TN-1315

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

Luigi Margarucci, PhD[1], Aidan Harrison, PhD[2], Jörg Baute, PhD[3], Richard Jack, PhD[4], Ramkumar Dhandapani, PhD[4], Sean Orlowicz[4], Samuele Scurati, PhD[5], and Bryan Tackett, PhD[4]

[1]Phenomenex Italy, Via M. Serenari, 15/D, 40013 Castel Maggiore (BO), Italy
[2]Phenomenex Ltd., Queens Avenue, Hurdsfield Ind. Est., Macclesfield, Cheshire SK10 2BN, UK
[3]Phenomenex Ltd. Deutshland, Zeppelinstr. 5, 63741 Aschaffenburg, Germany
[4]Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA
[5]DASP SRL Via Andrea Costa 1/A, 20131 Milan, Italy

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

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 C_{18}-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
Dimensions: 100 x 2.1 mm
Part No.: VH931002-0
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

Have questions or want more details on implementing this method? We would love to help!
Visit www.phenomenex.com/Chat to get in touch with one of our Technical Specialists

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

Have questions or want more details on implementing this method? We would love to help!
*Visit **www.phenomenex.com/Chat** to get in touch with one of our Technical Specialists*

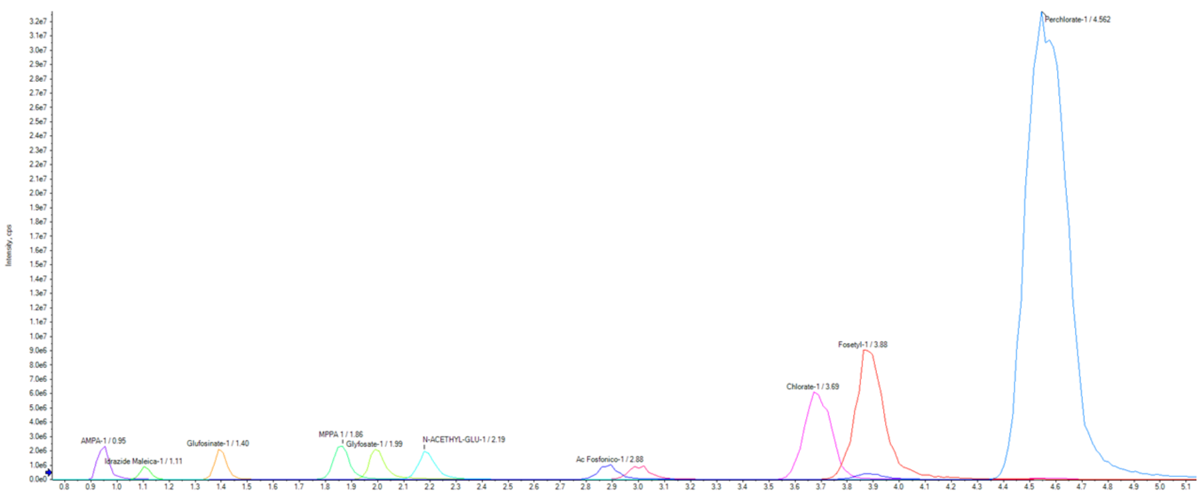
Results and Discussion

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 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.

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

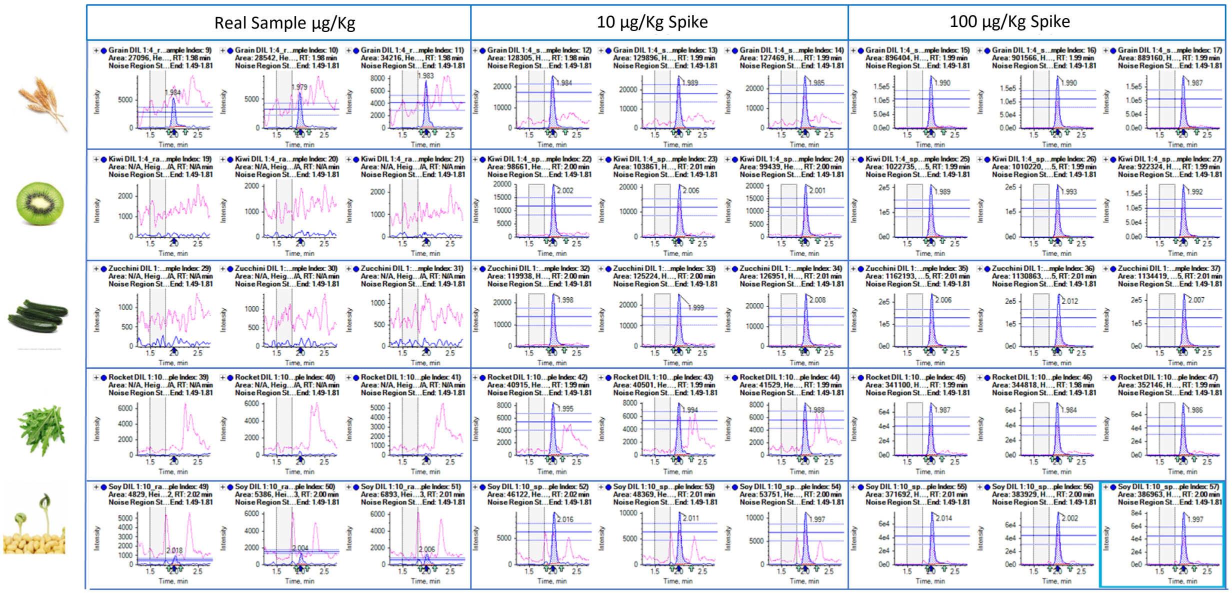


Analyte	RT (min)	Analyte	RT (min)
AMPA	0.95	Phosphonic Acid	2.9
MH	1.1	Ethephon	3
Glufosinate	1.4	Chlorate	3.7
MPPA	1.8	Fosetyl	3.9
Glyphosate	2	Perchlorate	4.5
N Acetyl Glu	2.2		

Have questions or want more details on implementing this method? We would love to help!
*Visit **www.phenomenex.com/Chat** to get in touch with one of our Technical Specialists*

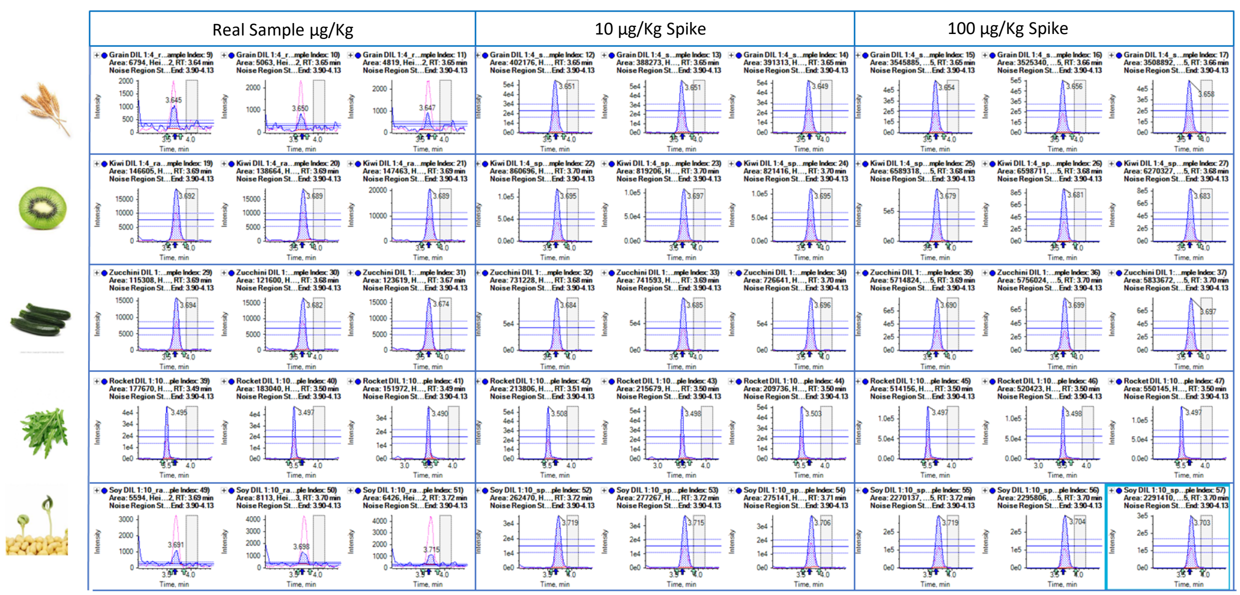
Figure 2. Real Matrix Analysis of Polar Pesticides.

Glyphosate



	Real Sample μg/Kg		10 μg/Kg Spike			100 μg/Kg Spike		
Matrix	Conc. μg/Kg	% CV	Conc. μg/Kg	% CV	% Recovery	Conc. μg/Kg	% CV	% Recovery
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

Chlorate

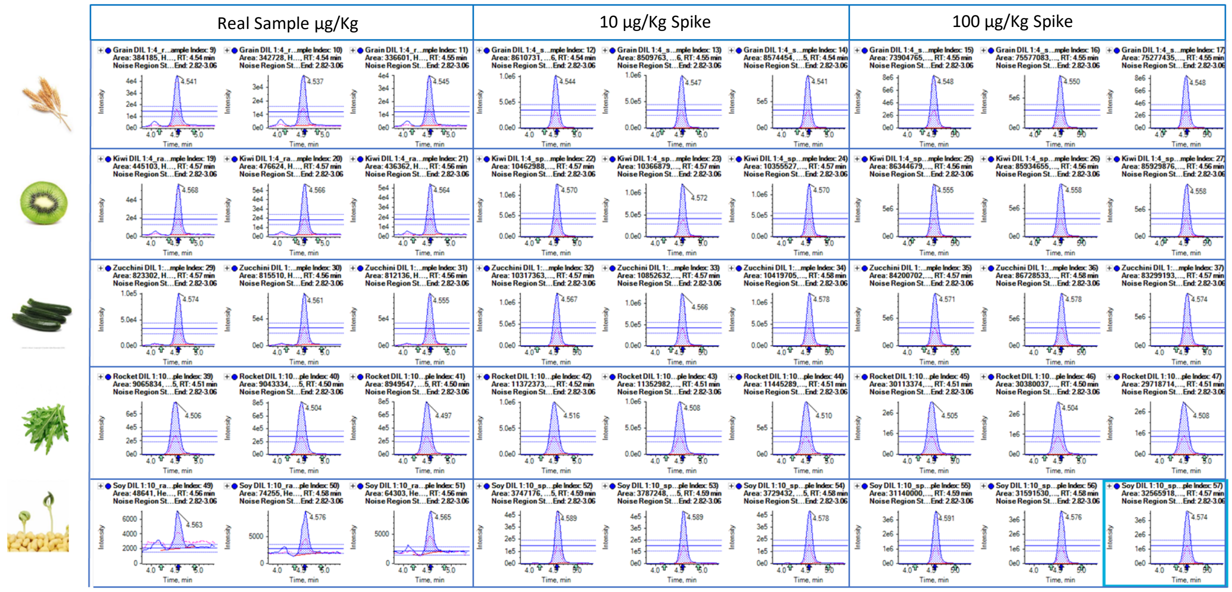


	Real Sample μg/Kg		10 μg/Kg Spike			100 μg/Kg Spike		
Matrix	Conc. μg/Kg	% CV	Conc. μg/Kg	% CV	% Recovery	Conc. μg/Kg	% CV	% Recovery
Grain DIL 1:4	0.15	24.12	9.41	3.25	92.6	87.14	0.91	86.99
Kiwi DIL 1:4	1.93	3.33	11.4	1.63	94.7	96.56	3.5	94.63
Zucchini DIL 1:4	1.92	4.85	11.3	1.28	93.8	92.35	3.34	90.43
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

Have questions or want more details on implementing this method? We would love to help!
*Visit **www.phenomenex.com/Chat** to get in touch with one of our Technical Specialists*

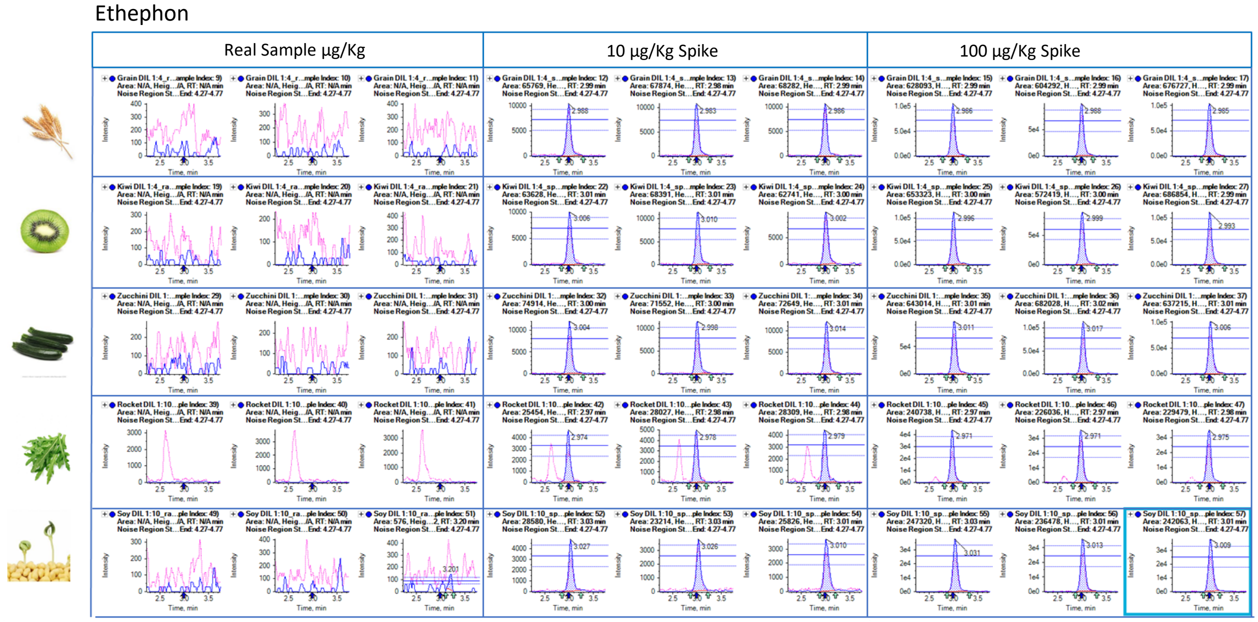
Figure 2 (cont'd). Real Matrix Analysis of Polar Pesticides.

Perchlorate



	Real Sample µg/Kg		10 µg/Kg Spike			100 µg/Kg Spike		
Matrix	**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

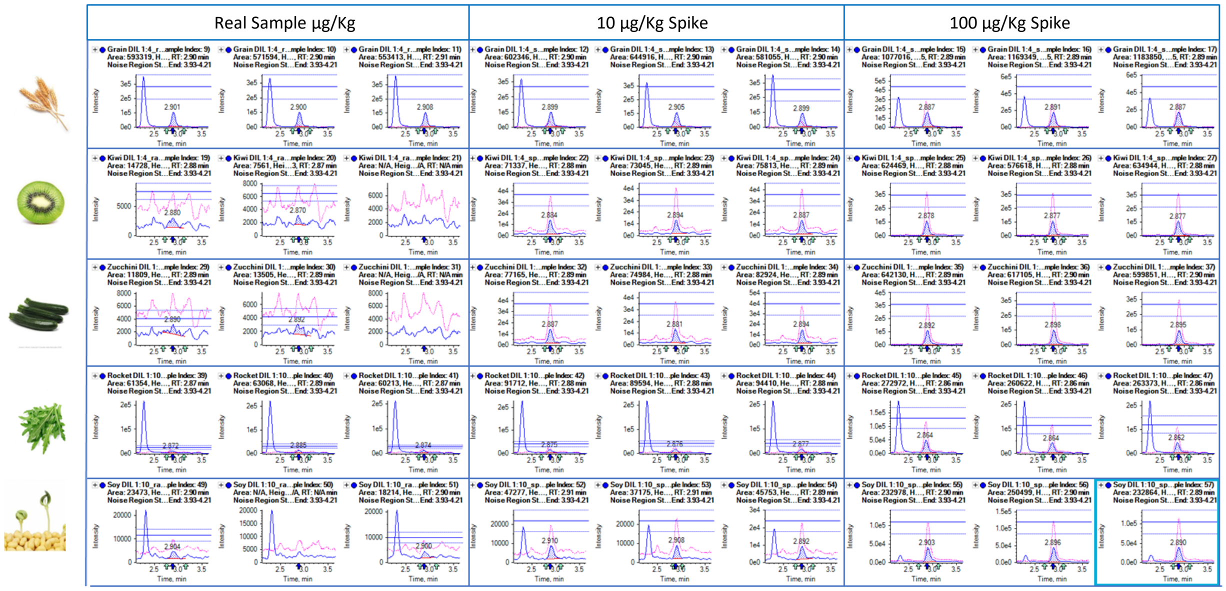


	Real Sample µg/Kg		10 µg/Kg Spike			100 µg/Kg Spike		
Matrix	**Conc. µg/Kg**	**% CV**	**Conc. µg/Kg**	**% CV**	**% Recovery**	**Conc. µg/Kg**	**% CV**	**% Recovery**
Grain DIL 1:4	0	0	9.43	2	94.3	89.12	5.8	89.12
Kiwi DIL 1:4	0	0	9.09	4.68	90.9	89.28	9.23	89.28
Zucchini DIL 1:4	0	0	10.23	2.35	102.3	91.6	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!
*Visit **www.phenomenex.com/Chat** to get in touch with one of our Technical Specialists*

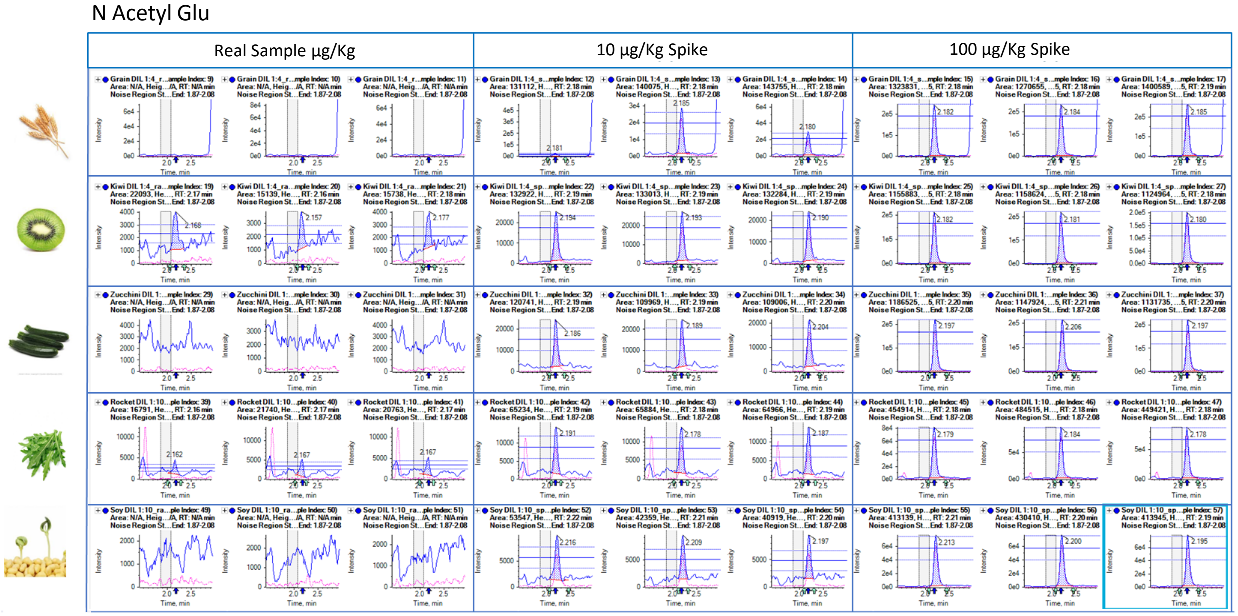
Figure 2 (cont'd). Real Matrix Analysis of Polar Pesticides.

Phosphonic Acid



	Real Sample µg/Kg		10 µg/Kg Spike			100 µg/Kg Spike		
Matrix	Conc. µg/Kg	% CV	Conc. µg/Kg	% CV	% Recovery	Conc. µg/Kg	% CV	% Recovery
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	4.46	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

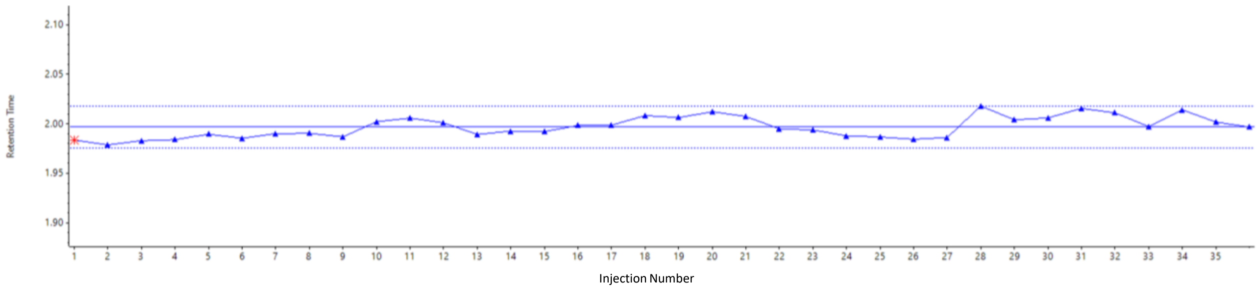
N Acetyl Glu



	Real Sample µg/Kg		10 µg/Kg Spike			100 µg/Kg Spike		
Matrix	Conc. µg/Kg	% CV	Conc. µg/Kg	% CV	% Recovery	Conc. µg/Kg	% CV	% Recovery
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

Have questions or want more details on implementing this method? We would love to help!
*Visit **www.phenomenex.com/Chat** to get in touch with one of our Technical Specialists*

Figure 3. Retention Time Stability of Glyphosate.



Conclusions

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 on implementing this method? We would love to help!
*Visit **www.phenomenex.com/Chat** to get in touch with one of our Technical Specialists*

Need a different column size or sample preparation format?

No problem! We have a majority of our available dimensions up on www.phenomenex.com, but if you can't find what you need right away, our super helpful Technical Specialists can guide you to the solution via our online chat portal www.phenomenex.com/Chat.

Australia
t: +61 (0)2-9428-6444
auinfo@phenomenex.com

Austria
t: +43 (0)1-319-1301
anfrage@phenomenex.com

Belgium
t: +32 (0)2 503 4015 (French)
t: +32 (0)2 511 8666 (Dutch)
beinfo@phenomenex.com

Canada
t: +1 (800) 543-3681
info@phenomenex.com

China
t: +86 400-606-8099
cninfo@phenomenex.com

Czech Republic
t: +420 272 017 077
cz-info@phenomenex.com

Denmark
t: +45 4824 8048
nordicinfo@phenomenex.com

Finland
t: +358 (0)9 4789 0063
nordicinfo@phenomenex.com

France
t: +33 (0)1 30 09 21 10
franceinfo@phenomenex.com

Germany
t: +49 (0)6021-58830-0
anfrage@phenomenex.com

Hong Kong
t: +852 6012 8162
hkinfo@phenomenex.com

India
t: +91 (0)40-3012 2400
indiainfo@phenomenex.com

Indonesia
t: +62 21 5019 9707
indoinfo@phenomenex.com

Ireland
t: +353 (0)1 247 5405
eireinfo@phenomenex.com

Italy
t: +39 051 6327511
italiainfo@phenomenex.com

Japan
t: +81 (0) 120-149-262
jpinfo@phenomenex.com

Luxembourg
t: +31 (0)30-2418700
nlinfo@phenomenex.com

Mexico
t: 01-800-844-5226
tecnicomx@phenomenex.com

The Netherlands
t: +31 (0)30-2418700
nlinfo@phenomenex.com

New Zealand
t: +64 (0)9-4780951
nzinfo@phenomenex.com

Norway
t: +47 810 02 005
nordicinfo@phenomenex.com

Poland
t: +48 22 104 21 72
pl-info@phenomenex.com

Portugal
t: +351 221 450 488
ptinfo@phenomenex.com

Singapore
t: +65 6559 4364
sginfo@phenomenex.com

Slovakia
t: +420 272 017 077
sk-info@phenomenex.com

Spain
t: +34 91-413-8613
espinfo@phenomenex.com

Sweden
t: +46 (0)8 611 6950
nordicinfo@phenomenex.com

Switzerland
t: +41 (0)61 692 20 20
swissinfo@phenomenex.com

Taiwan
t: +886 (0) 0801-49-1246
twinfo@phenomenex.com

Thailand
t: +66 (0) 2 566 0287
thaiinfo@phenomenex.com

United Kingdom
t: +44 (0)1625-501367
ukinfo@phenomenex.com

USA
t: +1 (310) 212-0555
www.phenomenex.com/chat

All other countries/regions
Corporate Office USA
t: +1 (310) 212-0555
www.phenomenex.com/chat

www.phenomenex.com

Phenomenex products are available worldwide. For the distributor in your country/region, contact Phenomenex USA, International Department at international@phenomenex.com

BE-HAPPY™ GUARANTEE

Your happiness is our mission. Take 45 days to try our products. If you are not happy, we'll make it right.
www.phenomenex.com/behappy

Terms and Conditions
Subject to Phenomenex Standard Terms and Conditions, which may be viewed at www.phenomenex.com/phx-terms-and-conditions-of-sale.
Trademarks
BE-HAPPY is a trademark of Phenomenex. Venusil is a registered trademark of Bonna-Agela Technologies, Inc. SCIEX is a registered trademark of AB SCIEX Pte. Ltd.
Disclaimer
Comparative separations may not be representative of all applications.

FOR RESEARCH USE ONLY. Not for use in clinical diagnostic procedures.
© 2022 Phenomenex, Inc. All rights reserved.

TN49350922_W

Have questions or want more details on implementing this method? We would love to help!
*Visit **www.phenomenex.com/Chat** to get in touch with one of our Technical Specialists*

phenomenex