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 study, 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.

Materials and Methods

Sample Preparation: QuPPE-PO Method

Weigh Sample Homogenate into 50 mL Centrifuge Tube
Fresh fruits and vegetables (with high water content): 10 g ± 0.1 g
Previously re-hydrated dry fruit: e.g. 13.5 g ± 0.1 g (containing 5 g sample)
Dry commodities (e.g. herbs): 2 g ± 0.02 g

↓

Adjust Water Content of Sample to 10 mL
(Mandatory for matrices w. <80 % water.
If no IL-IS used mandatory for ALL matrices)
e.g. +10 mL water to 2 g of dried mint; +2 mL water to 10 g potato; +3.5 mLwater to 10 g garlic

↓

Add 100 µL Isotopically-labeled Internal Standard (IL-IS mix)

↓

Add Extraction Solvent (10 mL Methanol containing 1 % Formic Acid)

↓

Shake Thoroughly for 1 min to 15 min for Dry Commodities

↓

Preferably Freeze-out Extract until Completely Frozen
e.g. >90 min at -18 °C or ca. 30 min at -80 °C

↓

Centrifuge (5 min at >3,000 g but Preferably >10,000 g);
Preferably cryogenic centrifugation (e.g. at -10 °C)
(If centrifuge not refrigerated, proceed with centrifugation and following step to avoid redissolvation of matrix)

↓

dSPE to Remove Lipids for High Oil Content Samples:
(This step may be skipped if sample was centrifuged frozen at ≤ -10 °C and ≥ 20 min)
Transfer 4 mL raw extract into a tube containing 200 mg C18-sorbent, shake for 1 min and centrifuge (>3,000 g for 5 min)

↓

Withdraw Supernatant and Filter in into a Plastic Autosampler Vial
(Use syringe filter of 0.2 µm pore size; e.g. H-PTFE)
(Plastic vials are recommended as some compounds tend to interact with glass)
(Withdraw cold supernatant quickly after centrifugation to avoid that matrix components redissolve)

↓

LC-MS/MS and IC-MS/MS Analysis

LC-MS/MS Conditions

Column: Venusil® 3 µm HILIC
Dimension: 100 x 2.1 mm
Part No.: VH931002-0
Injection: A: 0.2 % Formic Acid in Water
B: 0.2 % Formic Acid in Methanol

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

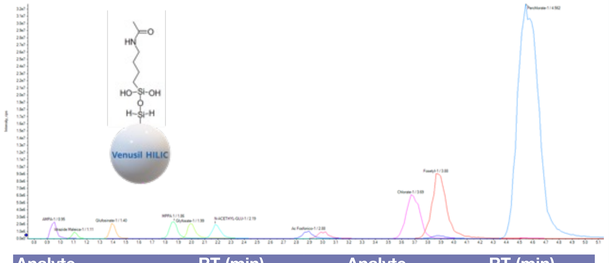
Flow Rate: 0.3 mL/min
Injection: 0.5 or 1 µL
Temperature: 40 °C
Detector: SCIEX® 7500

Sample Details

1. Matrix Extracted following QuPPe Method: Grain*, Kiwi*, Zucchini*, Rocket**, Soy**
2. Raw Matrix, 10ppb Spike, 100ppb Spike
3. Different Matrices Dilution depending on matrix composition (1:4* or 1:10**)
4. Each sample injected in triplicate

Results

Figure 1. Retention Profile of Polar Pesticides on a Venusil HILIC HPLC Column.

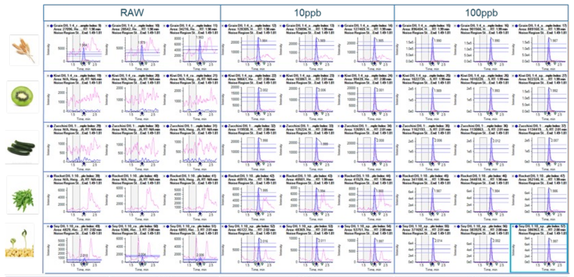


Analyte	RT (min)	Analyte	RT (min)
AMPA	0.95	Phosphonic Acid	2.9
MH	1.1	Ethephon	3
Glufosinate	1.4	Chlorate	3.7
MPPA	1.8	Fosetyl	3.9
Glyphosate	2	Perchlorate	4.5
N Acetyl Glu	2.2		

Results

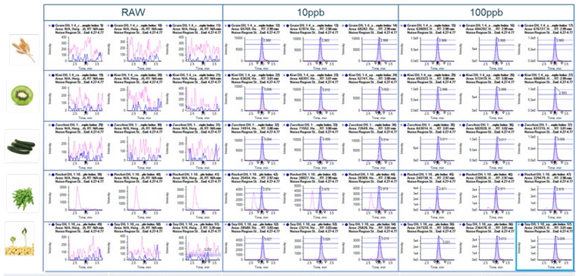
Figure 2. Real Matrix Analysis of Polar Pesticides.

Glyphosate



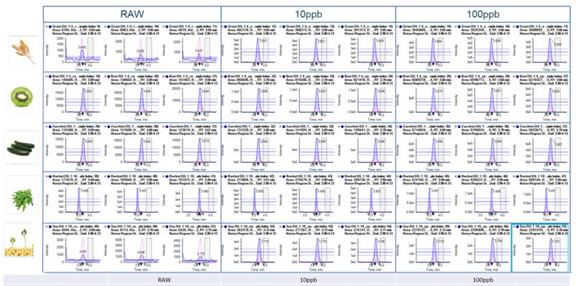
Matrix	RAW Mean	RAW CV	10ppb Mean	10ppb CV	10ppb Accuracy	100ppb Mean	100ppb CV	100ppb Accuracy
Grain DIL 1:4	3.27	14.7	14.46	2.32	111.9	96.67	2.56	93.4
Kiwi DIL 1:4	0	0	10.07	3.95	100.7	106.57	10.85	106.57
Zucchini DIL 1:4	0	0	10.75	3.99	107.5	99.02	1.86	99.02
Rocket DIL 1:10	0	0	10.17	4.17	101.7	86.41	4.66	86.41
Soy DIL 1:10	1.22	12.12	10.51	11.6	92.9	81.62	4.67	80.4

Ethephon



Matrix	RAW Mean	RAW CV	10ppb Mean	10ppb CV	10ppb Accuracy	100ppb Mean	100ppb CV	100ppb Accuracy
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

Chlorate

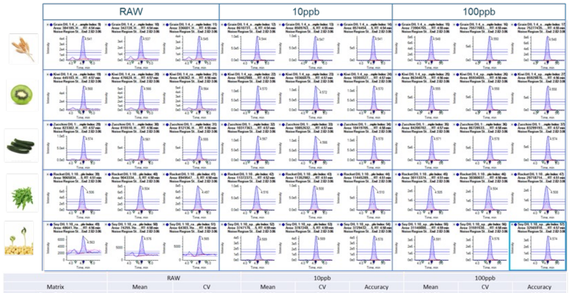


Matrix	RAW Mean	RAW CV	10ppb Mean	10ppb CV	10ppb Accuracy	100ppb Mean	100ppb CV	100ppb Accuracy
Grain DIL 1:4	0.15	24.12	9.41	3.25	92.6	87.14	0.91	86.99
Kiwi DIL 1:4	1.93	3.33	11.4	1.63	94.7	96.56	3.5	94.63
Zucchini DIL 1:4	1.92	4.85	11.3	1.28	93.8	92.35	3.34	90.43
Rocket DIL 1:10	32.65	4.88	41.2	0.91	85.5	103.25	6.28	70.6
Soy DIL 1:10	0.21	11.02	8.38	0.96	81.7	69.82	2.76	69.61

Phosphonic Acid

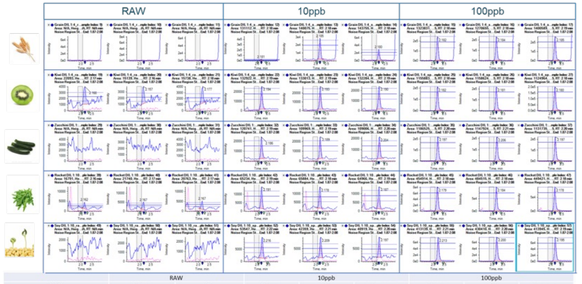
Matrix	RAW Mean	RAW CV	10ppb Mean	10ppb CV	10ppb Accuracy	100ppb Mean	100ppb CV	100ppb Accuracy
Grain DIL 1:4	101.2	2.39	111.49	5.31	102.9	202.46	3.69	101.26
Kiwi DIL 1:4	1.76	45.7	11.69	3.88	99.3	98.34	5.01	96.58
Zucchini DIL 1:4	2.12	10.61	12.63	3.43	105.1	99.37	4.45	97.25
Rocket DIL 1:10	24.67	4.10	35.72	1.19	110.5	105.95	1.38	81.28
Soy DIL 1:10	7.07	13.01	14.63	13.16	75.6	81.39	2.17	74.32

Percholrate



Matrix	RAW Mean	RAW CV	10ppb Mean	10ppb CV	10ppb Accuracy	100ppb Mean	100ppb CV	100ppb Accuracy
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

N Ac Glu

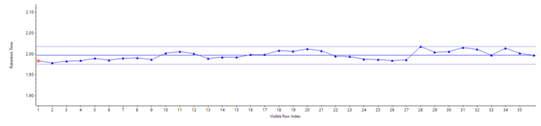


Matrix	RAW Mean	RAW CV	10ppb Mean	10ppb CV	10ppb Accuracy	100ppb Mean	100ppb CV	100ppb Accuracy
Grain DIL 1:4	0	0	15.53	3.31	155.3	143.63	6.33	143.63
Kiwi DIL 1:4	1.76	19.2	13.26	1.73	115	123.77	7.52	122.01
Zucchini DIL 1:4	0	0	9.81	7.13	98.1	100.03	2.64	100.03
Rocket DIL 1:10	4.81	13.72	16.2	2.31	113.9	115.35	0.29	110.54
Soy DIL 1:10	0	0	9.65	13.2	96.5	89.81	6.99	89.81

Results

Figure 3. Retention Time Stability of Glyphosate.

RT Stability



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 Venusil HILIC 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 Venusil HILIC 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 Venusil HILIC column, enhanced polar selectivity from the un-endcapped 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 Venusil HILIC column is a robust stationary phase selectivity for the analysis of polar pesticides by LC-MS/MS.

Conclusion

Venusil HILIC 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.

Have questions or want more details? We would love to help!
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