A Novel, and Versatile Hybrid HILIC and Ion Exchange Column for the Separation of a Wide Range of Polar Compounds

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Introduction

Hydrophilic interaction chromatography (HILIC) paired with mass spectrometry (MS) has increasingly become a powerful analytical tool. This is partly because of its ability to retain and separate challenging polar analytes where reversed-phase chromatography struggles. Additionally, the HILIC separations, operated under high organic conditions, are more compatible with MS detection and often lead to improved signal responses when compared to more aqueous conditions. Nevertheless, the method development using HILIC for a wide range of polar analytes with different physicochemical properties remains difficult.

We have developed a versatile hybrid HILIC/ion exchange column which offers multiple separation mechanisms, and have demonstrated its ability to not only retain and separate polar analytes including glyphosate and its metabolites, organic acids, and water-soluble vitamins, but also neutral and positive analytes such as amino acids, and carbohydrates.

Ion Exchange: Retention and Selectivity

The chromatographic analysis of highly polar, small organic herbicides like glyphosate is significantly challenging. On a reversed-phase column there is minimal retention and no resolution of the target analytes without derivatization. Whereas, on many other ion exchange or HILIC columns, poor peak shape and also long column conditioning requirements make these options difficult to adopt. Demonstrating the ion exchange characteristics of this phase, this method shows a robust LC-MS method for the separation and detection of glyphosate and its metabolites without the need for derivatization.

Figure 1: Polar Aromatic Herbicides

Organic acids represent a class of small, acidic polar compounds that are difficult to retain on a typical C18 reversed-phase column because of their charged carboxylic acid functional groups. Through ionic retention mechanisms, this phase shows retention and selectivity for these structurally similar small polar analytes.

Figure 2: Organic Acids

HILIC Retention

The dominant mode of retention in Hydrophilic Interaction Chromatography (HILIC) is partitioning between a water rich layer at the silica surface and an acetonitrile rich mobile phase. Secondary interactions from functionalities on the bonded phase can also add to the unique selectivity that HILIC offers for highly polar analytes.

To illustrate the retention of this phase in HILIC mode, a mixture of neutral glycolylated analytes were chosen to show the unique selectivity toward polar sugar groups. Alternatively, Thiamine, a positively charged polar molecule, is retained under HILIC conditions.

Figure 4: Water Soluble Vitamins

Several difficult applications were chosen to highlight the ease of use, robustness, and selectivity advantages of this novel phase. Through these applications the various retention mechanisms are demonstrated while maintaining MS compatibility through the use of MS friendly mobile phases.

Retention, selectivity, and symmetrical peak shapes are achieved for the polar herbicide glyphosate and its metabolites.

Water-soluble vitamins, organic acids, and amino acids are also well retained on this column.

This novel hybrid stationary phase provides highly effective solutions across a wide array of challenging analyses.

Conclusion