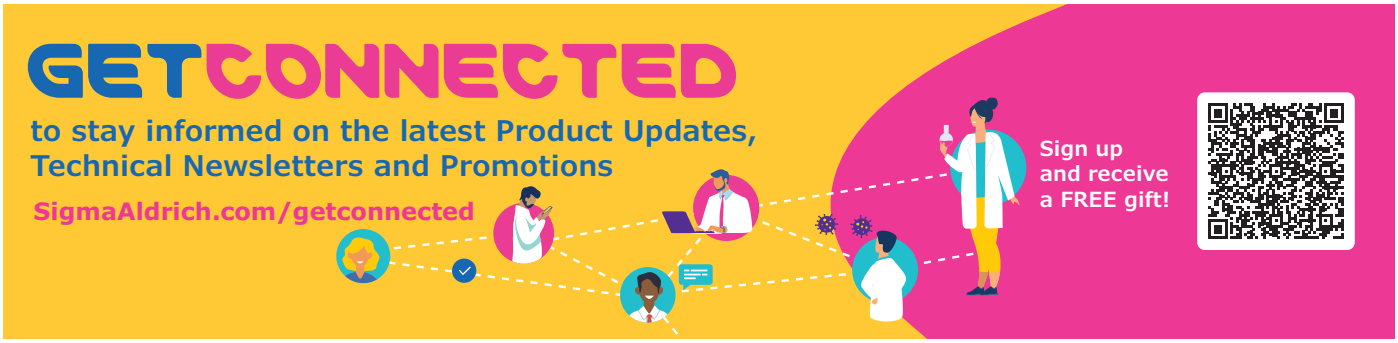
**Table 10:** Optimized MRM Parameters

| 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 12**.

## 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.




MZ ANALYSENTECHNIK

**AUTHORIZED DISTRIBUTOR**

MZ-Analysentechnik GmbH, Barcelona-Allee 17• D-55129 Mainz

Tel +49 6131 880 96-0, Fax +49 6131 880 96-20

e-mail: info@mz-at.de, www.mz-at.de

Merck KGaA
Frankfurter Strasse 250
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