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

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 application, 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 combinationsof 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

Micro LC Conditions

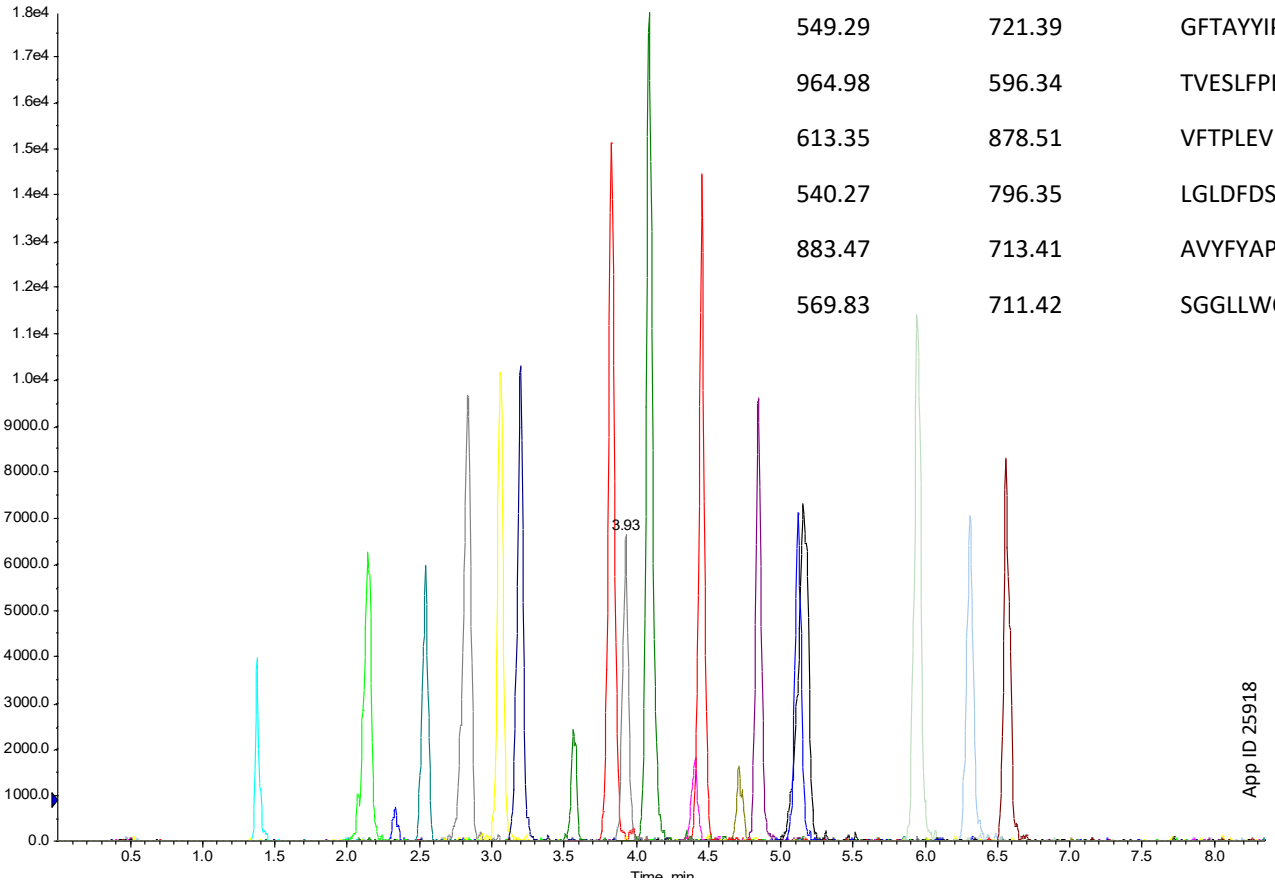
Column: Luna® Omega 3 µm Polar C18
Dimension: 50 x 0.3 mm
Part No.: 00B-4760-AC
Mobile Phase: A: Water with 0.1 % Formic Acid
B: Acetonitrile with 0.1 % Formic Acid

Gradient:

Time (min)	% B
0	3
10	40
12	80
14	80
15	3
20	3

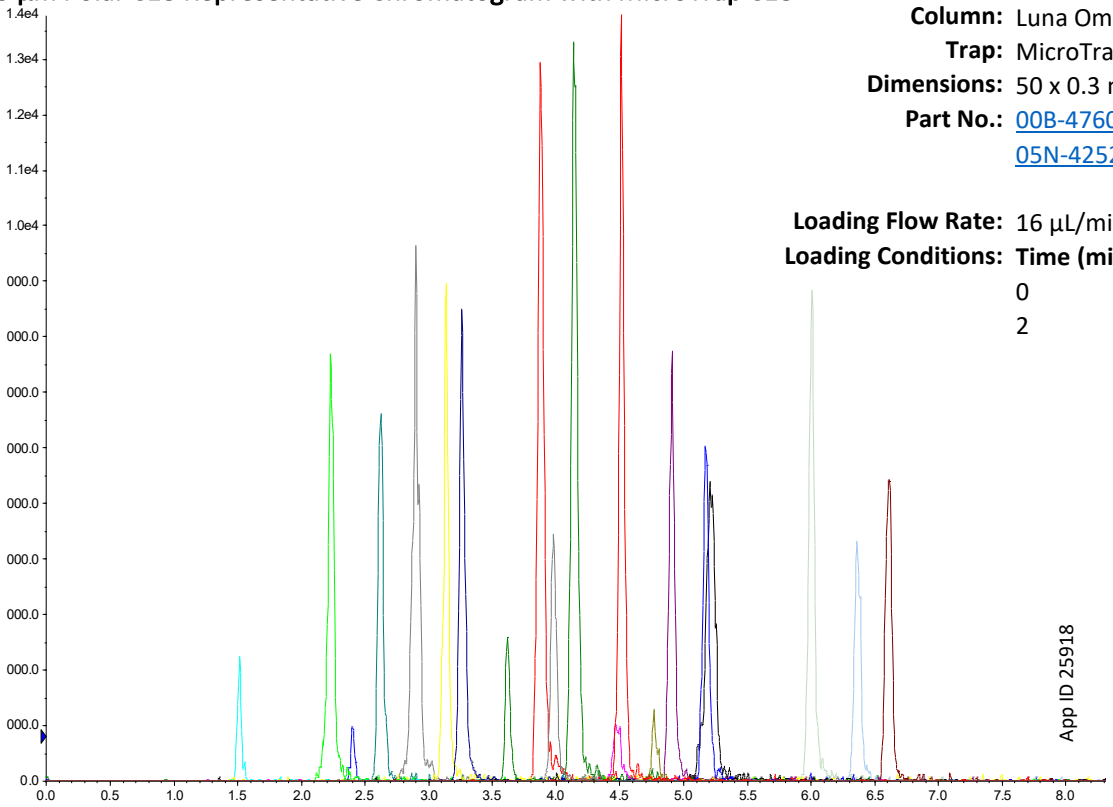
Flow Rate: 10 µL/min
Temperature: Ambient (25 °C)
Detector: MS/MS SCIEX® QTRAP® 5500
Injector Temp.: 4 °C
Column Temp.: 25 °C
Injection Volume: 1 µL
Sample: 20 stable-isotope-labeled (SIL) Peptide mix

Direct Injection on Luna Omega 3 µm Polar C18
Representative Chromatogram without Any Micro LC Trap



Precursor Mz	Product Mz	Peptide Sequence
485.25	856.41	IGNEQGVSR
491.27	769.37	LVGTPAEER
408.55	593.35	AETSELHTSLK
473.26	555.32	GAYVEVTAK
583.31	753.41	AVGANPEQLTR
519.8	422.29	LDSTSIPVAK
593.8	729.38	SAEGLDASASLR
739.36	999.51	YDSINNTEVSGIR
533.32	711.41	AGLIVAEGVTK
657.34	724.37	YIELAPGVDNSK
768.9	725.39	ALENDIGVPSDATVK
636.35	759.44	VGNEIQYVALR
677.86	649.41	DGTFAVDGPGVIAK
758.91	957.51	SPYVITGPGVVEYK
549.29	721.39	GFTAYYIPR
964.98	596.34	TVESLFPEEAETPGSAVR
613.35	878.51	VFTPLEVDVAK
540.27	796.35	LGLDFDSFR
883.47	713.41	AVYFYAPQIPLYANK
569.83	711.42	SGGLLWQLVR

Luna® Omega 3 µm Polar C18 Representative Chromatogram with MicroTrap C18



Column: Luna Omega 3 µm Polar C18
Trap: MicroTrap C18
Dimensions: 50 x 0.3 mm + 10 x 0.3 mm
Part No.: 00B-4760-AC
05N-4252-AC

Loading Flow Rate: 16 µL/min
Loading Conditions:

Time (min)	A%
0	100
2	100

Micro LC Column and Trap Selectivity Configuration Comparison Summary

Figure 1 shows the average peak height (blue), width (orange) and tailing (gray) of a Luna Omega Polar C18 column in line with a C18, Polar, or PS MicroTrap. LC conditions are the same as described above.

Figure 1. Luna Omega Polar Column with MicroTrap C18, Polar or PS Comparison

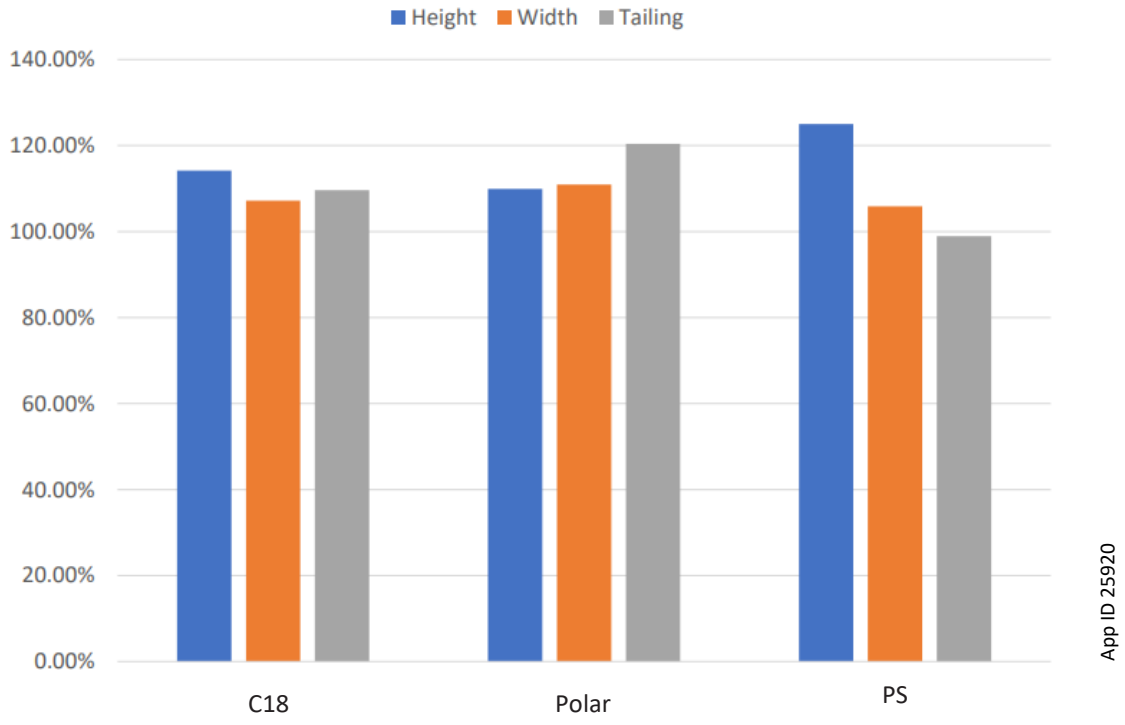


Figure 2. Area Recovery for Kinetex® XB-C18

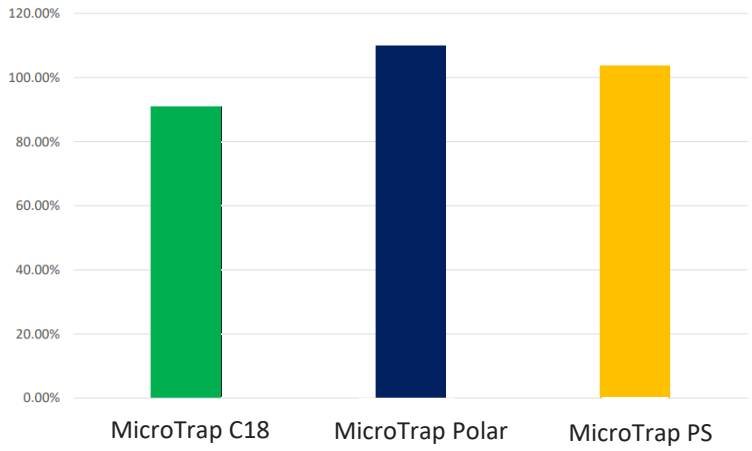


Figure 3. Area Recovery for Luna® Omega PS C18

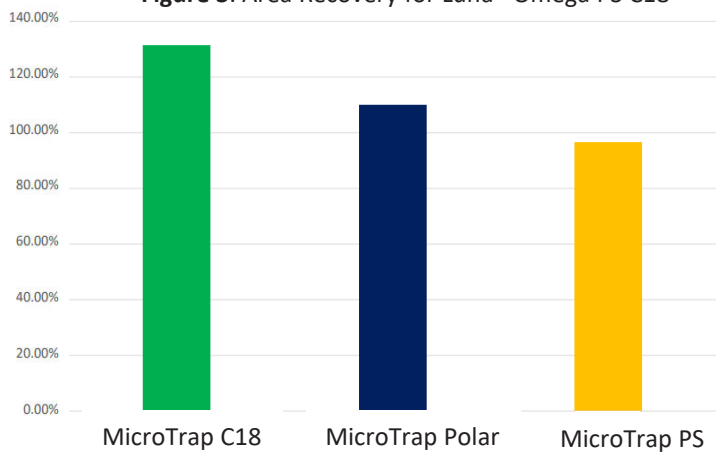
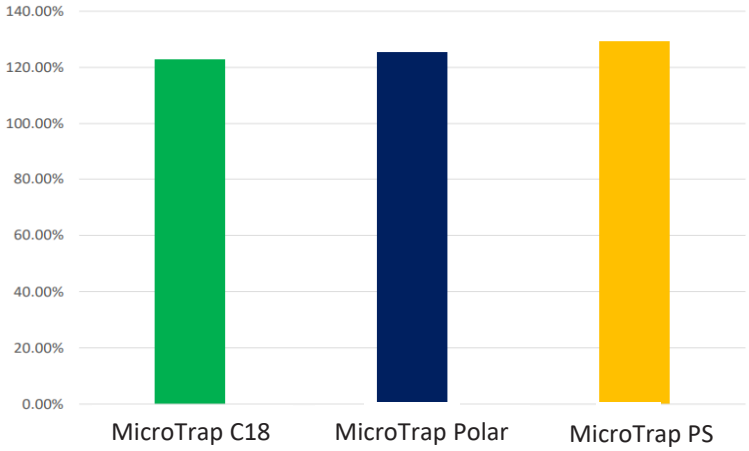


Figure 4. Area Recovery for Luna Omega Polar C18



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.

App ID 25920

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

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 differences in relative recovery, as seen in **Figures 3 – 4**. The relative difference in recovery illustrates the importance ofoptimizing micro LC column and trap selectivity combinations and demonstrates how using a different selectivitycombinations can yield improved recoveries depending upon the chemical characteristics of your compounds ofinterest. Figure **3** illustrates recovery improvements that can be obtained by varying the selectivity between thecolumn and trap. Generally, unlike guard columns, a media difference between the two separation devices can beadvantageous.

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

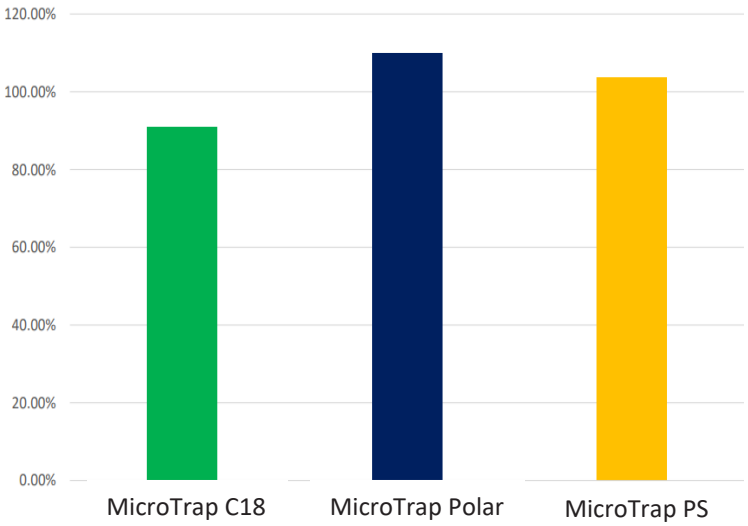


Figure 6. Height Recovery for Kinetex XB-C18

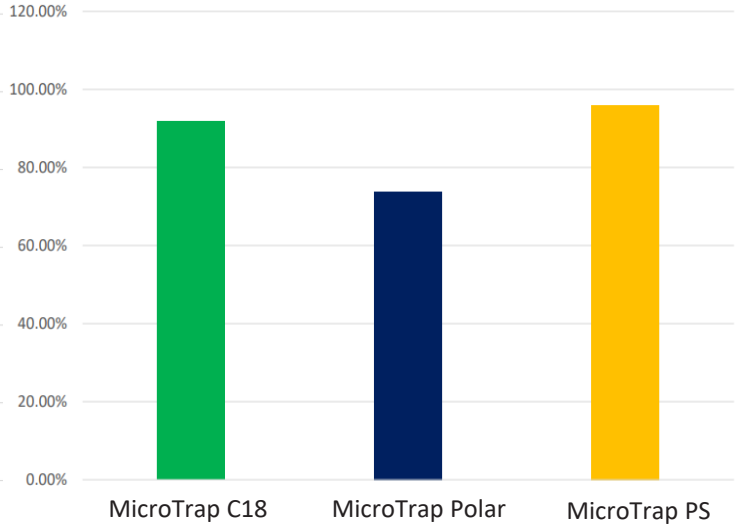


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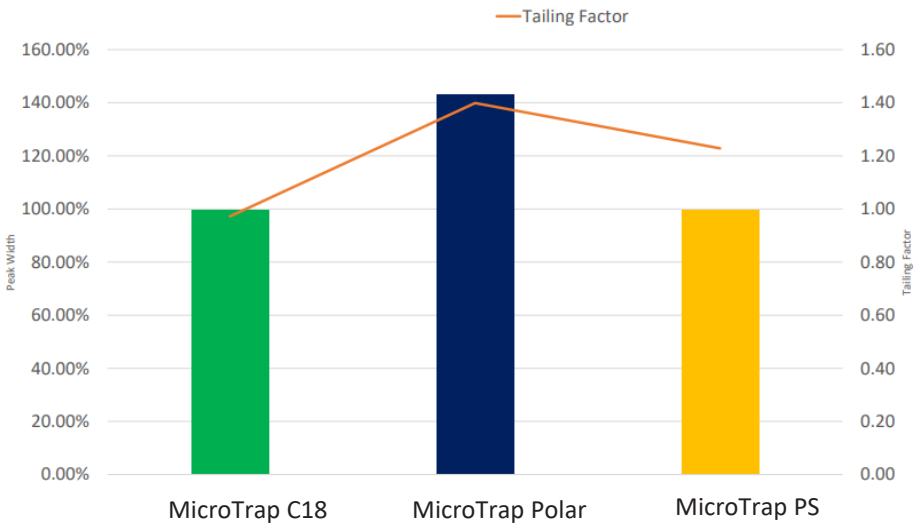
Figure 7. Peak Width & Tailing for Kinetex XB-C18



Conclusion

In conclusion, 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 micro LC column and trap selectivity configurations and the impact on optimization of your micro LC separation performance.

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