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Optimal Separation of Polar Anionic Pesticides from Fruits and Vegetables with Unique HPLC Column Selectivity

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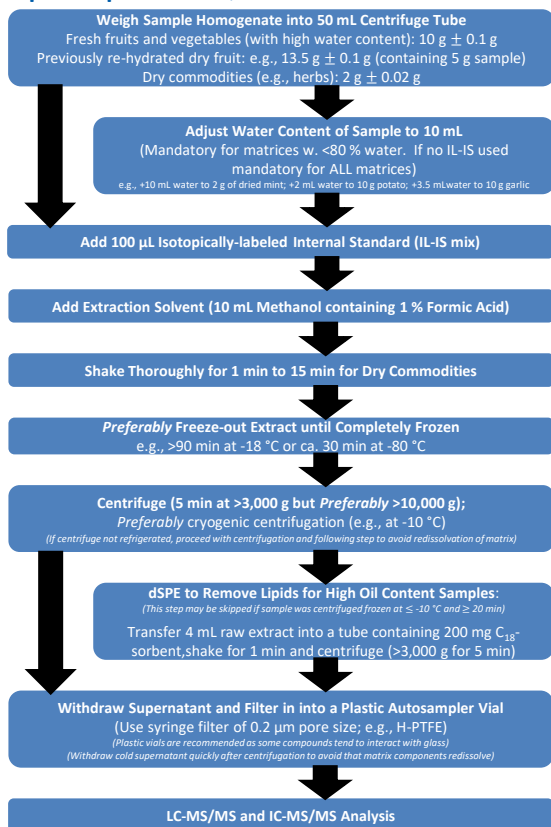
Introduction

Analysis of polar pesticides presents multiple challenges including adequate retention, separation of critical pairs, and reproducibility, to name a few. In addition, food matrices can add additional challenges due to the presence of complex matrix components including pigments, fats, and sugars that can interfere with the analyte of interest.

Often, polar, anionic analytes like Glyphosate will utilize QuEChERS or QuPPE sample preparation techniques, followed by HILIC LC-MS/MS methods for chromatographic retention and separation. Historically, these methods are not user friendly, and lack reproducibility necessary for a commercial application.

In this technical note, we are presenting a unique HPLC selectivity that provides optimal separation of various anionic polar pesticide classes including Glyphosate, Chlorate, Perchlorate, Ethephon, Phosphoric Acid-based pesticides, and N-Ac-Glu pesticides. The study demonstrates robust polar pesticide analysis from real sample matrix.

Sample Preparation: QuPPE-PO Method



LC-MS/MS Conditions

Column: Luna™ 3 µm Polar Pesticides

Dimensions: 100 x 2.1 mm

Part No.: [00D-4798-AN](#)

Mobile Phase: A: 0.2 % Formic Acid in Water
B: 0.2 % Formic Acid in Acetonitrile

| Gradient: | Time (min) | %B |
|-----------|------------|----|
| | 0 | 2 |
| | 0.5 | 2 |
| | 6 | 20 |
| | 7 | 90 |
| | 9 | 90 |
| | 9.1 | 2 |

Flow Rate: 0.3 mL/min

Injection Volume: 0.5 or 1 µL

Temperature: 40 °C

Detector: SCIEX® 7500

Sample Details

1. Matrix extracted following QuPPE-PO Method: Grain*, Kiwi*, Zucchini*, Rocket**, Soy**
2. Raw matrix, 10 ppb spike, 100 ppb spike
3. Different matrices dilution depending on matrix composition (1:4* or 1:10**)
4. Each sample injected in triplicate

Table 1. MRM Transitions

| Analyte | Q1 (m/z) | Q3 (m/z) |
|-----------------|---------------|----------------------|
| AMPA | 110 | 63, 79 |
| MH | 111.1 | 53, 55, 82 |
| Glufosinate | 180 | 63, 85, 95 |
| MPPA | 151 | 63, 107, 133 |
| Glyphosate | 168 | 63, 79, 81, 124, 150 |
| N Acetyl Glu | 222 | 59, 63, 136 |
| Phosphonic Acid | 81 | 63, 79 |
| Ethephon | 107, 143, 145 | 107, 79 |
| Chlorate | 83, 85 | 67, 69 |
| Fosetyl | 109.1 | 81, 63 |
| Perchlorate | 99, 101 | 83, 85 |



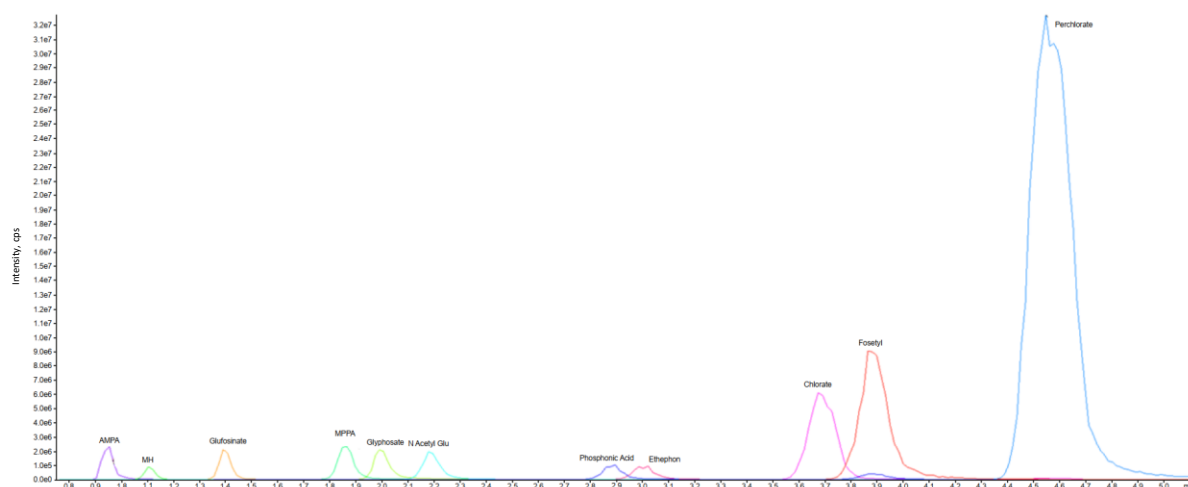
Results and Discussion

Column selectivity plays an important role in providing enhanced chromatographic resolution for critical pairs. In addition, retention of extremely polar analytes are very challenging. In this study, we present optimal separation of polar pesticides on a Luna™ Polar Pesticides HPLC Column, which is a versatile selectivity with amide functionality that can be run in normal, reverse and HILIC mode. In this study, we have utilized the polar interactions in the Luna Polar Pesticides stationary phase in reverse phase mode to obtain enhanced retention of polar pesticides. The chromatogram of standards on a SCIEX® 7500 shows excellent retention and selectivity for polar pesticides (**Figure 1**).

Optimal concentration of 0.2 % Formic Acid in the mobile phase provided a great balance of peak shape and retention. Traditional reverse phase columns do not retain analyte like Glyphosates, which can fall in the ion suppression zone in real samples and hence can show false positive or negative. With the Luna Polar Pesticides HPLC column, enhanced polar selectivity from the un-encapped silica base and from the Amide ligand provides excellent retention which is evident from retention factor for polar pesticides that ranges from 0.7 to 6.6.

Real samples like Grains, Kiwi, Zucchini, Rocket, and Soy were analyzed with this method followed by spiking them with a known concentration of polar pesticides. The method proved to be precise, robust, and accurate for the polar pesticides (**Figure 2**). In addition, retention time stability of Glyphosate is presented as a representative in **Figure 3**. Consistent and robust retention of Glyphosate proves that the Luna Polar Pesticides HPLC column is a robust stationary phase selectivity for the analysis of polar pesticides by LC-MS/MS.

Figure 1. Retention Profile of Polar Pesticides on a Luna Polar Pesticides HPLC Column in Under 6 Minutes.

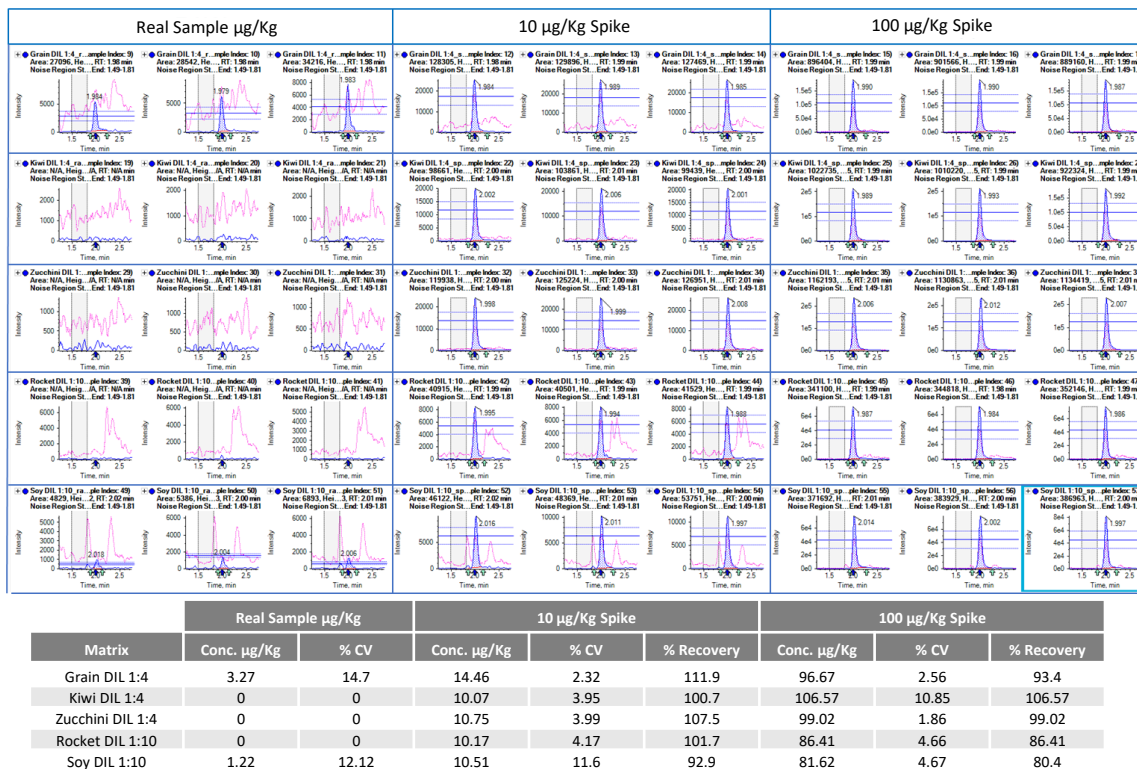


| Analyte | RT (min) | Analyte | RT (min) |
|--------------|----------|-----------------|----------|
| AMPA | 0.95 | Phosphonic Acid | 2.9 |
| MH | 1.1 | Ethephon | 3 |
| Glufosinate | 1.4 | Chlorate | 3.7 |
| MPPA | 1.8 | Fosetyl | 3.9 |
| Glyphosate | 2 | Perchlorate | 4.5 |
| N Acetyl Glu | 2.2 | | |



Figure 2. Real Matrix Analysis of Polar Pesticides.

Glyphosate



Chlorate

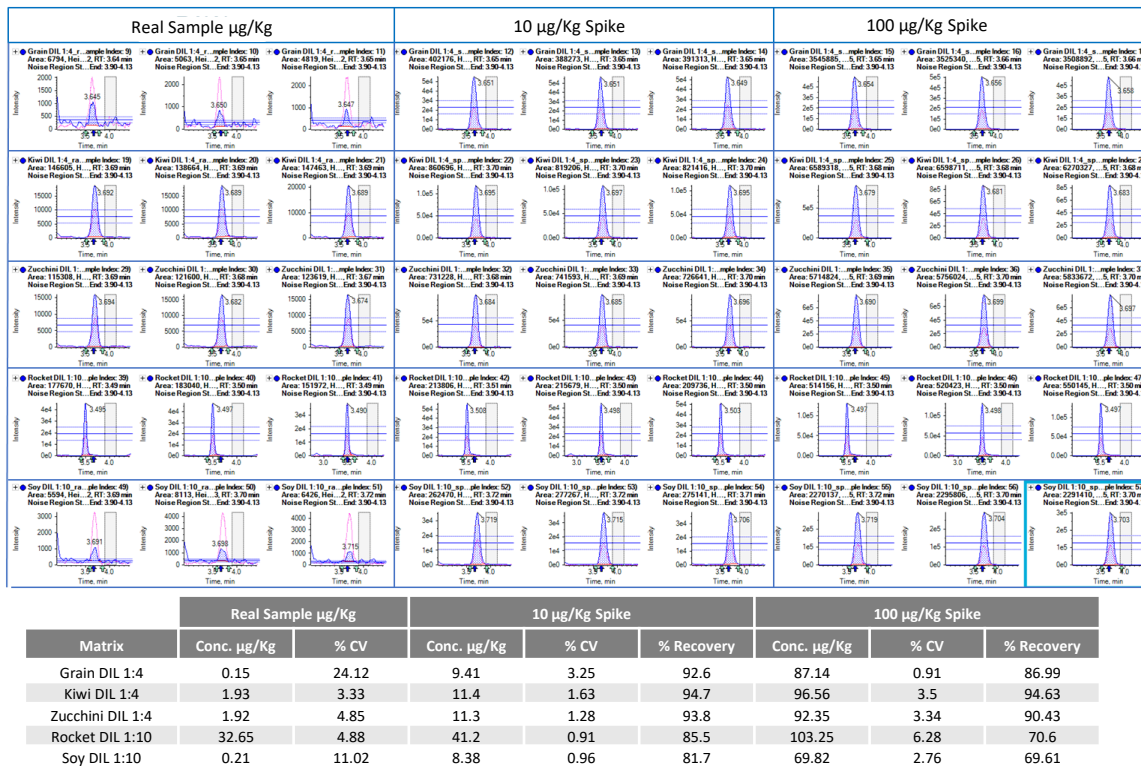
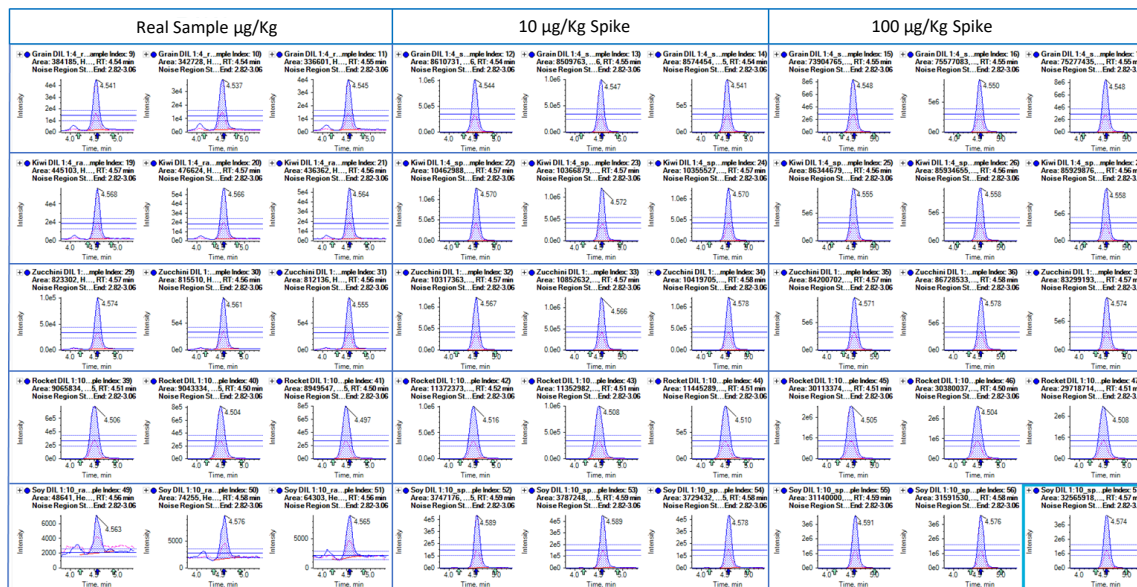


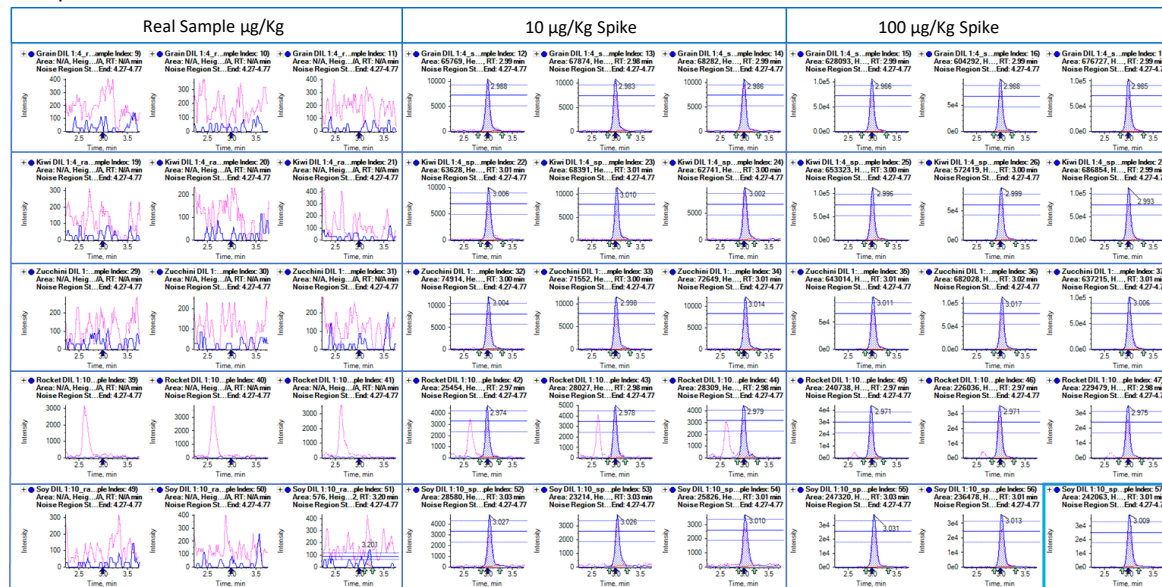
Figure 2 (cont'd). Real Matrix Analysis of Polar Pesticides.

Perchlorate



| Matrix | Real Sample µg/Kg | | | 10 µg/Kg Spike | | | 100 µg/Kg Spike | | |
|------------------|-------------------|-------|--|----------------|------|------------|-----------------|------|------------|
| | Conc. µg/Kg | % CV | | Conc. µg/Kg | % CV | % Recovery | Conc. µg/Kg | % CV | % Recovery |
| Grain DIL 1:4 | 0.44 | 8.59 | | 10.96 | 0.64 | 105.2 | 101.29 | 2.49 | 100.85 |
| Kiwi DIL 1:4 | 0.47 | 5.75 | | 11.01 | 1.98 | 105.4 | 104.04 | 1.03 | 103.57 |
| Zucchini DIL 1:4 | 0.9 | 0.98 | | 11.56 | 2.61 | 106.6 | 101.71 | 2 | 100.81 |
| Rocket DIL 1:10 | 33.24 | 2.34 | | 41.72 | 0.69 | 84.8 | 112.44 | 0.23 | 79.2 |
| Soy DIL 1:10 | 0.14 | 18.04 | | 8.8 | 0.52 | 86.6 | 73.98 | 2.8 | 73.84 |

Ethephon

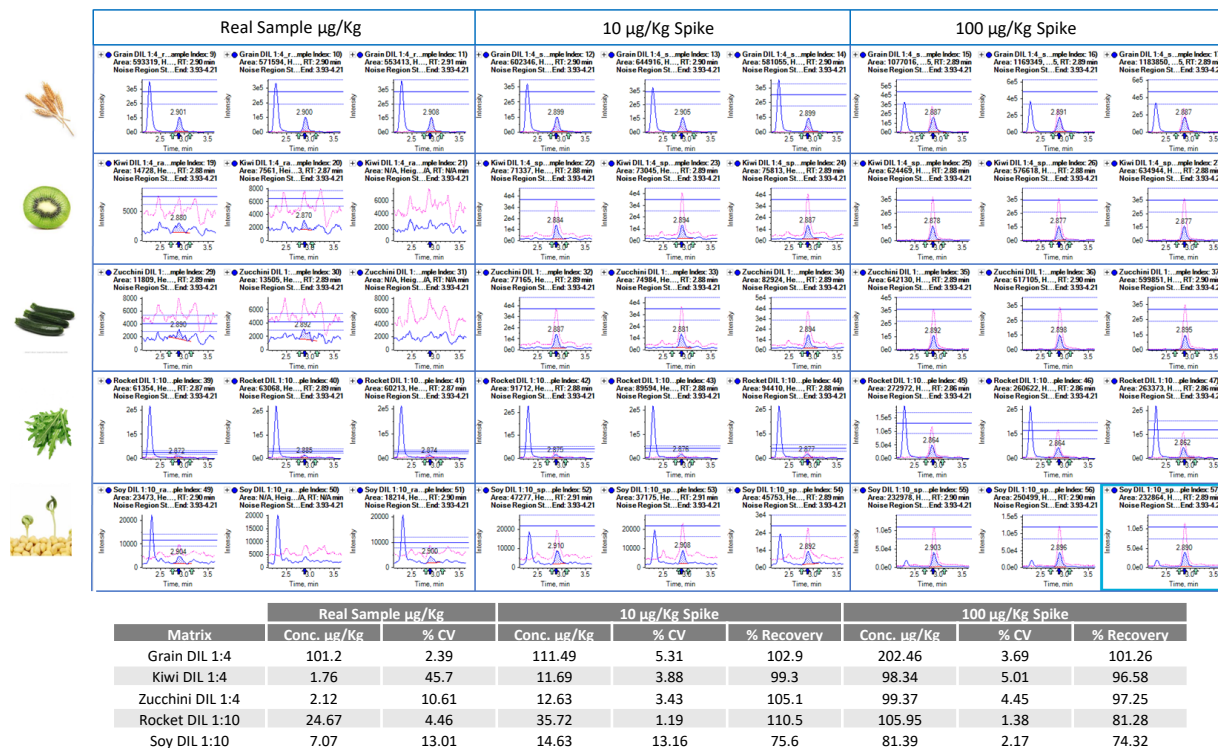


| Matrix | Real Sample µg/Kg | | | 10 µg/Kg Spike | | | 100 µg/Kg Spike | | |
|------------------|-------------------|------|--|----------------|-------|------------|-----------------|------|------------|
| | Conc. µg/Kg | % CV | | Conc. µg/Kg | % CV | % Recovery | Conc. µg/Kg | % CV | % Recovery |
| Grain DIL 1:4 | 0 | 0 | | 9.43 | 2 | 94.3 | 89.12 | 5.8 | 89.12 |
| Kiwi DIL 1:4 | 0 | 0 | | 9.09 | 4.68 | 90.9 | 89.28 | 9.23 | 89.28 |
| Zucchini DIL 1:4 | 0 | 0 | | 10.23 | 2.35 | 102.3 | 91.6 | 3.73 | 91.6 |
| Rocket DIL 1:10 | 0 | 0 | | 9.55 | 5.77 | 95.5 | 81.25 | 3.31 | 81.25 |
| Soy DIL 1:10 | 0.2 | 0 | | 9.06 | 10.37 | 88.6 | 84.71 | 2.24 | 84.51 |

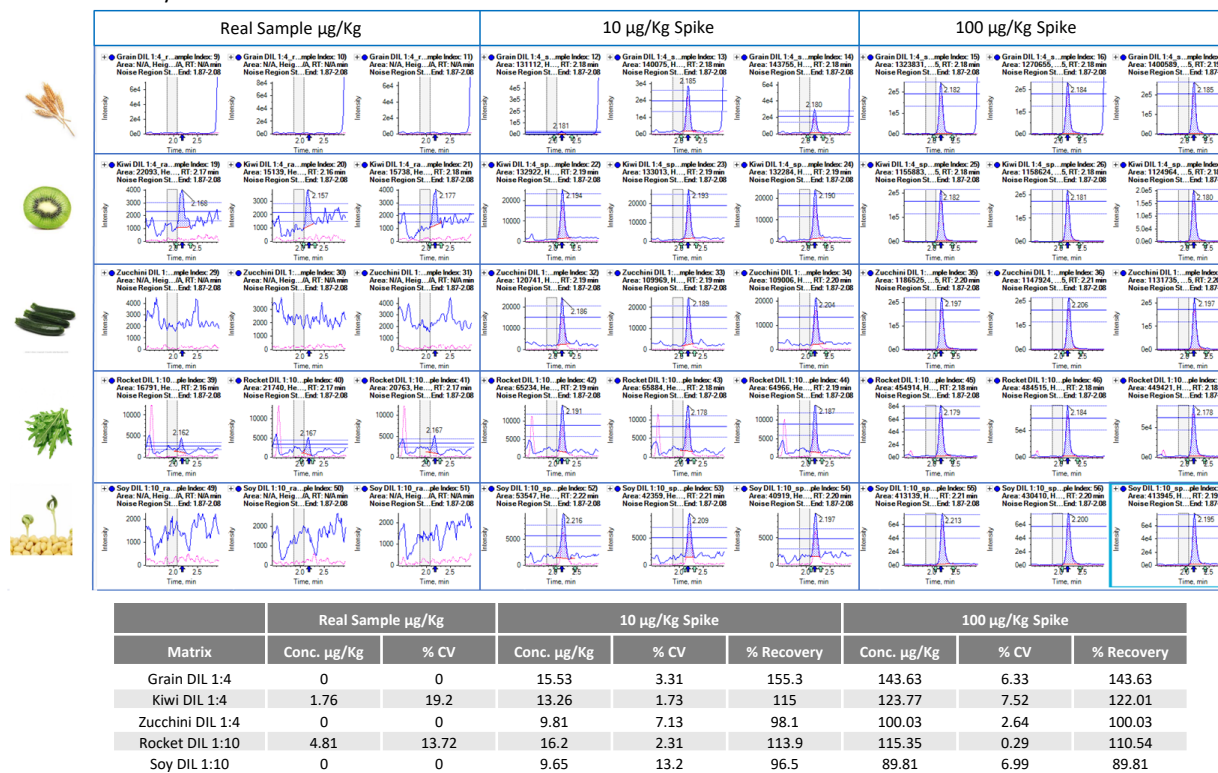
Have questions or want more details on implementing this method? We would love to help!
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Figure 2 (cont'd). Real Matrix Analysis of Polar Pesticides.

Phosphonic Acid

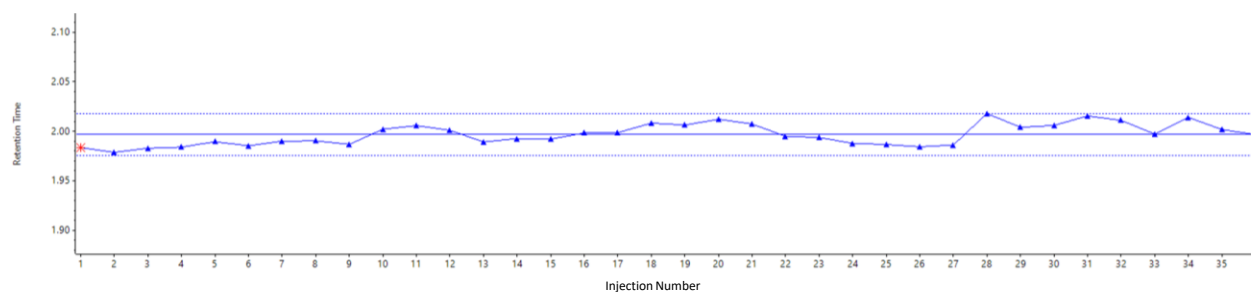


N Acetyl Glu



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Figure 3. Retention Time Stability of Glyphosate.



Conclusions

The Luna™ Polar Pesticides column is a versatile HPLC column selectivity that provides enhanced retention and selectivity for polar pesticides. Reproducible retention, optimal selectivity, and precise and accurate results prove that the Venusil HILIC column is the ideal choice for polar pesticide analysis. In addition to providing consistent retention, the Venusil HILIC column offers short run time of less than 6 minutes for high throughput analysis. Unlike traditional HILIC methods, the method demonstrated here provides stable retention in reverse phase by utilizing polar interactions from the stationary phase. Thus, the developed method is easy to adopt in labs running routine polar pesticide analysis.

Ordering Information

| Luna 3 µm Analytical Columns (mm) | | | | | | SecurityGuard™ ULTRA Cartridges* |
|-----------------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|----------------------------------|
| Phase | 30 x 2.1 | 50 x 2.1 | 100 x 2.1 | 150 x 2.1 | 150 x 3.0 | 3/pk |
| Polar Pesticides | 00A-4798-AN | 00B-4798-AN | 00D-4798-AN | 00F-4798-AN | 00F-4798-Y0 | AJ0-8789 |

For ID: 2.1-4.6 mm

*SecurityGuard ULTRA Cartridges require holder, Part No.: [AJ0-9000](#)



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