# Sharper Peaks and Higher Sensitivity: Advancing Positively Charged Surface Stationary Phase Technology with 2 µm Particles

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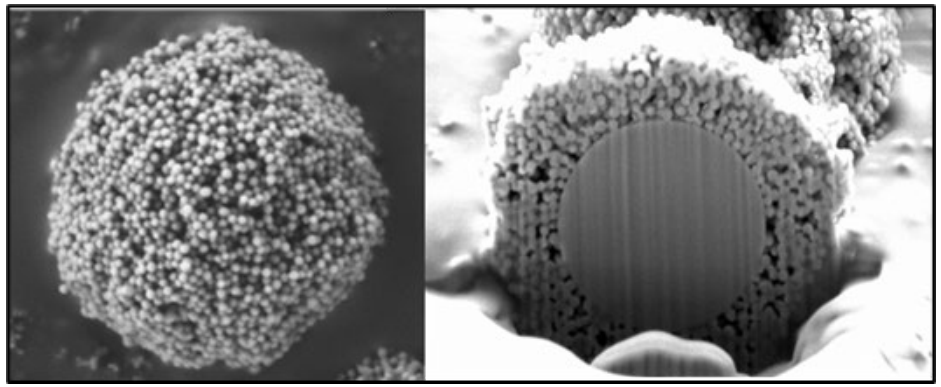
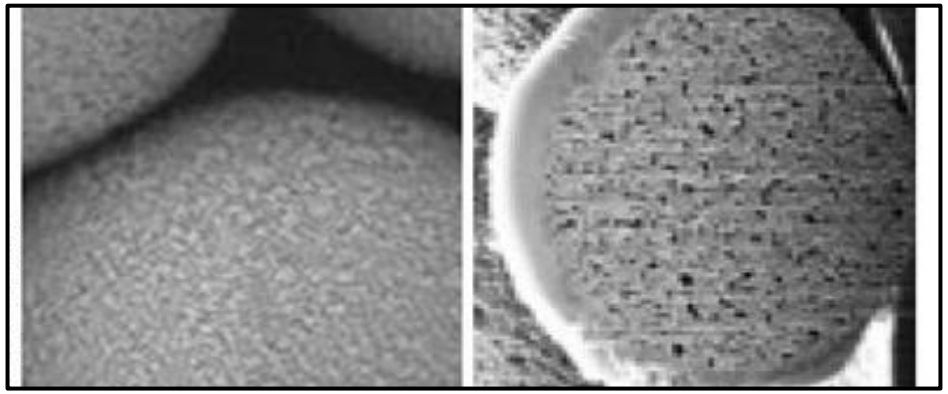
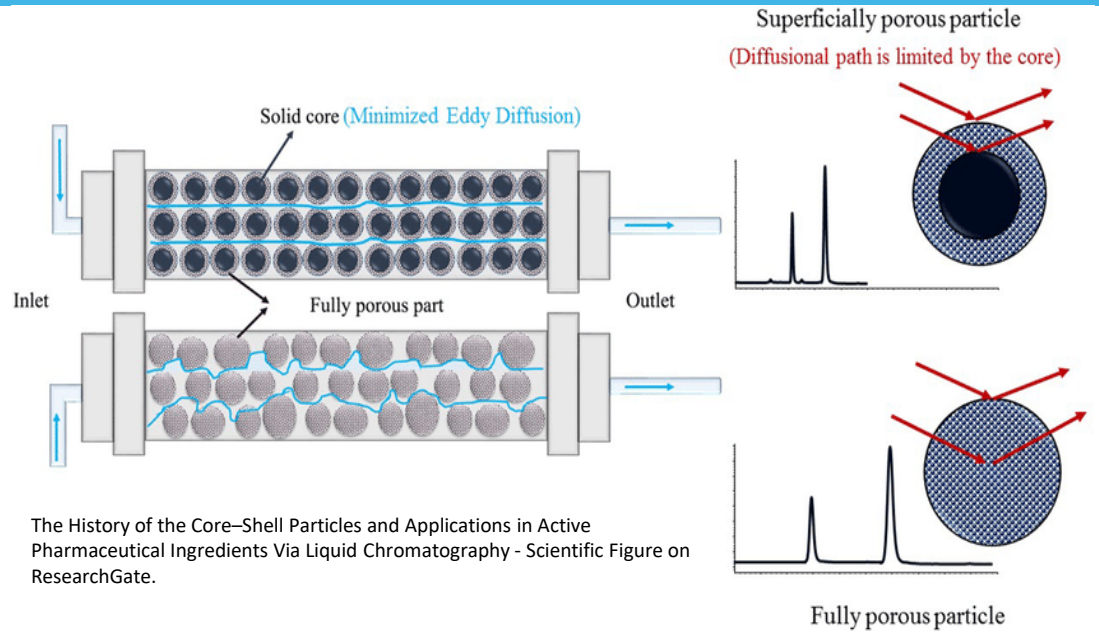
# HALO® vs. FPP

## Superficially Porous Particle (SPP)



## Fully Porous Particle (FPP)

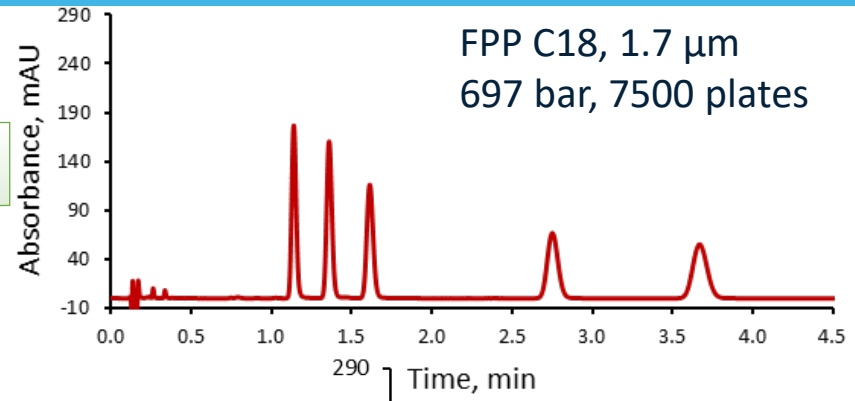


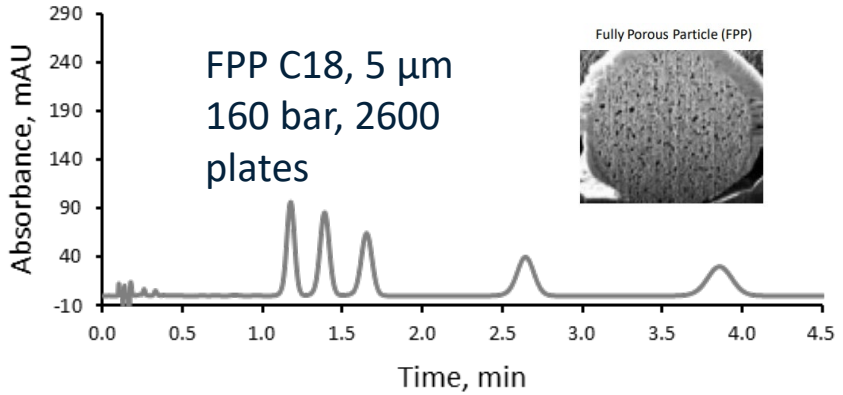



The History of the Core–Shell Particles and Applications in Active Pharmaceutical Ingredients Via Liquid Chromatography - Scientific Figure on ResearchGate.

- Higher Efficiency/ Peak Capacities: HALO® provides a **30-50% increase in efficiency over FPPs** of the same particle size
- Faster Analysis Times: Due to enhanced mass transfer
- Great Performance at Lower Back Pressures: HALO® allows for performance similar to smaller FPPs but at significantly lower back pressures

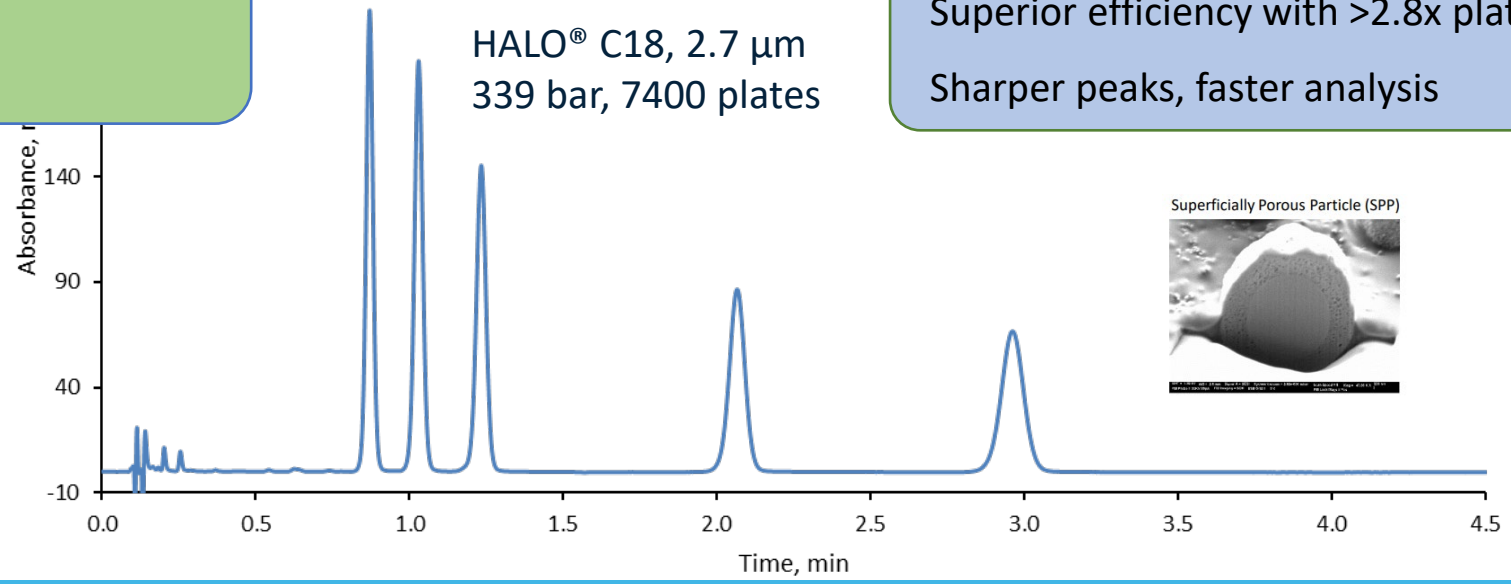
# Power of Fused-Core® Technology




High performance with <1/2 the back pressure

Faster analysis

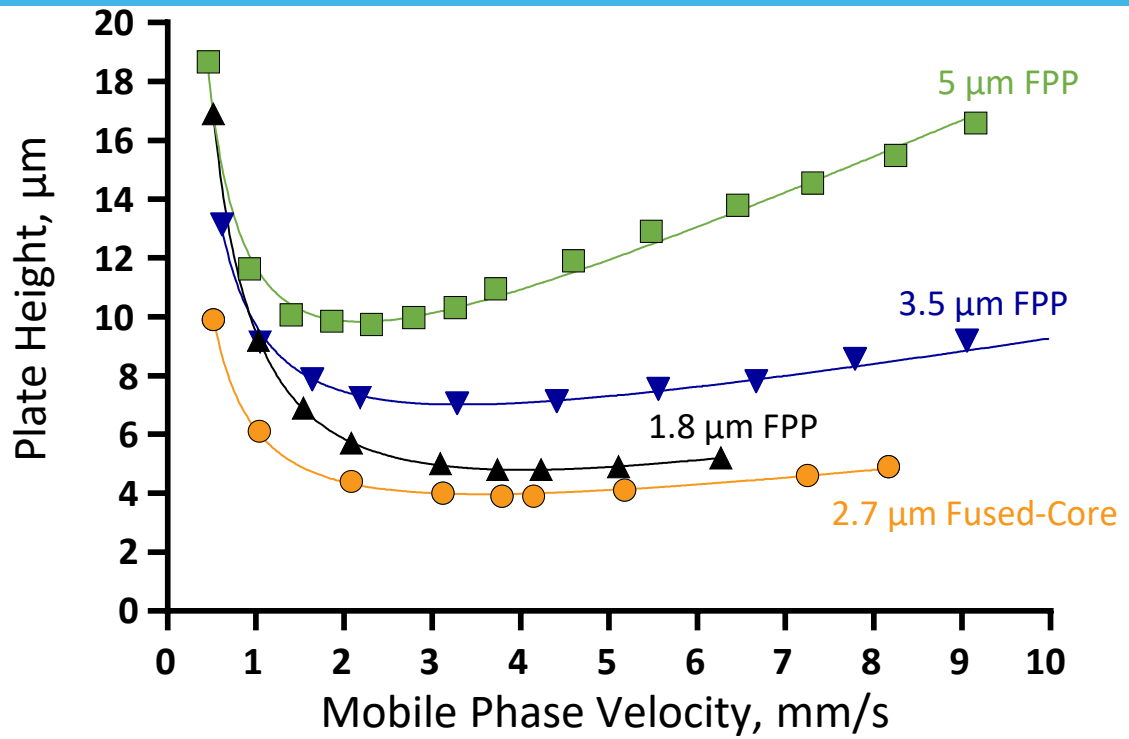



Superior efficiency with >2.8x plates!

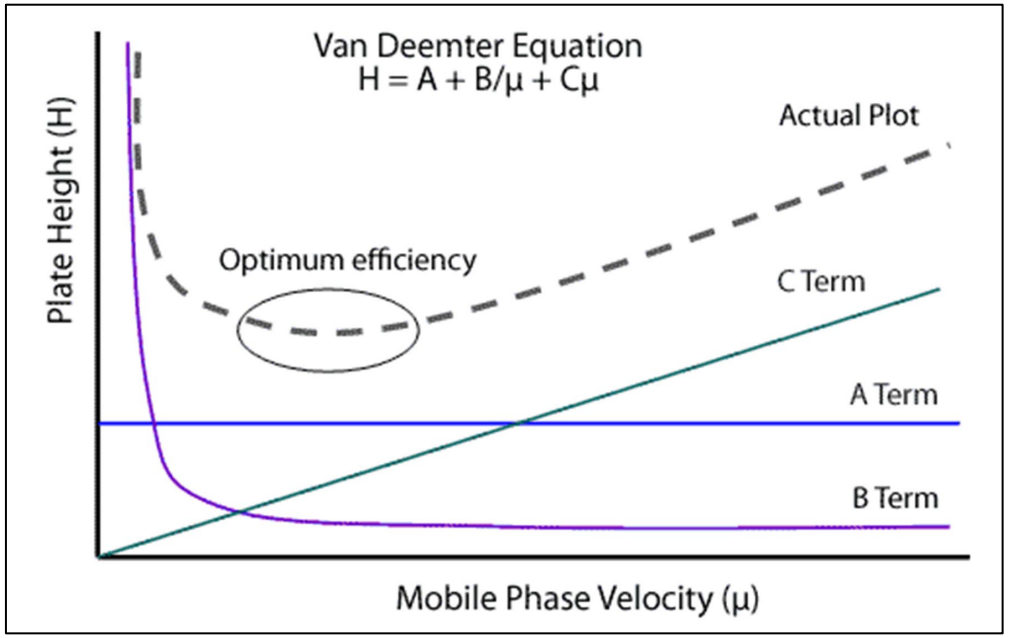
Sharper peaks, faster analysis

# How HALO® Benefits Separations?

## Speed and Efficiency




J.J. DeStefano, T.J. Langlois, & J.J. Kirkland, ***J. Chromatogr. Sci.***, 2008, 46(3), 254-260




$$H = A + \frac{B}{\mu} + C\mu$$

**van Deemter Equation**

**H** = height equivalent to theoretical plate

**A** = eddy diffusion term (particle size and how well bed was packed) **30 - 40% smaller**

**B** = longitudinal diffusion term **25 - 30% smaller**

**C** = resistance to mass transfer term (kinetics of the analyte b/w mobile phase and stationary phase)

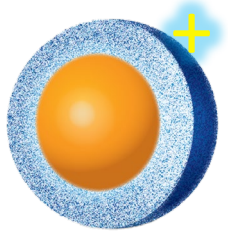
**µ** = mobile phase linear velocity ($L/t_0$)

# Positively Charged Surface Stationary Phase

# Positively Charged Surface Particle Technology (PCS)


## How PCS Works

- Must run at pH < 5
- Basic analytes are positively charged and are repelled by the positively charged surface of the particles
- This enables higher sample loading and more symmetrical peak shapes

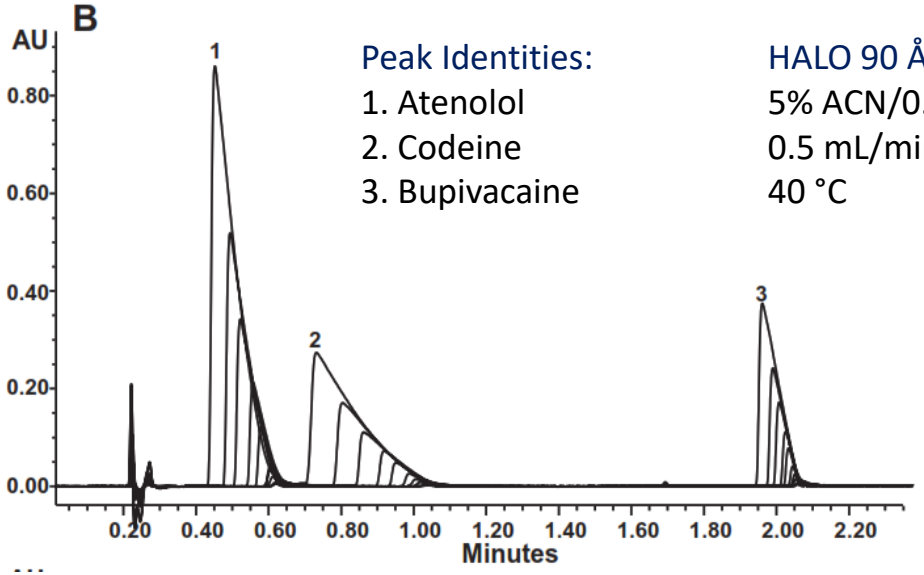


# When do we need a Positively Charged Stationary Phase?

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

# 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.

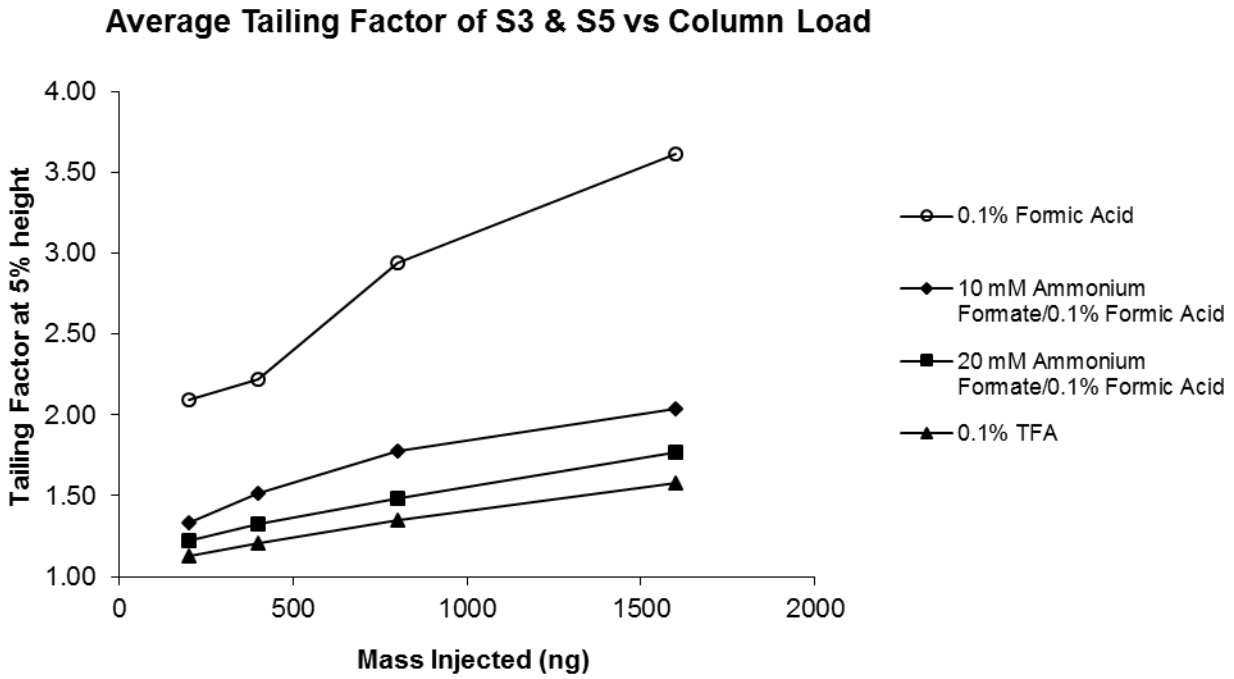


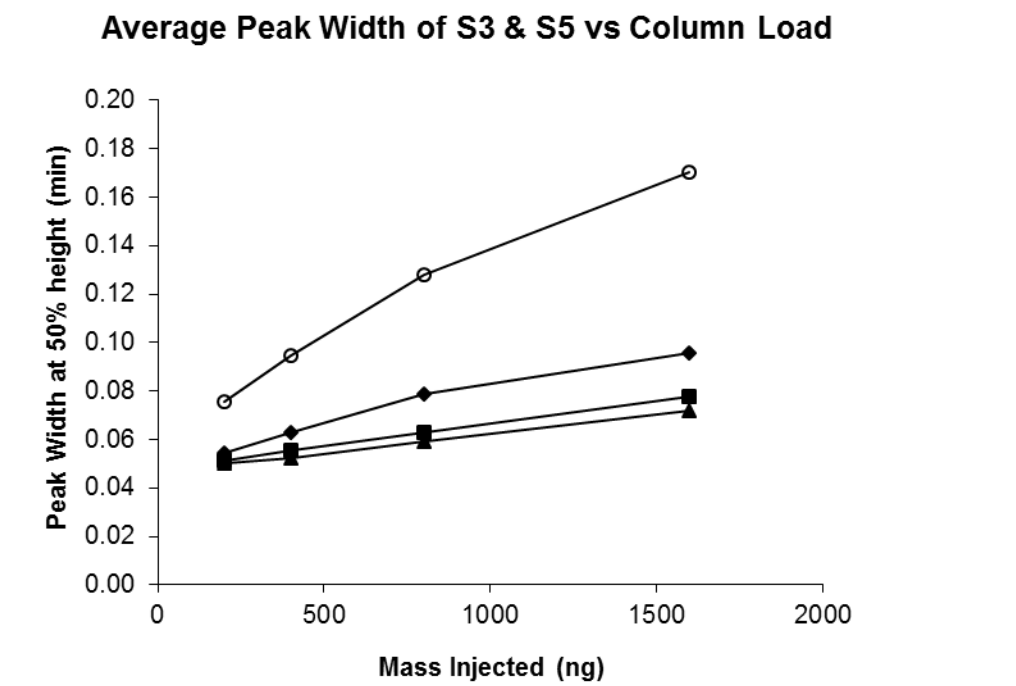

Peak Identities:
1. Atenolol
2. Codeine
3. Bupivacaine

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

# Load Effects for Peptides Comparing Acids



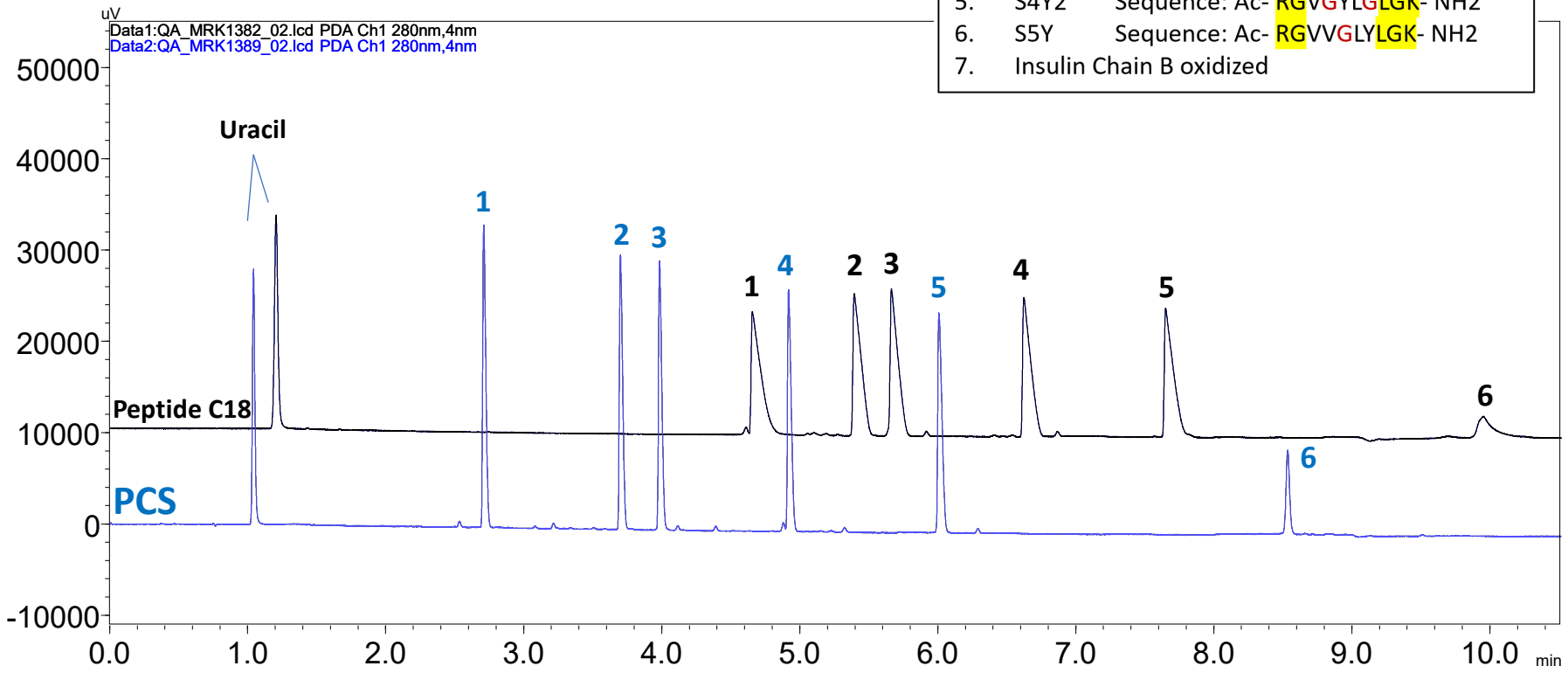

Reference: Johnson, D.J., Boyes, B.E., Orlando, R.C. The Use of Ammonium Formate as a Mobile-Phase Modifier for LC-MS/MS Analysis of Tryptic Digests. **2013** *J. Biomol.Tech.*, 24, 187-197.

# 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

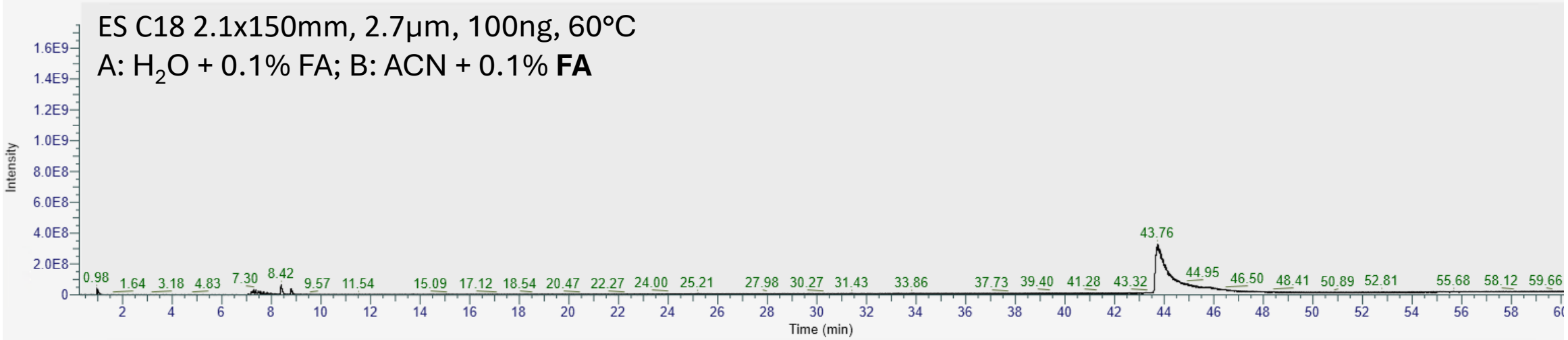


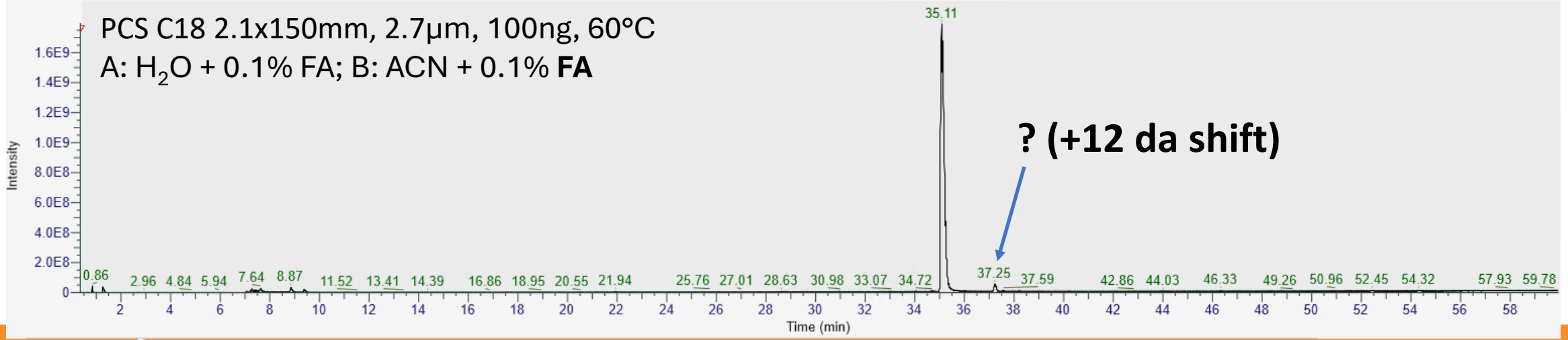

- Gradient separation of 6 peptides
- Decrease in retention time for PCS C18
- Improved peak widths and reduced tailing

# Application of the PCS on 2µm Particles

# Semaglutide ES-C18 vs PCS C18

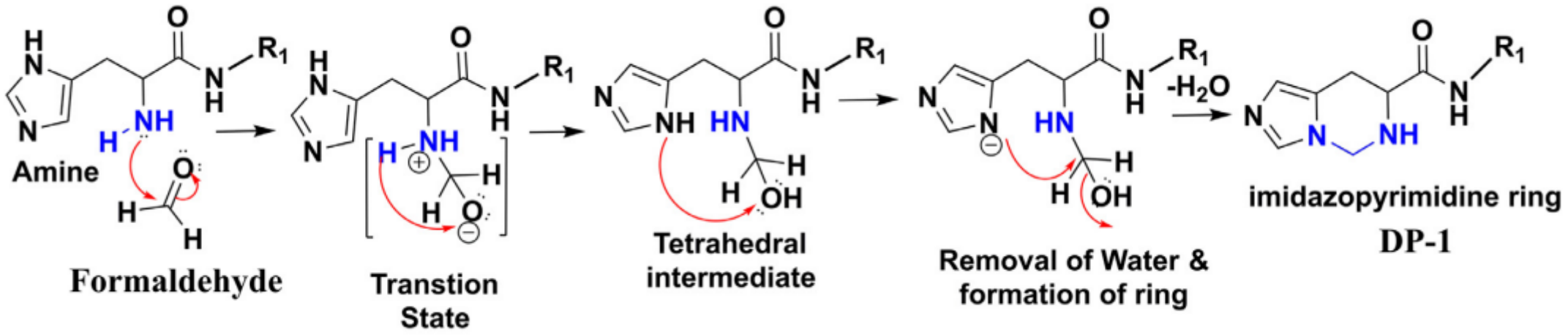
## Compounded Semaglutide Sample



# N-Terminal Modification of Liraglutide

- **N-terminal Histidine sensitive to Formaldehyde exposure in Liraglutide**



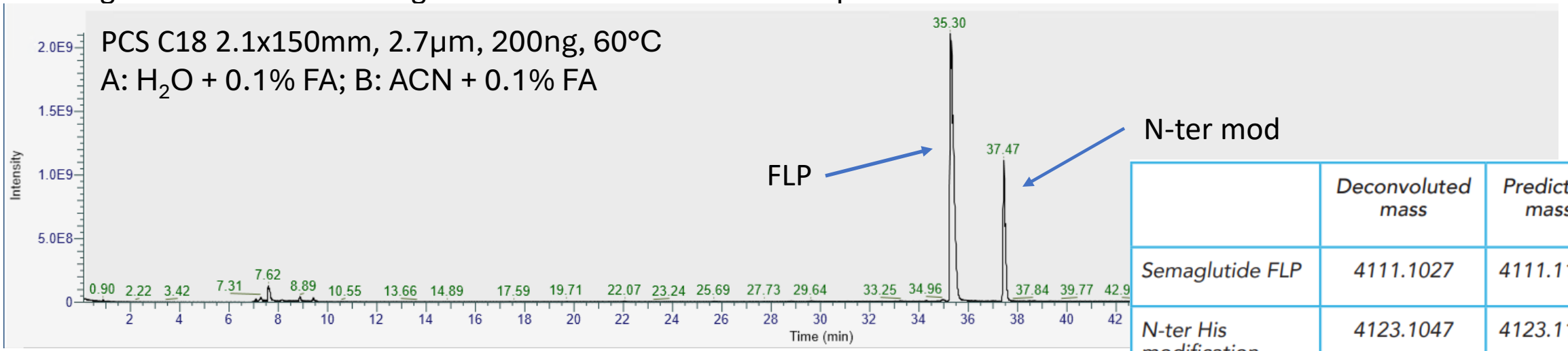

- **Semaglutide also has N-terminal Histidine**
- **Is Semaglutide also sensitive to formaldehyde?**

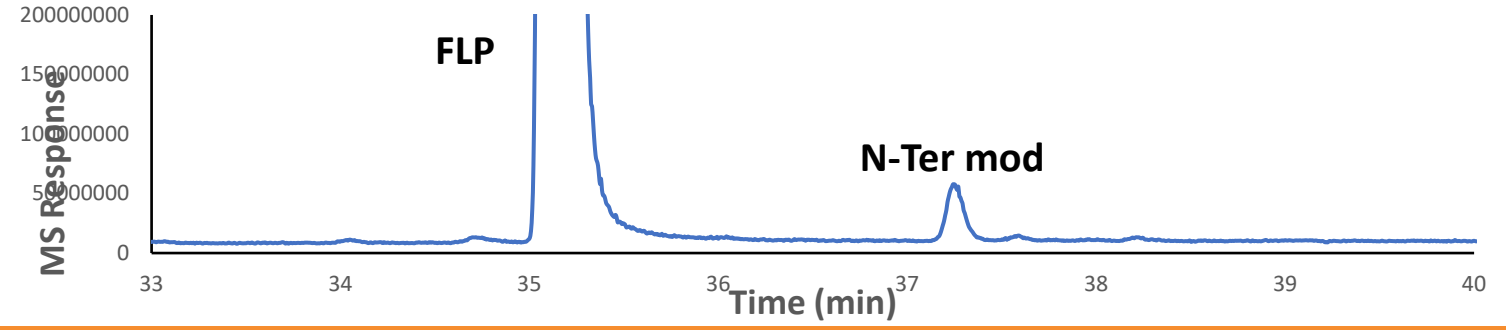
Sheikh, AR *et Al.* **J. Pharmaceutical Sciences** 113(2024) pp3246-3254

# Semaglutide in Tris buffer

- Tris buffer manufactured from Nitromethane and Formaldehyde in a 1:3 Molar Ratio
- Tris buffer can also thermally degrade back into formaldehyde

- 1mg Research Grade Semaglutide in 1ml 10mM Tris-HCl at pH 8.0. Heated 24hr at 40°C

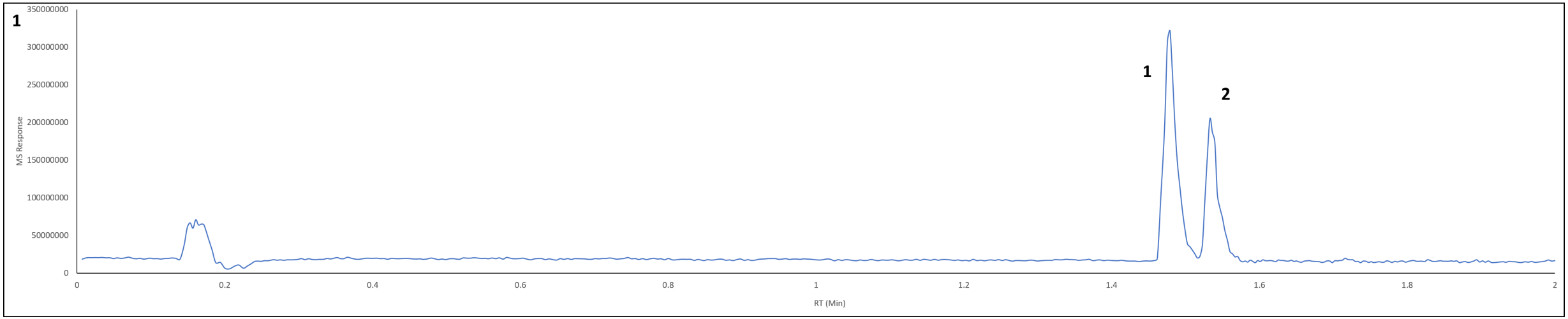


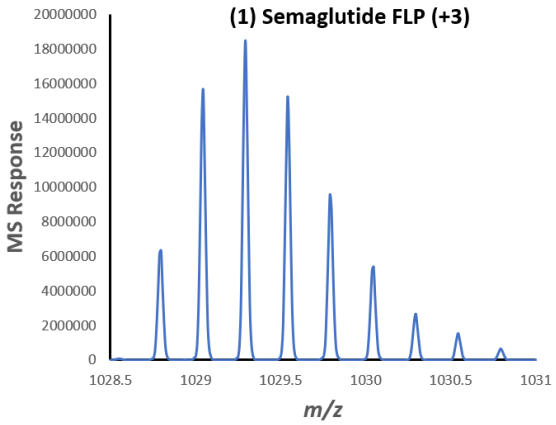



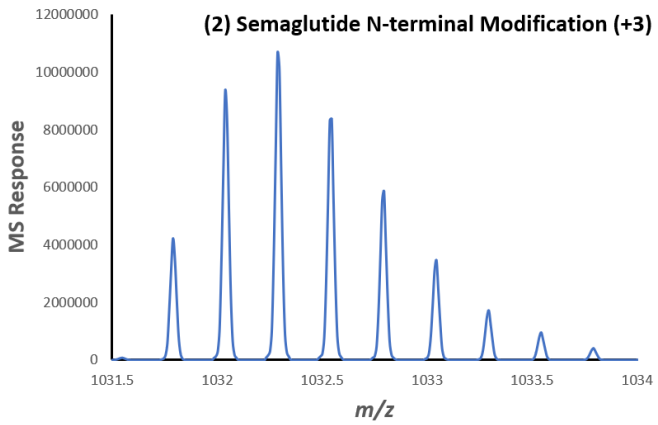

| | Deconvoluted mass | Predicted mass | ppm mass error |
|---|---|---|---|
| Semaglutide FLP | 4111.1027 | 4111.1154 | 3.09 |
| N-ter His modification | 4123.1047 | 4123.1154 | 2.60 |

| | Deconvoluted EIC | Integrated TIC |
|---|---|---|
| Compounded Semaglutide | 1.42% | 1.77% |
| Ozempic | 0.24% | 0.47% |

# Rapid Impurity Detection in Semaglutide

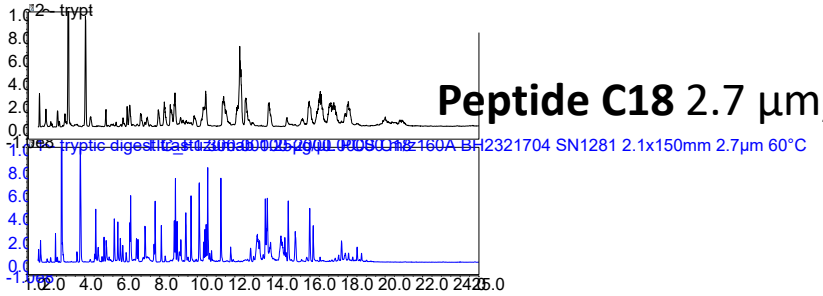



| Column Type/Sample | Retention Time (Mins) | 50% Peak Width (Seconds) | Tailing Factor (EP) |
|---|---|---|---|
| 2.0µm PCS Semaglutide FLP | 1.476 | 0.72 | 1.34 |
| 2.0µm PCS N-terminal Mod | 1.533 | 0.96 | 1.77 |
| | | | |
| 2.7µm PCS Semaglutide FLP | 1.457 | 1.14 | 1.4 |
| 2.7µm PCS N-terminal Mod | 1.513 | 1.26 | 1.69 |

# Trastuzumab Tryptic Digest:
## Higher Peak Capacity with HALO® PCS C18 Peptide




**Peptide C18** 2.7 µm, 2.1 x 150 mm

$n_{PC}$ = **170**

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

**HALO® PCS C18 Peptide** 2.7 µm, 2.1 x 150 mm

$n_{PC}$ = **424**

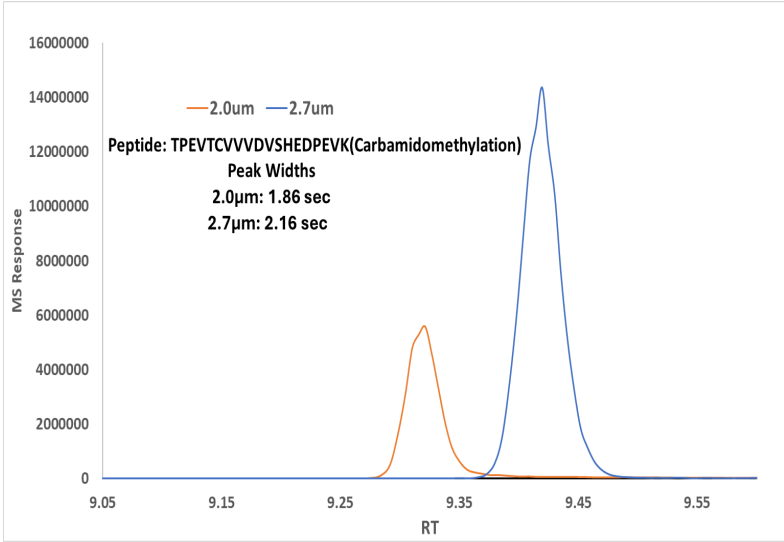
**MarvelXACT Post-Column Plumbing:**

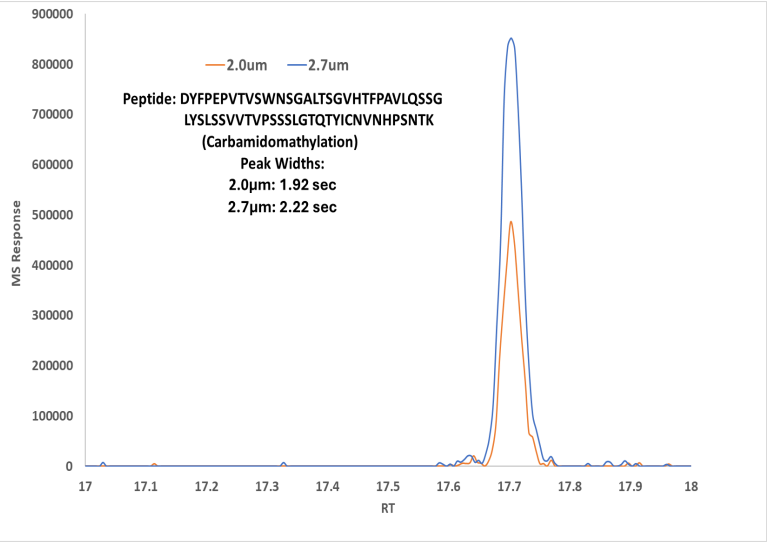
1. 50 µm x 350 mm from column to diverter valve
2. 50 µm x 350 mm from diverter valve to union
3. 50 µm x 150 mm from grounding union to HESI II

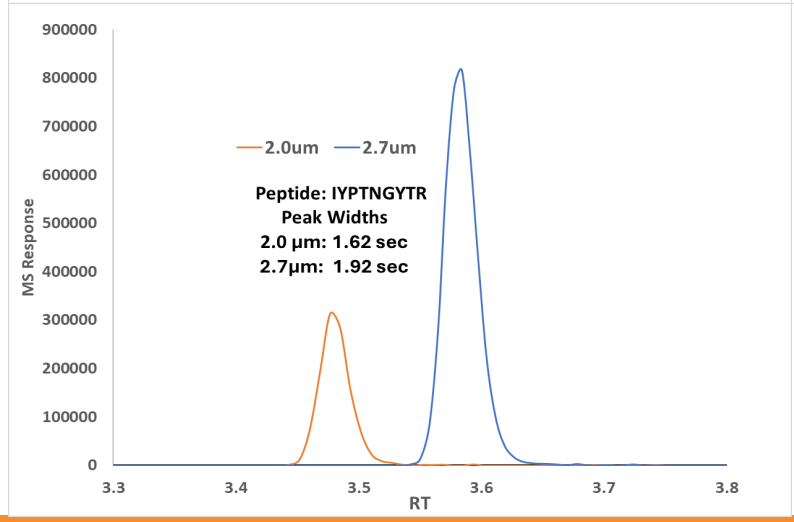
$n_{PC}$ based on ID peptides

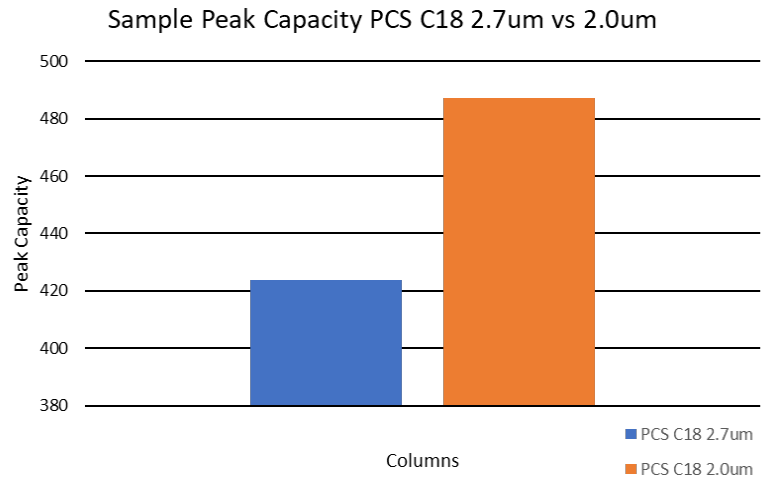
# Trastuzumab Tryptic Digest:
## Higher Peak Capacity on 2µm

- Compared HALO® 160 Å PCS C18 columns with identical chemistry but different particle sizes (2.7 µm vs. 2 µm) for trastuzumab tryptic digest analysis
- Moving to 2 µm particles increased total ion current by **1.4×**, indicating improved analyte response and MS sensitivity
- Smaller particles produced **~15% narrower peak widths**, enhancing chromatographic efficiency
- Narrower peaks led to improved resolution of peptides within the complex digest
- Peak capacity (based on eight representative peptides) showed a **substantial increase (Δ = 64)** with the 2 µm column
- Overall, the 2 µm particle size significantly improves separation efficiency and information recovery in complex peptide analyses



# Summary

- Combining PCS (positively charged surface) technology with smaller particle sizes significantly improves LC–MS separation performance
- PCS phases reduce unwanted secondary interactions (via pKa-dependent behavior), leading to better peak shape, retention consistency, and robustness for basic and amphoteric analytes
- Pairing PCS with **2 µm particles** amplifies benefits through higher efficiency, narrower peaks, and increased peak capacity
- Enhanced performance enables improved resolution and deeper characterization of complex samples
- Demonstrated effectiveness across diverse applications (e.g., antibiotics, peptide mapping, semaglutide impurity analysis)
- Overall, this approach boosts **sensitivity, resolution, and throughput** under MS-friendly conditions, making it ideal for modern LC–MS workflows and high-quality therapeutic analysis

# Questions?

Photo by Jamie Street on Unsplash

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