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Simultaneous Analysis of Anionic and Cationic Polar Pesticides by Reversed Phase LC-MS/MS

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Introduction

The analysis of highly polar pesticides in foodstuffs is an essential aspect of food safety and quality control. Highly polar pesticides, also known as water-soluble pesticides, are compounds with a high affinity for water and are particularly challenging to analyze in food samples due to their physicochemical properties. Therefore, they are not included in conventional multiresidue methods, and specific methods should be developed.

Highly polar pesticides analysis in food is subjected to regulatory requirements and guidelines established by national and international authorities. These regulations aim to protect consumers from the potential health risks associated with pesticide residues in food. Maximum residue limits (MRLs) are set for different pesticides in various food commodities to ensure that the levels of these compounds do not exceed acceptable safety thresholds.

Analyzing highly polar pesticides in fruits, vegetables, and other aliments involves several steps. First, sample preparation techniques are employed to extract the target compounds from the food matrix. Due to the water-soluble nature of these pesticides, extraction methods that maximize their recovery while minimizing interference from co-extracted substances are necessary. Different approaches had been used, although currently, Quick Polar Pesticides (QuPPe) is a simple analysis approach that covers the extraction of polar pesticides from food commodities.

After sample preparation, instrumental analysis techniques are employed to detect and quantify the target pesticides. Liquid chromatography (LC) coupled with tandem mass spectrometry (MS/MS) is the most common approach for the analysis of highly polar pesticides. LC-MS/MS provides high sensitivity, selectivity, and accuracy, enabling the identification and quantification of multiple pesticides simultaneously in complex food matrices.

Analyzing highly polar pesticides in foodstuffs by LC can present several challenges. Because polar pesticides exist in anionic and cationic forms, their analysis is typically performed using different separation techniques. For example, a reversed phase separation is typically used for anionic pesticides such as Glyphosate, while a HILIC separation is typically used for cationic pesticides such as Nereistoxin. In this technical note, a method to demonstrate the separation of a suite of anionic and cationic pesticides with fast and robust application switching with a single, mixed mode Luna 3 µm Polar Pesticides column.

Sample Preparation

Individual polar pesticide standard stock solutions were prepared in a suitable solvent (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.

LC 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 Methanol

Gradient:

Time (min)	%B
0	2
6	20
7	90
9	95
9	75
9.1	2

Flow Rate: 0.3 mL/min
Injection Volume: 2 µL
Temperature: 40 °C
LC System: Agilent® 1290 Infinity
Detection: MRM
Detector: Agilent 6460 Triple Quad

Table 1. MRM Transitions and Parameters.

Analyte	Polarity	Precursor Ion (m/z)	Product Ion (m/z)	Fragmentor Voltage (V)	Collision Energy (eV)
Aminomethylphosphonic Acid (AMPA)	Negative	110.0	79.0 63.1	110	30 20
Glyphosate	Negative	168.1	150.0 78.9 63.0	90	10 30 35
2-Hydroxyethylphosphonic Acid (HEPA)	Negative	125.0	95.0 79.0	110	10 35
Phosphonic Acid	Negative	81.1	79.0 63.1	50	20 30
Ethephon	Negative	143.0	107.0 79.1	50	10 20
Chlorate	Negative	83.0	67.0 51.0	50	20 35
Fosetyl-Al	Negative	109.1	81.0 63.1	50	10 35
Perchlorate	Negative	99.0	82.9 67.0	130	30 35
Nicotinic Acid	Positive	163.1	132.1 130.0 106.1 84.0 80.1	100	10 20 10 20 20
Triethanolamine	Positive	150.0	132.0 70.0	100	10 20
Nereistoxin	Positive	150.0	105.0 71.0 61.0	75	20 40 20
1,2,4-Triazole	Positive	70.0	43.0	50	20
Dimethoate	Positive	230.0	157.0 125.0	75	20 20
Glufosinate	Positive	182.0	136.0 119.1	100	10 15

Results and Discussion

In **Figures 1** and **2**, a total ion chromatogram obtained with the proposed experimental conditions for each of the developed methods for negative ionization, positive ionization, and a method for all pesticides where polarity is switched with a standard at a concentration level of 0.100 mg/L are shown.

The study of the stability of the retention times was carried out by performing the following experiments, which included 120 injections of pesticide standards. The first part of the study consisted of a first round of 10 injections of the anionic pesticides (ESI - mode), then a second round of 10 injections of the cationic pesticides (ESI + mode), and finally a third round of 10 injections of the anionic pesticides at a concentration of 0.100 mg/L (ESI - mode). Prior to each batch of 10 injections, 3 volumes of the mobile phase A / B (10:90, v/v) were run through the Luna™ 3 µm Polar Pesticides column with the method developed for each polarity. The results are shown in **Table 2**.

A second part of the study has consisted 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 A / B (10:90, v/v) were run through the column with the method developed for each polarity, with the obtained results shown in **Table 3**.

Finally, the same experiment was carried out, but the standards were spiked in two extracts of zucchini and tangerine, after QuPPe extraction. The results are shown in **Table 4** and **5**.

Figure 1. Total Ion Chromatograms Obtained for Negative Ionization, Polarity Switching, and Positive Ionization of Pesticides at a Concentration of 0.100 mg/L.

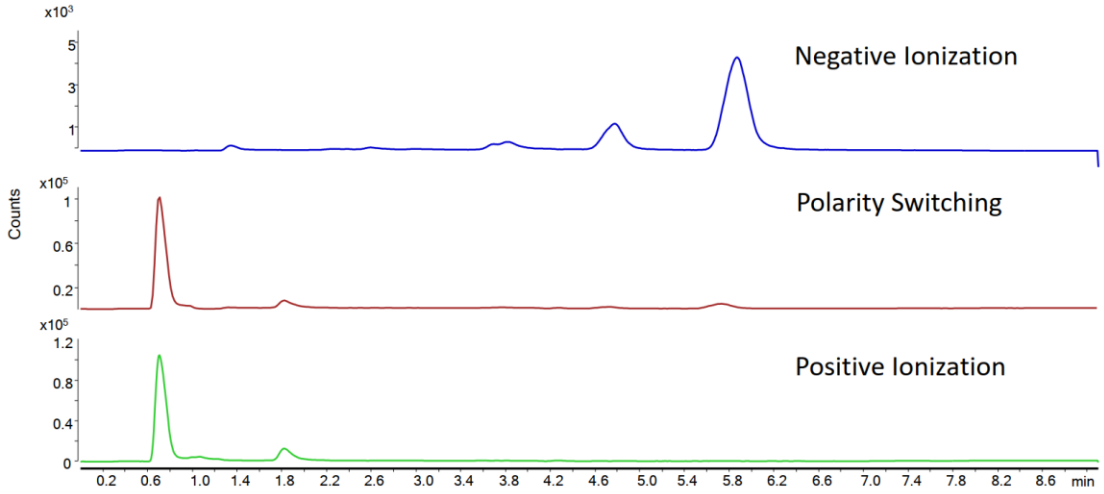

Figure 2. Extracted Total Ion Chromatograms for Negative Ion Mode Showing Anionic Pesticides (Left) and for Positive Ion Mode Showing Cationic Pesticides (Right).

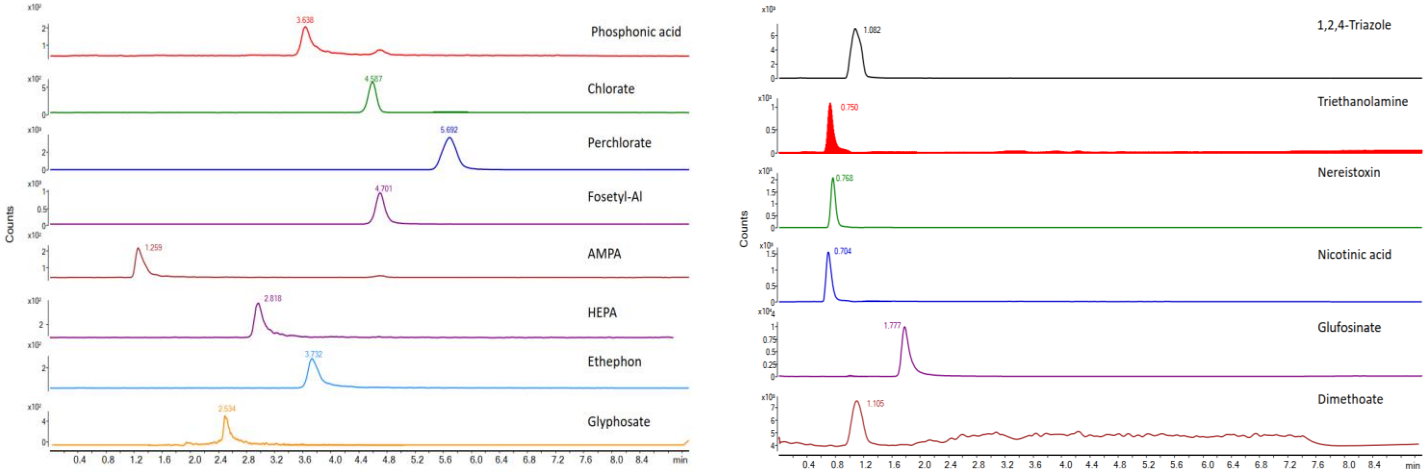

Table 2. Mean Retention Time and Variability (Expressed as Coefficient Variation in Parentheses) of the Retention Time of Polar Compounds.

Compound	Polarity	Retention Time and Coefficient Variation		
		1st Round	2nd Round	3rd Round
AMPA	Negative	1.274 (1.6)	-	1.280 (3.1)
Glyphosate	Negative	2.584 (1.5)	-	2.622 (1.6)
HEPA	Negative	2.849 (1.2)	-	2.865 (0.9)
Phosphonic Acid	Negative	3.679 (1.8)	-	3.703 (1.1)
Ethephon	Negative	3.732 (1.3)	-	3.745 (0.6)
Chlorate	Negative	4.572 (1.7)	-	4.552 (0.7)
Fosetyl-Al	Negative	4.619 (0.5)	-	4.625 (0.5)
Perchlorate	Negative	5.565 (1.3)	-	5.553 (0.6)
Nicotinic Acid	Positive	-	0.703 (0.0)	-
Triethanolamine	Positive	-	0.731 (0.0)	-
Neresitoxin	Positive	-	0.756 (0.5)	-
1,2,4-Triazole	Positive	-	1.074 (2.1)	-
Dimethoate	Positive	-	1.132 (0.8)	-
Glufosinate	Positive	-	1.814 (0.6)	-

Table 3. Mean Retention Time and Variability of the Retention Time of 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 4. 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

Table 5. Results of Tangerine 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	1.280 (2.7)	11.64	-	-	1.239 (3.5)	7.05
Glyphosate	Negative	2.495 (1.3)	9.58	-	-	2.423 (3.6)	7.39
HEPA	Negative	3.803 (0.9)	9.64	-	-	3.678 (1.5)	7.71
Phosphonic Acid	Negative	3.545 (0.9)	4.03	-	-	3.492 (2.4)	18.51
Ethephon	Negative	3.615 (0.5)	2.79	-	-	2.731 (1.8)	15.02
Chlorate	Negative	4.609 (0.6)	1.02	-	-	4.524 (1.7)	8.26
Fosetyl-Al	Negative	4.614 (0.6)	0.54	-	-	4.581 (0.8)	3.87
Perchlorate	Negative	5.770 (0.3)	0.54	-	-	5.634 (0.8)	1.11
Nicotinic Acid	Positive	-	-	0.681 (0.0)	9.72	0.680 (0.0)	12.42
Triethanolamine	Positive	-	-	0.723 (5.9)	3.67	0.720 (6.0)	0.79
Neresitoxin	Positive	-	-	0.812 (0.4)	2.56	0.812 (0.0)	3.26
1,2,4-Triazole	Positive	-	-	0.864 (0.4)	8.78	0.859 (0.8)	3.48
Dimethoate	Positive	-	-	1.004 (0.3)	8.79	0.982 (0.8)	17.95
Glufosinate	Positive	-	-	1.621 (2.0)	16.04	1.606 (2.0)	18.72

Conclusions

According to these results, the following conclusions can be highlighted: 1) The Luna™ 3 µm Polar Pesticide column allows the simultaneous determination of cationic and anionic compounds using a single chromatographic method. 2) The tested column provides a suitable stability of the retention times after a short conditioning stage. 3) The retention time of the compounds is not affected by the matrix, showing the robustness of the proposed methodology.

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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