Increased Efficiency of Protein and Peptide Separations by Varying Particle Size, Column Dimension, and Pore Size of Superficially Porous Particle Columns

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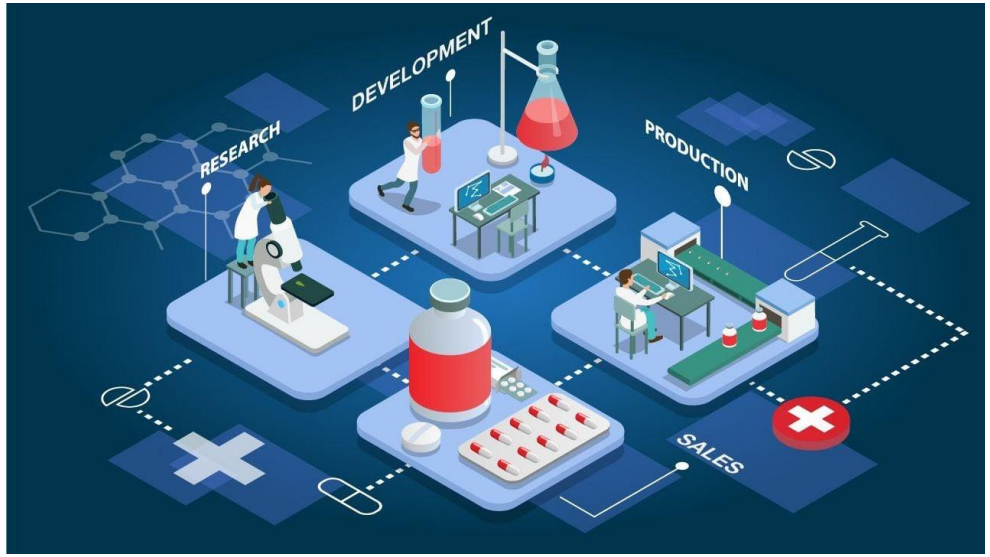
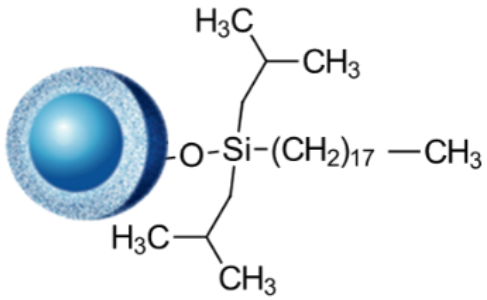
Proteins & Peptides: Column Technology

- Biopharmaceutical drugs are becoming more complex
  - These drugs are monitored throughout the development process
  - They must be tested for the safety of patients
- Column Technology must evolve
  - To evaluate more complex samples
  - To learn more of the drugs being developed
  - Help develop safe drugs through research
  - To be more efficient



What was Employed

- 1.5 column ID
  - To increase ionization efficiencies
  - To reduce solvent usage
  - Potentially reduce sample usage
- 2µm particle size
  - To increase efficiency through packing
  - Increase to ionization efficiencies
  - To reduce peak widths
- Pore Size
  - Using a 160Å pore size to allow access to the stationary phase



ES-C18 Structure

Application Sample

- Sample – An aliquot of Trastuzumab drug product underwent tryptic digest
  - Diluted with 50mM ammonium bicarbonate
    - To 1.5M Guanidine prior to digestion
  - Standard digest conditions were used for an overnight digestion @ 37C
  - After digest sample was adjusted to 0.5% formic acid
  - Acetonitrile was added to make solution 2% ACN
    - To aid in solubility
  - Stock concentration: 21mg/mL
    - Final concentration: 1.25µg mAb/µL

Standard UHPLC Setup

TEST CONDITIONS:

Column: HALO 160 Å ES-C18 , 2.7 µm, 2.1 x 150 mm
Mobile Phase A: Water, 0.1% DFA
Mobile Phase B: Acetonitrile, 0.1% DFA
Gradient:

Time	%B
0.5	2
60.5	50
61.0	70
65.0	70
65.5	2
70.0	Stop

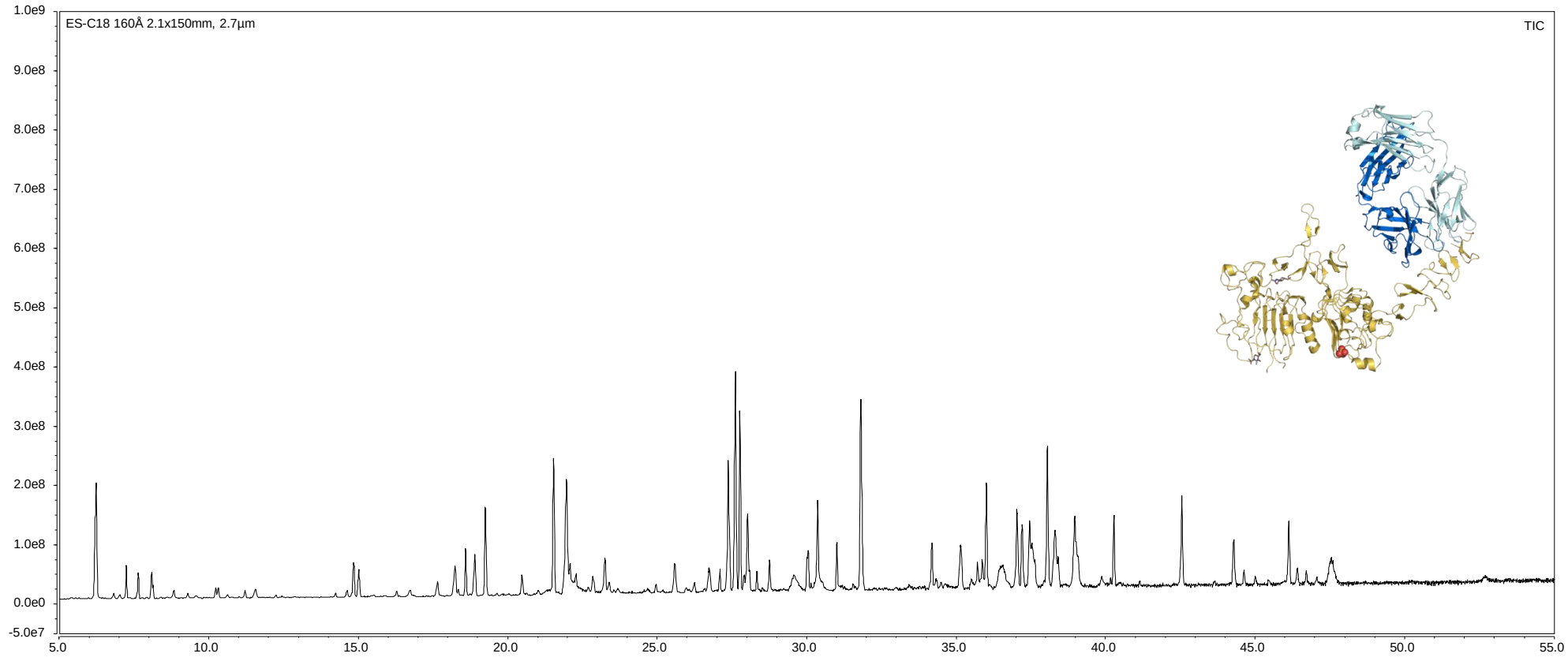
Flow Rate: 0.4 mL/min
Temperature: 60 °C
Injection Volume: 1 µL
Sample: 1mg/mL Trastuzumab tryptic digest
Sample Solvent: 1.5M Guanidine HCl/0.5% Formic Acid/~50mM Tris pH 7.8
LC System: Shimadzu Nexera X2

Tubing Optimization:

50µm x 600mm Column to Diverter Valve
50µm x 350mm Diverter Valve to Ground
50µm x 100mm Ground to Source

MS CONDITIONS:

System: ThermoFisher Q Exactive
Spray Voltage (kV): 3.8
Capillary temperature: 320 °C
Sheath gas: 35
Aux gas: 10
RF lens: 50



Decreasing Column ID

TEST CONDITIONS:

Column: HALO 160 Å ES-C18 , 2.7 µm, 1.5 x 150 mm
Mobile Phase A: Water, 0.1% DFA
Mobile Phase B: Acetonitrile, 0.1% DFA
Gradient:

Time	%B
0.5	2
60.5	50
61.0	70
65.0	70
65.5	2
70.0	Stop

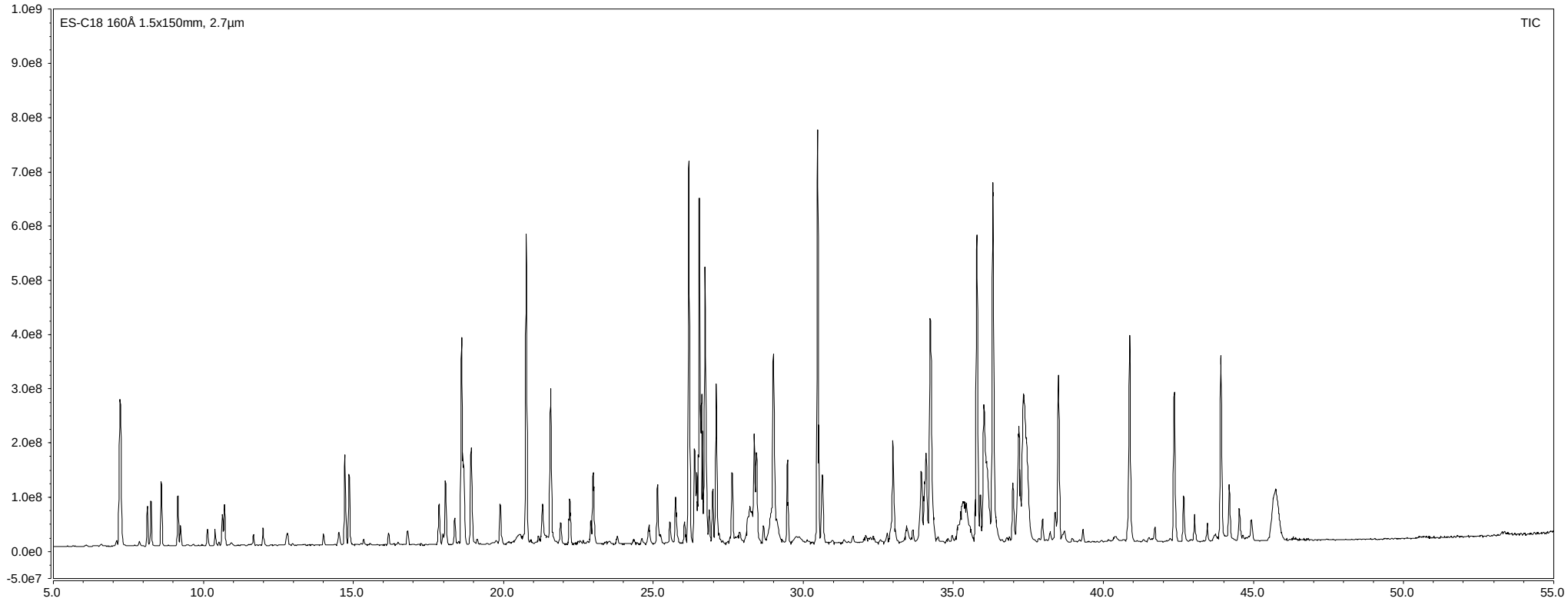
Flow Rate: 0.2 mL/min
Temperature: 60 °C
Injection Volume: 1 µL
Sample: 1mg/mL Trastuzumab tryptic digest
Sample Solvent: 1.5M Guanidine HCl/0.5% Formic Acid/~50mM Tris pH 7.8
LC System: Shimadzu Nexera X2

Tubing Optimization:

50µm x 600mm Column to Diverter Valve
50µm x 350mm Diverter Valve to Ground
50µm x 100mm Ground to Source

MS CONDITIONS:

System: ThermoFisher Q Exactive
Spray Voltage (kV): 3.8
Capillary temperature: 320 °C
Sheath gas: 35
Aux gas: 10
RF lens: 50



Comparing the Change in ID

HALO®

TEST CONDITIONS:

Column: HALO 160 Å ES-C18 , 2.7 µm, 1.5 x 150 mm
Column: HALO 160 Å ES-C18 , 2.7 µm, 2.1 x 150 mm
Mobile Phase A: Water, 0.1% DFA
Mobile Phase B: Acetonitrile, 0.1% DFA
Gradient:

Time	%B
0.5	2
60.5	50
61.0	70
65.0	70
65.5	2
70.0	Stop

Flow Rate: 0.2 mL/min for 1.5 mm ID
0.4 mL/min for 2.1 mm ID
Temperature: 60 °C
Injection Volume: 1 µL
Sample: 1mg/mL Trastuzumab tryptic digest
Sample Solvent: 1.5M Guanidine HCl/0.5% Formic Acid/~50mM Tris pH 7.8
LC System: Shimadzu Nexera X2

Tubing Optimization:

50µm x 600mm Column to Diverter Valve
50µm x 350mm Diverter Valve to Ground
50µm x 100mm Ground to Source

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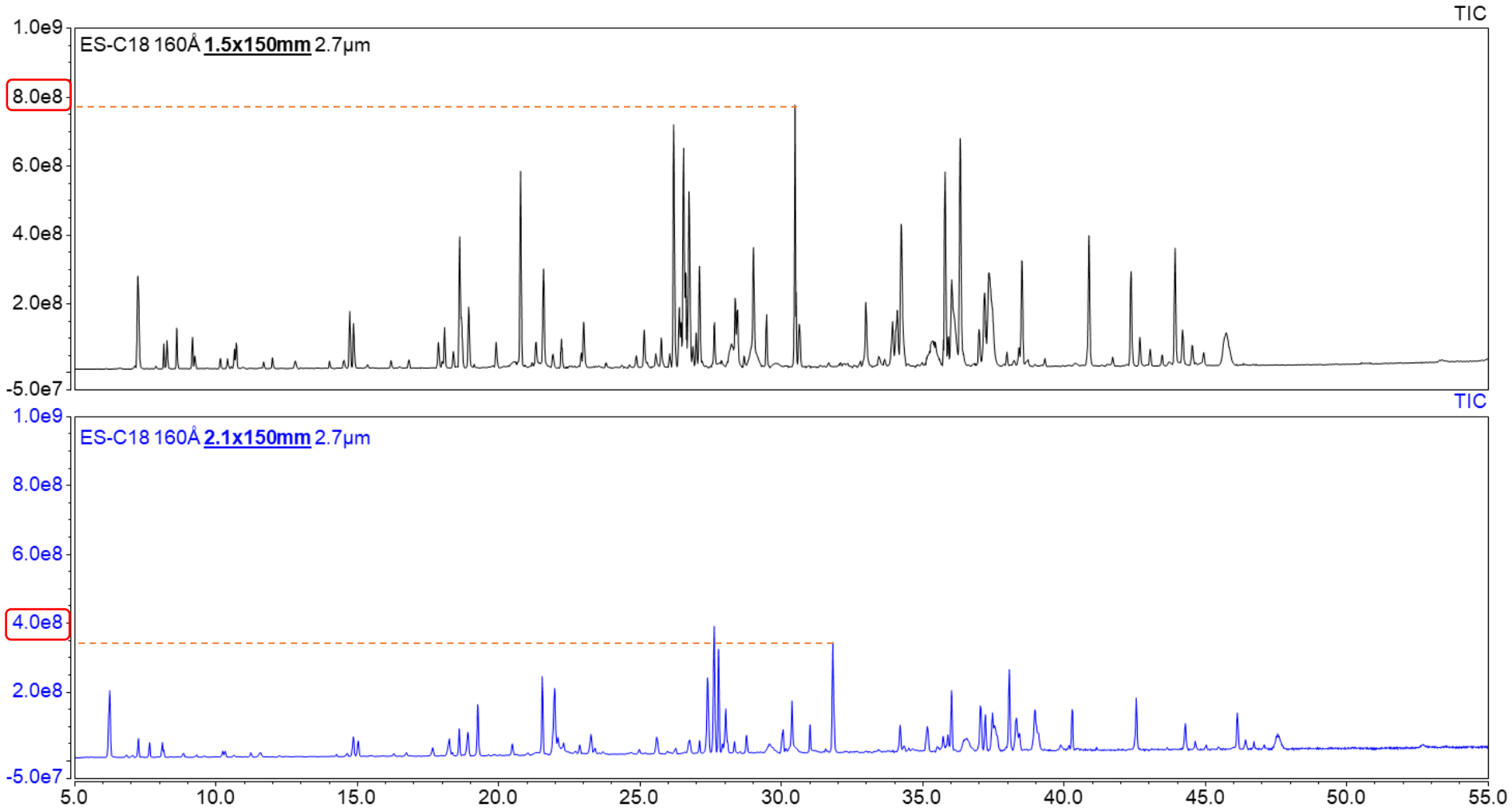
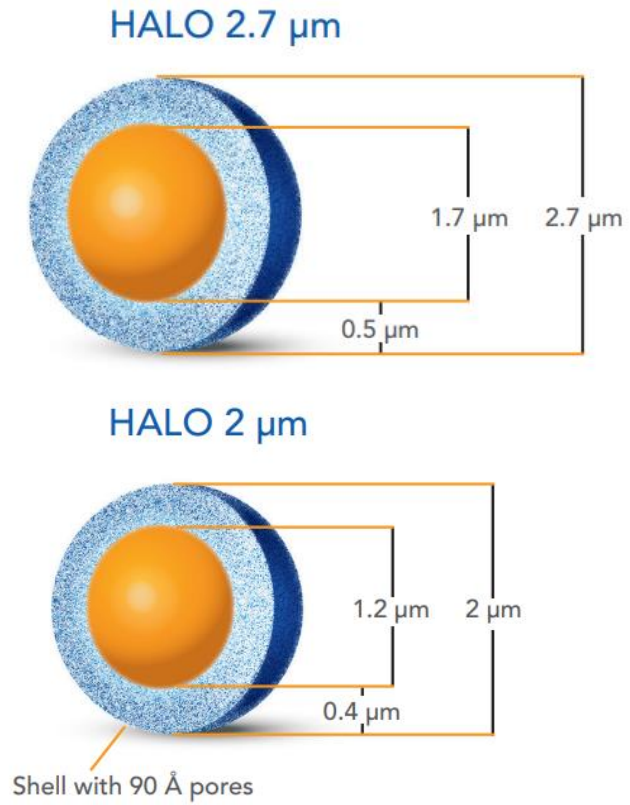
Spray Voltage (kV): 3.8
Capillary temperature: 320 °C
Sheath gas: 35
Aux gas: 10
RF lens: 50



Changing Particle Size

- Pros of changing from 2.7µm to 2µm
  - Increased efficiency
  - Increased surface area through better column packing
  - Decreased peak width increased peak capacity
- Cons of changing from 2.7µm to 2µm
  - Increased backpressure
    - This can impact peak band spreading
  - More wear and tear on the system
  - May require a specialized system (UHPLC)
    - Higher pressure rated systems (Up to 1000 bar)



Decreased Particle Size on 2.1mm ID

TEST CONDITIONS:

Column: HALO 160 Å ES-C18 , 2.0 µm, 2.1 x 150 mm
Mobile Phase A: Water, 0.1% DFA
Mobile Phase B: Acetonitrile, 0.1% DFA
Gradient:

Time	%B
0.5	2
60.5	50
61.0	70
65.0	70
65.5	2
70.0	Stop

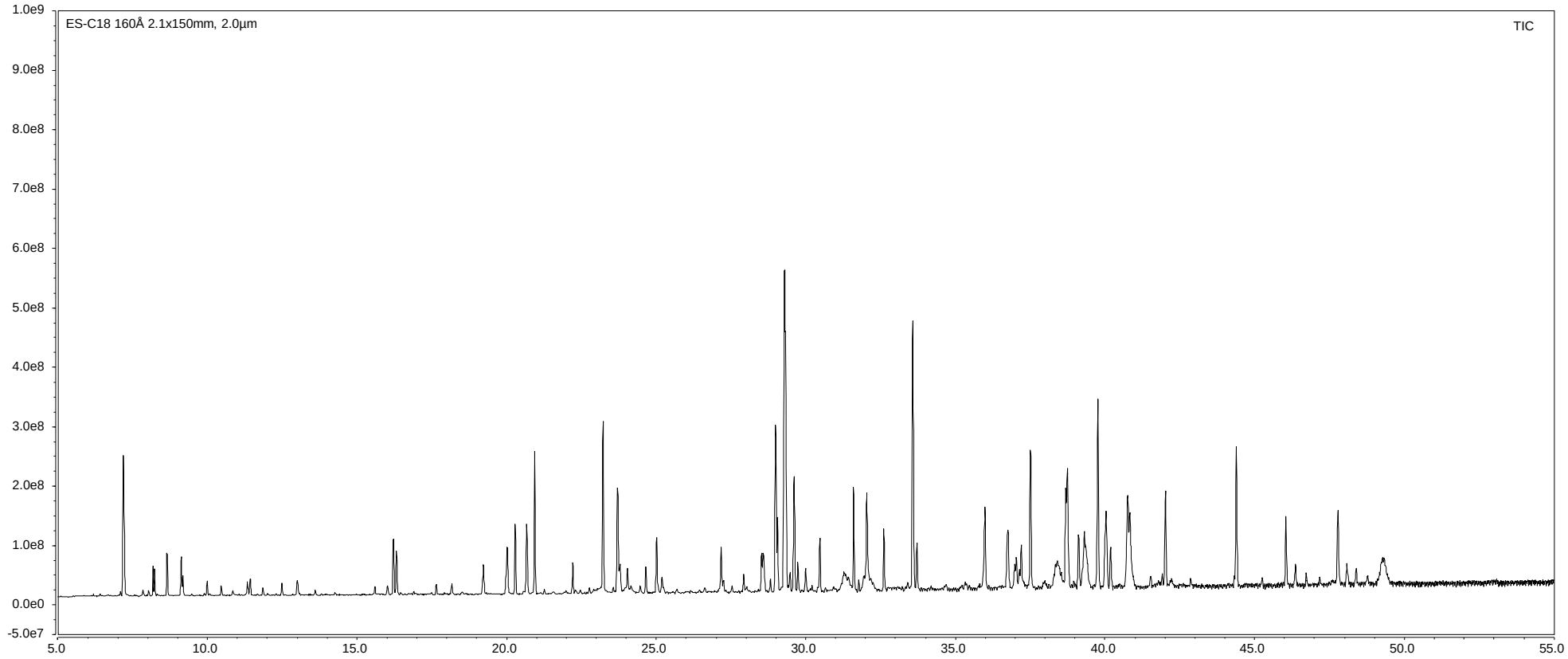
Flow Rate: 0.4 mL/min
Temperature: 60 °C
Injection Volume: 1 µL
Sample: 1mg/mL Trastuzumab tryptic digest
Sample Solvent: 1.5M Guanidine HCl/0.5% Formic Acid/~50mM Tris pH 7.8
LC System: Shimadzu Nexera X2

Tubing Optimization:

50µm x 600mm Column to Diverter Valve
50µm x 350mm Diverter Valve to Ground
50µm x 100mm Ground to Source

MS CONDITIONS:

System: ThermoFisher Q Exactive
Spray Voltage (kV): 3.8
Capillary temperature: 320 °C
Sheath gas: 35
Aux gas: 10
RF lens: 50



Comparing Particle Size on 2.1mm ID

TEST CONDITIONS:

Column: HALO 160 Å ES-C18 , 2.7 µm, 2.1 x 150 mm
Column: HALO 160 Å ES-C18 , 2.0 µm, 2.1 x 150 mm
Mobile Phase A: Water, 0.1% DFA
Mobile Phase B: Acetonitrile, 0.1% DFA
Gradient:

Time	%B
0.5	2
60.5	50
61.0	70
65.0	70
65.5	2
70.0	Stop

Flow Rate: 0.4 mL/min
Temperature: 60 °C
Injection Volume: 1 µL
Sample: 1mg/mL Trastuzumab tryptic digest
Sample Solvent: 1.5M Guanidine HCl/0.5% Formic Acid/~50mM Tris pH 7.8
LC System: Shimadzu Nexera X2

Tubing Optimization:

50µm x 600mm Column to Diverter Valve
50µm x 350mm Diverter Valve to Ground
50µm x 100mm Ground to Source

MS CONDITIONS:

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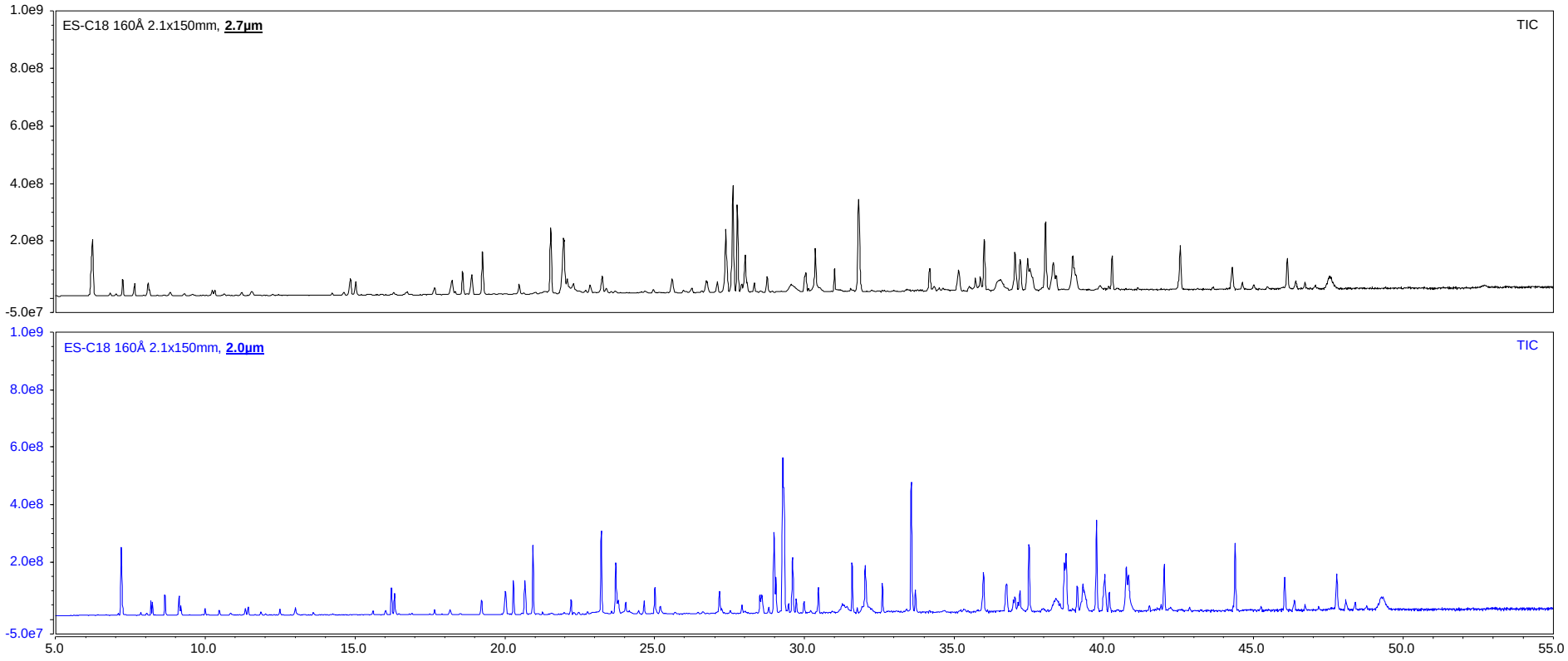
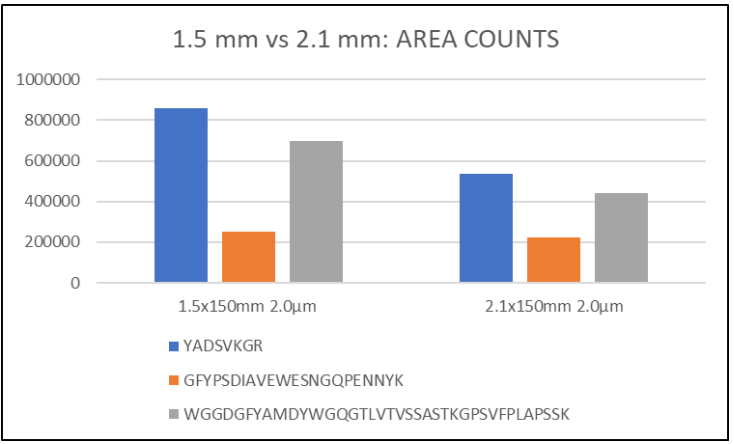
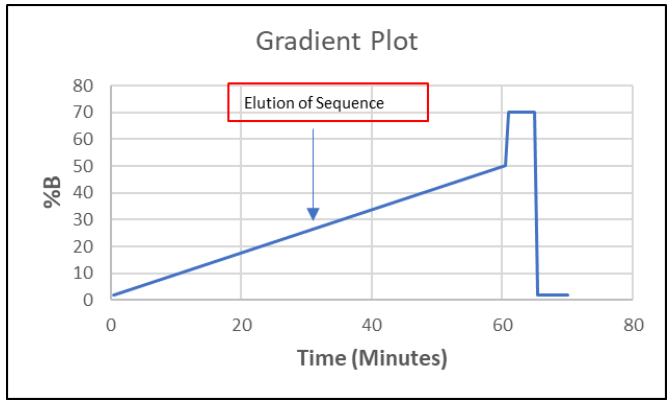
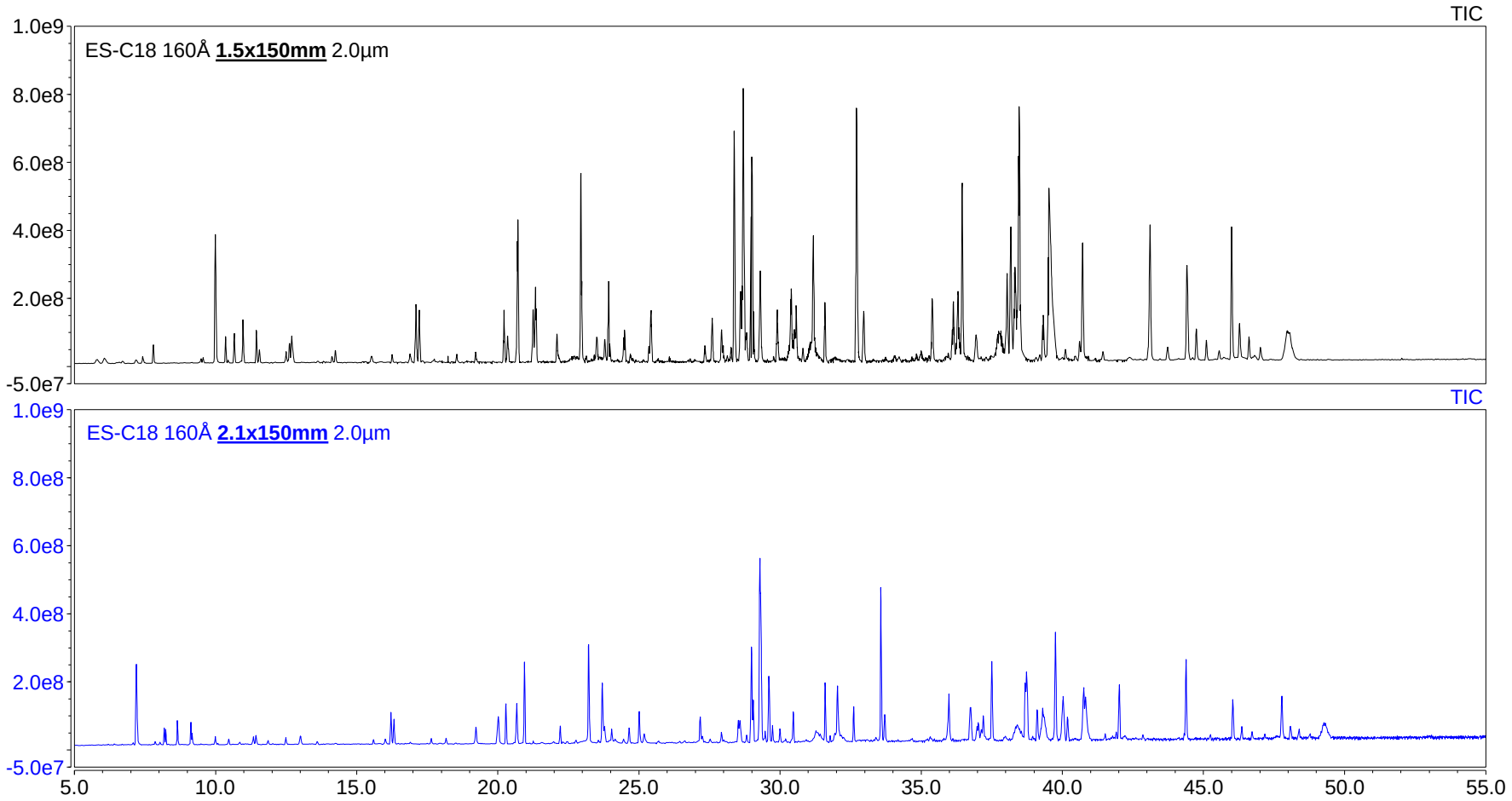
Spray Voltage (kV): 3.8
Capillary temperature: 320 °C
Sheath gas: 35
Aux gas: 10
RF lens: 50



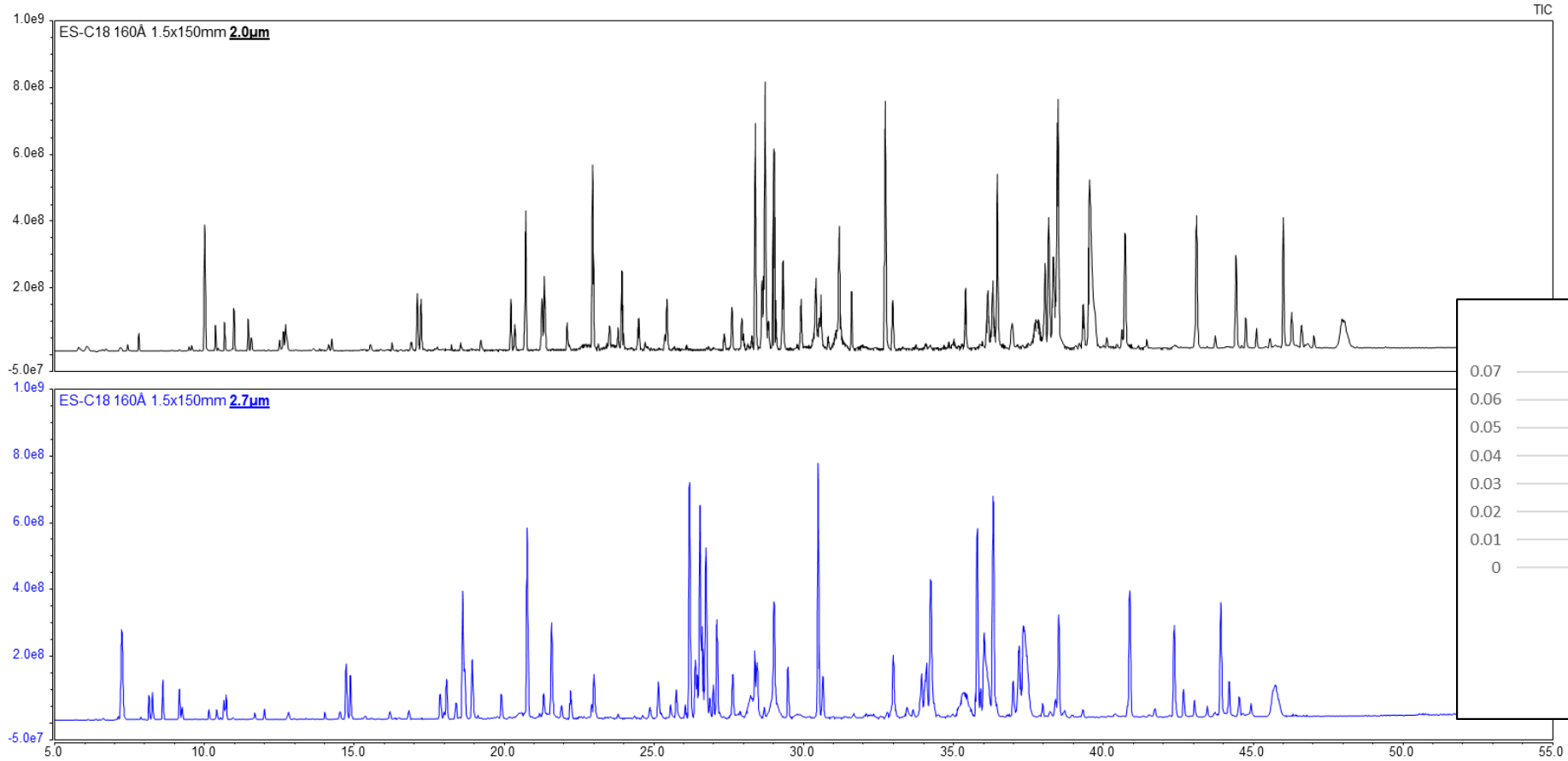
Compare of Particle and ID Size

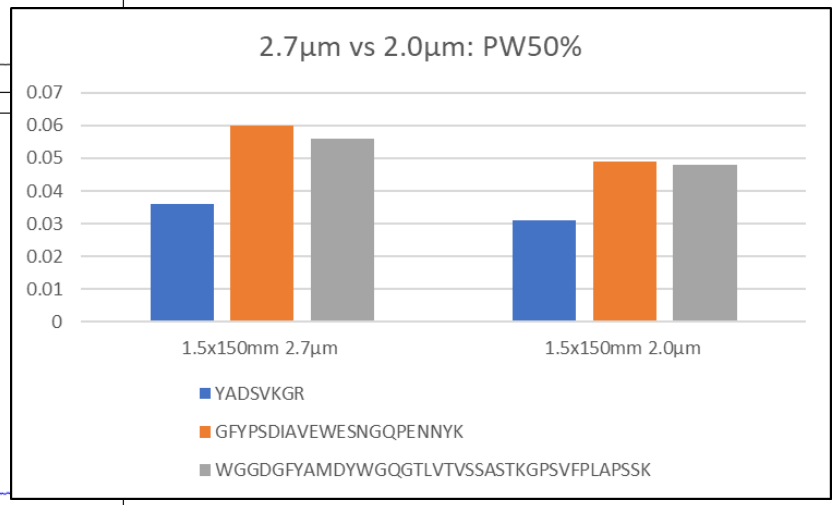






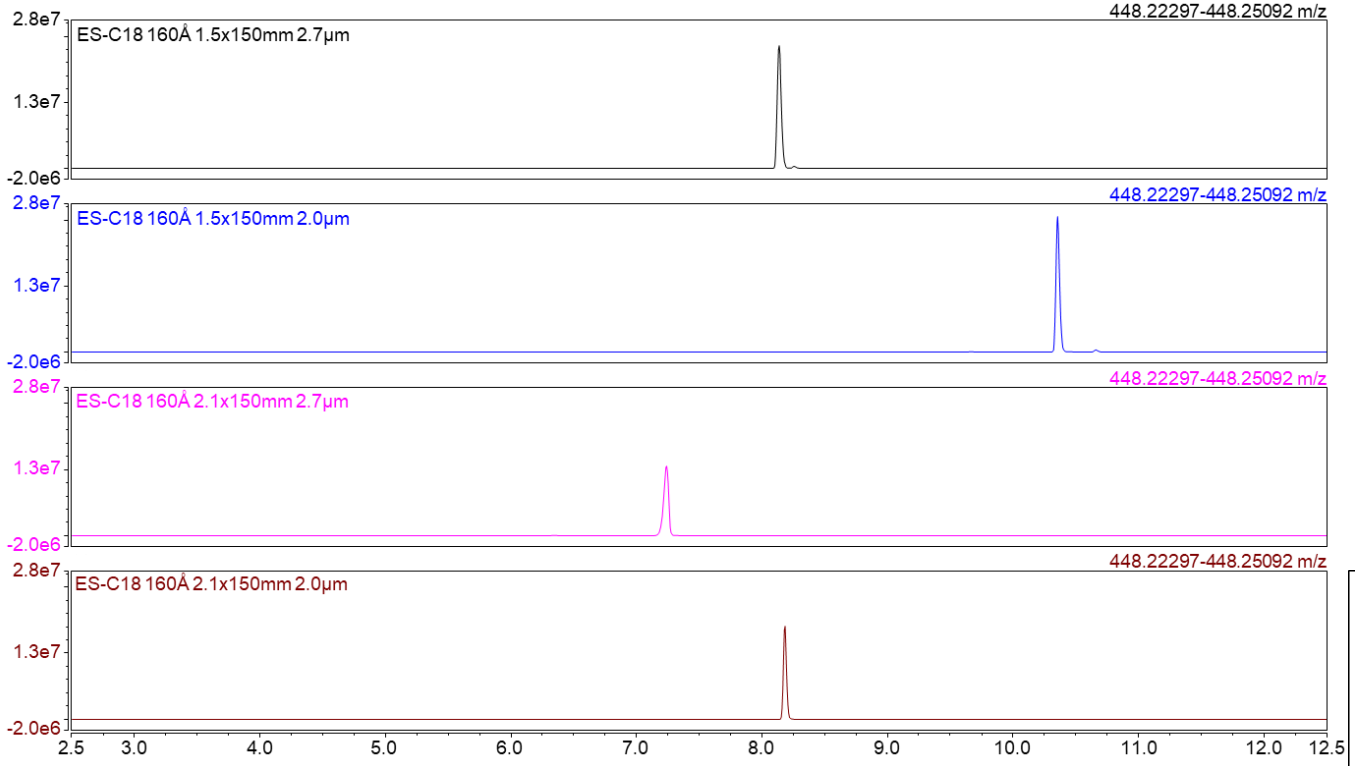
Compare of 1.5ID and Particle Size

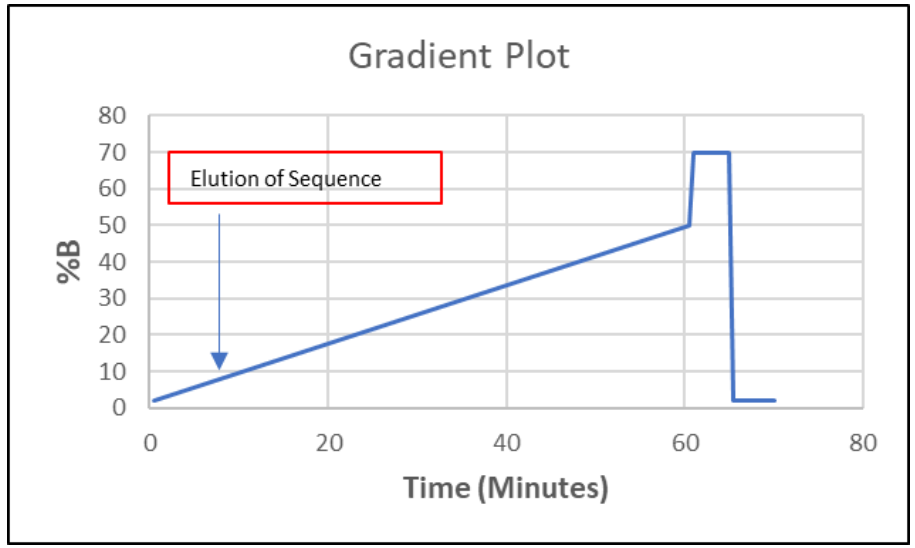


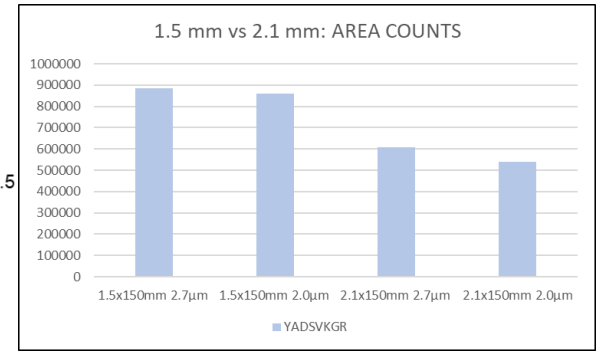


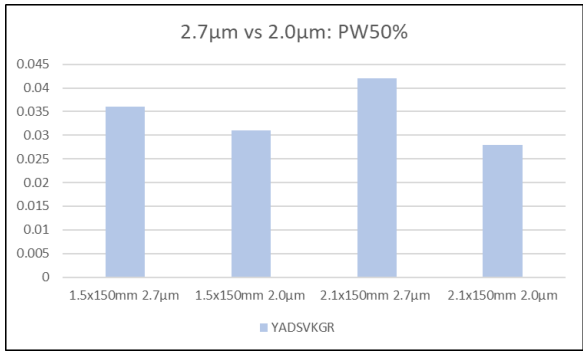
Closer Look at Sequences

YADSVKGR Sequence



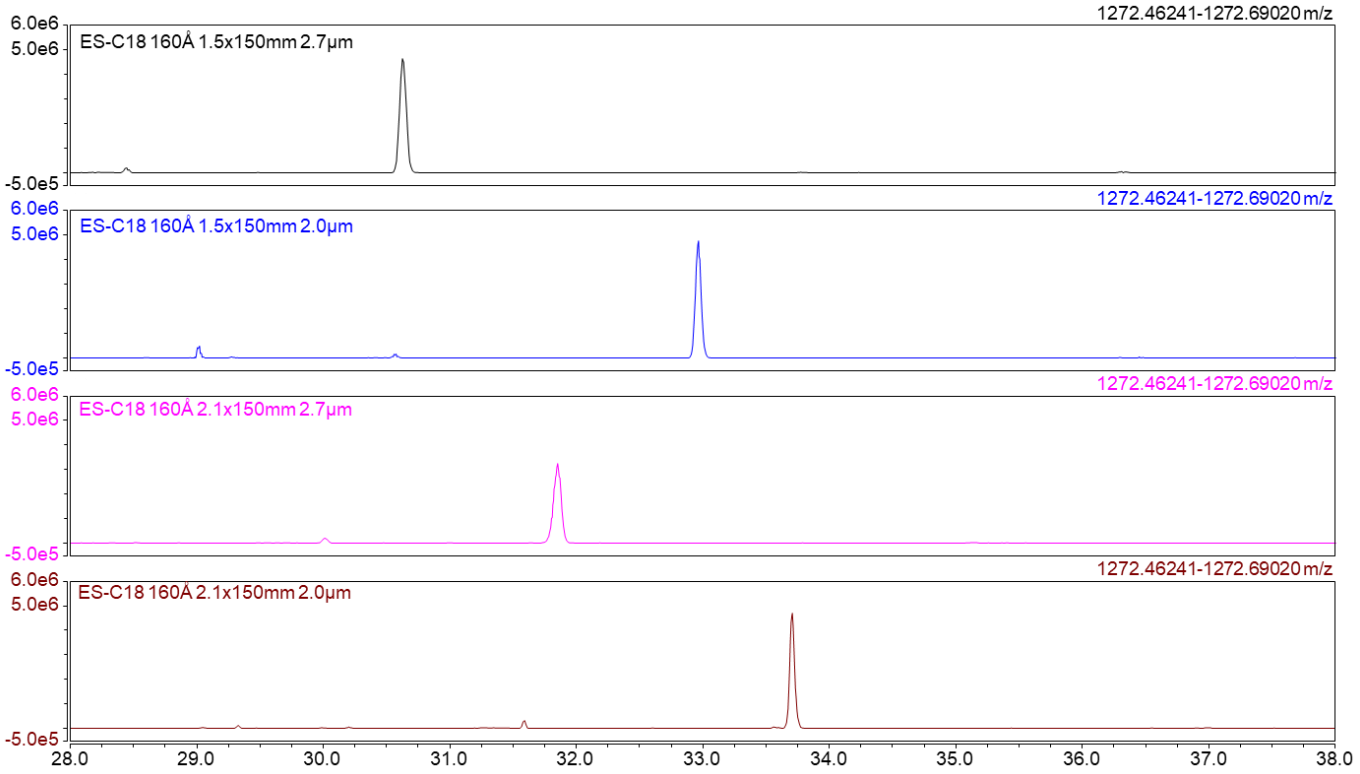


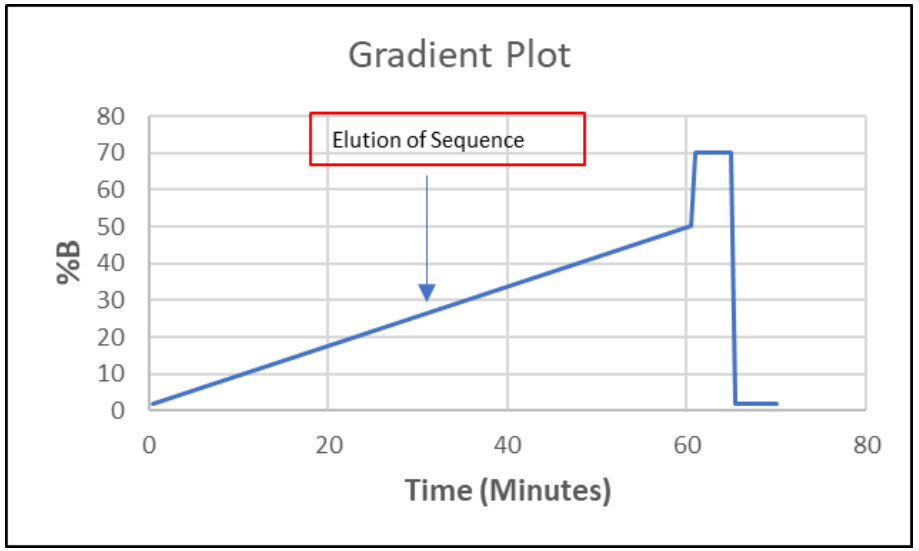


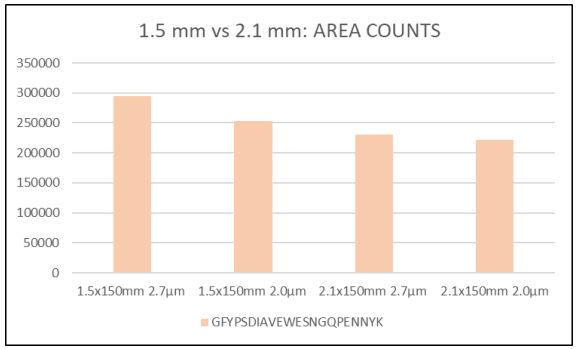


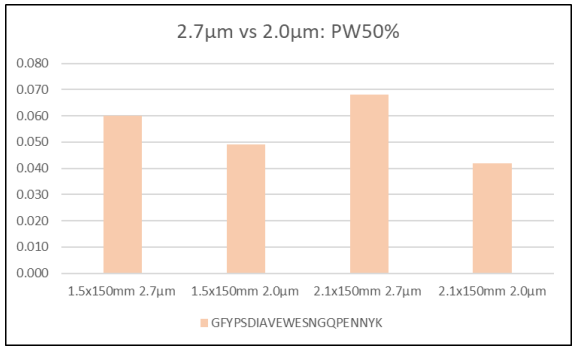
Closer Look at Sequences

GFYPSDIAVEWESNGQPENNYK Sequence



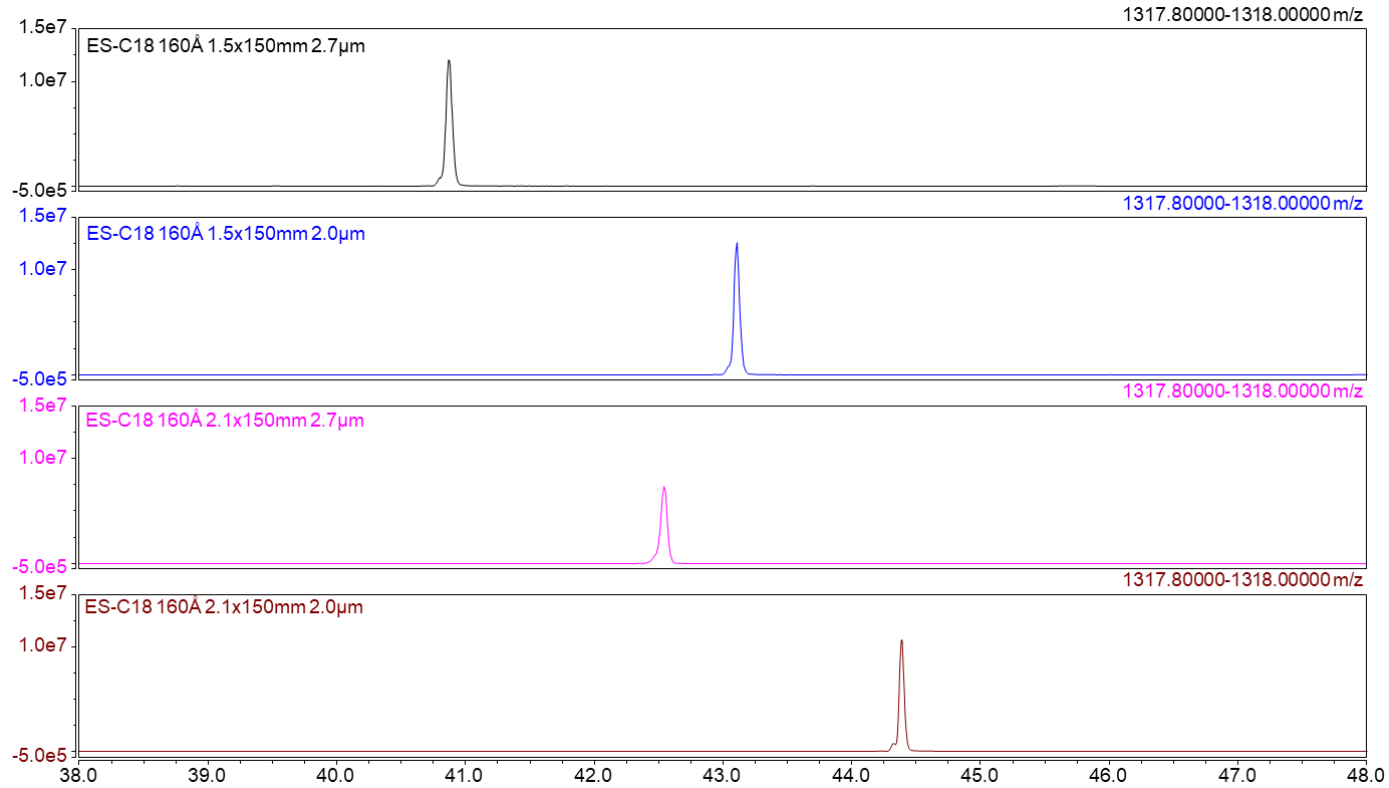


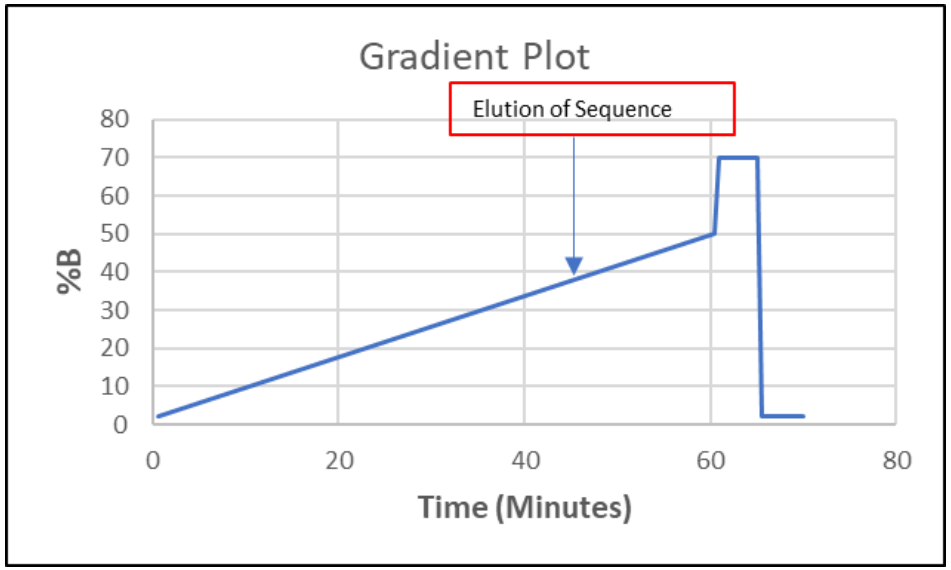


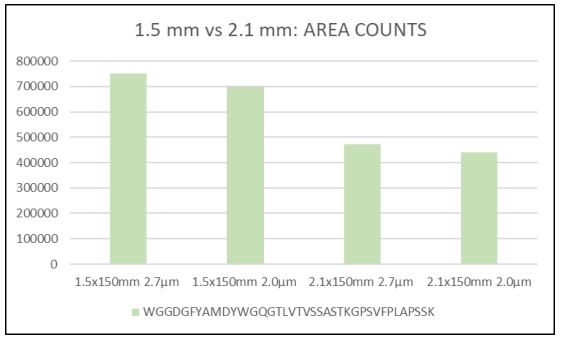


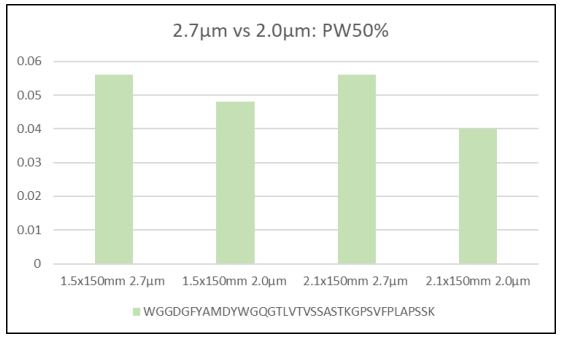
Closer Look at Sequences

WGGDGFYAMDYWGQGTLVTVSSASTKGPSVFPLAPSSK Sequence

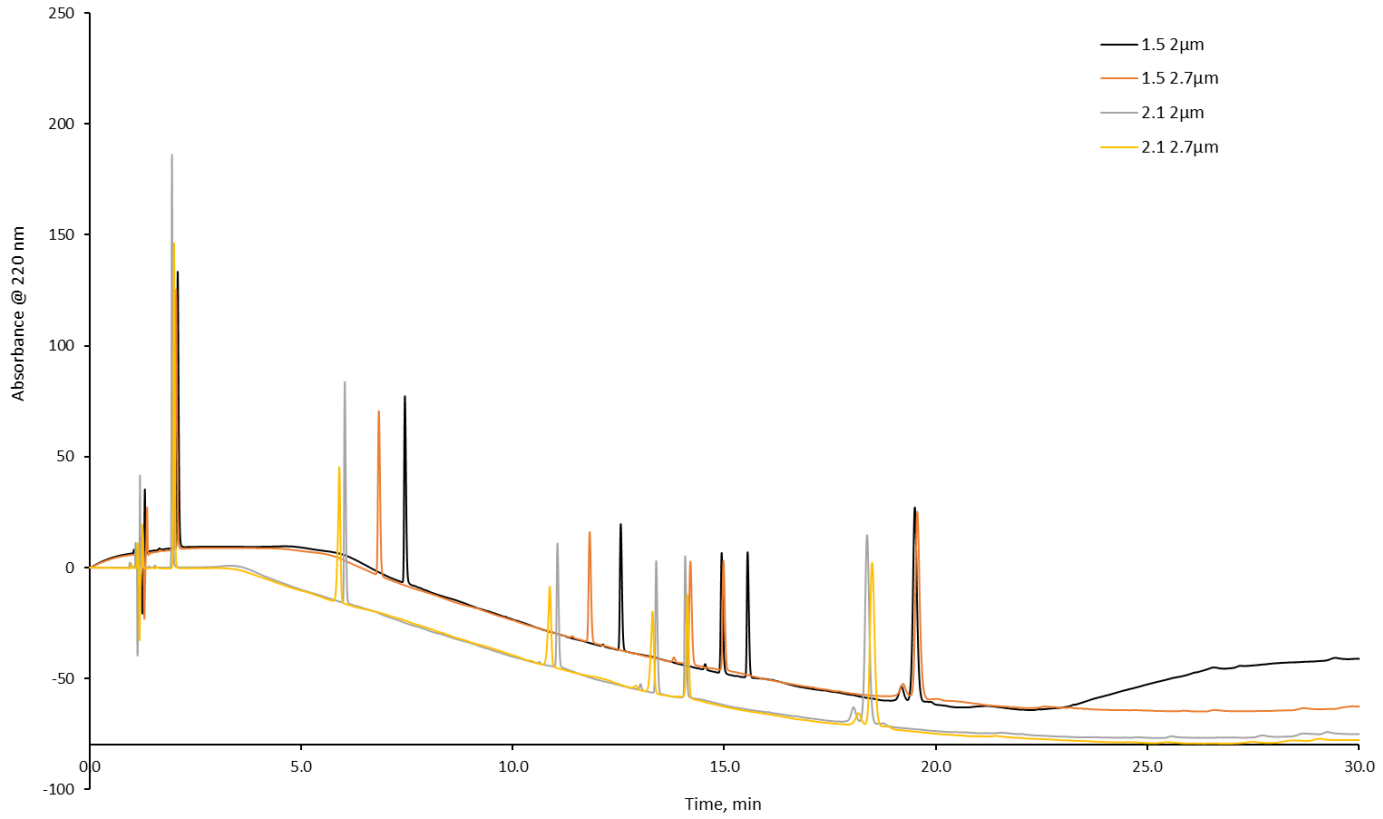


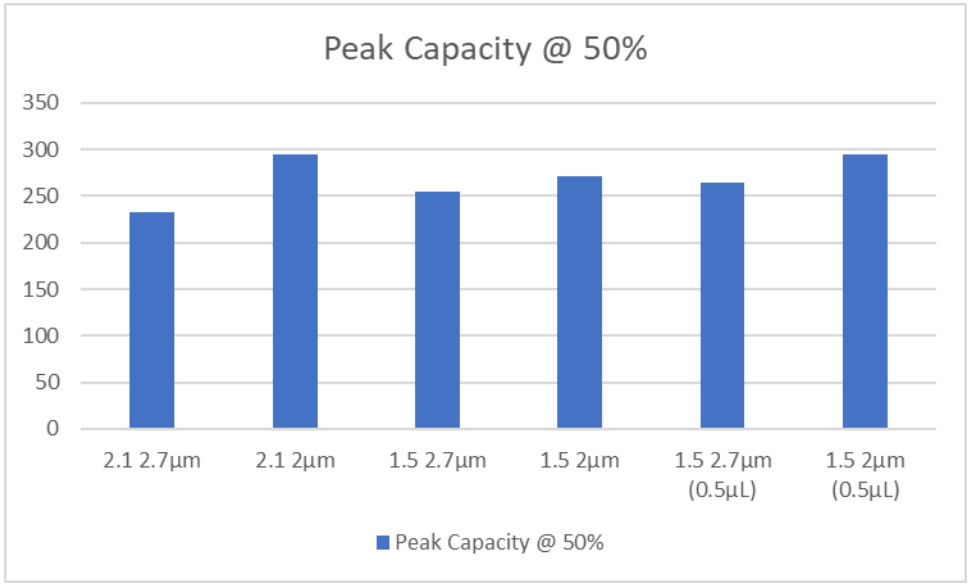






Peak Capacity and How it Changes



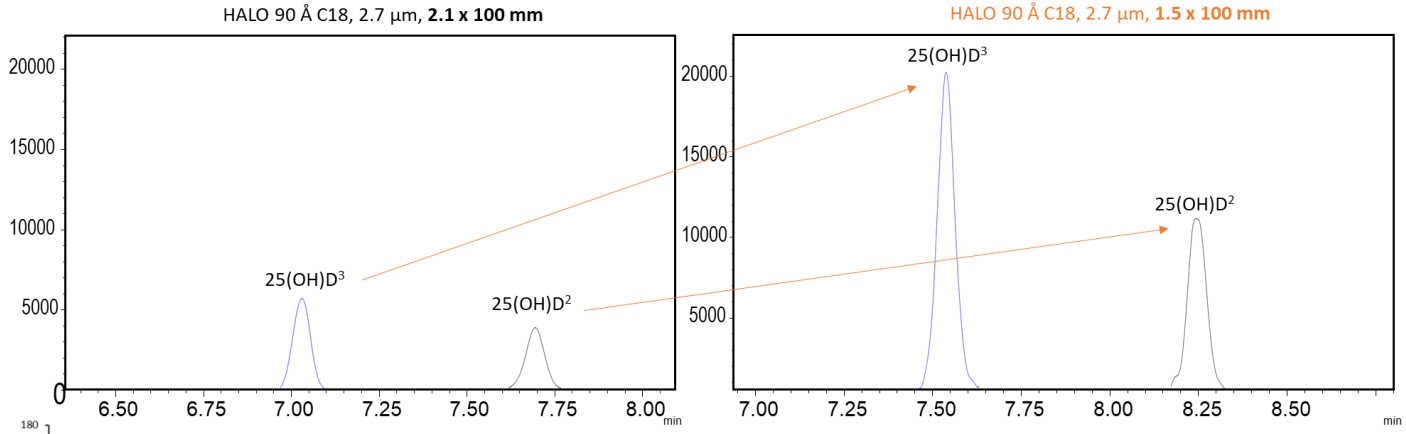


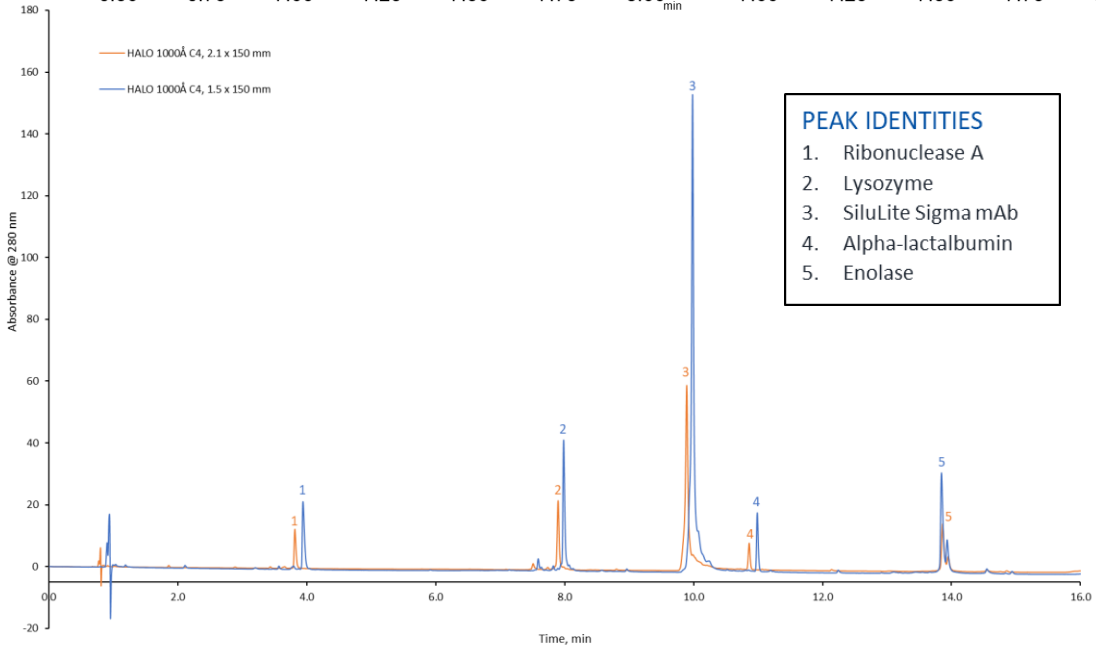
(RT P6 – RT P1)/ AVG (PW @50%) = Peak Capacity

Where Else Are These Changes Seen?

- Increased ionization efficiencies
  - Peptides and small bio molecules on MS
  - Small molecule on MS
- Decreased peak widths
  - Peptides and small bio molecules on MS
  - Small molecule on MS
  - Small molecule on UV
  - Bio separations on UV
- Increased sensitivity
  - Seen for all sample types under UV conditions

Other Examples

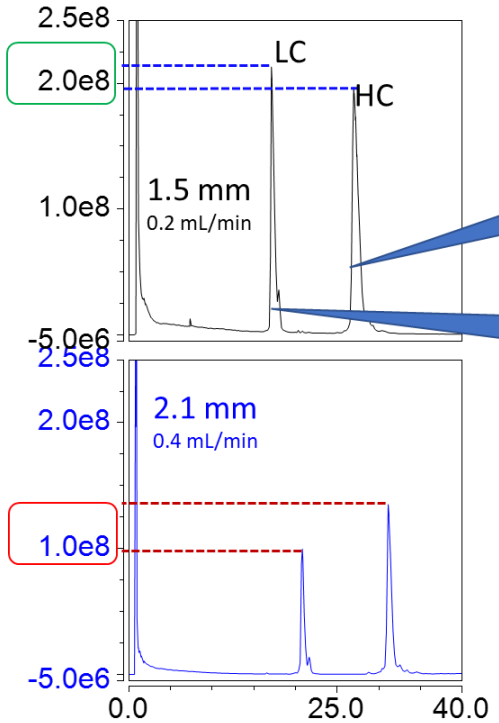




PEAK IDENTITIES

1. Ribonuclease A
2. Lysozyme
3. SiluLite Sigma mAb
4. Alpha-lactalbumin
5. Enolase

Reduced and Alkylated Trastuzumab using HALO 1000 Å Diphenyl



Heavy Chain (HC)
Area is 2.3x larger

Light Chain (LC)
Area is 2.7x larger

In Summary

- MS separation demands of biotherapeutic drugs has become more challenging
  - This requires LC/MS technology to evolve
  - Column technology can help by
    - Decreasing particle size
      - Increasing efficiency
      - Increased peak capacity for complex separations
    - Decreasing column ID
      - Increasing sensitivity/ionization
      - Reducing solvent usage

- These perks are not for MS only
  - UV separations receive the perks of smaller IDs and particle sizes

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