Positively Charged Surface Chemistry for Biological Separations

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Overview

- The advantages of a positively charged surface column with Fused-Core® technology
- Improvement of peak shape for basic compounds using MS-friendly mobile phases for both small molecule and peptide analysis. Why is using low ionic strength mobile phase desirable?
- How a positively charged surface column chemistry benefits LC & LCMS separations including loading capacity improvement gains.
- Applications of the PCS Peptide phase and comparisons

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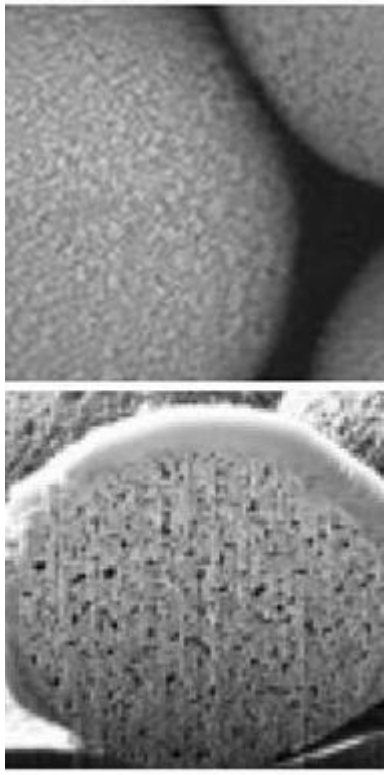
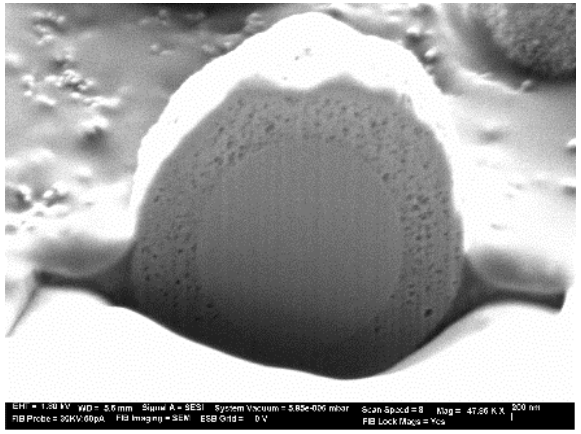
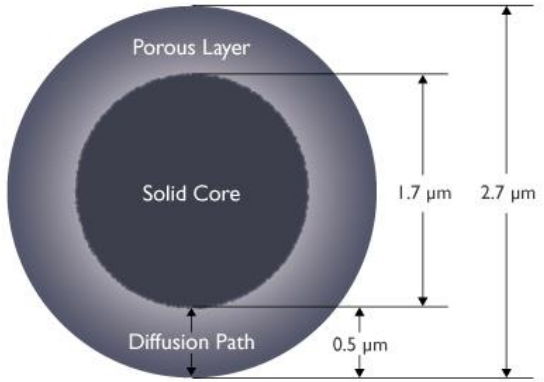
Fused-Core® Technology



Fully Porous Particle (FPP)

HALO 90 Å, 2.7 µm





Superficially Porous Particle (SPP)

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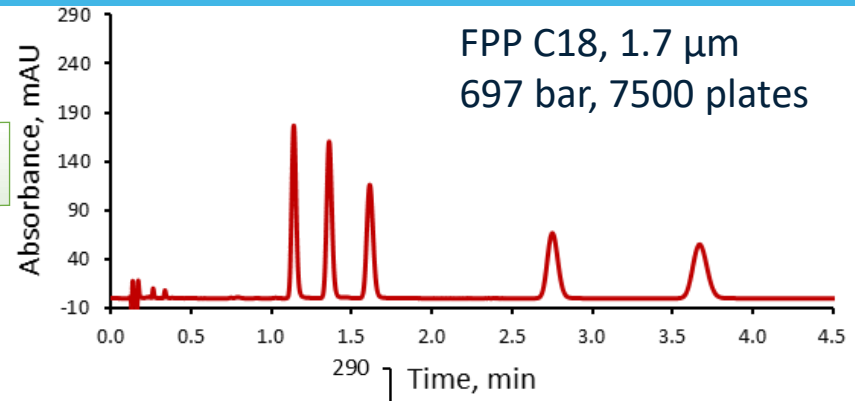
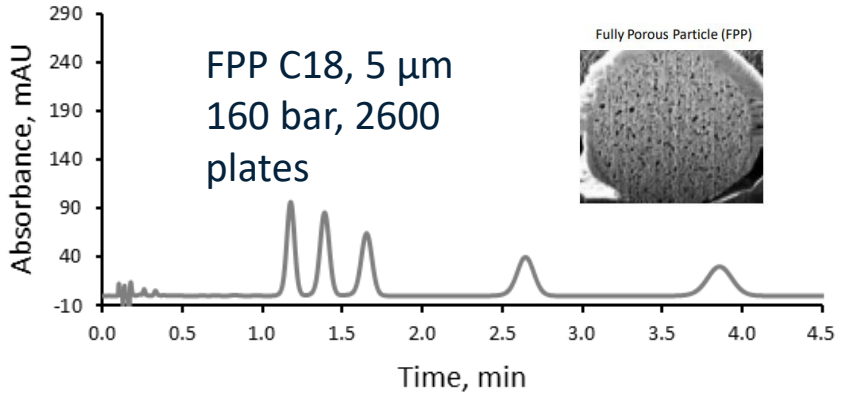
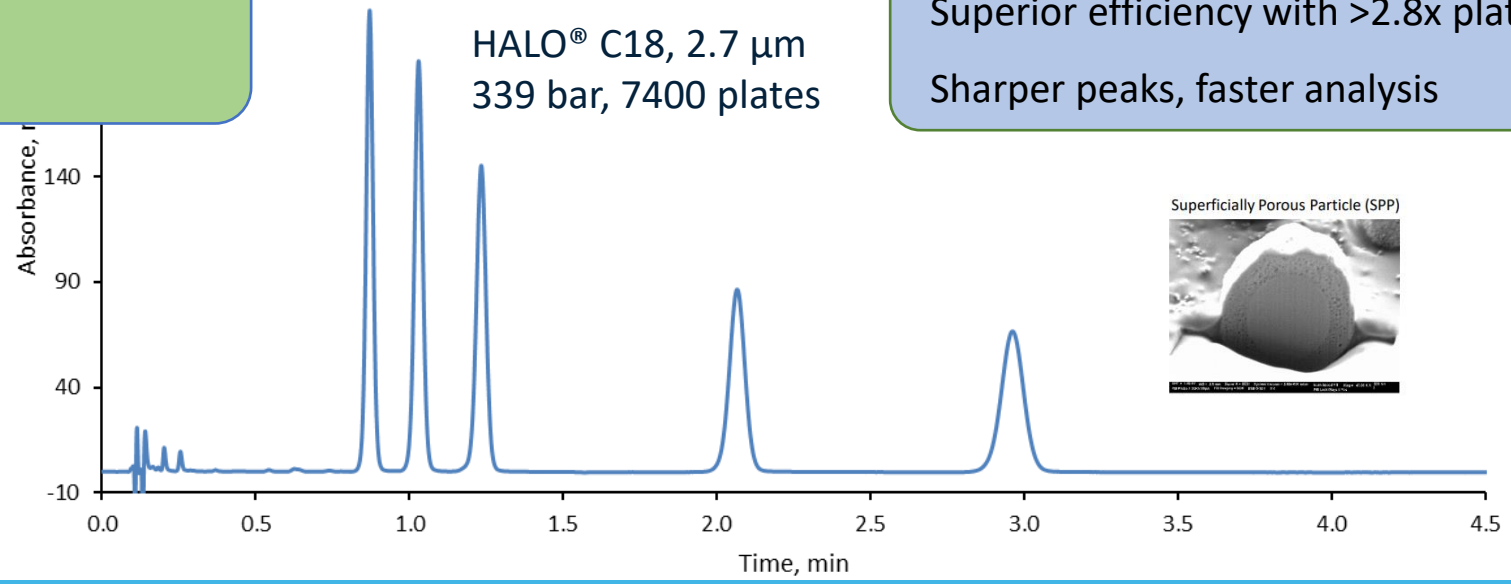
Power of Fused-Core® Technology





High performance with <1/2 the back pressure

Faster analysis



Superior efficiency with >2.8x plates!

Sharper peaks, faster analysis

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Positively Charged Surface Stationary Phase

When do we need a Positively Charged Stationary Phase?

- When running low ionic strength mobile phase with formic acid for LC and LC-MS applications for
  - Basic compounds
  - Peptides
  - Protein digests

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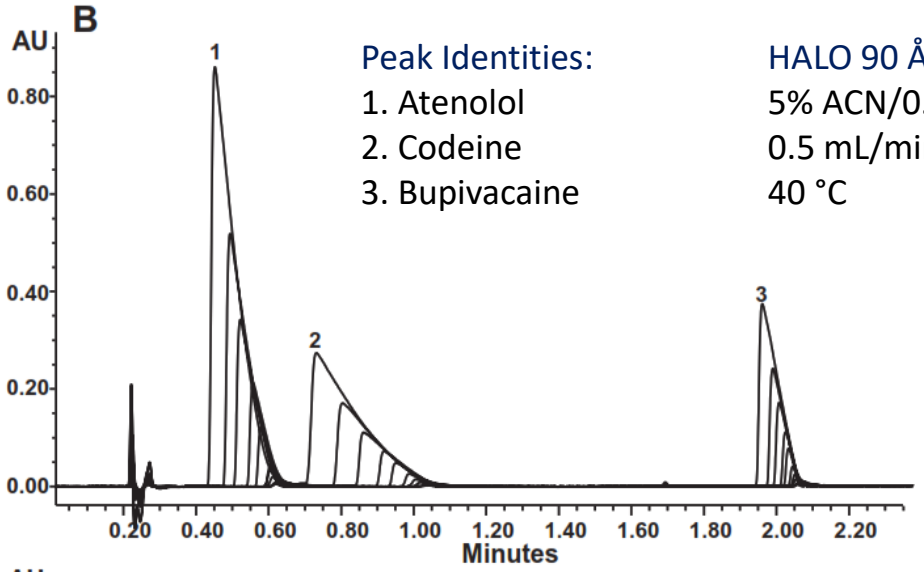
Why do we need a Positively Charged Stationary Phase?

- When basic compounds are run at low pH, they gain a proton and become positively charged.
- At low sample loads, the tailing will be symmetrical using formic acid containing mobile phases.
- At high sample loads, the tailing will become significant and the peak shape will suffer.



Peak Identities:
1. Atenolol
2. Codeine
3. Bupivacaine

HALO 90 Å C18, 2.7 µm, 2.1 x 50 mm
5% ACN/0.1% formic acid for 1 min, then 5-95% ACN/0.1% formic acid in 3 min
0.5 mL/min; 230 nm
40 °C

J. Chromatogr. A 1228 (2012) 221-231

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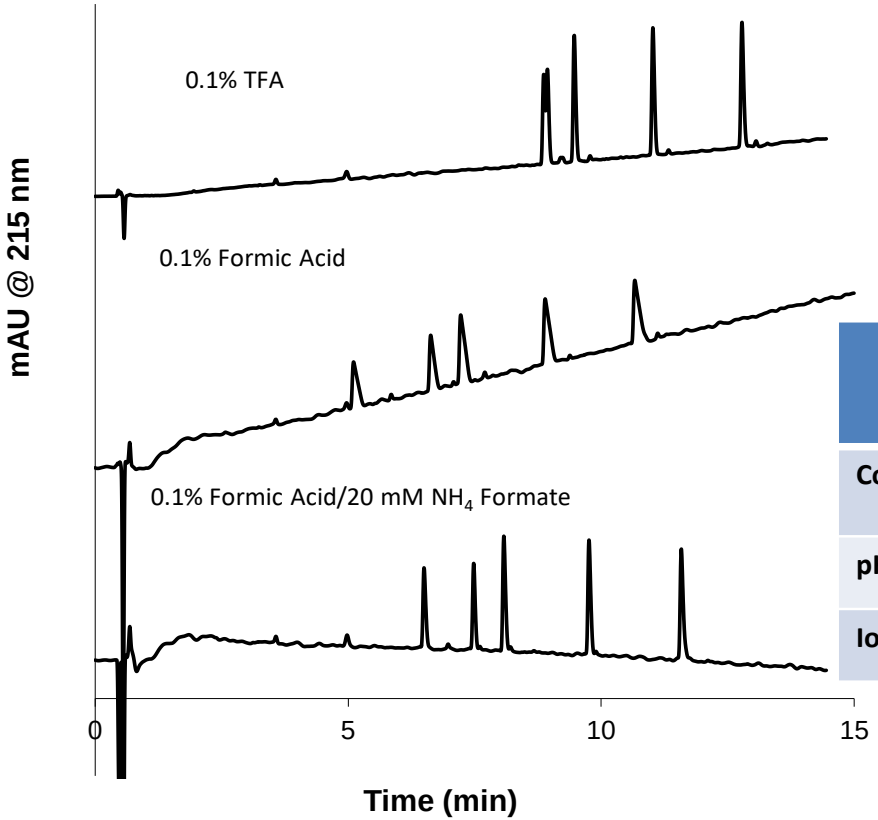
Peptide Separations in Acidic MP

Improving Retention and Peak Shape Using Ammonium Formate



Column: Halo Peptide ES-C18, 4.6 x 100 mm; Flow rate: 2.0 mL/min; T= 30 C;
A: Water/acid modifier; B: ACN/0.1% TFA or Formic Acid
Gradient: 1.5% to 26% B in 15 min.; Injection: 8 µL (800 ng) of synthetic peptides S1-S5

Synthetic Peptide Retention Standards:

S1 RGAGGLGLGK-Am
S2 Ac-RGGGGLGLGK-Am
S3 Ac-RGAGGLGLGK-Am
S4 Ac-RGVGGLGLGK-Am
S5 Ac-RGVVGLGLGK-Am

	TFA 0.1%	Formic Acid 0.1%	Ammonium Formate	AF/FA
Concentration	**0.013 M**	**0.026 M**	**0.020 M**	**20 mM AF 26 mM FA**
pH	**1.8**	**2.7**	**c. 7**	**3.3**
Ionic strength	**26 mM**	**4.4 mM**	**40 mM**	**c. 44 mM**

McCalley, D. V., Effect of buffer on peak shape of peptides in reversed-phase high performance liquid chromatography. *J Chromatogr* **2004,** *1038* (1-2), 77-84.
Schuster, S. A.; Boyes, B. E.; Wagner, B. M.; Kirkland, J. J., Fast high performance liquid chromatography separations for proteomic applications using Fused-Core® silica particles. *J Chromatogr* **2012,** 1228, 232-241.

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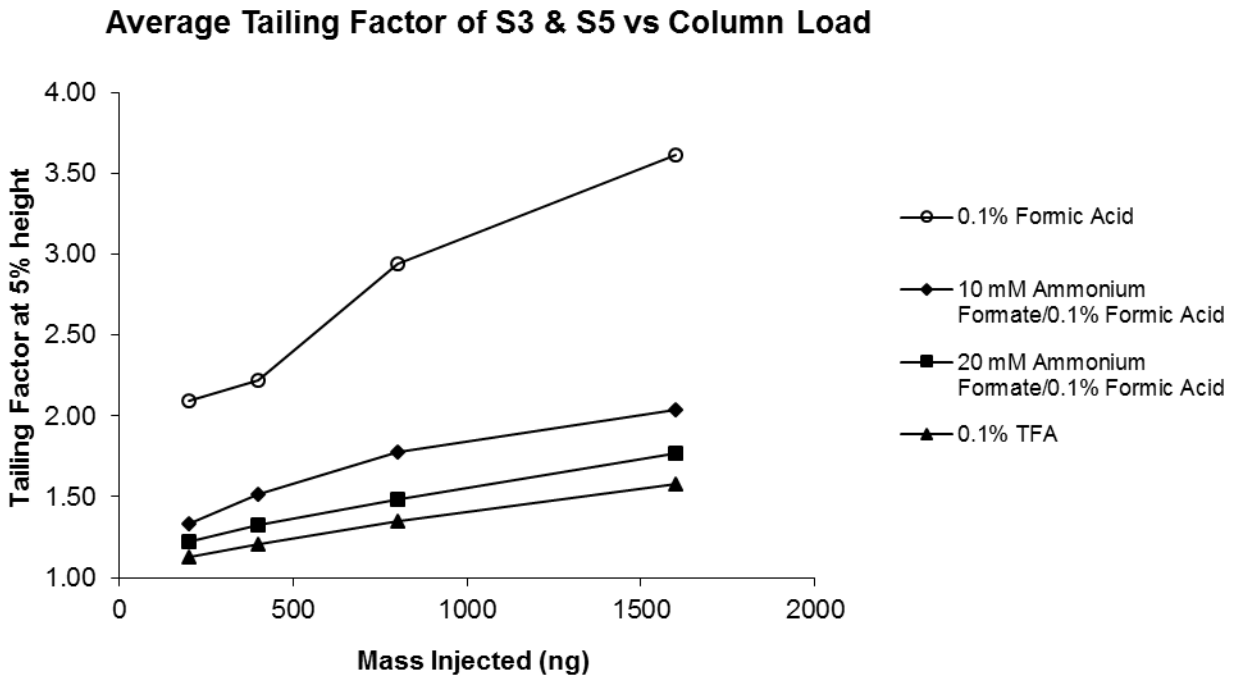
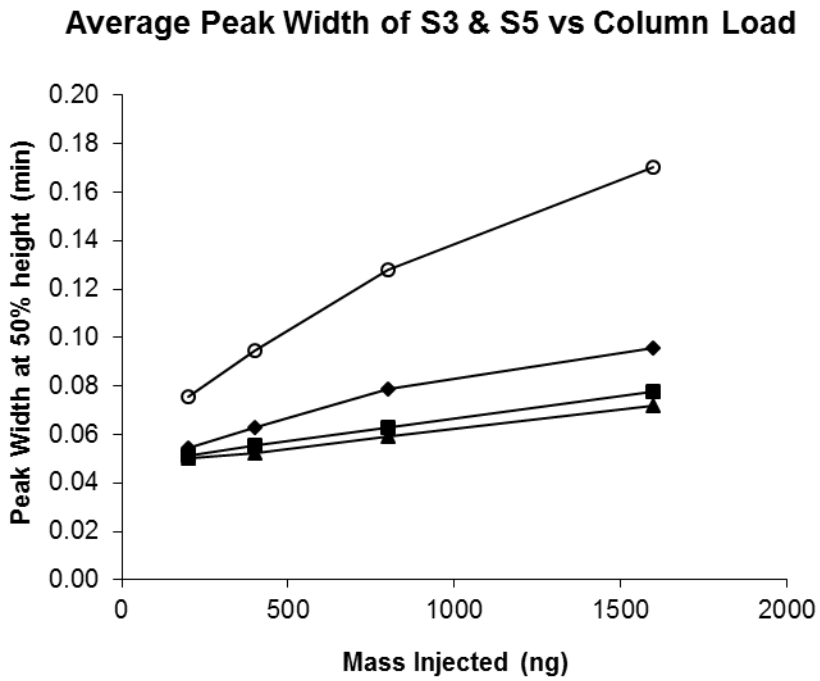
Load Effects for Peptides Comparing Acids





Reference: Johnson, D.J., Boyes, B.E., Orlando, R.C. The Use of Ammonium Formate as a Mobile-Phase Modifier for LC-MS/MS Analysis of Tryptic Digests. **2013** *J. Biomol.Tech.*, 24, 187-197.

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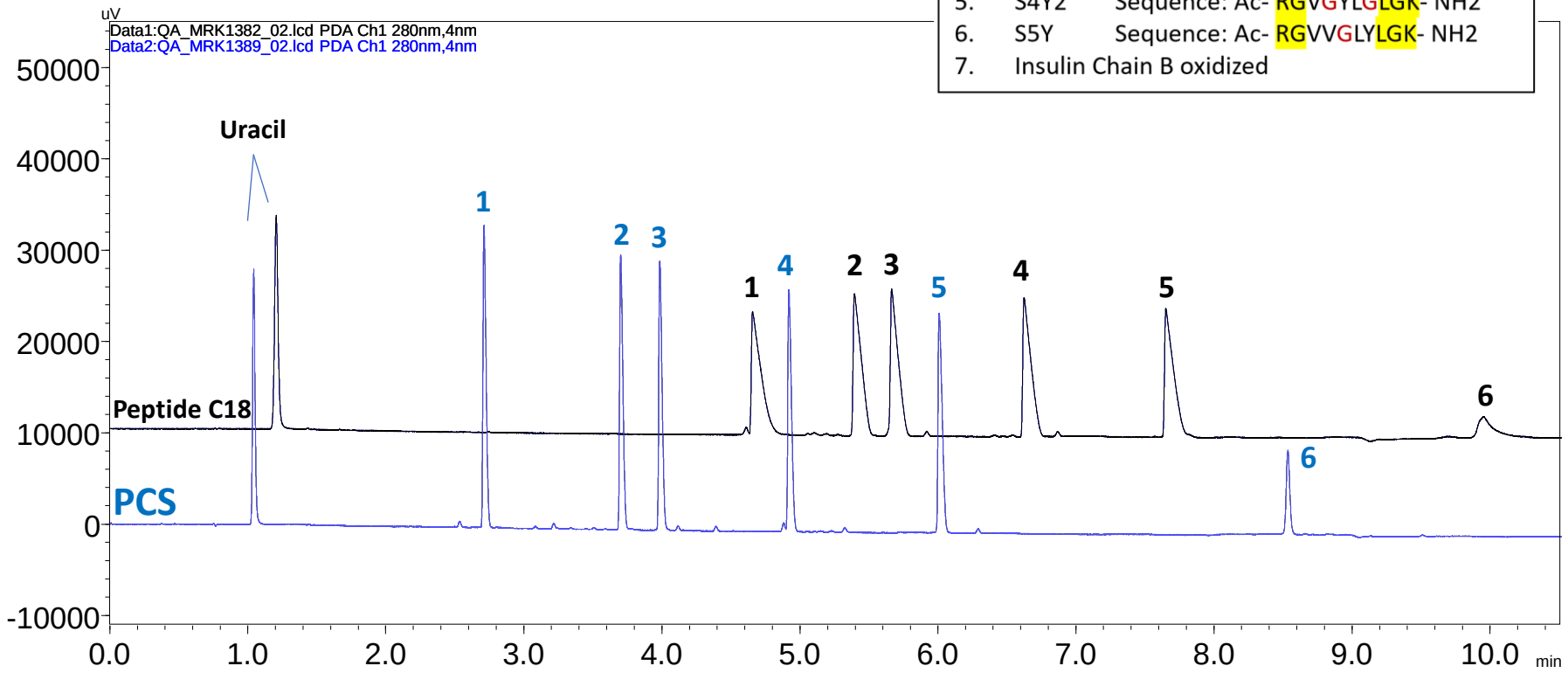
Example of HALO® PCS C18 Peptide

4.6x100mm, 1.50mL/min, 280nm, 5.0µL inj.
Gradient Separation, 0-35%B in 10min
Mobile Phase A = H_2O + 0.1% formic acid
Mobile Phase B = ACN + 0.1% formic acid

Peptide QA **4.6x100mm 2.7µm**

1. Uracil
2. S1Y Sequence: RGAGGLYLGK-NH2
3. S2Y Sequence: Ac- RGGGGLYLGK- NH2
4. S3Y Sequence: Ac- RGAGGLYLGK- NH2
5. S4Y2 Sequence: Ac- RGVGYLGLGK- NH2
6. S5Y Sequence: Ac- RGVVGLYLGK- NH2
7. Insulin Chain B oxidized



- Gradient separation of 6 peptides
- Decrease in retention time for PCS C18
- Improved peak widths and reduced tailing

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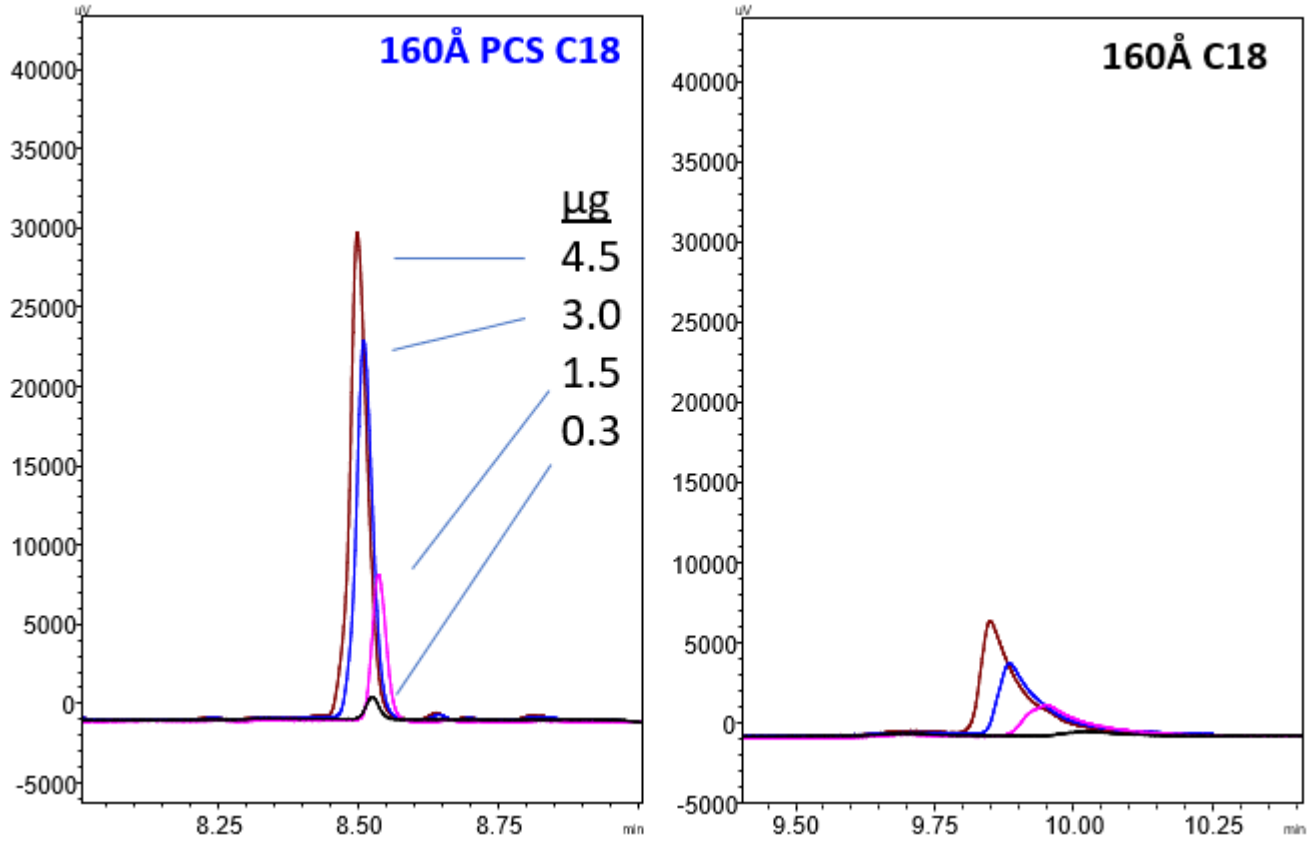
Loading Capacity Improvement Gains

Peptide Load Tolerance

1, 5, 10, and 15µL injections of synthetic peptides (0.3µg/µL peptides) on 4.6x100mm

Insulin Chain B; 3496 Da



1.5mL/min, 0-35%B in 10min, 30°C; 280nm A=0.1%Formic; B=0.1%Formic in ACN

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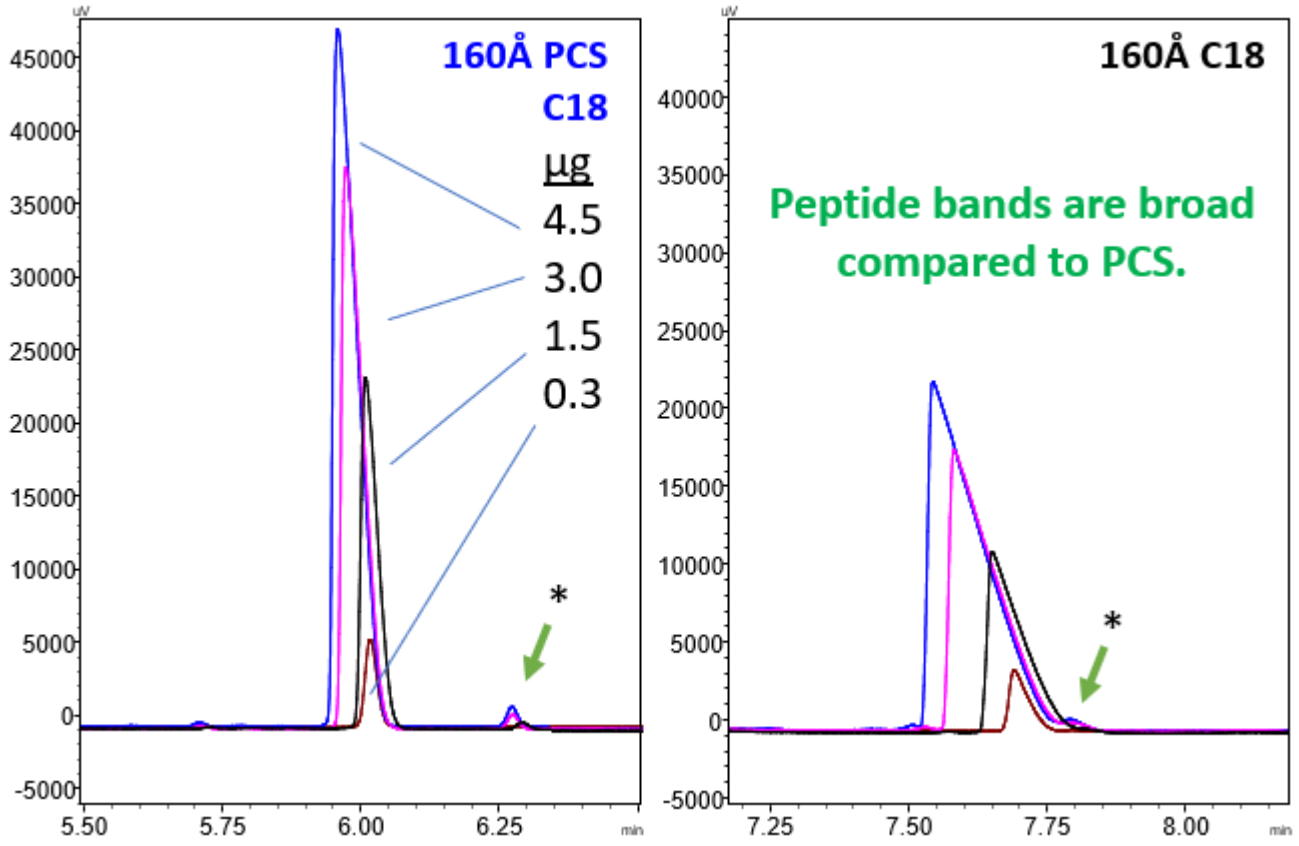
Peptide Load Tolerance (2)

1, 5, 10, and 15µL injections of synthetic peptides (0.3µg/µL peptides) on 4.6x100mm

Ac-RGVVGLYLGK-NH2 (1102 Da)



1.5mL/min, 0-35%B in 10min, 30°C; 280nm A=0.1%Formic; B=0.1%Formic in ACN

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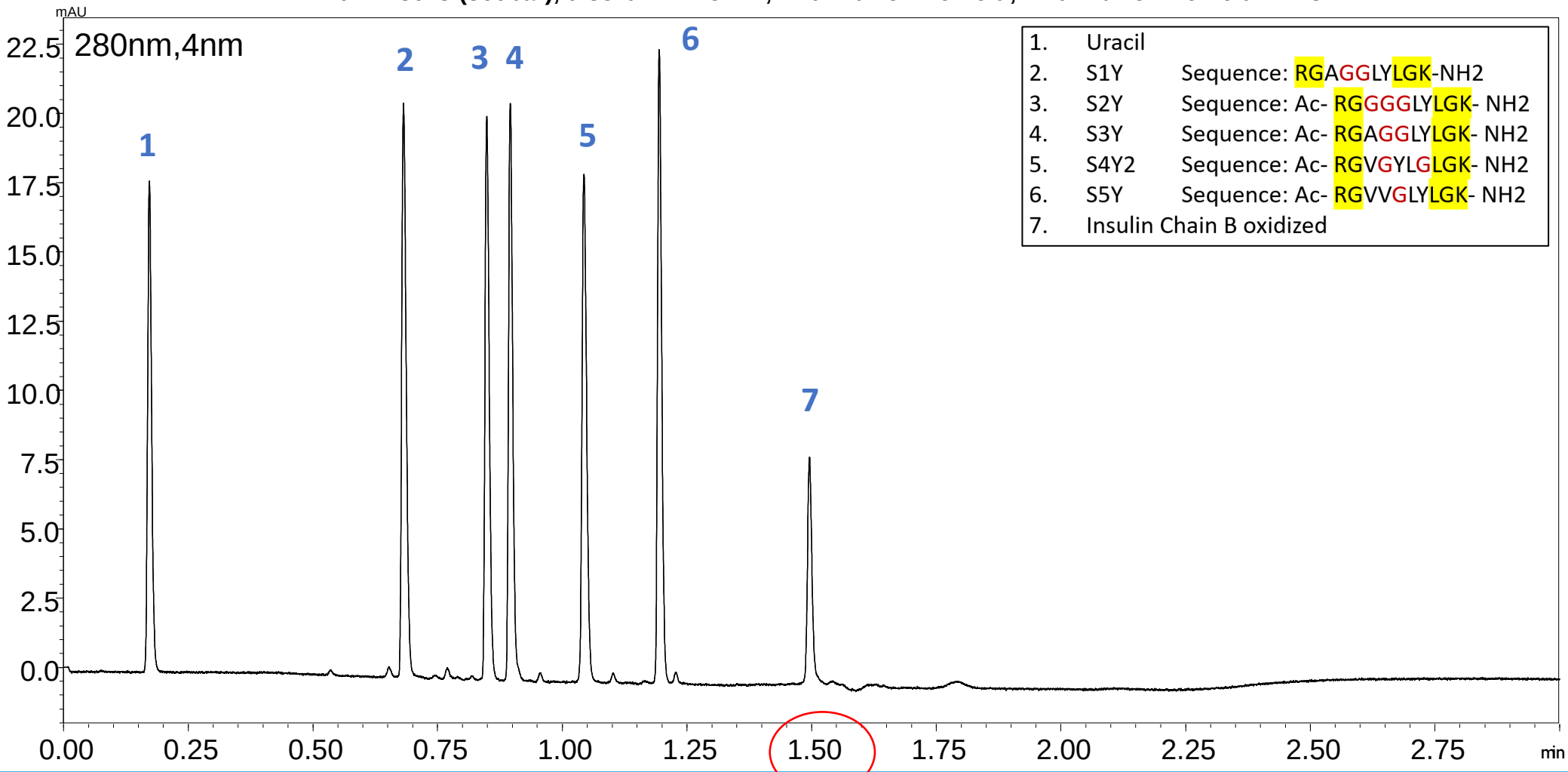
Applications of the PCS C18 Peptide Phase

HALO® PCS C18 Peptide: Rapid Separation

1µL of Synthetic Peptide Standard (0.3µg/µL) PCS 160Å 2.1x50mm 2.7µm

1mL/min 30°C (360bar), 0-35%B in 1.5min; A=0.1% Formic Acid; B=0.1% Formic Acid in ACN



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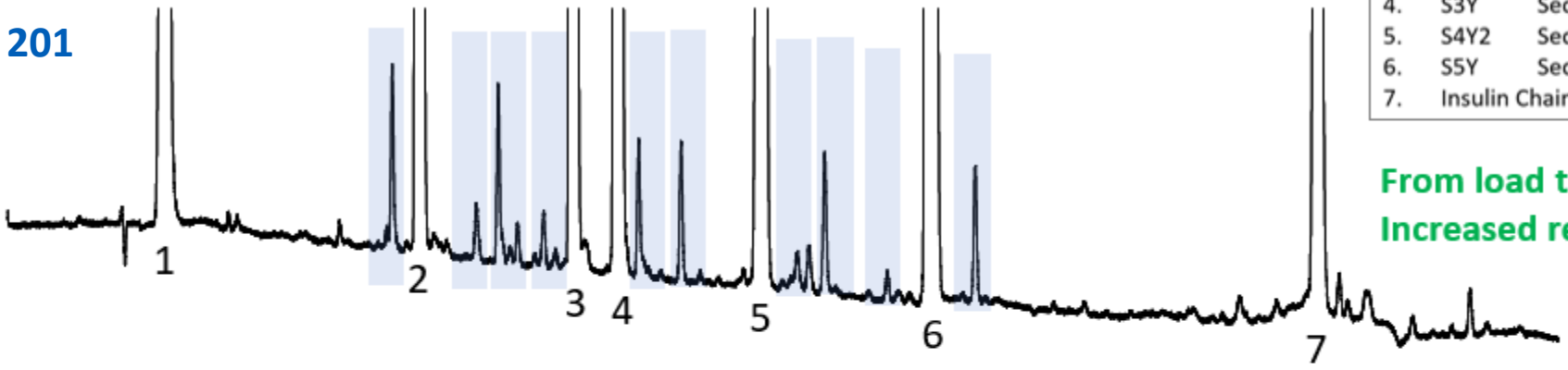
Low Abundance Peptide Analysis

15µL injection of Synthetic Peptide Standard (0.3µg/µL)
1.5mL/min, 0-35%B in 10min, 30°C; 280nm A=0.1%Formic; B=0.1%Formic in ACN

1. Uracil
2. S1Y Sequence: RGAGGLYLGK-NH2
3. S2Y Sequence: Ac- RGGGGLYLGK- NH2
4. S3Y Sequence: Ac- RGAGGLYLGK- NH2
5. S4Y2 Sequence: Ac- RGVGYLGLGK- NH2
6. S5Y Sequence: Ac- RGVVGLYLGK- NH2
7. Insulin Chain B oxidized

Peak Capacity: 201

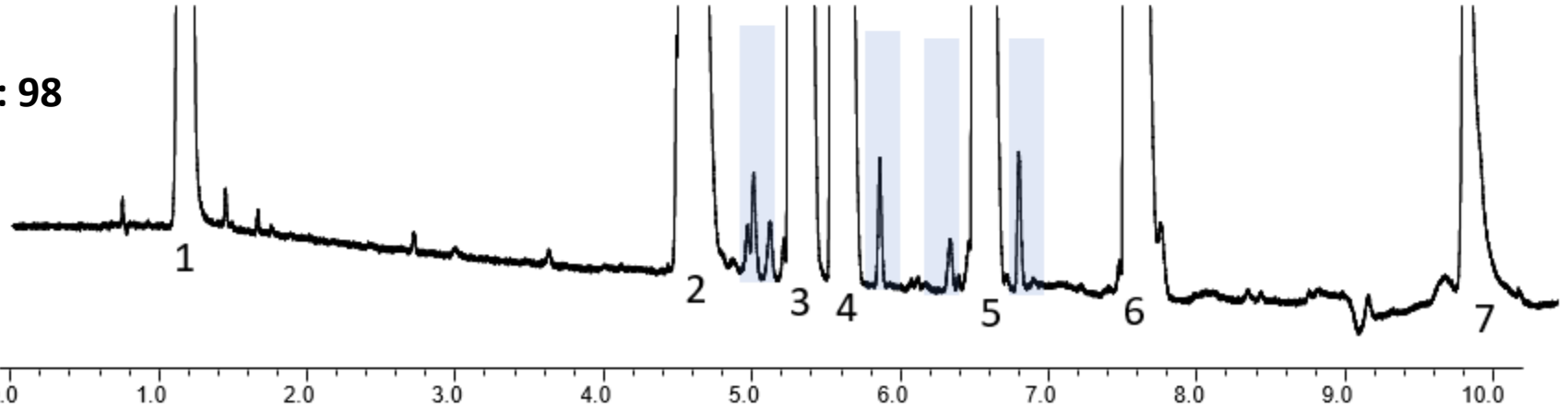
160Å PCS C18



From load tolerance advantage..
Increased resolution on PCS

Peak Capacity: 98

160Å C18



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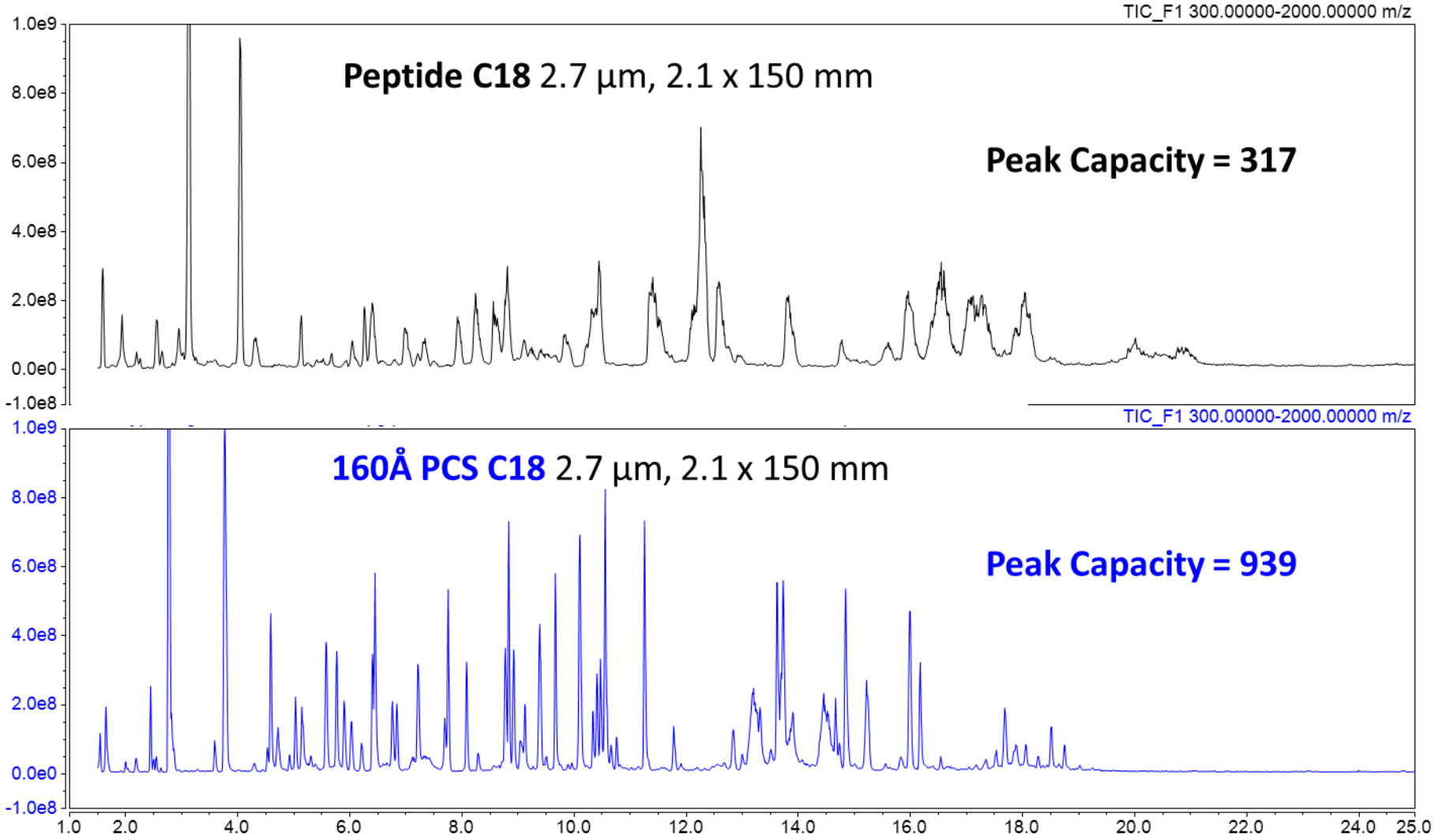
Trastuzumab Tryptic Digest: Higher Peak Capacity with 160 Å PCS C18



0.4mL/min; 60°C; 3-50% in 30 min;
A=0.1% Formic Acid in H_2O
B=0.1% Formic Acid in ACN
Shimadzu NexeraX2 -> divert valve ->
QExactive HF (res=240,000)

MarvelXACT

post-column plumbing: Peptide C18 and PCS C18

1) 50µmx350mm from column to divert
2) 50µmx350mm from divert valve to union
3) 50µmx150mm from grounding union to HESI II

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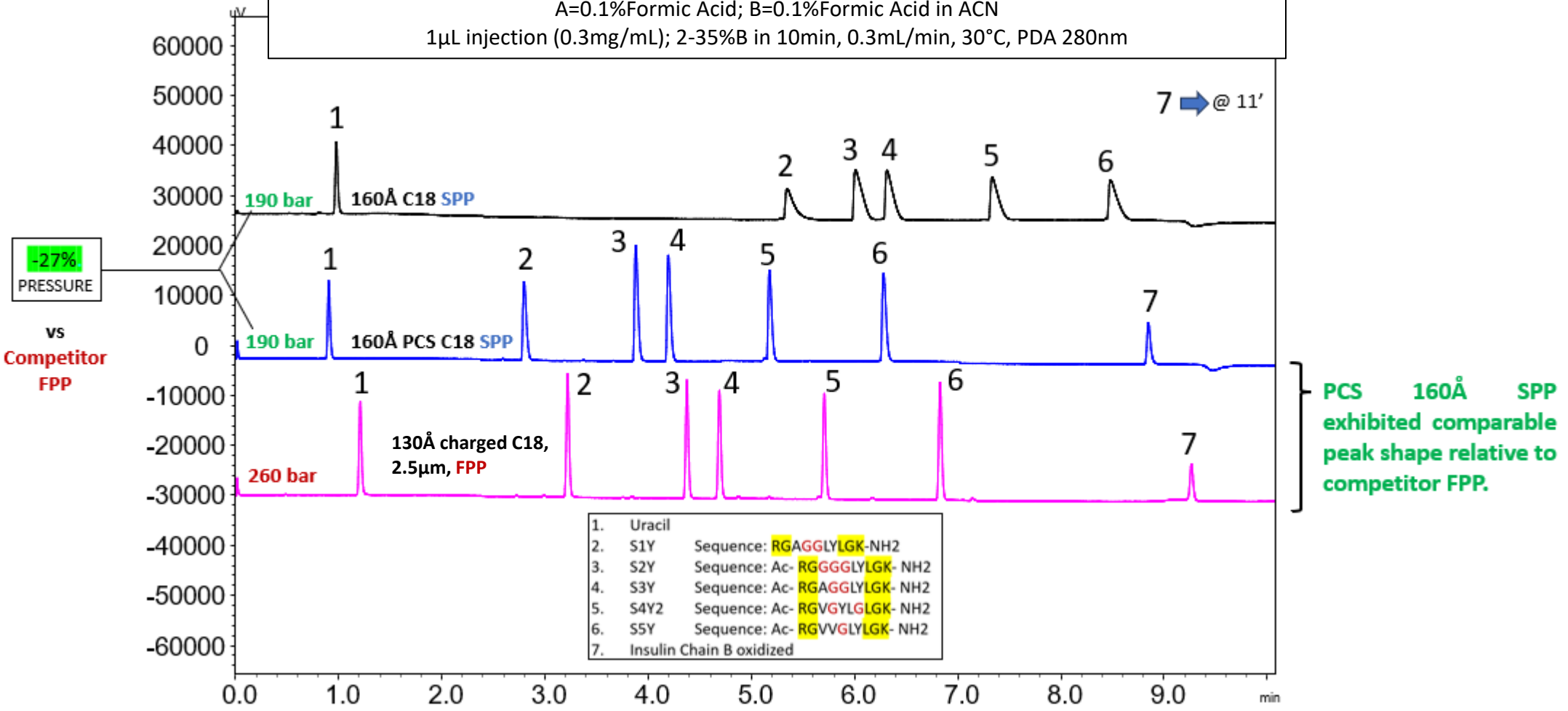
HALO 160Å PCS vs Competitor

Peptide Standard Analysis on 160Å PCS C18 2.1x100mm 2.7µm

A=0.1%Formic Acid; B=0.1%Formic Acid in ACN

1µL injection (0.3mg/mL); 2-35%B in 10min, 0.3mL/min, 30°C, PDA 280nm



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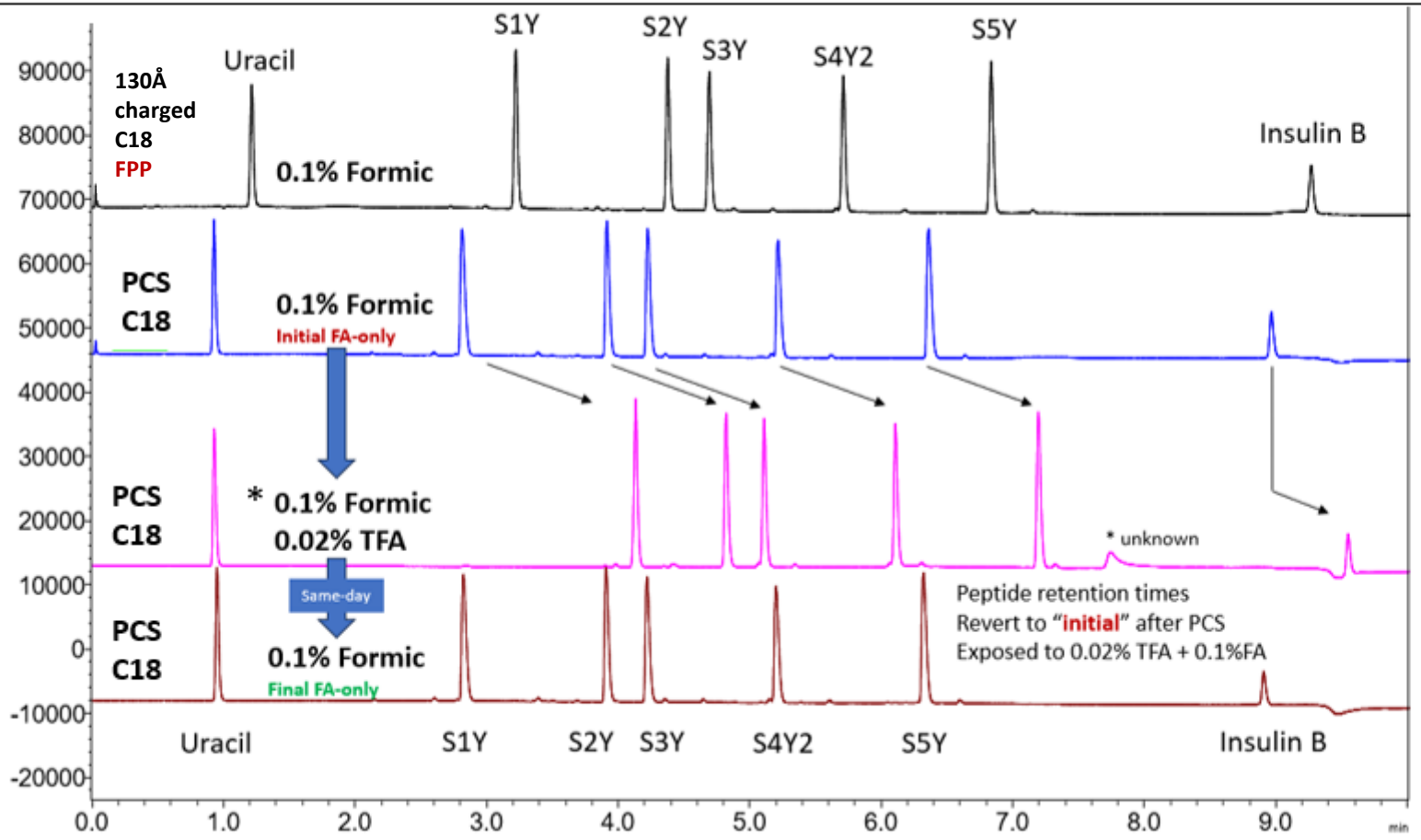
Using PCS with Trifluoracetic Acid

160Å PCS C18 2.1x100mm 2.7µm SPP versus Competitor 130Å 2.5µm FPP



1µl injection of peptide standard (0.3mg/mL), Gradient 2-35%B in 10min at 0.3mL/min 30°C, 280nm on NexeraX2

A=H2O + acid modifier(s)
B=ACN + acid modifier(s)

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Summary

- HALO® PCS C18 exhibits favorable peak shape for various small molecule bases/peptides in weakly acidic mobile phase (0.1% Formic).
- Improved peak shape vs traditional C18
- Improved load tolerance vs traditional C18
- Improved ionization efficiencies when using formic acid
- PCS C18 can be used with other acids such as TFA or DFA

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