Analysis of Anionic and Cationic Polar Pesticides Using a New Mixed Mode Column

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Introduction

The presence of highly polar pesticides in foodstuff raises concerns due to their potential adverse effects on human health. These pesticides can enter the food chain through various routes, including their application during crop production, contamination from neighboring fields, or environmental exposure. Therefore, accurate and sensitive analytical methods are required to detect and quantify these compounds in food samples. Polar pesticides exist in anionic and cationic forms and to date, have required different separation modes. In the case of negative charged compound like Glyphosate a QuECheRS or QuPPe sample preparation approach and a reversed phase separation while cationic pesticides like Nereistoxin typically require a HILIC separation. Both of these separations are typically followed with MS/MS detection. Here we demonstrate the separation of a suite of anionic and cationic pesticides with a single, mixed mode column.

Materials and Methods

Sample Preparation

Individual polar pesticide standard stock solutions were prepared in a suitable solvent (mostly Methanol, Water, or Acetonitrile) at a concentration of 1000 mg/L and were stored in amber screw-capped plastic vials in the darkness. From individual polar pesticide standard stock solutions, two mix-standards, anionic and cationic pesticides, were prepared at a concentration of 50 mg/L in Methanol, and used for the calibration, as needed. Stock and intermediate solutions were stored at -20 °C.

QuPPe-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 rj 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)

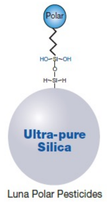
LC-MS/MS and IC-MS/MS Analysis

LC-MS/MS Conditions

Column: 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: 10 µL
Temperature: 40 °C
Detector: Agilent® G6460



Luna Polar Pesticides

Table 1. MS/MS Conditions and Transitions.

Compound	Retention Time (min)	Precursor Ion (m/z)	Product Ion (m/z)	Fragmentor Voltage (V)	Collision Energy (eV)
Aminomethylphosphonic Acid (AMPA)	1.275	110.0	79.0 63.1	110	30 20
Glyphosate	2.559	168.1	150.0 78.9 63.0	90	10 30 35
2-Hydoxyethylphosphonic Acid (HEPA)	2.850	125.0	95.0 79.0	110	10 35
Phosphonic Acid	3.647	81.1	79.0 63.1	50	20 30
Ethephon	3.757	143.0	107.0 79.1	50	10 20
Chlorate	4.603	83.0	67.0 51.0	50	20 35
Fosetyl-Al	4.709	109.1	81.0 63.1	50	10 35
Perchlorate	5.716	99.0	82.9 67.0	130	30 35
Nicotinic Acid	0.696	163.1	132.1 130.0 106.1 84.0 80.1	100	10 20 10 20 20
Triethanolamine	0.723	150.0	132.0 70.0	100	10 20
Neresitoxin	0.768	150.0	105.0 71.0 61.0	75	20 40 20
1,2,4-Triazole	1.074	70.0	43.0	50	20
Dimethoate	1.090	230.0	157.0 125.0	75	20 20
Glufosinate	1.823	182.0	136.0 119.1	100	10 15

Results From Reversed Phase Separation

Figure 1. Total Ion Chromatograms (TIC) of Negative Polarization (Top), Polarity Switching (Middle), and for Positive Ionization (Bottom) at a Concentration of 0.100 mg/L.

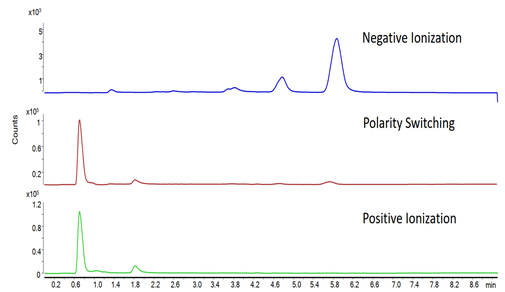
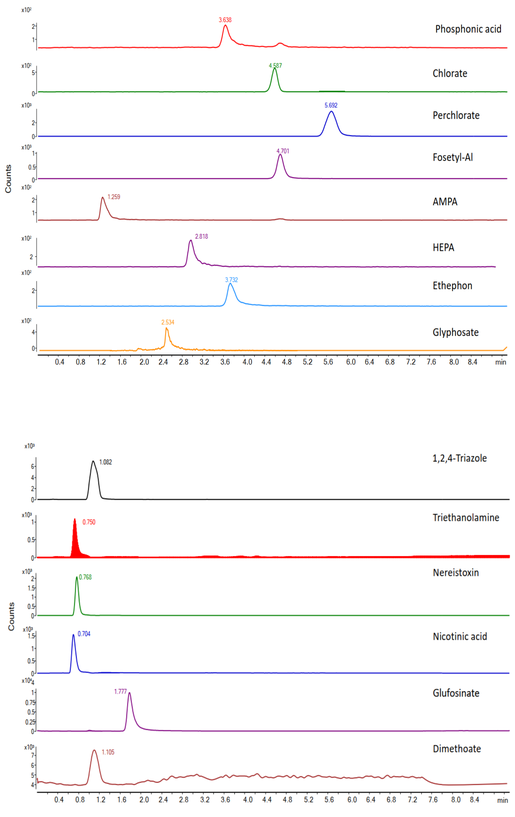


Figure 2. Extracted TIC for Negative Ion Mode Showing Anionic Pesticides (Top) and for Positive Ion Mode Showing Cationic Pestcides (Bottom).



Results From Reversed Phase Separation

In order to determine the retention time stability, a series of 10 injections of the anionic pesticides (ESI -mode), then 10 injections of the cationic pesticides (ESI+ mode), and finally 10 injections of all pesticides (ESI – and + simultaneously performing polarity switching), at a concentration of 0.100 mg/L. Prior to each batch of 10 injections, 3 volumes of the mobile phase 10:90 (A:B) were run through column with the method developed for each polarity, showing in the Table below.

Table 2. Mean Retention Time and Variability of the Retention Time of the Polar Compounds.

Compound	Polarity	1st Round		2nd Round		3rd Round	
		Retention Time (min) (RSD)	RSD_{Area}	Retention Time (min) (RSD)	RSD_{Area}	Retention Time (min) (RSD)	RSD_{Area}
AMPA	Negative	1.283 (2.5)	14.10	-	-	1.291 (2.0)	3.22
Glyphosate	Negative	2.574 (1.1)	8.88	-	-	2.572 (1.4)	16.99
HEPA	Negative	2.907 (1.8)	3.05	-	-	3.207 (2.4)	8.87
Phosphonic Acid	Negative	3.656 (0.6)	4.50	-	-	3.656 (0.9)	6.51
Ethephon	Negative	3.773 (1.0)	5.88	-	-	3.741 (0.4)	17.65
Chlorate	Negative	4.623 (1.1)	3.37	-	-	4.605 (0.7)	2.69
Fosetyl-Al	Negative	4.730 (1.0)	8.03	-	-	4.703 (0.6)	1.50
Perchlorate	Negative	5.749 (1.4)	8.87	-	-	5.647 (0.6)	1.17
Nicotinic Acid	Positive	-	-	0.701 (0.5)	3.12	0.697 (0.0)	0.74
Triethanolamine	Positive	-	-	0.723 (0.0)	3.41	0.733 (0.0)	5.40
Neresitoxin	Positive	-	-	0.768 (0.0)	3.13	0.764 (0.0)	0.81
1,2,4-Triazole	Positive	-	-	1.096 (1.0)	14.25	1.098 (2.9)	8.18
Dimethoate	Positive	-	-	1.077 (0.3)	10.00	1.079 (0.8)	7.79
Glufosinate	Positive	-	-	1.777 (1.5)	11.43	1.770 (1.7)	15.15

Table 3. Results of Zucchini Matrix Patterns.

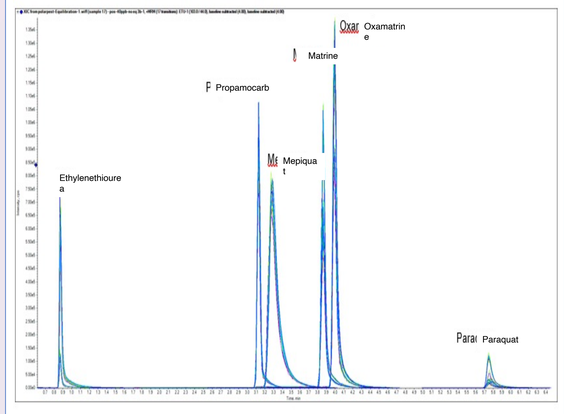
Compound	Polarity	1st Round		2nd Round		3rd Round	
		Retention Time (min) (RSD)	RSD_{Area}	Retention Time (min) (RSD)	RSD_{Area}	Retention Time (min) (RSD)	RSD_{Area}
AMPA	Negative	1.261 (3.1)	16.26	-	-	1.239 (3.7)	8.41
Glyphosate	Negative	2.505 (1.5)	2.55	-	-	2.504 (4.2)	12.62
HEPA	Negative	2.907 (0.8)	6.86	-	-	3.246 (2.6)	8.64
Phosphonic Acid	Negative	3.556 (0.8)	7.44	-	-	3.540 (2.4)	3.69
Ethephon	Negative	3.657 (0.7)	5.19	-	-	3.628 (1.3)	2.30
Chlorate	Negative	4.632 (0.8)	2.59	-	-	4.540 (1.5)	1.46
Fosetyl-Al	Negative	4.579 (0.5)	4.40	-	-	4.551 (1.0)	18.39
Perchlorate	Negative	5.910 (0.8)	5.20	-	-	5.688 (0.7)	1.55
Nicotinic Acid	Positive	-	-	0.682 (0.4)	3.85	0.680 (0.0)	2.84
Triethanolamine	Positive	-	-	0.700 (0.0)	4.91	0.700 (0.0)	3.15
Neresitoxin	Positive	-	-	0.812 (0.4)	1.87	0.809 (0.8)	1.15
1,2,4-Triazole	Positive	-	-	1.323 (5.2)	8.78	1.256 (4.3)	6.88
Dimethoate	Positive	-	-	1.045 (2.7)	5.64	1.049 (3.8)	6.02
Glufosinate	Positive	-	-	1.684 (2.5)	0.79	1.670 (2.5)	8.72

Results From HILIC Separation

We also investigated the retention time stability in the HILIC mode for select group of cationic pesticides as this is a common technique used in many laboratories. The Luna Polar Pesticides column shows excellent peak shape and retention time stability for these cationic pesticides using the same eluents using a spiked water sample.

Mobile Phase: A: 100 mM Ammonium Formate, pH 3.0 + Formic Acid in Water
B: Acetonitrile

Gradient: Time (min)	%B
0	97
0.5	97
4	70
5	40
6	40
6.1	97
10	97



Discussion

Normally a combination of reversed phase and HILIC conditions are used for the analysis of polar pesticides. One of the major advantages of HILIC is that the solvents are MS compatible. Here we show excellent peak shape and retention tine stability in both reversed phase and HILIC modes. In addition, application switching is fast and holds up between the separation modes and not affected by matrix effects.

Conclusion

The results we achieved by using this method for polar pesticides on real-life samples show that the Luna Polar Pesticides column is a feasible option to analyze both anionic and cationic pesticides compounds under reversed phase and HILIC conditions in a fast (see the retention times of the compounds) and robust way.

We showed that the column with its unique selectivity is a great asset to the group of columns used for this purpose. The method is easy and can be easily implemented into your routine polar pesticide analysis.

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