

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

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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.

Figure 2. Area Recovery for Kinetex® XB-C18

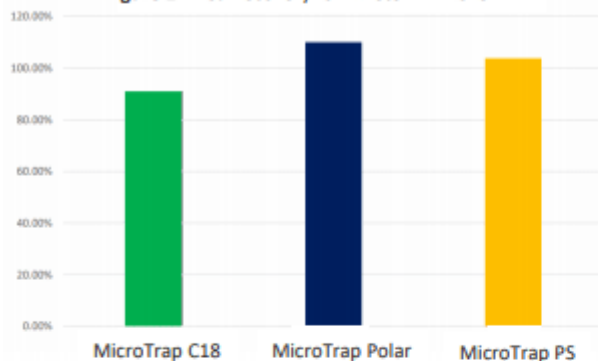


Figure 3. Area Recovery for Luna® Omega PS C18

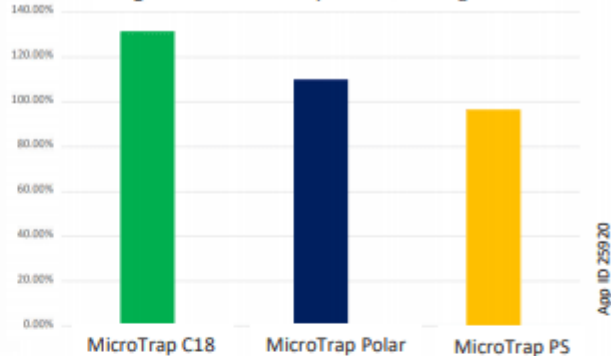
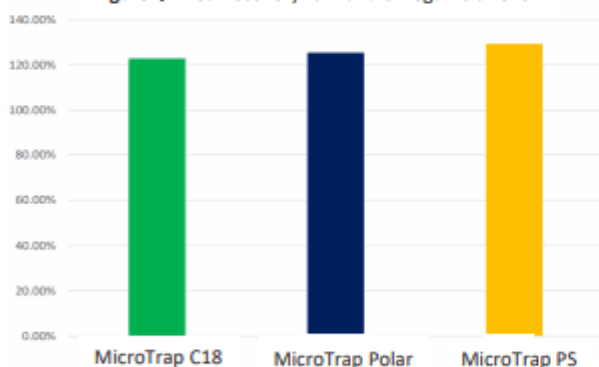


Figure 4. Area Recovery for Luna Omega Polar C18



Micro LC Conditions

Column: Kinetex 2.6 µm XB-C18 ([00B-4496-AC](#))
 Luna Omega 3 µm PS C18 ([00B-4758-AC](#))
 Luna Omega 3 µm Polar C18 ([00B-4760-AC](#))
Trap: MicroTrap C18 ([05N-4252-AC](#))
 MicroTrap Polar ([05N-4754-AC](#))
 MicroTrap PS ([05N-4753-AC](#))

Dimension: 50 x 0.3 mm + 10 x 0.3 mm

Mobile Phase: A: Water with 0.1 % Formic Acid
 B: Acetonitrile with 0.1 % Formic Acid

| Gradient: | Time (min) | % B |
|-----------|------------|-----|
| | 0 | 3 |
| | 10 | 40 |
| | 12 | 80 |
| | 14 | 80 |
| | 15 | 3 |
| | 20 | 3 |

Flow Rate: 10 µL/min

Temperature: Ambient (25 °C)

Detector: MS/MS SCIEX® QTRAP® 5500

Injector Temp.: 4 °C

Column Temp.: 25 °C

Injection Volume: 1 µL

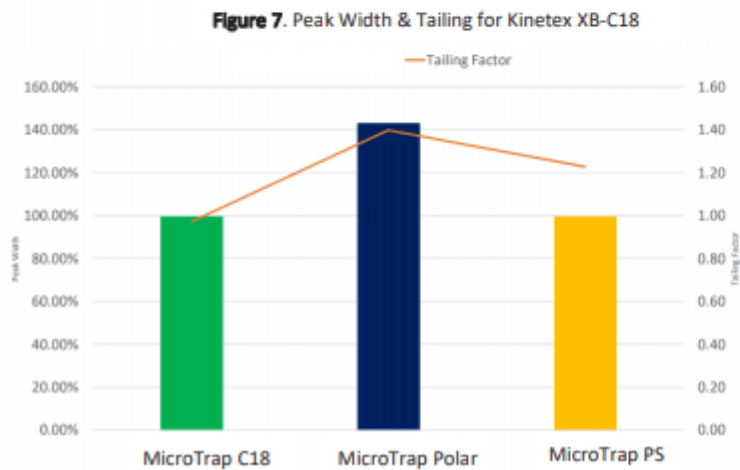
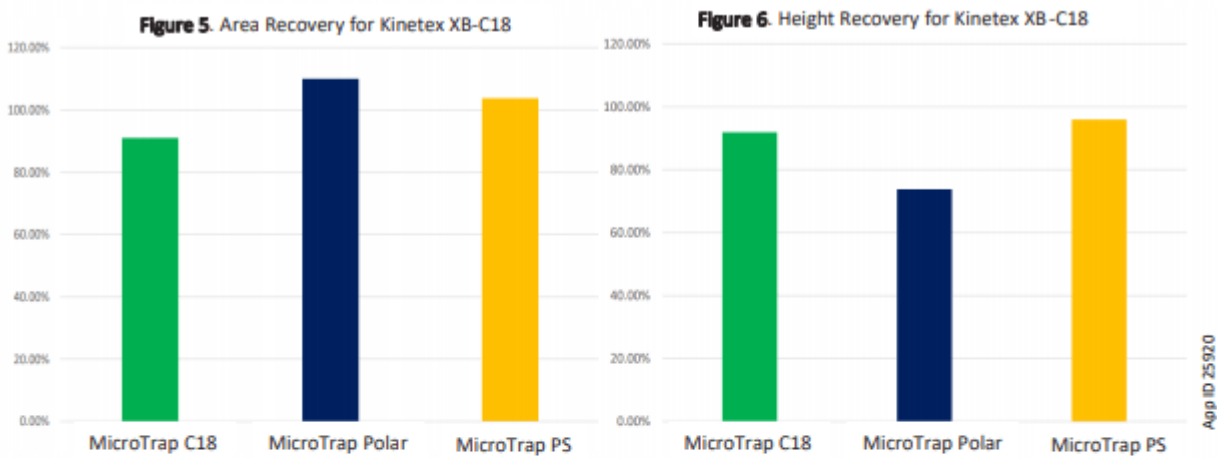
Sample: 20 stable-isotope-labeled (SIL) Peptide mix

Figures 2 – 4 show the average peptide peak area recovery when using a Kinetex XB-C18, a Luna Omega PS C18 or a Luna Omega Polar C18 column in line with a C18, Polar or a PS MicroTrap.

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.



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 in differences in relative recovery and separation performance as shown in 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

Roxana Eggleston-Rangel, Ryan Splitstone, and Jason Anspach
Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA

Overview

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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.

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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