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

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Introduction

Polar pesticides play a significant role in modern agriculture due to their affordability and high efficiency. However, their widespread use raises concerns as these pesticides are toxic and linked to cancer and other health concerns. Glyphosate, one of the most extensively discussed polar pesticides, has been found in numerous studies to be present in soil, crop products, animals that consume these products, humans, and even in freshwater sources. The emergence of concerns regarding their toxicity and potential carcinogenic effects highlights the importance of developing accurate and sensitive analytical methods to detect and quantify these compounds. Furthermore, it is particularly challenging to analyze polar pesticides due to their unique physicochemical properties, which encompass both anionic and cationic groups, making them an extremely difficult group of molecules to study.

Traditionally, two different separation modes are employed to analyze the positive and negative groups, necessitating the use of multiple columns. In this technical note, two integrated fast and robust methods for separating a suite of anionic and cationic pesticides using a single column are presented. The Luna Polar Pesticides column is employed for analyzing anionic pesticides in a reversed-phase mode, while it is utilized for analyzing cationic pesticides in a hydrophilic interaction liquid chromatography (HILIC) mode. Additionally, we demonstrate the equilibration time for each method and the impact of switching between the negative and positive modes on the precision and reproducibility of the analysis. By simplifying the analytical process and minimizing the need for multiple columns, this strategy offers a practical solution for accurately detecting and quantifying anionic and cationic pesticides.

Sample Preparation

Polar pesticide samples were diluted in Water / 1 % Formic Acid in Methanol (95:5, v/v) for Reversed Phase and 1 % Formic Acid in Acetonitrile / Water (95:5, v/v) for HILIC Mode.

LC Conditions for Reversed Phase

Column: Luna 3 µm Polar Pesticides
Dimensions: 100 x 2.1 mm
Part No.: 00D-4798-AN
Guard: SecurityGuard ULTRA for Luna Polar Pesticides: AJ0-8789
Mobile Phase: A: 0.3 % Formic Acid in Water
B: 0.3 % Formic Acid in Acetonitrile

Gradient:

Time (min)	%B
0	2
0.5	2
6	20
7	90
9	90
9.1	2
12	2

Flow Rate: 0.3 mL/min
Injection Volume: 2 µL
Temperature: 40 °C
Instrument: Agilent 1260
Detection: MRM
Detector: SCIEX 5500 Triple Quad

LC Conditions for HILIC Mode

Column: Luna™ 3 µm Polar Pesticides
Dimensions: 100 x 2.1 mm
Part No.: 00D-4798-AN
Guard: SecurityGuard™ ULTRA for Luna Polar Pesticides: AJ0-8789
Mobile Phase: A: 100 mM Ammonium Formate, adjusted to pH 3.0 with Formic Acid
B: Acetonitrile

Gradient:

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

Flow Rate: 0.3 mL/min
Injection Volume: 2 µL
Temperature: 40 °C
Instrument: Agilent® 1260
Detection: MRM
Detector: SCIEX® 5500 Triple Quad™

MRM Conditions for Reversed Phase

Polarity: Negative
Temperature: 450 °C
GS1: 50 psi
GS2: 50 psi
CUR: 20 psi
IS: -4500 V

MRM Conditions for HILIC Mode

Polarity: Positive
Temperature: 500 °C
GS1: 60 psi
GS2: 50 psi
CUR: 30 psi
IS: 5000 V

Table 1. Retention Times and MRM Transitions for Polar Pesticides.

Peak No.	Anionic Polar Pesticides	Retention Time (min)	Precursor Mass (m/z)	Fragment Mass (m/z)
1	Aminomethylphosphonic Acid (AMPA)	1.01	110	63 79
2	Maleic Hydrazide	1.21	111	82 55
3	Glufosinate	1.43	180	63 85
4	3-Methylphosphinicopropionic Acid (MPPA)	2.00	151	63 107
5	Glyphosate	2.08	168	63 79
6	N-Acetyl-Glufosinate	2.36	222	136 134
7	Phosphonic Acid	3.22	81	79 63
8	Ethephon	3.39	143 145	107 107
9	Chlorate	4.33	83 85	67 69
10	Fosetyl Al	4.60	109	63 81
11	Perchlorate	5.44	99 101	83 85
Peak No.	**Cationic Polar Pesticides**	**Retention Time (min)**	**Precursor Mass (m/z)**	**Fragment Mass (m/z)**
1	Ethylene Thiourea (ETU)	0.87	103	44 86
2	Propamocarb	3.13	189	102 74
3	Mepiquat	3.29	114	98 70
4	Matrine	3.87	249	150 176
5	Oxymatrine	3.99	265	247 136
6	Paraquat	5.75	93	171 77

Results and Discussion

Column equilibration time for each method was investigated in two sets of experiments. The equilibration study was performed in 4 steps (**Figure 1**). In the first step, 3 blanks followed by 10 samples containing a mix of select anionic polar pesticides were run in reversed phase mode. In step 2, the method was switched to HILIC mode running 3 blanks followed by 10 samples containing a mix of select cationic polar pesticides using the same column. In the third step, the method was switched back to reversed phase mode, repeating step 1. Finally in step 4, the method was switched back to HILIC mode, repeating step 2.

The relative standard deviation (%RSD) of retention time and peak area was calculated for 20 samples in negative mode, followed by 18 samples in negative mode, after removing the first injection from each sequence (**Figure 2**). The %RSD of retention time was less than 0.38 % and that of peak area was less than 15 %. With a 4-injection equilibration a significant improvement on %RSD of area count was observed (**Table 2**). The %RSD of retention time and peak area was calculated for 20 samples in positive mode, followed by 18 samples in positive mode, after removing the first injection from each sequence (**Figure 3**). The %RSD of retention time was less than 0.53 % and that of peak area was less than 14.2 % for all 20 samples. With 4 injection equilibration a significant improvement on %RSD of area count was observed (**Table 3**). The %RSD presented here are without internal standard. Use of deuterated internal standard can significantly reduce %RSD further. Switching between the negative and positive modes did not have any adverse effect on selectivity, retention profile, precision and reproducibility.

Figure 4 shows the stacked display of chromatograms from the second and last injections of both sets of experiments run in negative mode. The results are comparable from run to run, as well as between experiments. **Figure 5** shows the stacked display of chromatograms from the second and last injections of both sets of experiments run in positive mode. Again, there are comparable results from run to run and between experiments. Switching back and forth between negative and positive mode has no effect on the reproducibility and precision of the analysis.

Figure 1. Sequence of Experiments.

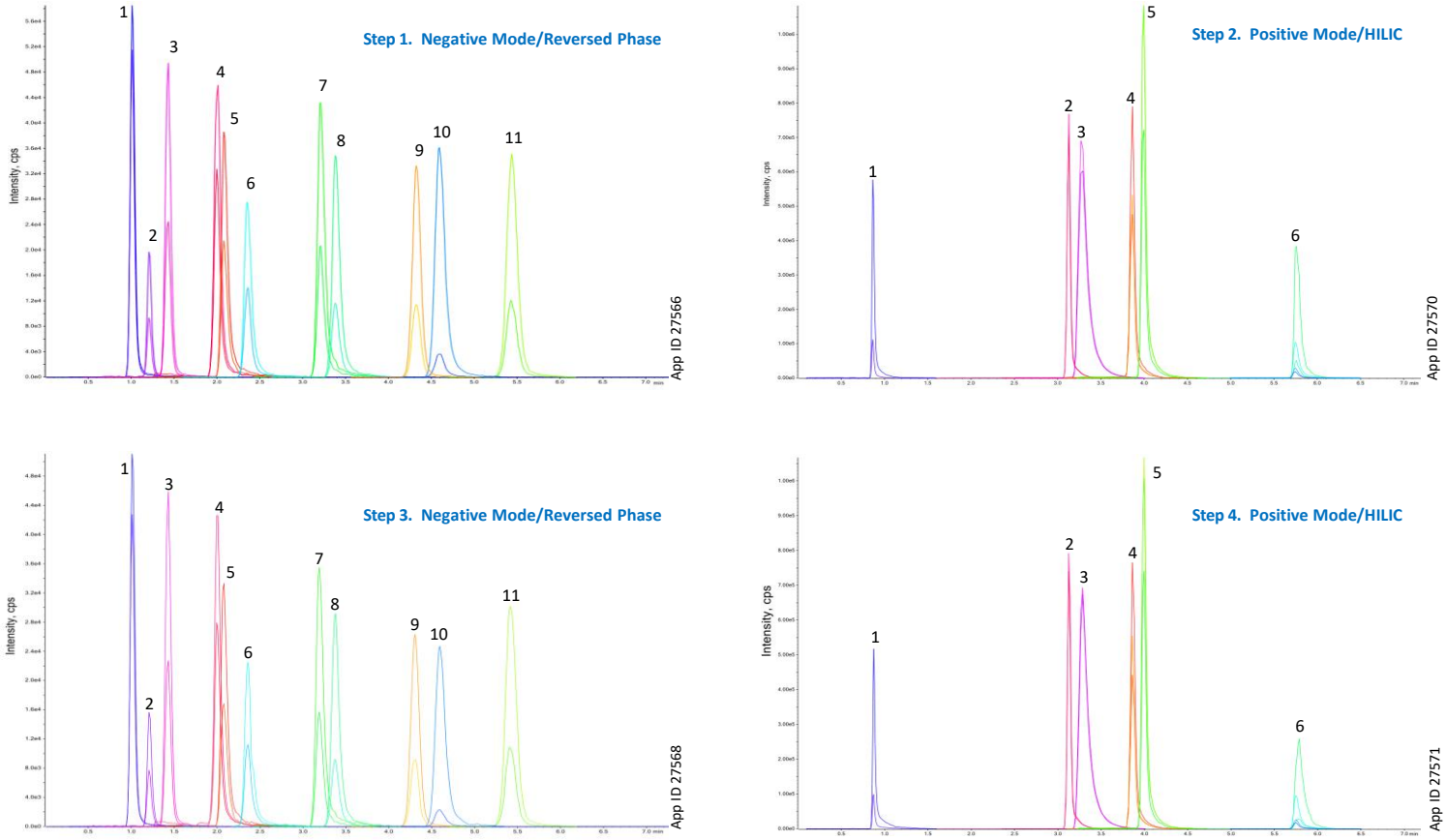


Figure 2. Overlay of Total Ion Chromatograms of 20 samples Run in Reversed Phase.

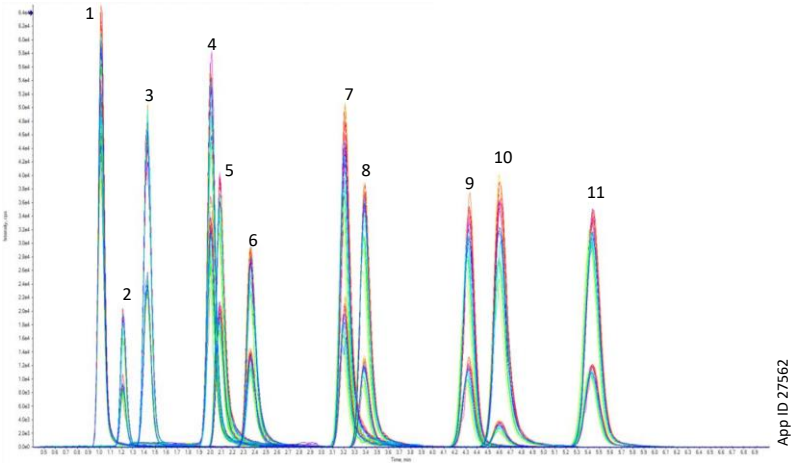


Table 2. Average Retention Time, %RSD Retention Time, and %RSD Peak Area Run in Reversed Phase.

Analyte	Precursor Mass (m/z)	Fragment Mass (m/z)	N=20 Injection			N=18 Injections		
			Average RT (min)	RT %RSD	Peak Area %RSD	Average RT (min)	RT %RSD	Peak Area %RSD
Aminomethylphosphonic Acid (AMPA)	110	63	1.01	0.36	7.37	1.01	0.38	5.92
		79	1.01	0.00	6.83	1.01	0.00	5.57
Maleic Hydrazide	111	82	1.21	0.25	6.30	4.32	0.21	9.82
		55	1.21	0.18	11.44	4.32	0.21	8.97
Glufosinate	180	63	1.43	0.16	4.91	3.39	0.15	8.65
		85	1.43	0.26	3.81	3.38	0.16	9.13
3-Methylphosphinicopropionic Acid (MPPA)	151	63	2.00	0.23	8.86	4.60	0.15	13.58
		107	2.00	0.18	9.97	4.60	0.15	13.15
Glyphosate	168	63	2.08	0.25	8.30	1.43	0.16	5.01
		79	2.08	0.21	7.65	1.43	0.23	3.20
N-Acetyl-Glufosinate	83	67	2.36	0.17	9.02	2.08	0.22	7.29
	85	69	2.36	0.19	8.81	2.08	0.18	6.87
Phosphonic Acid	99	83	3.20	0.25	11.48	1.21	0.27	5.84
	101	85	3.21	0.29	11.21	1.21	0.19	7.54
Ethephon	81	79	3.38	0.17	10.29	2.00	0.24	7.65
		63	3.39	0.18	10.16	2.00	0.19	9.12
Chlorate	143	107	4.32	0.23	10.63	2.36	0.18	7.93
	145	107	4.32	0.25	9.69	2.36	0.16	7.49
Fosetyl Al	222	136	4.60	0.16	14.90	5.44	0.13	5.21
		134	4.60	0.18	14.81	5.43	0.15	5.03
Perchlorate	109	63	5.43	0.17	5.10	3.21	0.24	9.94
		81	5.44	0.16	5.15	3.20	0.19	9.91

Figure 3. Overlay of Total Ion Chromatograms of 20 samples Run in HILIC Mode.

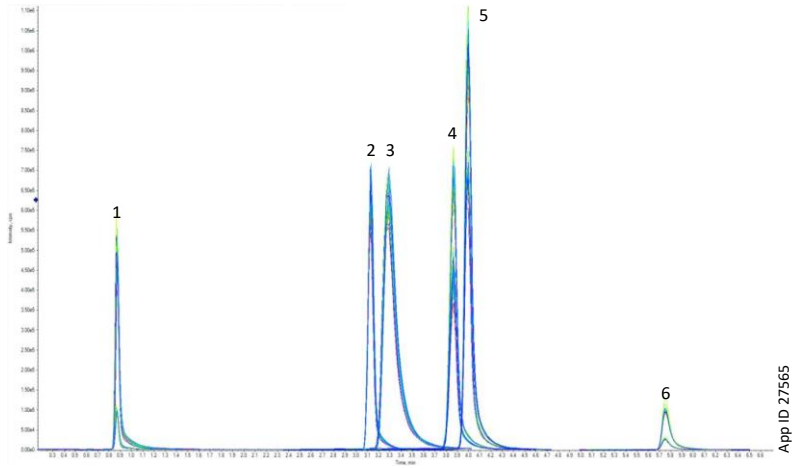


Table 3. Average Retention Time, %RSD Retention Time, and %RSD Peak Area Run in HILIC Mode.

Analyte	Precursor Mass (m/z)	Fragment Mass (m/z)	N=20 Injection			N=18 Injections		
			Average RT (min)	RT %RSD	Peak Area %RSD	Average RT (min)	RT %RSD	Peak Area %RSD
Ethylene Thiourea	103	44	0.87	0.51	5.71	0.87	0.53	4.08
		86	0.87	0.47	6.70	0.87	0.37	5.53
Propamocarb	114	98	3.12	0.10	11.29	3.12	0.08	4.06
		70	3.12	0.16	11.58	3.12	0.16	4.23
Mepiquat	189	102	3.28	0.12	11.66	3.28	0.12	3.11
		74	3.28	0.15	11.76	3.28	0.15	2.25
Matrine	249	150	3.86	0.13	14.16	3.86	0.13	4.89
		176	3.86	0.08	11.66	3.86	0.08	4.83
Oxymatrine	265	247	3.99	0.10	13.63	3.99	0.11	5.01
		136	3.99	0.00	13.24	3.99	0.00	4.81
Paraquat	93	171	5.75	0.00	8.79	5.75	0.00	5.58
		77	5.75	0.00	8.92	5.75	0.00	5.24

Figure 4. Experiments Run in Reversed Phase.

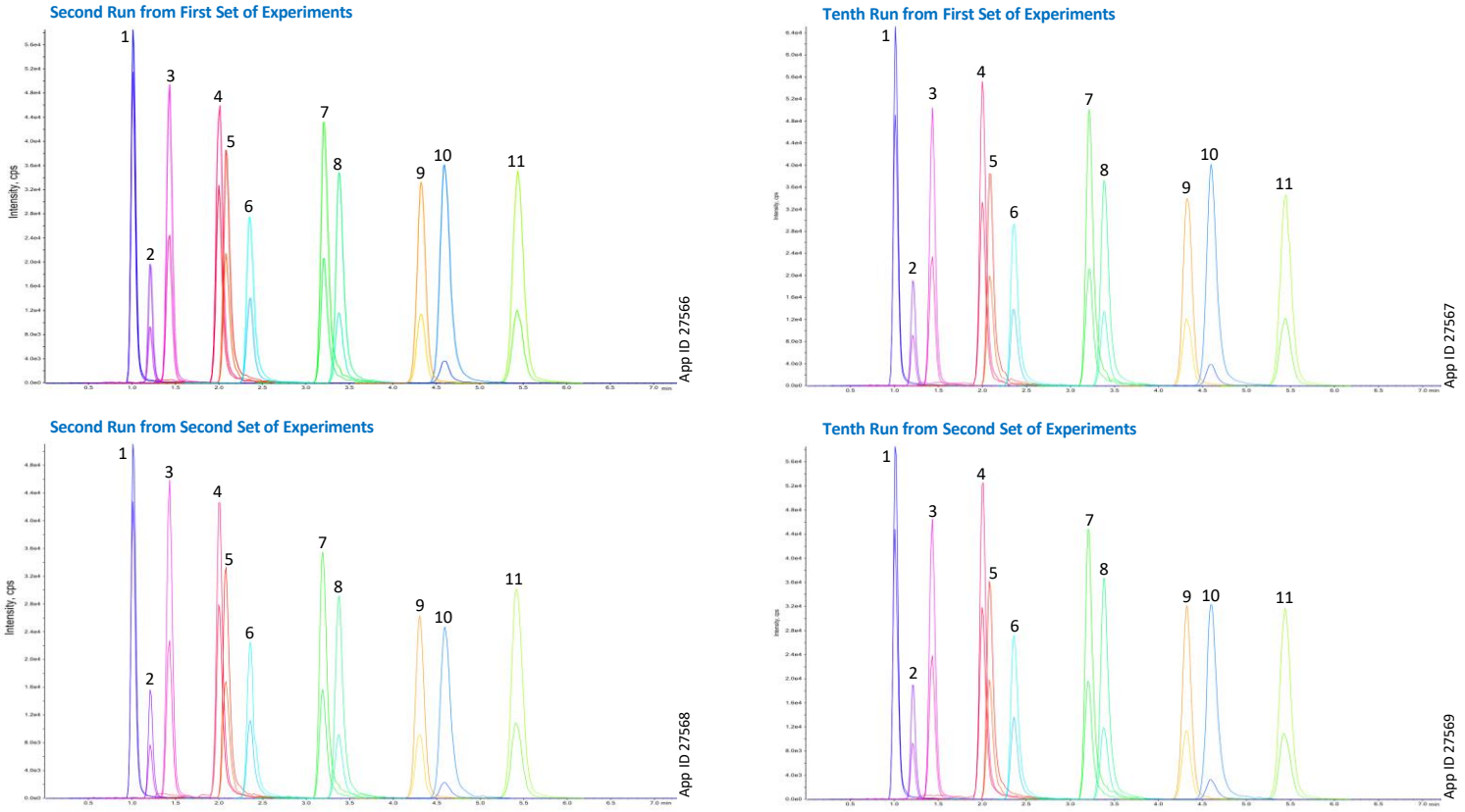
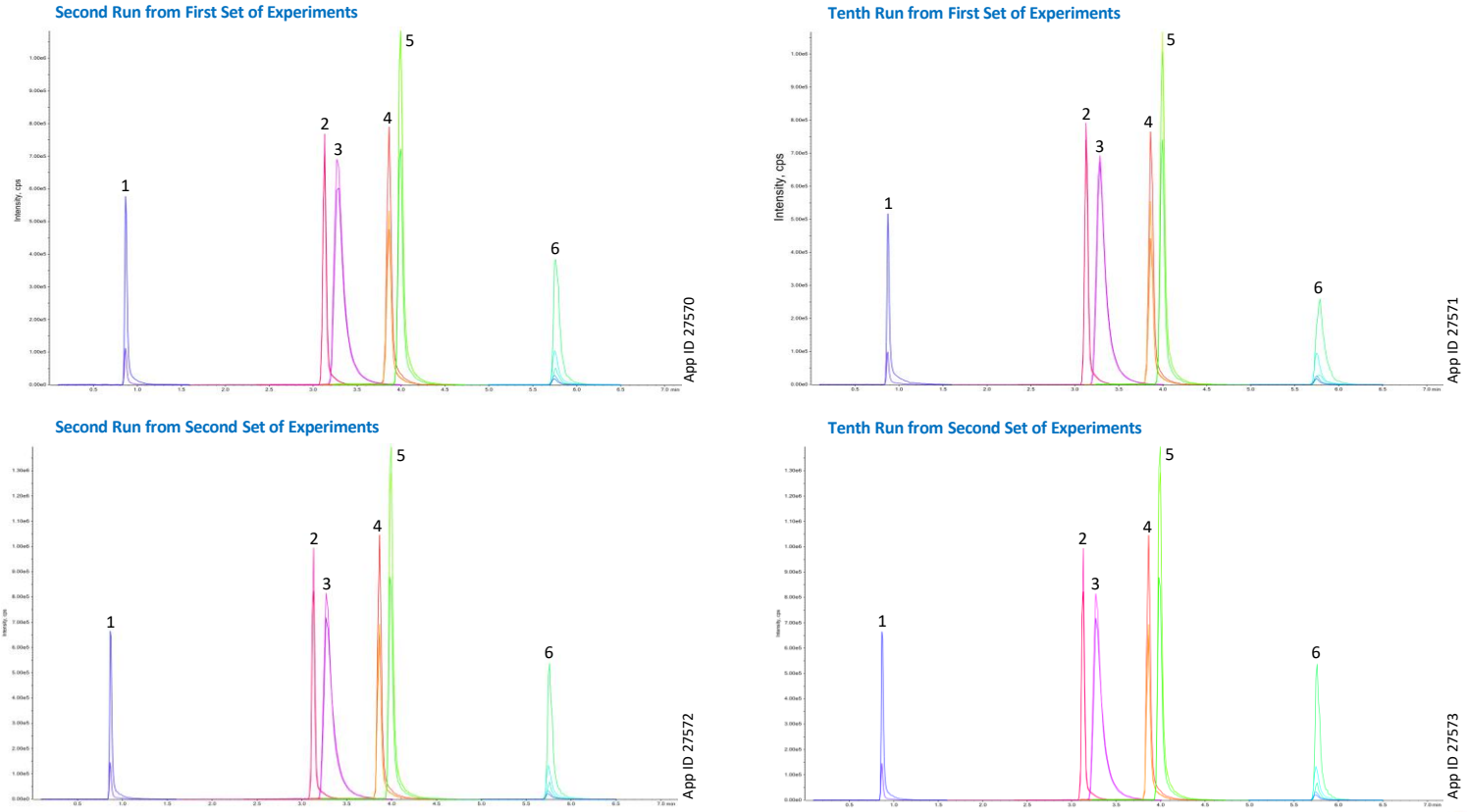


Figure 2. Experiments Run in HILIC Mode.



Conclusion

The application presented here demonstrates that injecting 3 blank samples is enough to reach equilibration and achieve reproducible results with high precision in both anionic and cationic polar pesticides analysis modes. Furthermore, switching back and forth between two different analysis modes had no adverse effect on the column and the obtained results.

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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