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 undersized 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-steel 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®X™ 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
- Low bleed

Figure 1: The Hybrid X™ Polar X 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. Figure 2 shows the separation of these water-soluble vitamins 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 <1.5% for both B3 and B9 (1st and 2nd peak), and 2.4% for B9 (3rd peak), respectively. The relatively low variability of 99% retention is obtained despite its high retention with a k’ about 30.

Figure 2: Separation of water-soluble vitamins B3, B9 and B12 on seven lots of hybrid IEX/HILIC columns

HPLC Flow Path Passivation

- Re-equilibration between single mobile phase conditions, retention can be improved by re-equilibrating under polar conditions
- Seeps open the ‘tongue’ of Restek’s LC Passivation Solution

Figure 3: The Polar X Hybrid Column offers Ion Exchange and HILIC retention mechanisms

LC-MS/MS Analysis of Glyphosate and Other Polar Contaminants in Food with a Novel Ion Exchange/HILIC Column

Xiaoning Lu, Dan Li, Connor Flannery
Restek Corporation

Detection of Glyphosate, AMPA and Glufosinate in Food

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

Figure 6: Analyte retention over 500 injections. 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 was built. The HILIC system passivated column 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 sensitivity 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.