LC-MS/MS Analysis of Glyphosate and Polar Contaminants in Food Using a Hybrid Ion-Exchange/HILIC Column

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Introduction

Glyphosate is a broad-spectrum herbicide widely used throughout the world. The International Agency for Research on Cancer classified glyphosate as a probable human carcinogen. The safety residual level of glyphosate in food is regulated in USA, Europe, as well as other regions. Direct analysis of underivatized glyphosate, however, can be challenging due to its minimum retention on a reversed-phase or Hydrophilic interaction chromatography (HILIC) column and severe adsorption onto the stainless flow path. On the other hand, glyphosate and other polar anionic contaminants tend to strongly retain on an anion-exchange column and require high salt mobile phase to elute, which is not friendly to mass spectrometric analysis.

These challenges motivated the development of a hybrid ion exchange/HILIC column that offers balanced retention of glyphosate as well as other polar contaminants. With the column, an LC-MS/MS method has been established for the detection of glyphosate, aminomethylphosphonic acid (AMPA), glufosinate, as well as other 14 polar contaminants in various food matrices in a single run. Additionally, the non-specific binding of glyphosate and other chelating compounds onto stainless steel is minimized with a simple passivation solution.

Hybrid Ion Exchange/HILIC Column

The hybrid ion exchange/HILIC column (Raptor™ Polar X, Restek) is built on a single ligand that is comprised of multiple functionalities including ion-exchange and polar chemical moieties (patent-pending). The hybrid ligand can be applied to separate a wide range of compounds through ion exchange, HILIC, or the combination (mixed mode) of ion exchange and HILIC. There are many features and benefits the hybrid column offers, some of which are listed below:

- Single ligand
- High reproducibility
- Balanced retentions
- Versatile separations
- Fast equilibration
- MS friendly conditions
- Sharp peak shape
- Core-shell particles

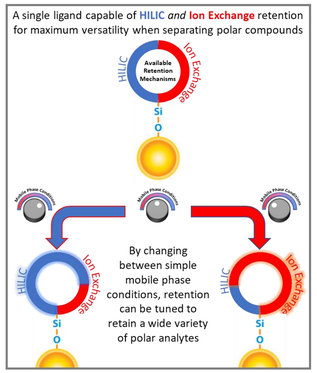
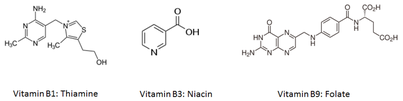


Figure 1:The Polar X Hybrid Column offers Ion Exchange and HILIC retention mechanisms

Lot-to-lot variability of the hybrid IEX/HILIC column

The hybrid IEX/HILIC is built on a single and pure ligand that is comprised of multiple functionalities for chromatography. A reproducible process has been developed to create and immobilize the hybrid ligand onto core-shell particles. Fig. 2 shows the separation of three water-soluble vitamin Bs through ion exchange mechanisms, on seven lots of hybrid columns made at different times by multiple operators. The variability (RSD) of the retention across the seven lots of column is <1.0% for both B1 and B3 (1st and 2nd peak), and 2.4% for B9 (3rd peak), respectively. The relatively low variability of B9's retention is obtained despite its high retention with a k' about 30.



Vitamin B1: Thiamine | Vitamin B3: Niacin | Vitamin B9: Folate

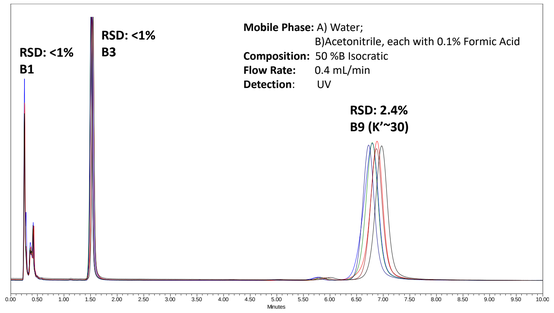
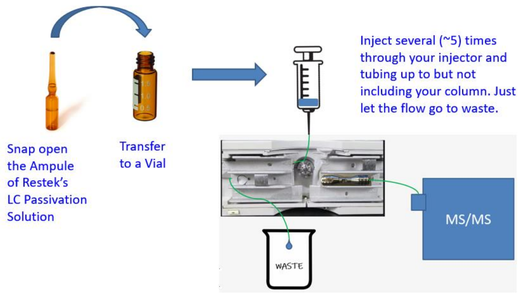


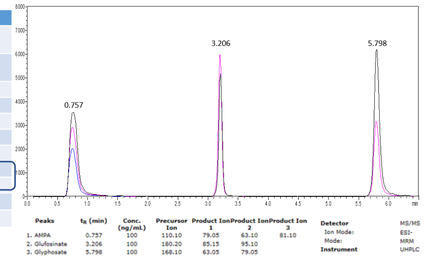
Figure 2: Separation of water soluble vitamins B1, B3 and B9 on seven lots of hybrid IEX/HILIC columns

HPLC Flow Path Passivation



LC-MS/MS Analysis of Glyphosate, AMPA and Glufosinate

Method Conditions:		
MPA:	0.5% formic acid in water	
MPB:	0.5% formic acid in ACN	
Flow:	0.5 mL/min	
Gradient:	Time	%B
	0.0	65
	5.0	10
	6.5	10
	6.51	65
	8.0	65
Oven	35 °C	
Inj.:	5 µL	

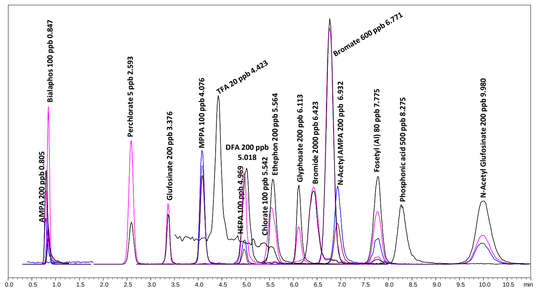


Peaks	t_R (min)	Conc. (ng/mL)	Precursor Ion	Product Ion 1	Product Ion 2	Product Ion 3	Detector
1. AMPA	0.757	100	110.10	79.05	63.10	81.10	Ion Mode:
2. Glufosinate	3.206	100	180.20	85.15	95.10		Mode:
3. Glyphosate	5.798	100	168.10	63.05	79.05		Instrument

MS/MS
ESI-
MRM
UHPLC

Fig. 3: LC-MS/MS separation of glyphosate, AMPA and glufosinate on a hybrid IEX/HILIC column (2.1x30 mm, 2.7 µm). Note the sharp peak shapes, fast equilibrium time (1.5 min) between runs, and short cycle time (8 min).

LC-MS/MS Analysis of 17 Polar Contaminants in One Run

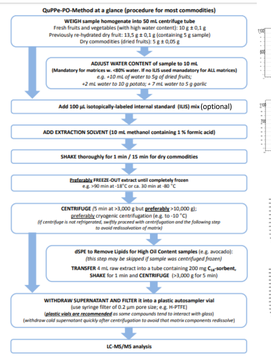


Method Conditions:		
MPA:	0.5% formic acid in water	
MPB:	0.5% formic acid in acetonitrile	
Flow:	0.5 mL/min	
Gradient:	Time	%B
	0.0	65
	5.0	10
	11.5	10
	11.51	65
	13	65
Oven Temp:	35 °C	
Injection Vol:	1 µL	

Glyphosate, AMPA and glufosinate and other 14 polar food contaminants are chromatographically resolved on a short hybrid IEX/HILIC column (2.1x30 mm, 2.7 µm) within 11 min under simple and MS friendly mobile phase conditions. These analytes are retained and separated predominantly through ion exchange interactions with the hybrid column.

Fig. 4, LC-MS/MS separation of glyphosate, AMPA and glufosinate and other 14 polar food contaminants on a hybrid IEX/HILIC column (2.1x30 mm, 2.7 µm).

Detection of Glyphosate, AMPA and Glufosinate in Food



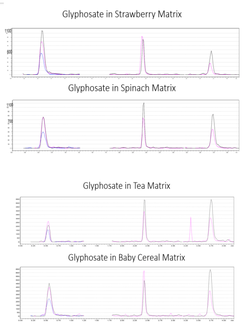


Fig. 5: QuPPe sample preparation and LC-MS/MS detection of glyphosate, AMPA and glufosinate in various food matrices.

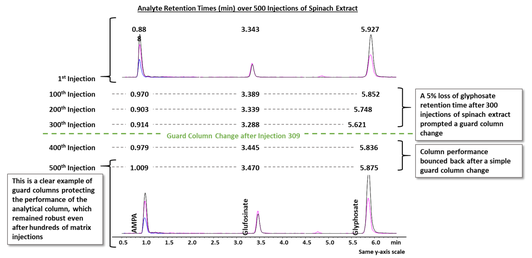


Fig. 6: Analyte retention over 500 injection. The data demonstrate a guard column protects and extends the column lifetime beyond 500 injections.

Conclusion

- To overcome the many challenges in the analysis of glyphosate and other polar contaminants in food, a novel hybrid ion exchange/HILIC column as well as a simple HPLC system passivation solution has been developed. The newly developed column offers simplicity in LC-MS mobile phase conditions, high lot-to-lot and run-to-run consistency, and versatility in the separation of a wide range of polar analytes.
- An LC-MS/MS method has been established for the analysis of glyphosate, AMPA and glufosinate as well as other 14 difficult polar contaminants in a single run.
- The applicability of the column and LC-MS/MS method has been verified with various food matrices.

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