

# 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 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

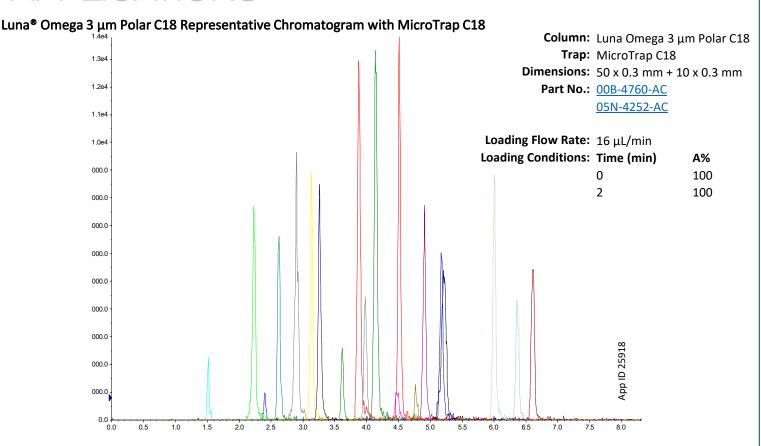
MicroTrap PS: 10 x 0.3 mm





Column: Luna* Omega 3 μm Polar C18   485.25   856.41   IGNEQGVSR	Micr	o LC Conditio	ns						Precurso	or Mz	Product M	Z	Peptide Sequence
Part No.: 008-4750-AC   491.27   769.37   LYGTPAEER					m Polar C1	8			485.25		856.41		IGNEQGVSR
Mobile Phase: A: Water with 0.1 % Formic Acid B: Acetonitrile with 0.1 % Formic Acid B: Acetonitrili with 0.1 % Formic Acid B: Aceto									404.27		760.27		
B: Acetonitrile with 0.1 % Formic Acid  Gradlent: Time (min) % 8 473.26 555.32 GAYVEVTAK 0 3 555.32 GAYVEVTAK 10 40 583.31 753.41 AVGANPEQLITR 12 80 519.8 422.29 LDSTSIPVAK 14 80 519.8 729.38 SAEGLDASASLR 20 3 739.36 999.51 VDSINNTEVSGIR  Flow Rate: 10 μL/min  Temperature: Ambient (25 °C) 533.32 711.41 AGLIVAEGVTK  Detector: MS/MS SCIEX* QTRAP* 5500 657.34 724.37 YIELAPGVDNSK Injector Temp:: 4 °C Column Temp:: 25 °C Injector Journer 1 μL Sample: 20 stable-isotope-labeled (SIL) Peptide mix 636.35 759.44 VGNEIQYVALR  Direct Injection on Luna Omega 3 μm Polar C18 677.86 649.41 DGTFAVDGPGVIAK  Representative Chromatogram without Any Micro LC Trap 758.91 957.51 SPYVITGPGVVEYK 1.644 964.98 596.34 TVESLFPEEAETPGSAVR 1.644 964.98 596.98 596.34 TVESLFPEEAETPGSAVR 1.644 964.98 596.98 596.98 596.98 596.98 596.98 596.98 596.98 596.98 596.98 596.98 596.98 596.98 596.98 596.98								491.27		769.37		LVGTPAEEK	
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Flow Rate: 10 μL/min   Temperature: Ambient (25°C)   533.32   711.41   AGLINAEGVTK   Detector: MS/MS SCIEX® QTRAP® 5500   657.34   724.37   YIELAPGVDNSK   Injector Temp: 4 °C   768.9   725.39   ALENDIGVPSDATVK   Injection Volume: 1 μL   Sample: 20 stable-isotope-labeled (SIL) Peptide mix   636.35   759.44   VGNEIQYVALR													
Detector: MS/MS SCIEX® QTRAP® 5500   657.34   724.37   YIELAPGVDNSK     Injector Temp.: 4 °C   768.9   725.39   ALENDIGVPSDATVK     Injection Volume: 1 μL   Sample: 20 stable-isotope-labeled (SIL) Peptide mix   636.35   759.44   VGNEIQYVALR     Direct Injection on Luna Omega 3 μm Polar C18   677.86   649.41   DGTFAVDGPGVIAK     Representative Chromatogram without Any Micro LC Trap   758.91   957.51   SPYVITGPGVVEYK     1.844   1.644   964.98   596.34   TVESLFPEEAETPGSAVR     1.594   1.644   540.27   796.35   LGLDFDSFR     1.344   1.644   1.644   540.27   796.35   LGLDFDSFR     1.344   1.644   540.27   796.35   LGLDFDSFR     1.344   1.644   569.83   711.42   SGGLLWQLVR     1.644   1.		Flow Rate	: 10 μL/n	nin					/39.36		999.51		YDSINNTEVSGIR
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## 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

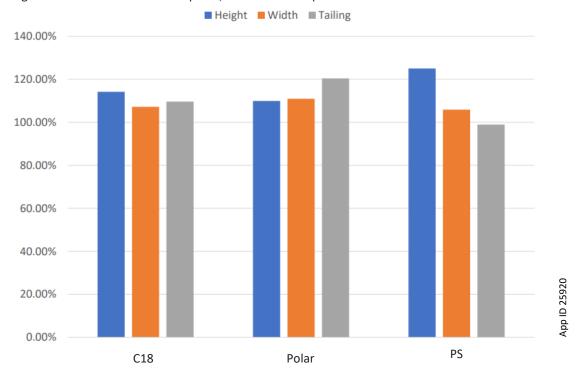




Figure 3. Area Recovery for Luna® Omega PS C18

## APPLICATIONS

Figure 2. Area Recovery for Kinetex® XB-C18

100.00%

80.00%

40.00%

MicroTrap C18 MicroTrap Polar MicroTrap PS

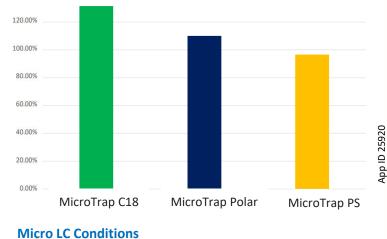
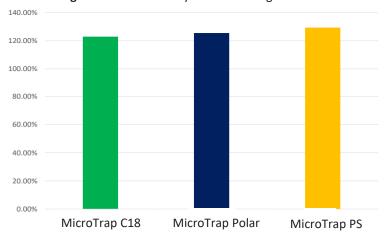


Figure 4. Area Recovery for Luna Omega Polar C18



**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.

**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:**  $50 \times 0.3 \text{ mm} + 10 \times 0.3 \text{ mm}$ 

Mobile Phase: A: Water with 0.1 % Formic Acid

B: Acetonitrile with 0.1 % Formic Acid

Gradient:	Time (min)	% В
	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.:  $4\,^{\circ}\text{C}$ Column Temp.:  $25\,^{\circ}\text{C}$ Injection Volume:  $1\,\mu\text{L}$ 

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 the column and trap. Generally, unlike guard columns, a media difference between the two separation devices can beadvantageous.

140.00%





**Figures 5 – 7** compare the trap performance of MicroTrap C18, MicroTrap Polar, and MicroTrap PS in combination with the Kinetex $^{\circ}$  2.6  $\mu$ m XB-C18 and under the same chromatographic conditions.

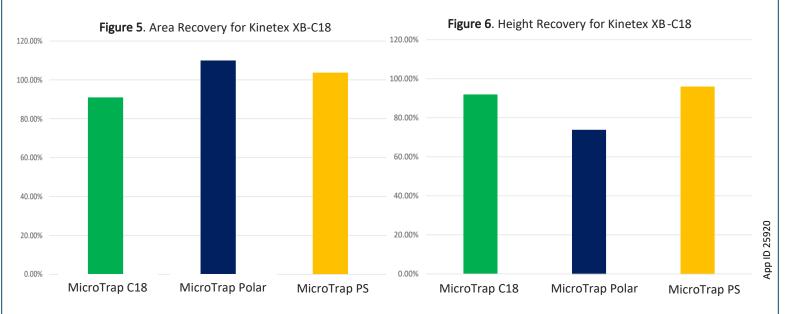
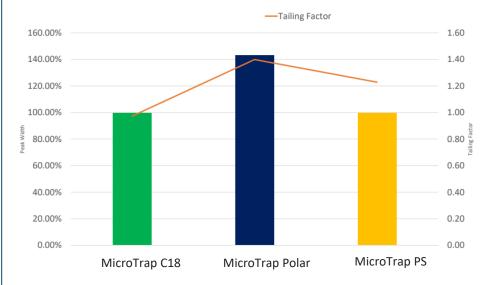


Figure 7. Peak Width & Tailing for Kinetex XB-C18



## **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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## PPLICATIONS

## Need a different column size or sample preparation format?

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