Comparison of Micro LC Columns with Additional LC-MS/MS Insights

Article Written Based on Technical Application by – Roxana Eggleston-Rangel, Ryan Splitstone, and Jason Anspach, PhD.

Micro LC uses columns with internal diameters (I.D.) that are typically within the range of 0.3 – 0.5-mm and packed with the same chromatographic materials used in traditional analytical column dimensions. As is the case in analytical scale LC separations, the chromatographic media impacts the chromatographic performance and selectivity of the separation and can be optimized. A common practice in micro LC is to use a trap-and-elute injection mode. The use of a trap-and elute injection mode allows for both the significant decrease in injection time and the protection of the column from sample contamination. Micro LC traps can influence the recovery and analytes from the sample based upon the stationary phase chemistry chosen.

In this article, we investigate the influence of different micro LC column and trap stationary phase selectivity combinations on chromatographic performance, sample recovery, and selectivity. We compared micro LC columns in a 50 x 0.3 mm column dimension packed with three different micro LC stationary phase chemistries: Kinetex® XB-C18, Luna® Omega PS C18, and Luna Omega Polar C18.

The comparison was generated using a sample of 20 stable-isotope-labeled (SIL) peptides under general reversed-phase mobile phase conditions and MS/MS detection using a SCIEX® 5500 QTRAP®. This application highlights the differences in chromatographic performance and recovery when different combinations of micro LC column and trap

selectivity are combined in trap-and-elute mode. Adjusting the column and trap selectivity combination is a useful method development tool for the optimization of your micro LC separation.

In Figure 2, the MicroTrap Polar in combination with the Kinetex XB-C18 column delivers

the highest peak area recovery for this example. The specific combination and differences between the column and trap's media can result in difference in relative recovery, as seen in Figures 3 – 4. The relative difference in recovery illustrates the importance of optimizing micro LC column and trap selectivity combinations and demonstrates how using a different selectivity combinations can yield improved recoveries depending upon the chemical characteristics of your compounds of interest. Figure 3 illustrates recovery improvements that can be obtained by varying the selectivity between the column and trap. Generally, unlike guard columns, a media difference between the two separation devices can be advantageous.

Figures 5 – 7 compare the trap performance of MicroTrap C18, MicroTrap Polar, and MicroTrap PS in combination with the Kinetex® 2.6 µm XB-C18 and under the same chromatographic conditions.

Figure 5. Area Recovery for Kinetex XB-C18

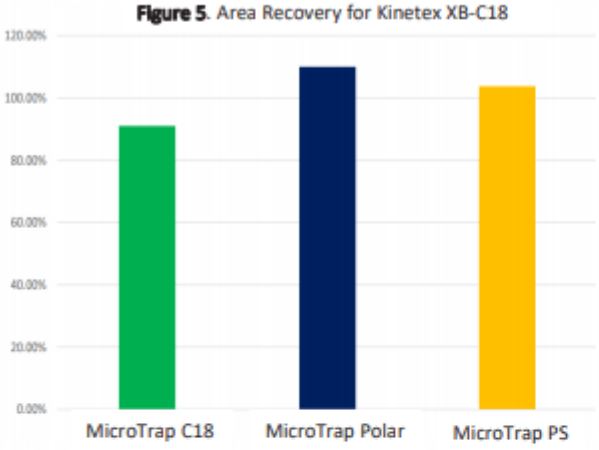


Figure 6. Height Recovery for Kinetex XB-C18

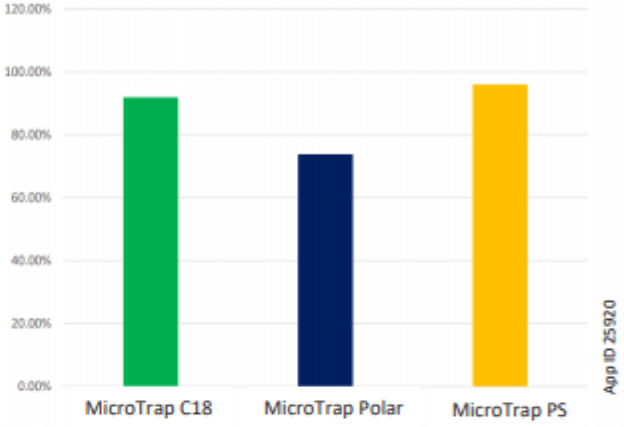
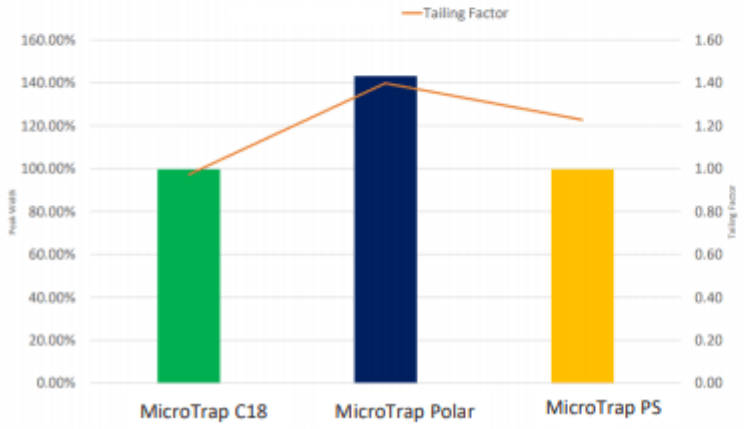


Figure 7. Peak Width & Tailing for Kinetex XB-C18



Similarly to analytical scale LC, the performance and optimization of your separation is affected by the chosen stationary phase chemistry. The specific combination of column and trap media can result indifferences in relative recovery and separation performance as shown Figures 5 – 6. Figure 5, the combination of the MicroTrap Polar and Kinetex XB-C18 yielded the highest overall average peak area. However, the MicroTrap PS and Kinetex XB-C18 combination provided the best peak shape and recovery performance for this given separation as seen in Figure 7. This application demonstrates the performance differences

between different column and trap selectivity configurations and the impact on optimization of your micro LC separation performance.

To explore the original full Technical Application, click here or the image below.

APPLICATIONS

Comparison of Three Unique and Complementary Micro LC Columns and Three Trap Selectivities Under Reversed Phase LC-MS/MS Conditions

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Overview

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Kinetex 2.6 µm XB-C18: A C18 that is modified with protective iso-butyl side chains for improved analysis of polar compounds.

Luna Omega PS C18: The surface contains a positive charged ligand which improves peak shape for basic compounds, while the C18 ligand promotes general reversed phase hydrophobic retention.

Luna Omega Polar C18: The C18 ligand provides general hydrophobic interactions while a polar modified particle surface provides enhanced polar compound retention

Micro LC Trap Phases & Dimension

MicroTrap C18: 10 x 0.3 mm

MicroTrap Polar: 10 x 0.3 mm

MicroTrap PS: 10 x 0.3 mm

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> Technical Tip – How to Improve Your Micro LC Column's Performance

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