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

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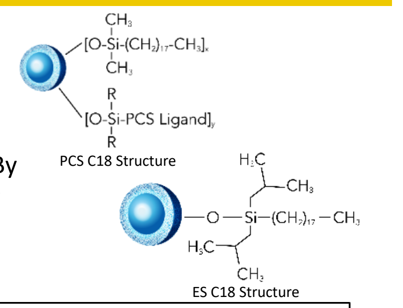
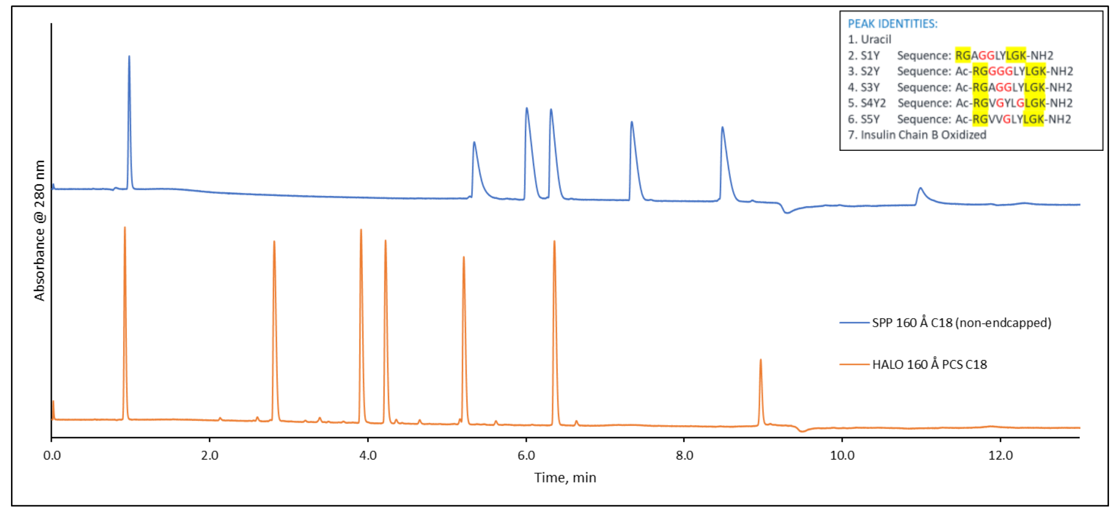
Introduction

Traditional C18 HPLC columns are widely used for separating compounds from small molecules to biopharmaceuticals. However, basic compounds often exhibit unwanted interactions with silica, impacting peak shape and efficiency. Positively charged surface (PCS) stationary phases mitigate these interactions, improving separations for basic analytes under acidic mobile phase conditions, such as formic acid. While PCS technology is established on 2.7 µm particles, its implementation on smaller particles introduces significant performance gains. By reducing the superficially porous particle size from 2.7 µm to 2.0 µm, improved resolution and enhanced efficiency across a range of basic compounds is observed, from small molecules (90 Å phase) to peptides (160 Å phase).

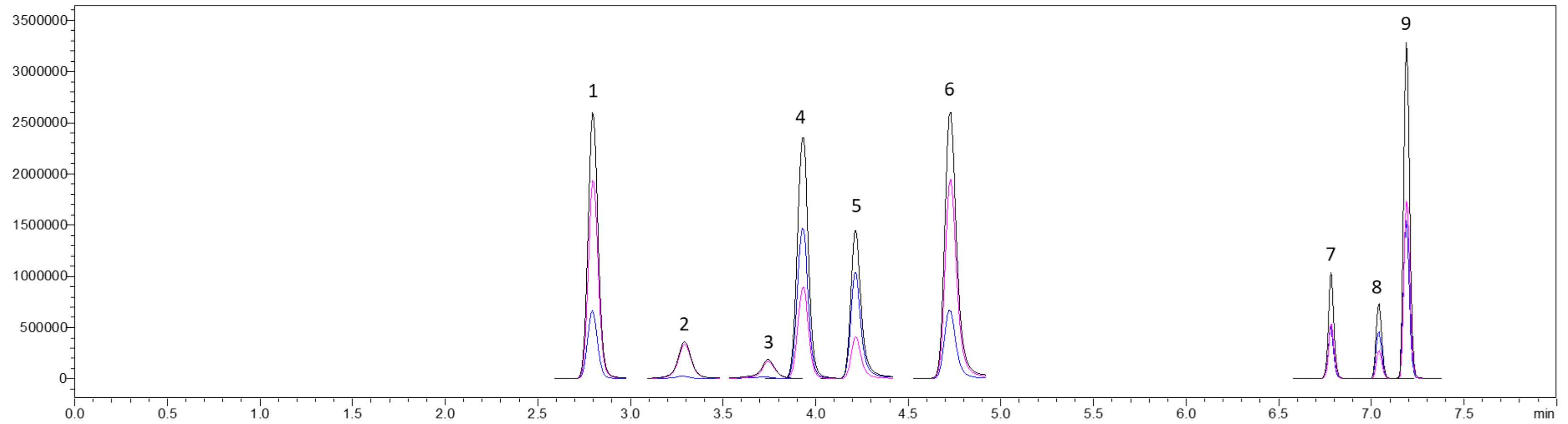




TEST CONDITIONS:
Column: HALO 160 Å PCS-C18 , 2.7 µm, 2.1 x 100mm
Part Number: 92812-617
Comparison Column: SPP 160 Å C18, 2.7 µm, 2.1 x 100mm
Mobile Phase A: Water/ 0.1% Formic Acid
Mobile Phase B: Acetonitrile/ 0.1% Formic Acid
Gradient: Time %B
0.0 2
10.0 35
Flow Rate: 0.3 mL/min
Temperature: 30 °C
Injection Volume: 1.0 µL
Wavelength: PDA, 280 nm
Flow Cell: 1 µL
Data Rate: 100 Hz
Response Time: 0.025 sec.
LC System: Shimadzu Nexera X2

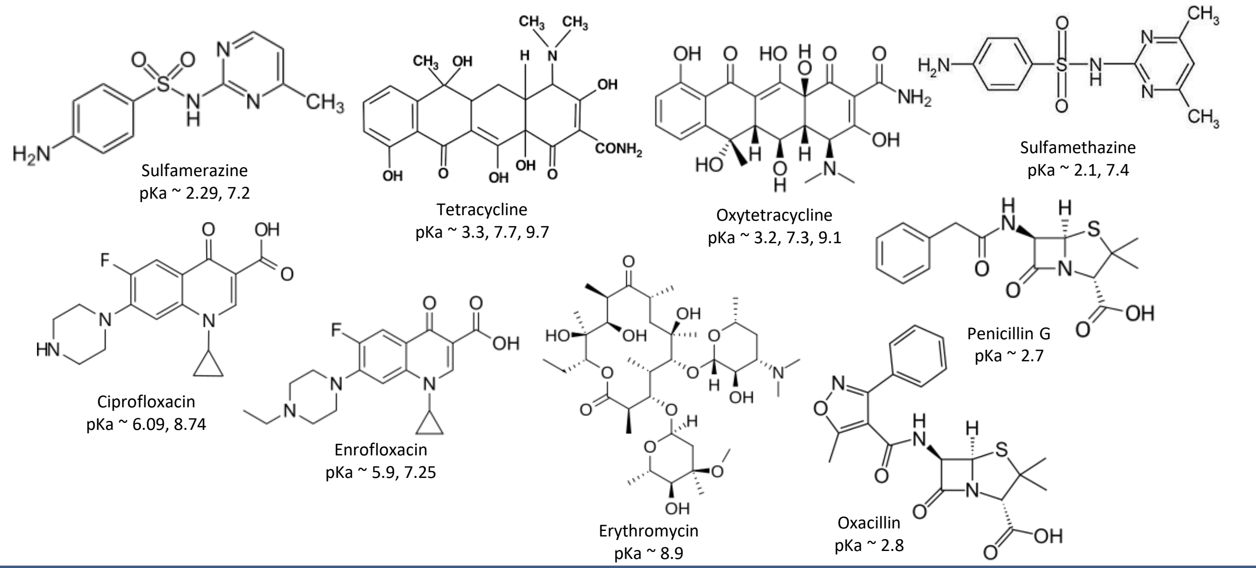
Positively charged surface technologies are designed to deliver improved peak shapes for basic compounds observed with standard stationary phase technologies. This works by reducing secondary interactions from the basic compounds, like above, where basic residue peptides are compared on a standard technology vs the PCS technology. The reduced interactions improve tailing and increase loading capacity. This benefit is particularly valuable for LC-MS workflows where weaker ion pairs such as formic acid are commonly used as a mobile phase additive.

Evaluating the Use of Positively Charged Technology for Polyionic Compounds



TEST CONDITIONS:
Column: HALO 90 Å PCS C18, 2 µm, 2.1 x 50 mm
Part Number: 91882-417
Mobile Phase A: Water/0.1% formic acid
Mobile Phase B: Methanol/0.1% formic acid
Gradient: Time %B
0.00 6
5.50 19
6.00 64
8.00 95
8.01 6
12.00 6
Flow Rate: 0.4 mL/min
Pressure: 300 bar
Temperature: 27 °C
Injection Volume: 0.5 µL
Sample: 0.2 - 17 µg/mL
Sample Solvent: 98/2 water/methanol
LC System: Shimadzu Nexera X2

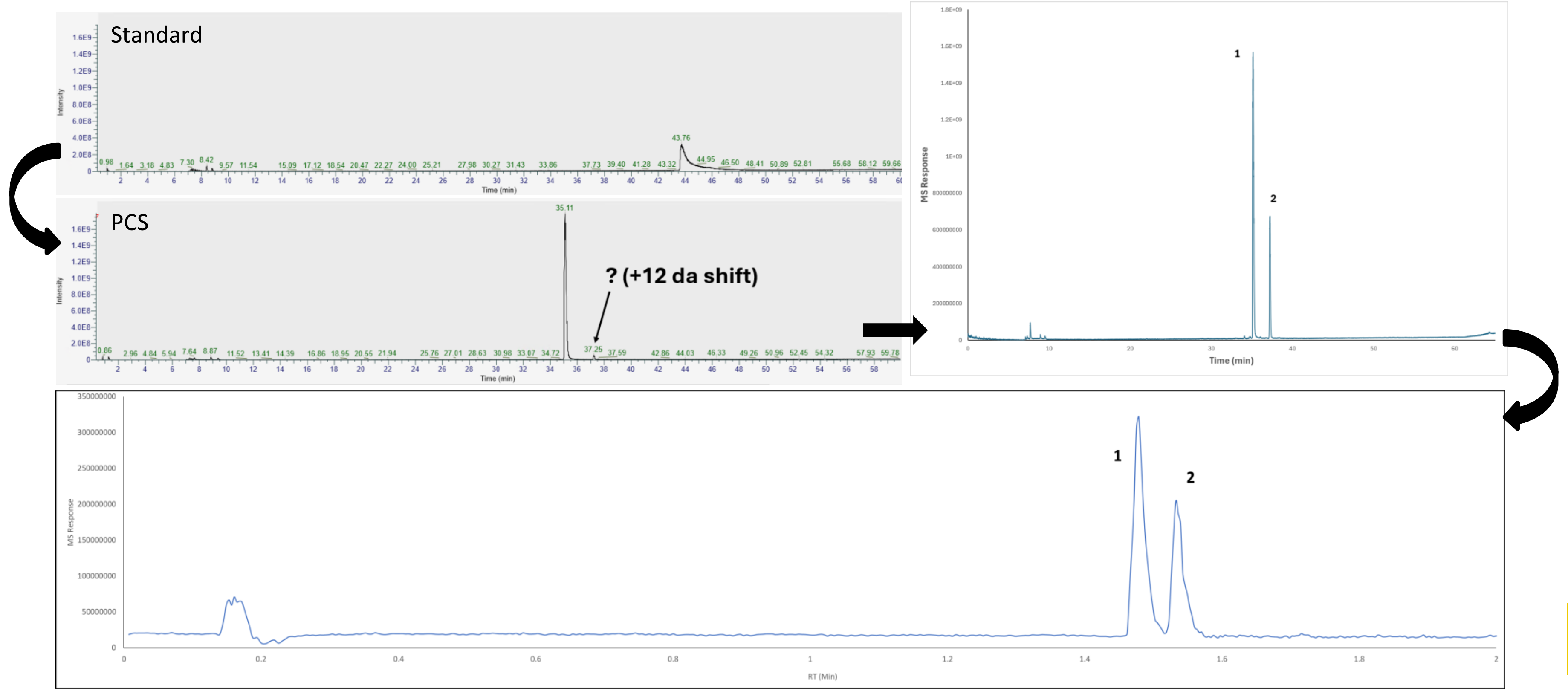
MS CONDITIONS:
System: Shimadzu 8060
Detection Mode: DUIS ESI + 1 kV
Nebulizer Gas Flow: 3 L/min
Interface Temperature: 150 °C
DL Temperature: 300 °C
Heat Block Temperature: 200 °C
Drying Gas Flow: 5 L/min

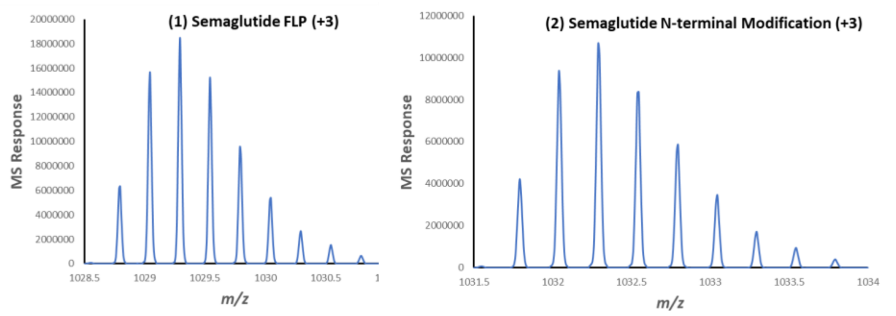


The separation of antibiotics presents a significant chromatographic challenge due to their diverse structures, multiple ionizable functional groups, and wide range of pKa values, which result in complex charge states under typical LCMS conditions. In this work, PCS technology is used to address these challenges by leveraging pKa-dependent retention mechanisms. Under low pH conditions with formic acid as a mobile phase modifier, many antibiotics, particularly those with basic functionalities, are protonated and positively charged. On conventional stationary phases, these species can interact with residual negatively charged silanol groups, leading to peak tailing and poor efficiency. In contrast, PCS technology introduces a controlled positive charge, creating electrostatic repulsion with protonated analytes and thereby reducing secondary interactions. This results in improved peak shape, particularly for compounds with higher pKa values, while retention is governed more predictably by hydrophobic interactions and residual ionization state. Compounds with lower pKa values, which are more likely to be neutral under these conditions, exhibit retention behavior driven primarily by their hydrophobicity. The use of a smaller 2 µm particle size further enhances chromatographic performance by increasing the number of theoretical plates, reducing band broadening, and yielding sharper peaks compared to larger particle formats such as 2.7 µm. Additionally, formic acid serves a dual role by maintaining protonation of both the analytes and the stationary phase ligands while also providing excellent compatibility with electrospray ionization mass spectrometry. As a volatile modifier, formic acid supports efficient ionization in positive mode with minimal ion suppression compared to stronger, nonvolatile acids. Together, the combination of PCS technology, optimized particle size, and MS-friendly mobile phase conditions enables improved efficiency, selectivity, and peak shape for the separation of structurally diverse antibiotics.

Rapid Impurity Detection in Semaglutide Enhanced by PCS Technology

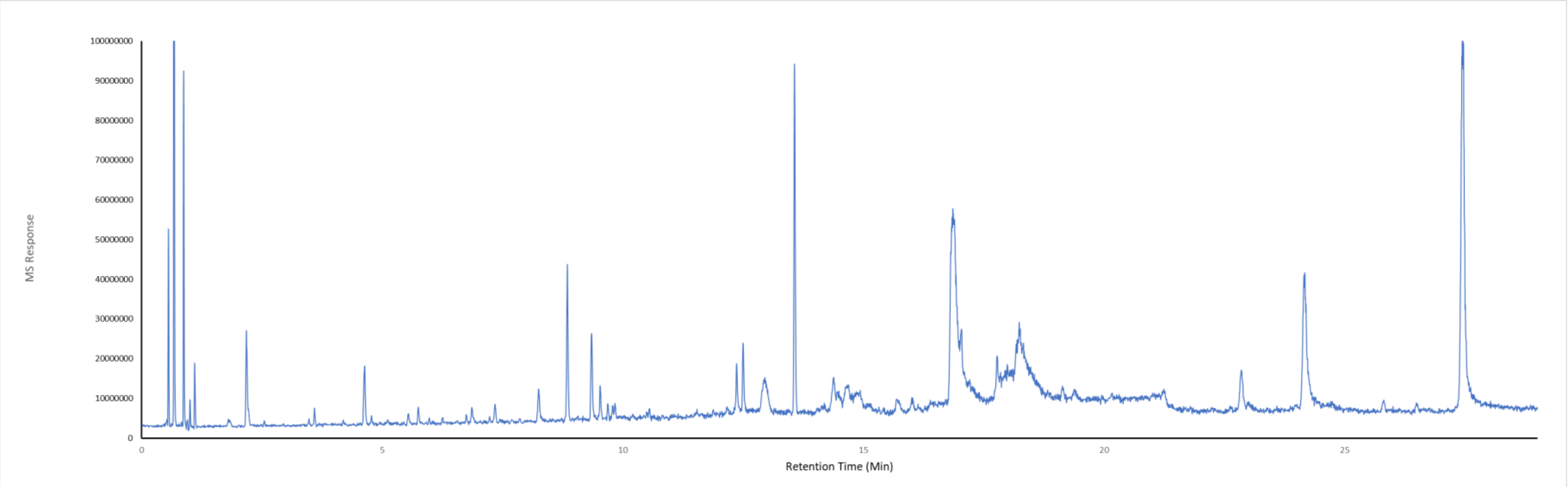
To demonstrate the applicability of this technology to real-world samples, a compounded formulation of semaglutide was analyzed using a high-resolution mass spectrometry system. When evaluated on a conventional peptide stationary phase under formic acid conditions, the chromatogram exhibited poor peak shape, limiting both resolution and interpretability. Upon transitioning to PCS technology, peak shape improved dramatically, revealing an impurity that had not been observed on the standard phase. Further investigation suggested that this impurity corresponded to cyclization, and modification of the N-terminal histidine. To confirm this hypothesis, the sample was subjected to forced degradation to induce formation of the suspected impurity. Analysis of the degraded sample showed a clear increase in the impurity peak, supporting the proposed theory. While the initial PCS method provided excellent resolution between the intact semaglutide and the cyclized impurity, the run time was approximately 60 minutes. By optimizing the gradient and leveraging a smaller particle size to maximize plate count and efficiency, the method was significantly accelerated, achieving baseline separation of both species in under 2 minutes while also decreasing the peak width by 27%. This rapid separation highlights the potential of PCS technology not only for analytical characterization but also as a practical, high-throughput method for quality assurance of semaglutide therapies.

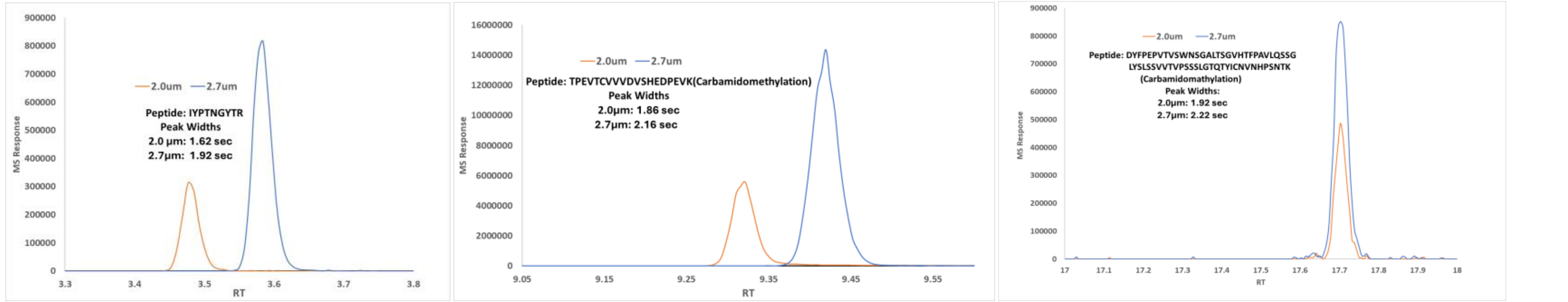




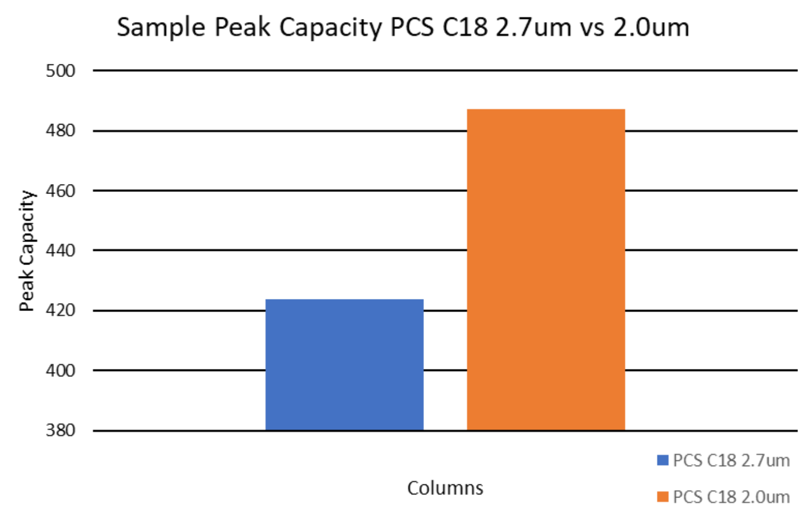
Column Type/Sample	Retention Time (Mins)	50% Peak Width (Seconds)	Tailing Factor (EP)
2.0µm PCS Semaglutide FLP	1.476	0.72	1.34
2.0µm PCS N-terminal Mod	1.533	0.96	1.77
2.7µm PCS Semaglutide FLP	1.457	1.14	1.4
2.7µm PCS N-terminal Mod	1.513	1.26	1.69

Improving Resolution and Sensitivity in Trastuzumab Digests Using Smaller Particle PCS Columns





A separation of a trastuzumab tryptic digest was performed on a HALO® 160 Å PCS C18 column using a Thermo Fisher Q-Exactive HF to evaluate the impact of particle size on performance in complex peptide analyses. Two columns packed with identical PCS chemistry but differing particle sizes (2.7 µm and 2 µm) were compared. Transitioning to the smaller 2 µm particle size resulted in a 1.4× increase in total ion current, indicating improved analyte response and MS sensitivity. Performance was further assessed by evaluating peak capacity using eight representative peptides identified within the digest, with calculations based on measured peak widths.



Testing Conditions:
Column: HALO 160 Å PCS C18, 2.0 µm, 2.1 x 100 mm
Part Number: 91182-617
Mobile Phase: A: Water + 0.1% Formic Acid
B: Acetonitrile + 0.1% Formic Acid
Gradient:
Time %B
0.0 3
30.0 50
35.0 3

Flow Rate: 0.3 mL/min
Back Pressure: 330 bar
Temperature: 60 °C
Injection: 1µL of 200 ng/mL Trastuzumab Digest
Sample Solvent: H_2O
LC System: Shimadzu Nexera X2
MS System: HS Orbitrap

MS Conditions:
Polarity: Positive
Resolution: 120k
AGC Target: 3e6
Max IT: 200ms
Scan Range: 200-2000 *m/z*
Sheath Gas Flow Rate: 25
Aux Gas Flow Rate: 10
Sweep Gas Flow Rate: 1
Spray Voltage: 4.5kv
Capillary Temp: 350°C
Aux Gas Heater Temp: 300°C
S-Lens RF level: 60

The 2 µm column produced significantly narrower peaks (approximately 15% reduction in peak width), leading to improved resolution and a substantial increase in peak capacity relative to the 2.7 µm column, with an overall difference of 64. This gain highlights the advantage of smaller particle sizes for extracting more information from highly complex peptide mixtures.

Conclusions

These results demonstrate that combining positively charged surface stationary phase technology with smaller particle sizes provides a highly effective strategy for improving LCMS separations. By leveraging pKa-dependent behavior, PCS phases reduce undesired secondary interactions under formic acid conditions, leading to improved peak shape, more consistent retention, and better overall performance for basic and amphoteric analytes.

When paired with 2 µm particles, these benefits are amplified through increased efficiency, narrower peak widths, and higher peak capacity, enabling greater resolution and deeper insight into complex samples. This is evident across applications ranging from small molecule antibiotics to peptide mapping and real-world samples such as semaglutide, where PCS enabled impurity detection and rapid, high-resolution separations.

Overall, PCS technology combined with smaller particle sizes enhances sensitivity, resolution, and throughput under MS-friendly conditions, making it a powerful solution for modern LC–MS workflows, including those requiring fast and reliable quality analysis of complex therapeutics.