Nano-Capillary HPLC with Fused-core Particles for Proteomics Applications

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Purpose of Study

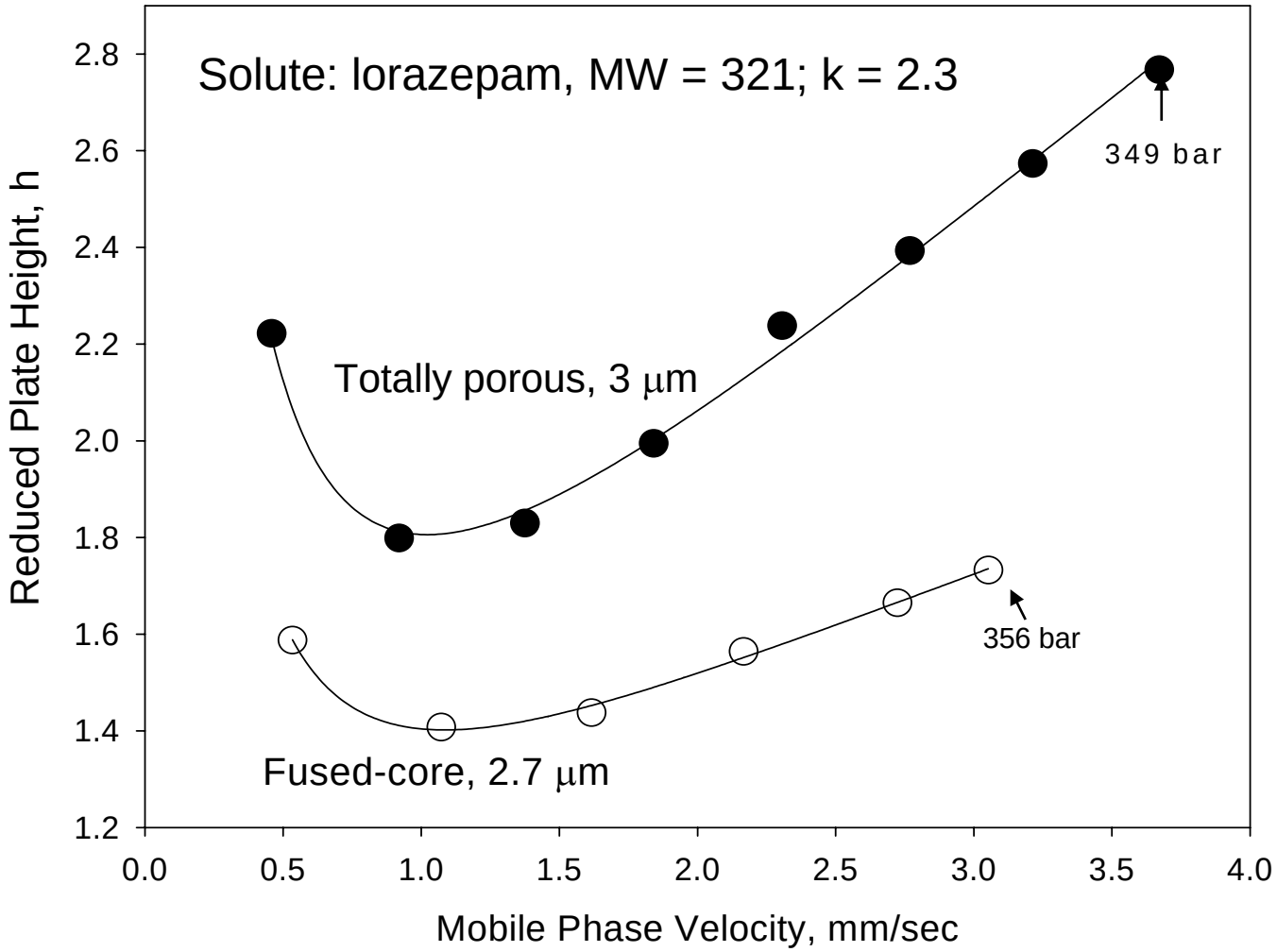
Illustrate the characteristics of superficially porous fused-core particles for high-efficiency separations in proteomic applications by capillary HPLC

Characteristics of Halo Fused-core Particles

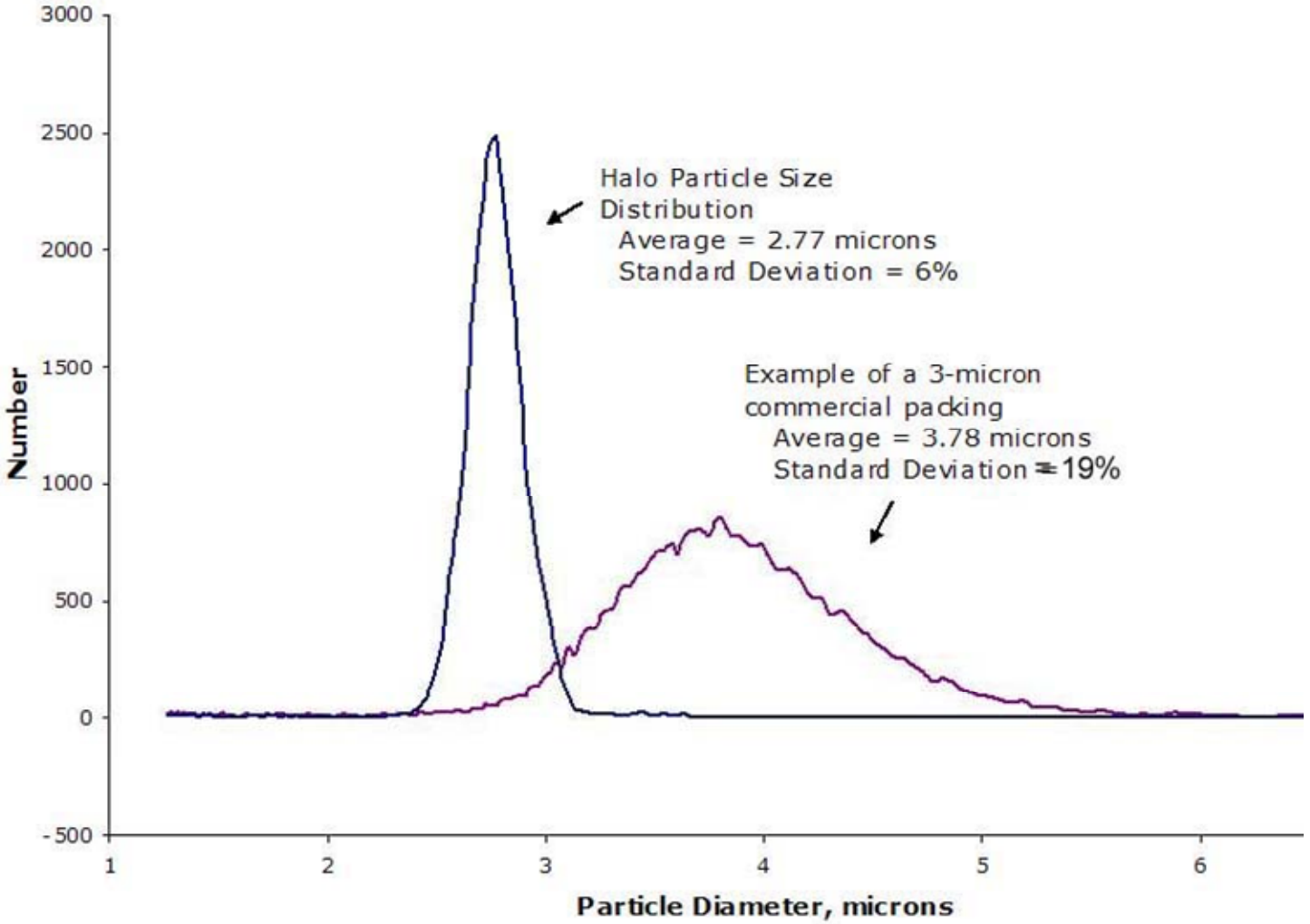
Silica ----------High-purity Type B
Overall diameter----------2.7 um
Solid core diameter----------1.7 um
Porous shell thickness----------0.5 um
Ave. pore diameter----------9 nm
Total surface area, nitrogen---150 sq.m/g
Porous shell surface area-----220 sq.m/g
Pore volume----------0.27 mL/g
Particle density----------1.3 cc/g
Particle porosity----------0.37

Fused-core *vs.* Totally Porous Particles

Columns: 4.6 x 150 mm; Temperature: 22 ^{o}C
Halo C18, 2.7 μm; 41/59 ACN/0.02 M phosphate buffer, pH 3.5
Ace C18, 3.0 μm; 43.5/56.5 ACN/0.02 M phosphate buffer, pH 3.5

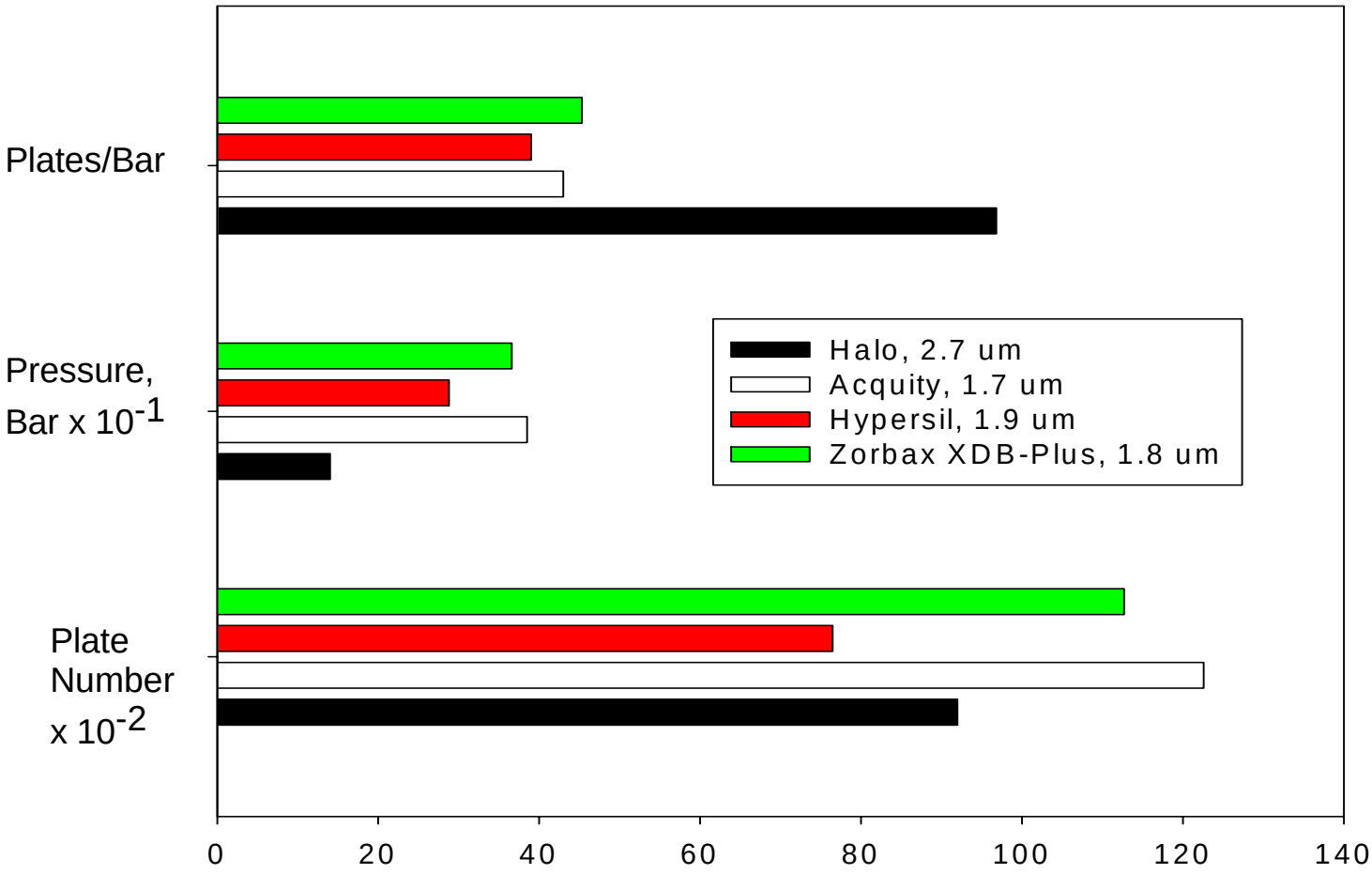


Particle Size Distributions



Performance of Small-particle Columns

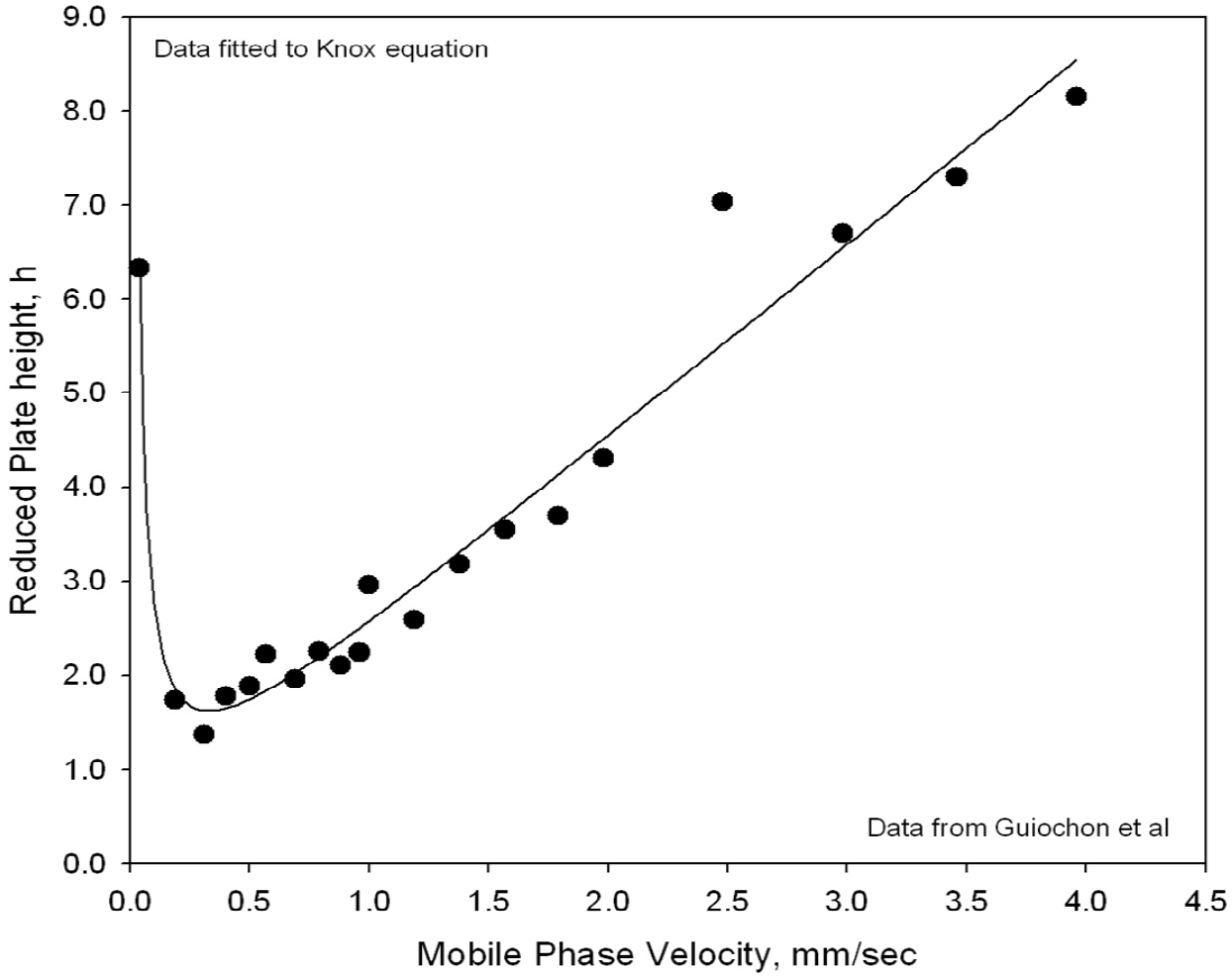
Columns: 50 x 2.1 mm; solute: acenaphthalene; Temp. 25 °C
Mobile phase: 70% ACN/30% water; Agilent 1100
All data at the plate height minimum

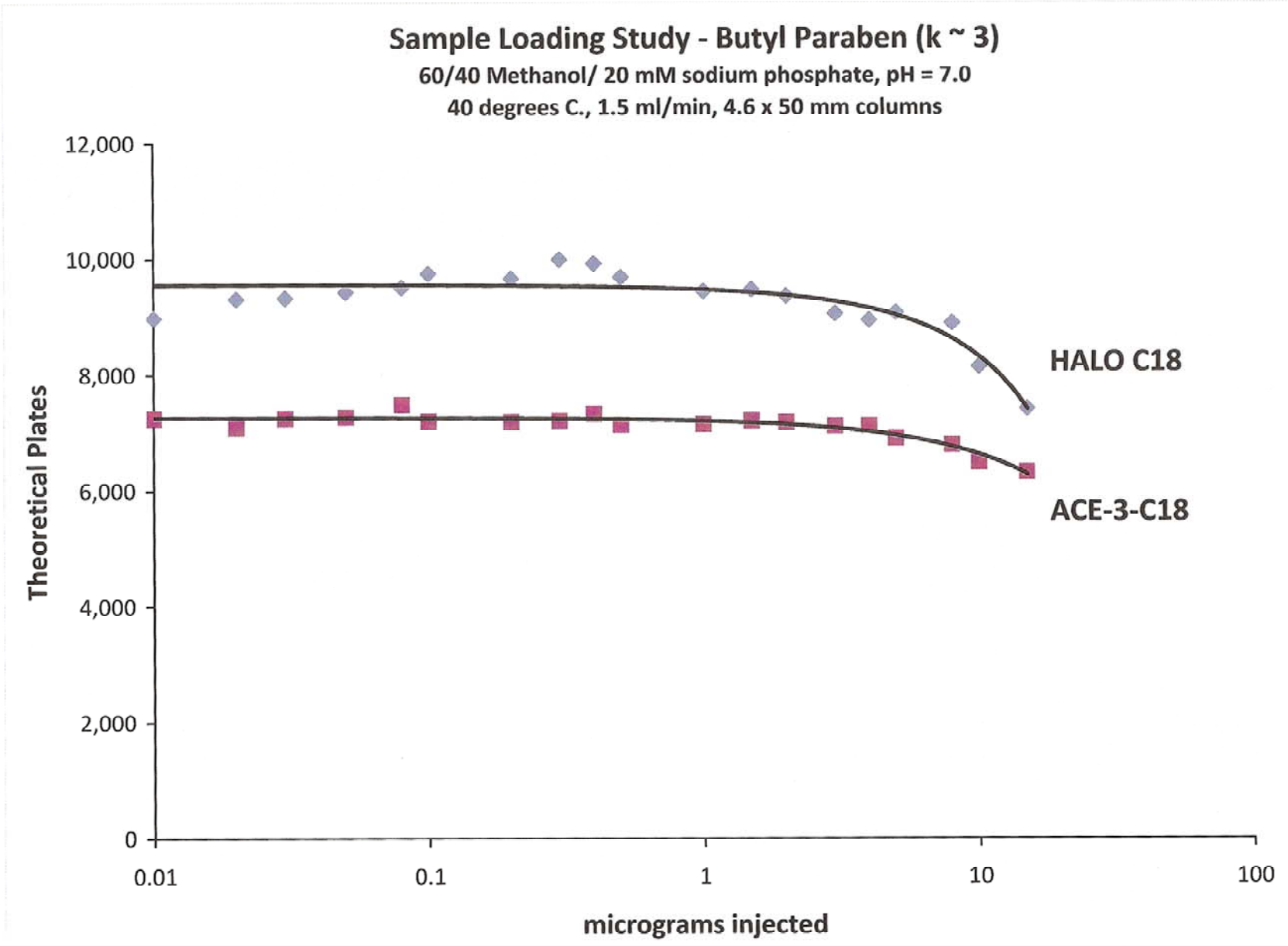


Van Deemter Plot for Kallidin, 1205 Da

Column: 50 x 4.6 mm Halo C18; Temperature: 60°C

Mobile Phase: water/ACN/TFA/ 82/18/0.1 v/v %




Sample Loading Study - Butyl Paraben (k ~ 3)
60/40 Methanol/ 20 mM sodium phosphate, pH = 7.0
40 degrees C., 1.5 ml/min, 4.6 x 50 mm columns
Theoretical Plates
12,000
10,000
8,000
6,000
4,000
2,000
0
0.01
0.1
1
10
100
micrograms injected
HALO C18
ACE-3-C18

Four Tryptic Peptides on Halo C18 Nano Column

Conditions:

Column - 0.1 x 150 mm 2.7u 90A Halo C18
Solvent A - 0.1% Formic Acid in Water
Solvent B - 100% Acetonitrile
Gradient - 5-45% B in 20 minutes
Flow Rate - 500 nl/min
Pressure - 2250 PSI
Temp - Room Temperature

Sample:

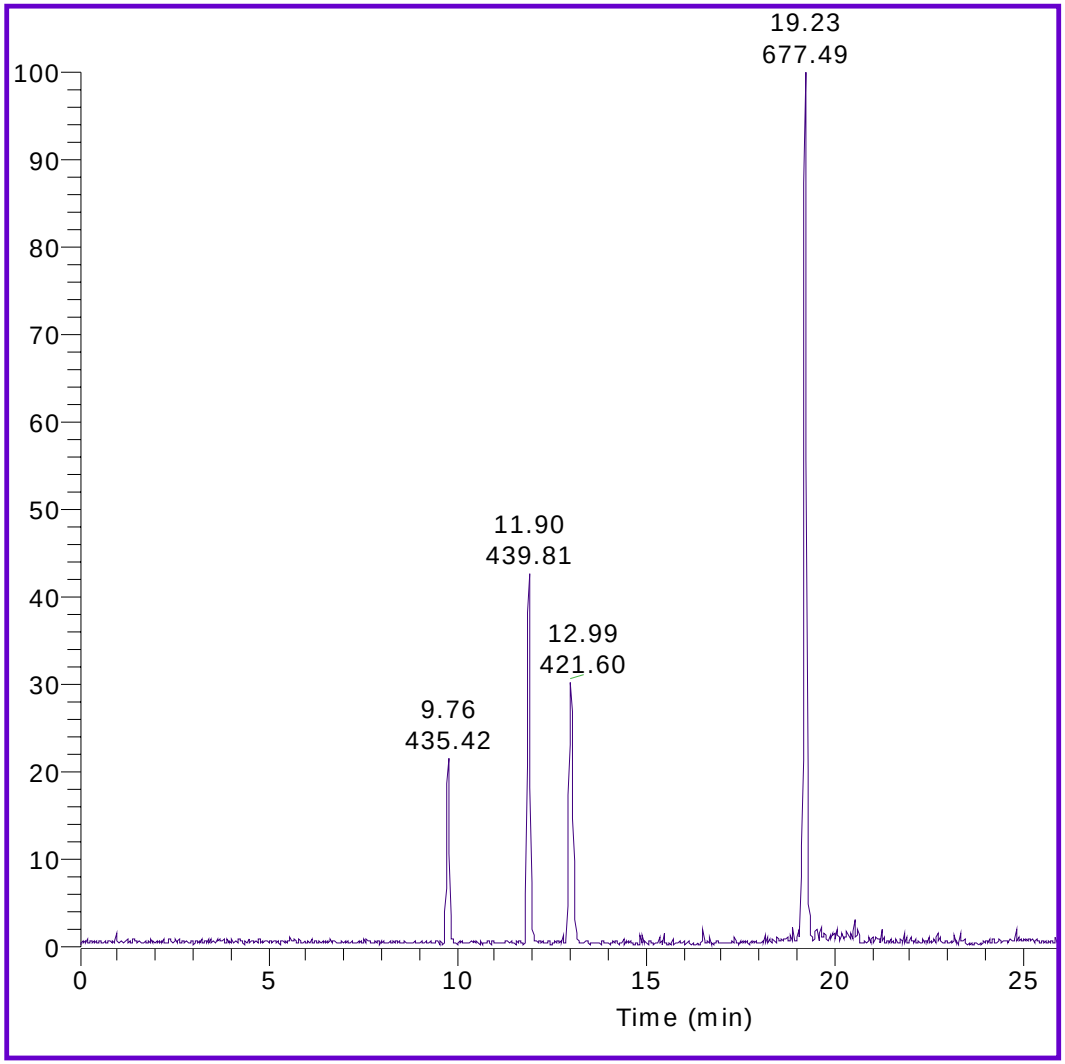
Volume - 100 nl
Amount - 100 fmol each peptide

Peptides:

TGGFLRR	**(MW - 869)**	**MH^{2+} - 435**
YIYGSFK	**(MW - 877)**	**MH^{2+} - 439**
ISRPPGFSPFR	**(MW - 1262)**	**MH^{3+} - 421**
GFVFTLTVPSGR	**(MW - 1353)**	**MH^{2+} - 677**

Instrumentation:

HPLC - Paradigm MS2-NC MDLC
ESI - ADVANCE Nanospray Source
MS - LCQ Deca MS



Six Bovine Protein Digest on Halo C18 Column

Conditions:

Column - 0.2 x 150 mm 2.7u 90A Halo C18
Solvent A - 0.1% Formic Acid in Water
Solvent B - 100% Acetonitrile
Gradient - 5-45% B in 80 minutes
Flow Rate - 1 ul/min
Pressure - 1270 PSI
Temp - Room Temperature

Sample:

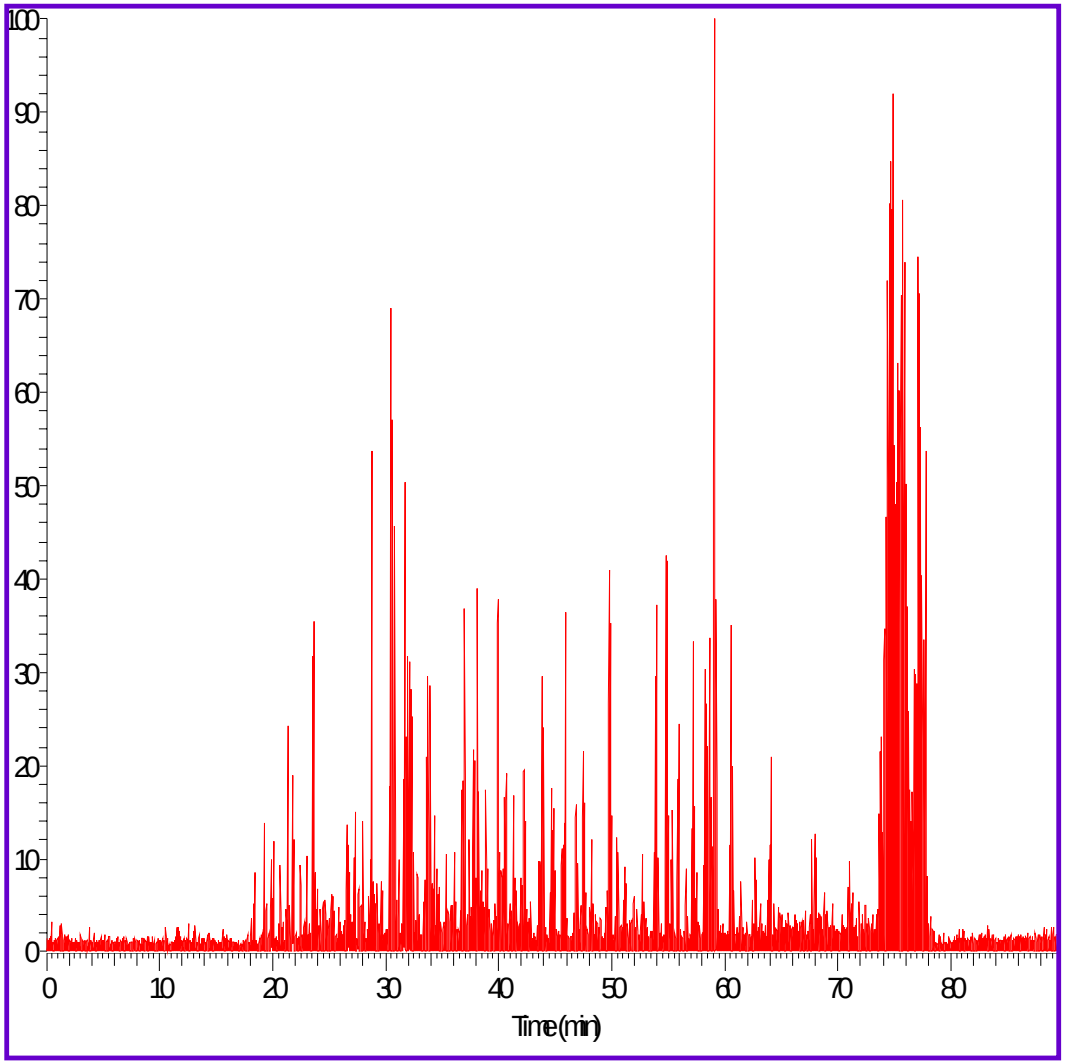
Volume - 100 ul (Trap Column Load)
Amount - 10 fmol each protein digest

Proteins:

Beta Lactoglobin	**(MW - 18,300)**
Alpha Casein	**(MW - 23,600)**
Carbonic Anhydrase	**(MW - 29,200)**
Glutamate Dehydrogenase	**(MW - 55,500)**
Serum Albumin	**(MW - 69,300)**
Lactoperoxidase	**(MW - 79,800)**

Instrumentation:

HPLC - Paradigm MS4-NC MDLC
ESI - ADVANCE Nanospray Source
MS - LCQ Deca MS/MS



Robustness of Halo C18 Capillary Column

Conditions:

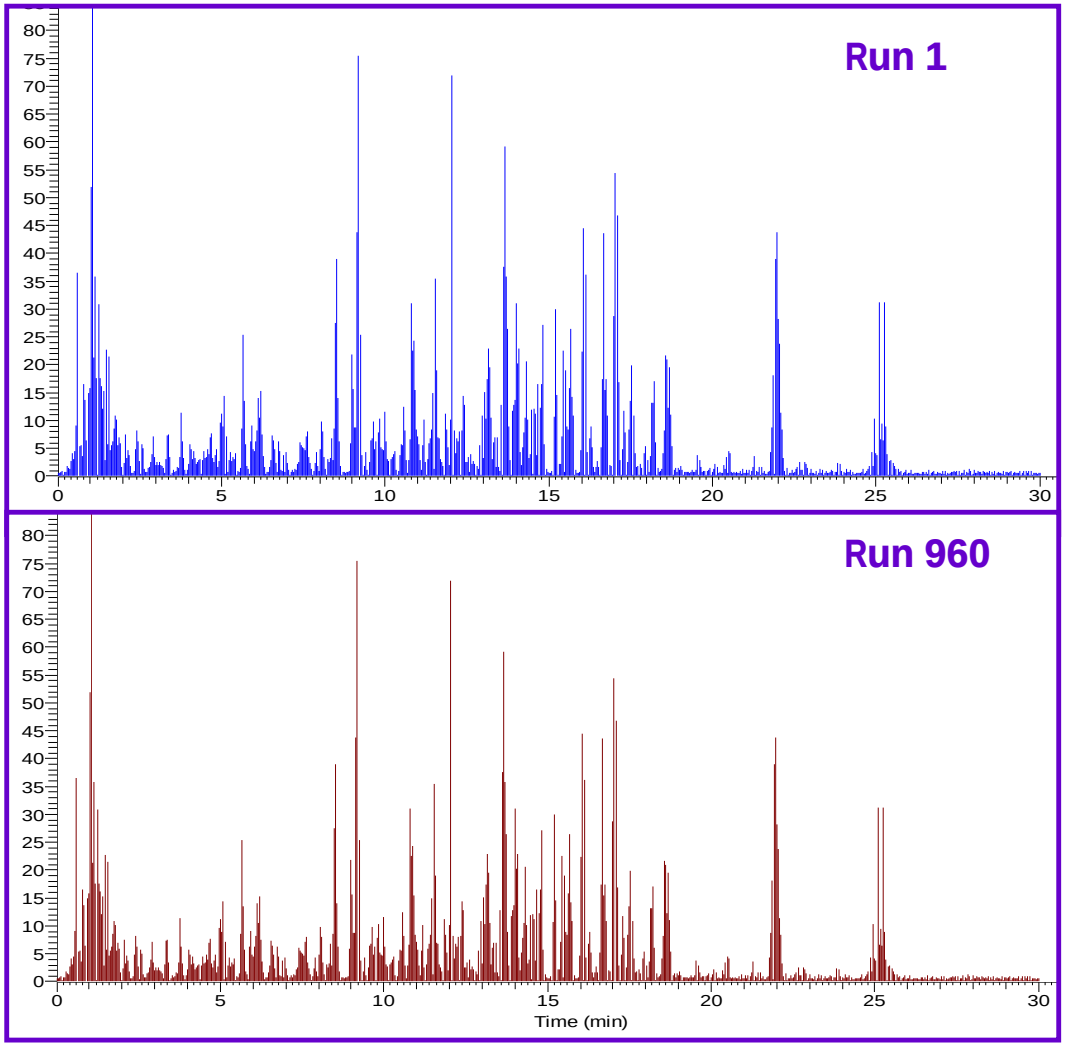
Column - 0.2 x 150 mm 2.7u 90A Halo C18
Solvent A - 0.1% Formic Acid in Water
Solvent B - 100% Acetonitrile
Gradient - 5-35% B in 20 minutes
Flow Rate - 2 ul/min
Pressure - 2340 PSI
Temp - Room Temperature

Sample:

Volume - 100 ul (Trap Column Load)
Amount - 100 fmol BSA Tryptic Digest

Instrumentation:

HPLC - Paradigm MS3-NC MDLC
ESI - ADVANCE Nanospray Source
MS - LCQ Deca MS/MS



Three Minute Biomarker Quantitation

Conditions:

Column - 0.2 x 50 mm 2.7u 90A Halo C18
Solvent A - 0.1% Formic Acid in Water
Solvent B - 100% Acetonitrile
Gradient - 5-45% B in 2 minutes
Flow Rate - 20 ul/min
Pressure - 4690 PSI
Temp - 40C

Sample:

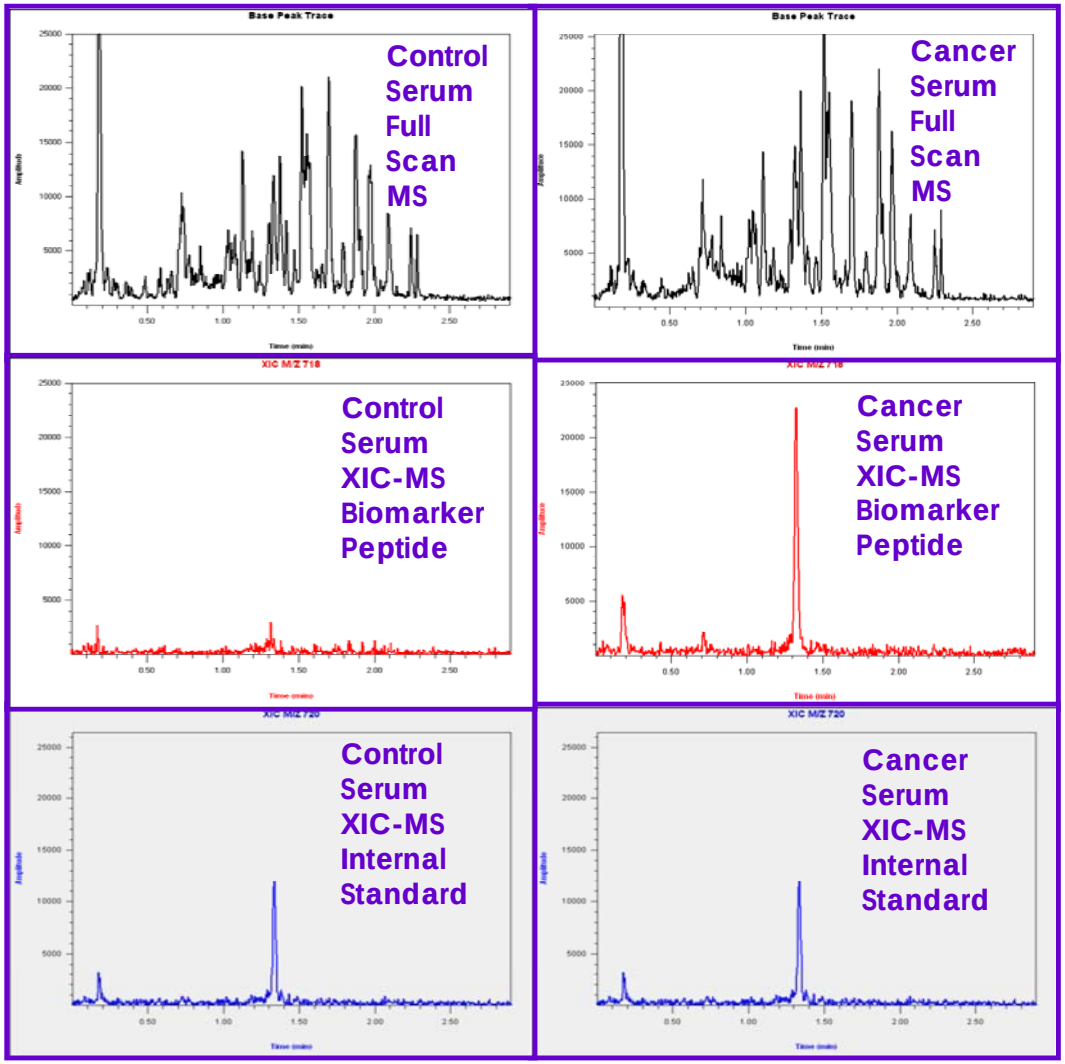
Volume - 100 ul (Trap Column Load)
Amount - 100 amol internal standard

Sample 1 - Prep Digest Control Patient Serum

Sample 2 - Prep Digest Cancer Patient Serum

Instrumentation:

HPLC - Paradigm MS4-NC MDLC
ESI - ADVANCE Nanospray Source
MS - Paradigm MX1 TOF MSD



20 Intact Proteins (5-80 kD) on Halo C8 vs Micra C18

Conditions:

Column 1 - 1x50 mm 2.7u 90A Halo C8
Pressure - 1280 PSI

Column 2 - 1x50 mm 1.5u NPS Micra C18
Pressure - 4130 PSI

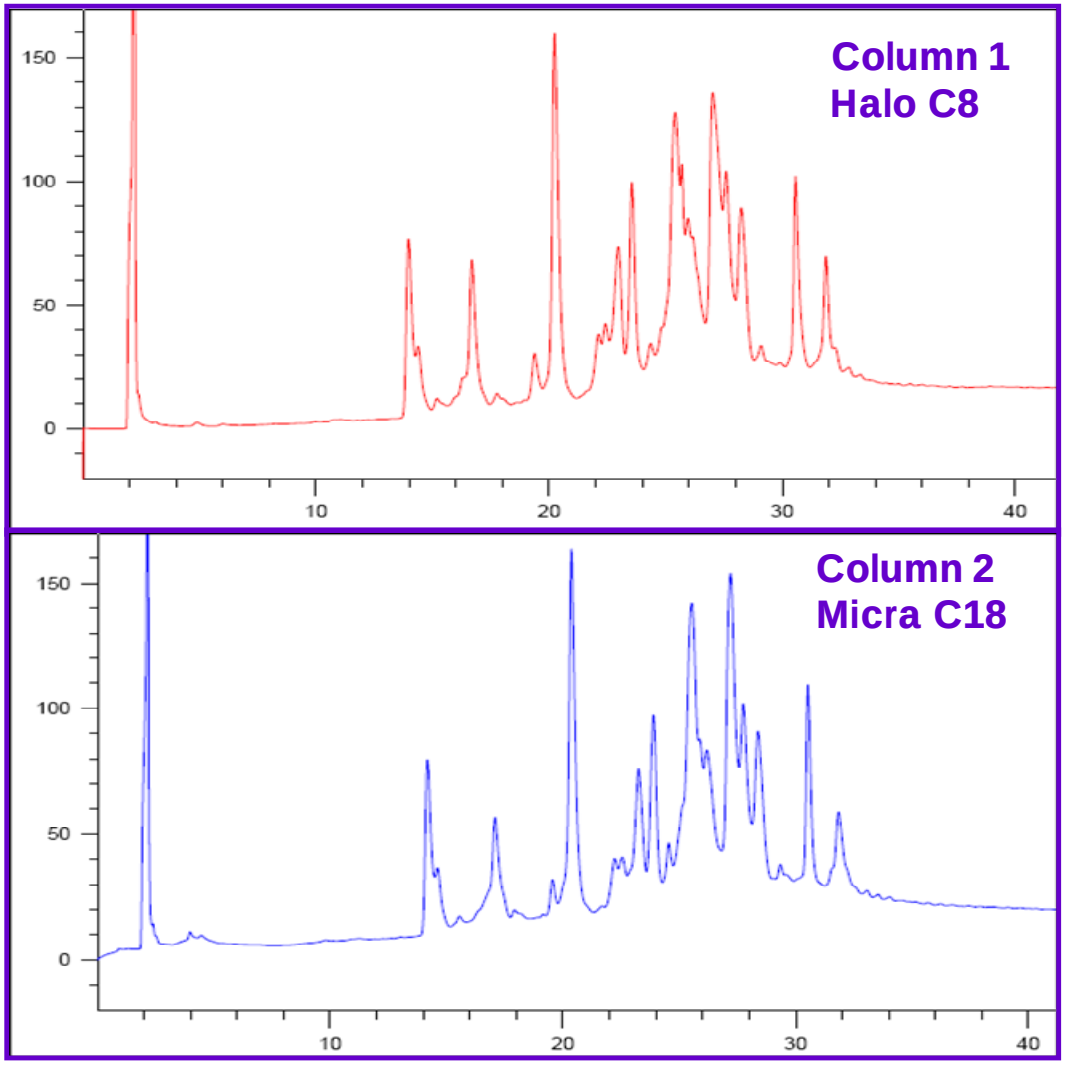
Solvent A - 0.1% TFA in Water
Solvent B - 0.1% TFA in Acetonitrile
Gradient - 5-65% B in 30 minutes
Flow Rate - 50 ul/min
Temp - 40 C

Sample:

Volume - 10 ul
Amount - 1 pmol each protein

Instrumentation:

HPLC - Paradigm MS2-NC MDLC
ESI - ADVANCE Nanospray Source
MS - Paradigm MX1 TOF MSD



Conclusions

- Highly efficient separations of proteomic samples are feasible with fused-core particles in capillaries
- Rugged capillary columns of fused-core particles provide highly reproducible separations
- Capillaries with fused-core particles appear useful for the rapid screening of biomarkers using LCMS
- Other types of Halo fused-core columns allow additional selectivity options for difficult separations
- Fused-core particles with larger pores are anticipated for separating larger peptides and proteins