Advanced Core Shell Particle Technologies Applied to (Bio) Analytical Separations

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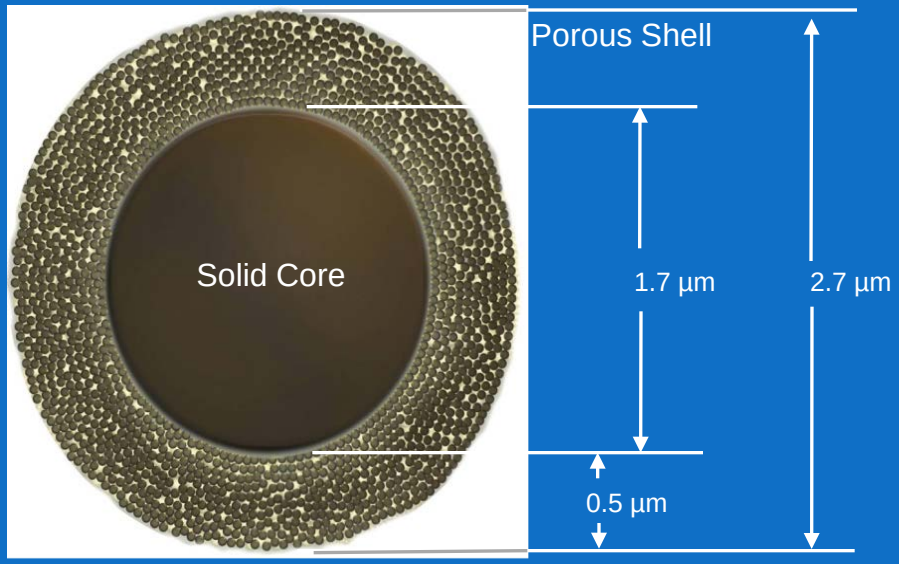
Agenda

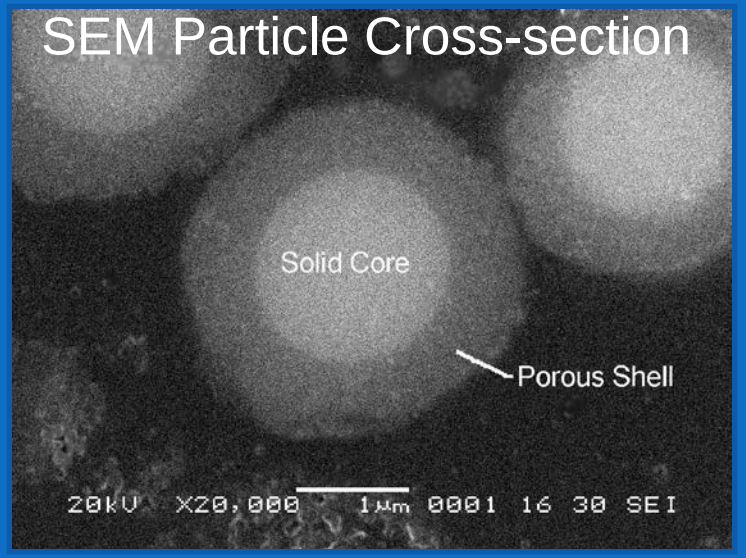
- Fused-Core Particles for highly efficient separations
- Products for Biomolecule Separations
  - Reversed Phase Materials
    - 160 Å Peptide
    - 400 Å Protein
  - Polar HILIC (Penta-HILIC) Materials
- Recent Biomolecule Application Examples

Faster HPLC Separations

- Smaller Particle Packed Beds
  - Totally Porous (including flow through)
  - Not Porous (Pellicular)
  - Partly Porous (Superficially Porous)
- Monolithic Materials
- Open Tubular Columns (channels)

Superficially Porous (Fused-Core®) Particles





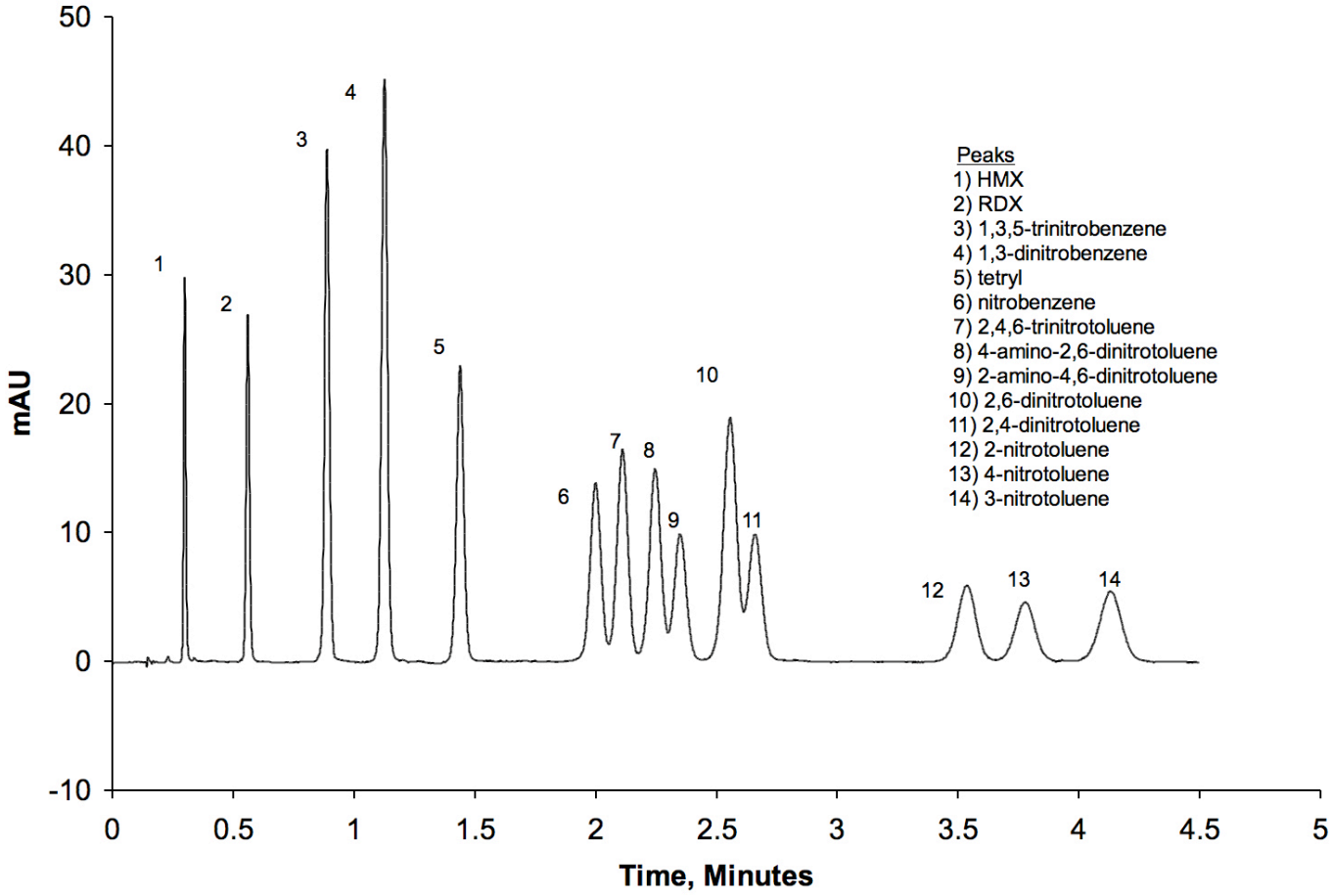
- Low back pressure due to the particle design (solid core with a porous shell)
- No need for specialized HPLC equipment
- Not necessary to filter samples and mobile phase since frits are not as small as needed for sub-2-µm
- High resolution is maintained at high flow rates (flat C-term in van Deemter plot)

SPP History!

Year	Who	Particle Name	Core (µm)	Shell (µm)	Total Size (µm)
2001	Agilent Technologies	Poroshell® 300	4.5	0.25	5.0
2007	Advanced Materials Technology	HALO®	1.7	0.5	2.7
2007	Supelco Sigma-Aldrich	Ascentis Express®	1.7	0.5	2.7
2009	Phenomenex	Kinetex®	1.9 1.25	0.35 0.23	2.6 1.7
2010	Agilent Technologies	Poroshell® 120	1.7	0.5	2.7

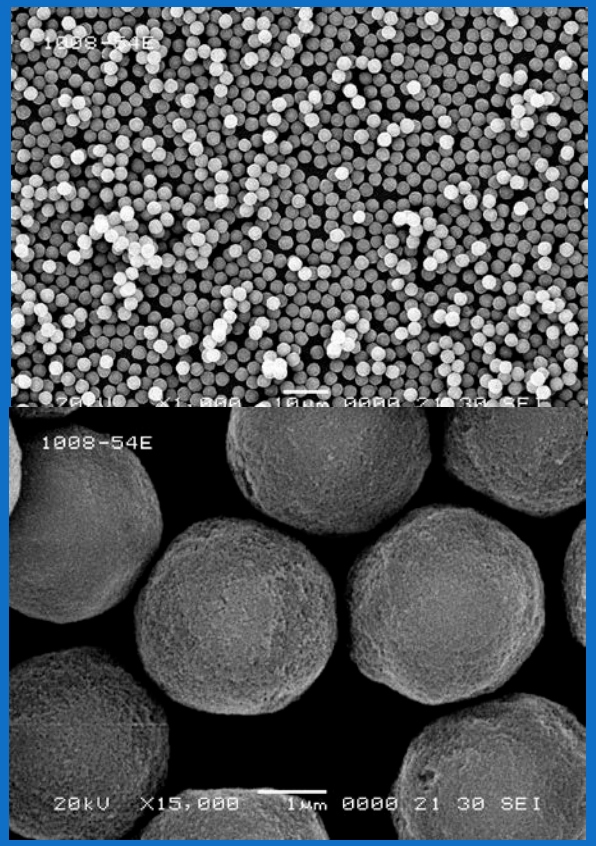
Separation of Explosives (c. 2007 EAS)

Column: 4.6 x 50 mm Halo C18; Mobile phase: 27% Methanol/73% Water; Temperature: 40ºC; Flowrate: 3.3 ml/min; Detector: UV @ 254 nm; Pressure: 343 bar; Agilent 1100

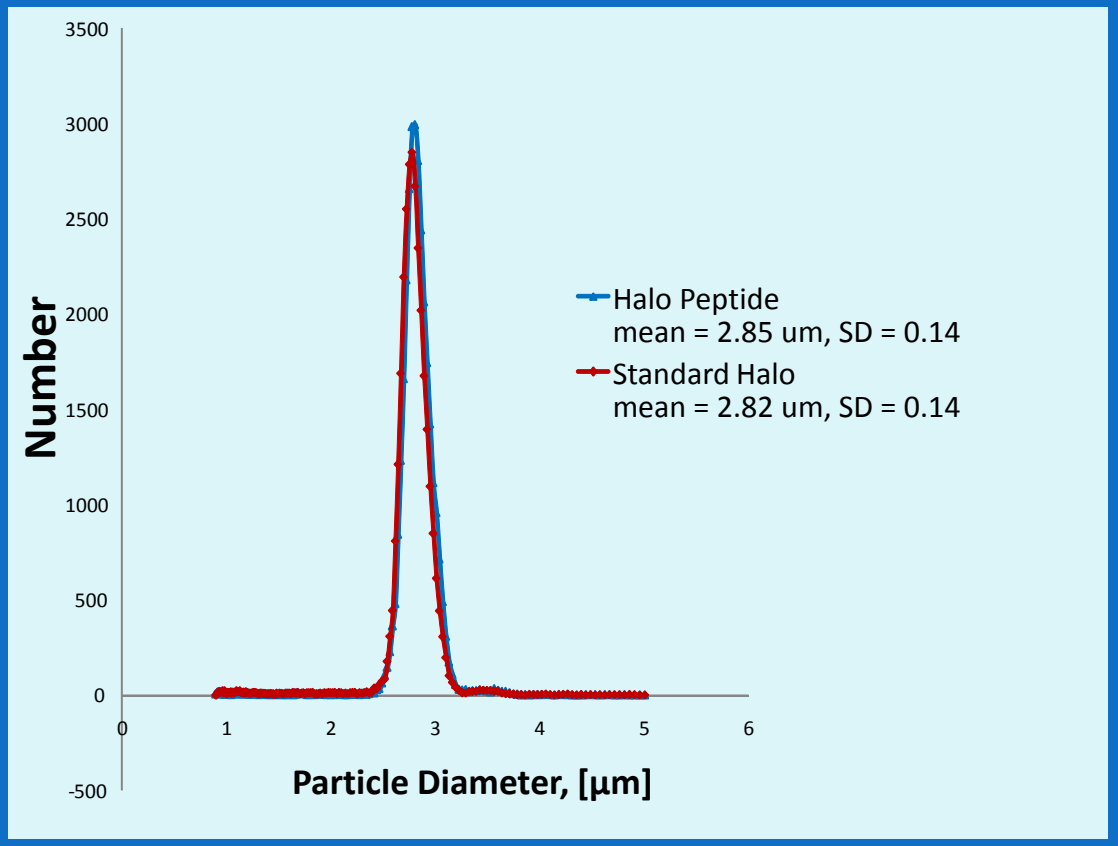


Fused-Core Particle Analysis

Electron Micrographs of Halo Peptide

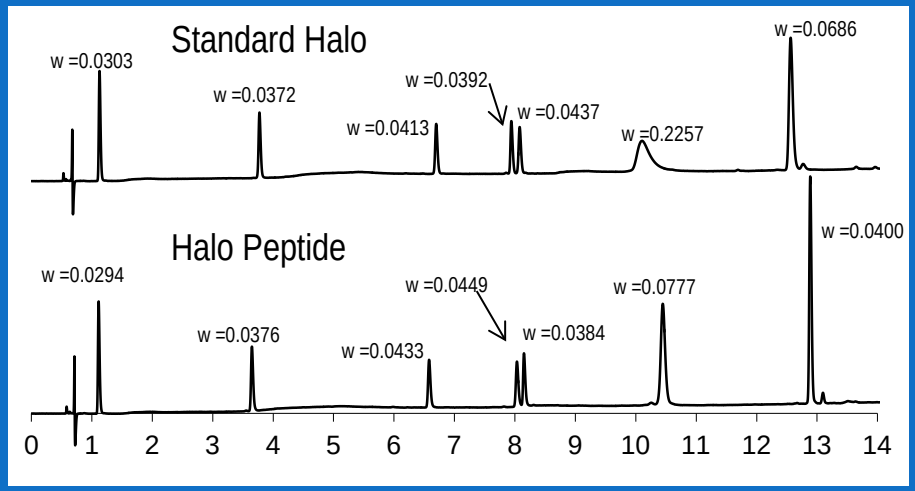


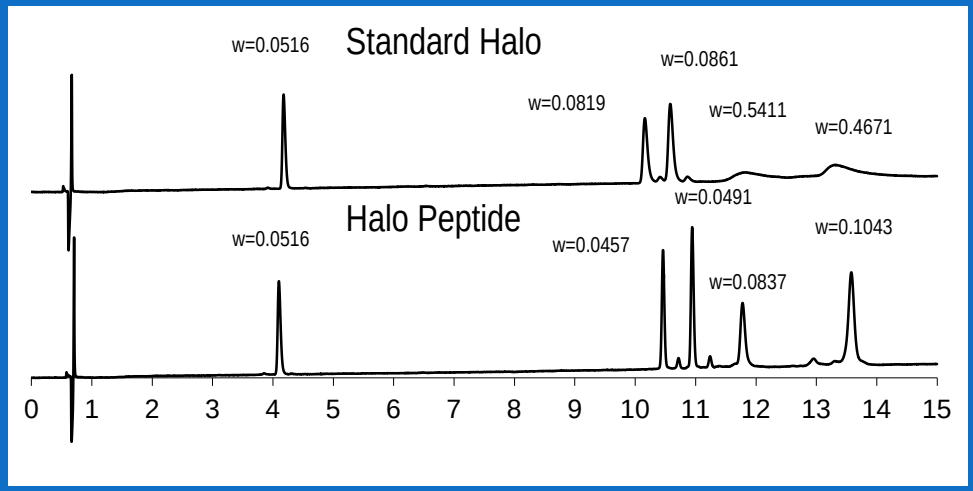
Standard Halo vs. Halo Peptide



Halo Peptide ES-C18
Protein and Peptide Separations

Column: 4.6 x 100 mm; Flow rate: 1.5 mL/min; Temperature: 30° C
A: 0.1% TFA/10% ACN, B: 0.1% TFA/70% ACN
Gradient: 0% to 50% B in 15 min.; Injection volume: 5 µL





Sample 1
Gly-Tyr, Val-Tyr-Val, Met-enk, Angiotensin II, Leu-enk
Ribonuclease, Porcine Insulin

Sample 2
Leu-enk
Bovine Insulin, Human Insulin, Cytochrome C, Lysozyme

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ORIGINAL ARTICLE

Fused-core particle technology in high-performance liquid chromatography: An overview

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Peak Identities:

1.	Ribonuclease A	13.7 kDa
2.	Bovine insulin	5.7 kDa
3.	Human insulin	5.8 kDa
4.	Cytochrome c	12.4 kDa
5.	Lysozyme	14.3 kDa

Bovine insulin
Human insulin
lysozyme
cytochrome *c*
ribonuclease A
90 Å pores

Column: 4.6 x 100 mm HALO C18 and
4.6 x 100 HALO Peptide ES-C18
Injection: 5 µL
Detection: 215 nm
Temperature: 30 °C

Flow rate: 1.5 mL/min
Mobile Phase A: water/0.1% TFA
Mobile Phase B: acetonitrile/0.1% TFA
Gradient: 25-42% B in 10 min

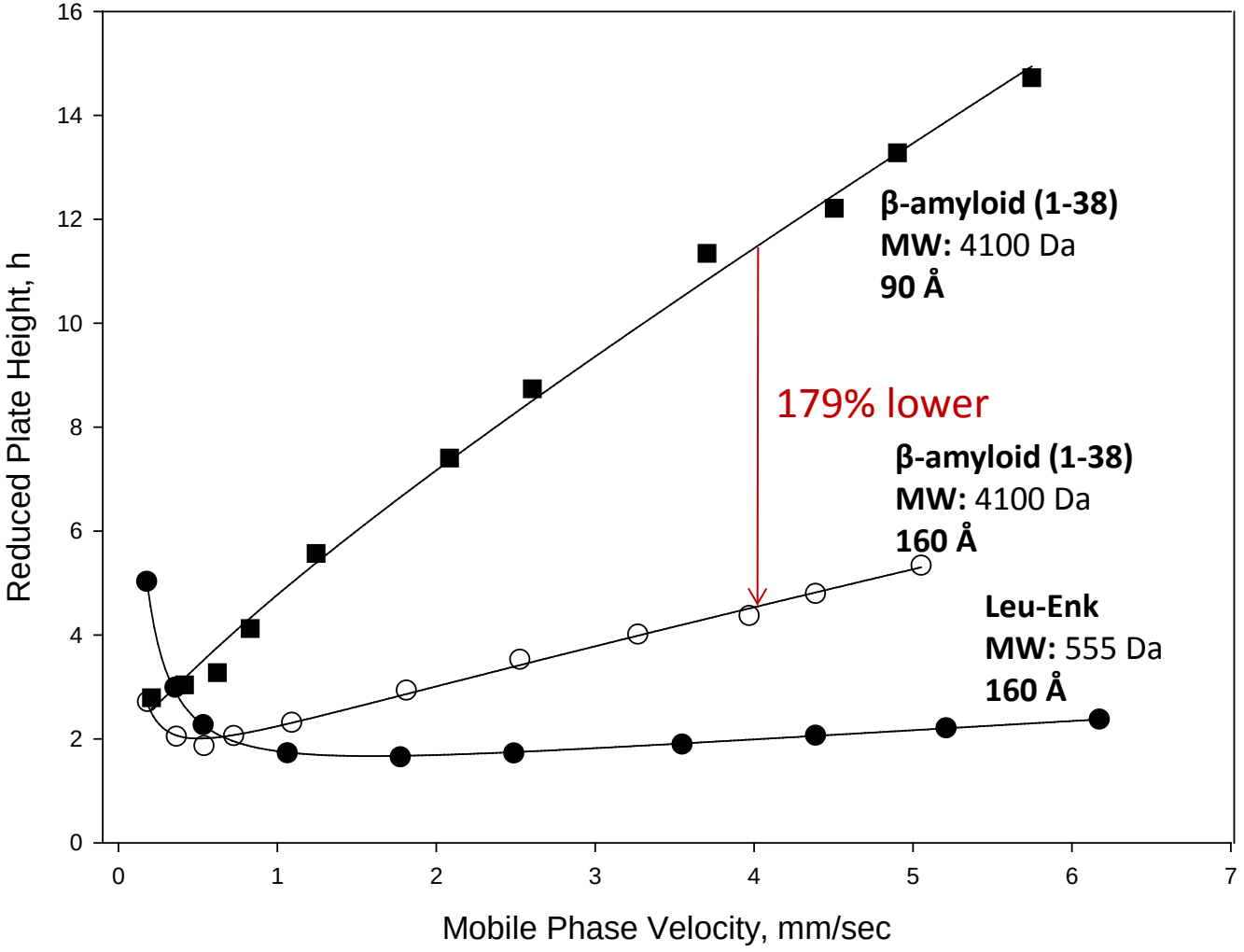
lysozyme
Bovine insulin
ribonuclease A
Human insulin
cytochrome *c*
160 Å pores

Absorbance (mAU)

0 1 2 3 4 5 6 7 8 9 10

Time (min.)

Effect of Pore Size on Efficiency



Columns: 4.6 x 100 mm HALO C18, 2.7 µm, 90 Å
4.6 x 100 mm HALO Peptide ES-C18, 2.7 µm, 160 Å

Mobile Phase: Leu-Enk: 21% ACN/79% Water/0.1% TFA
β-amyloid (1-38) 160 Å : 29% ACN/71% Water/0.1% TFA
β-amyloid (1-38) 90 Å : 27% ACN/73% Water/0.1% TFA

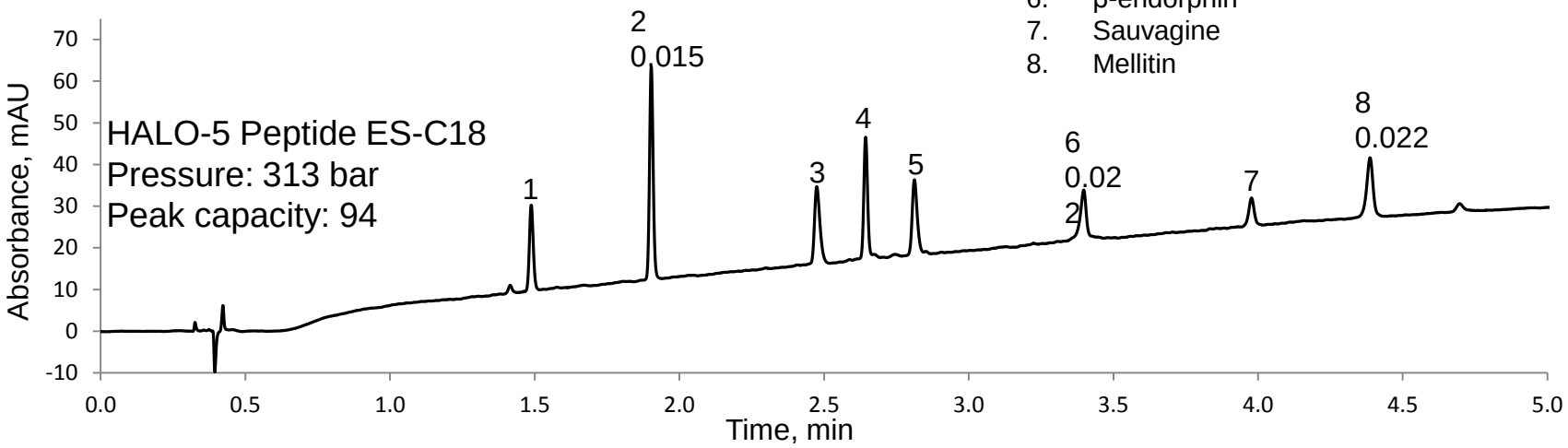
Temperature: 60 °C
Detection: 215 nm

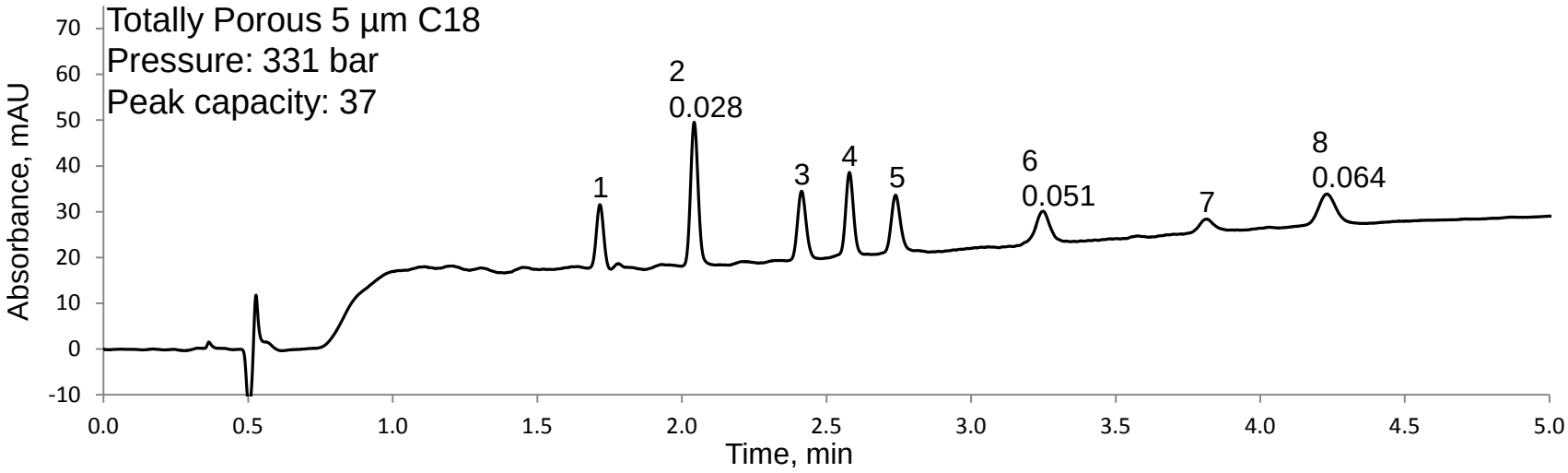
Peptide Separations: Fused-Core compared to Totally Porous

Columns: 4.6 x 150 mm
Instrument: Agilent 1100
Injection Volume: 5 µL
Detection: 215 nm
Temperature: 40 °C

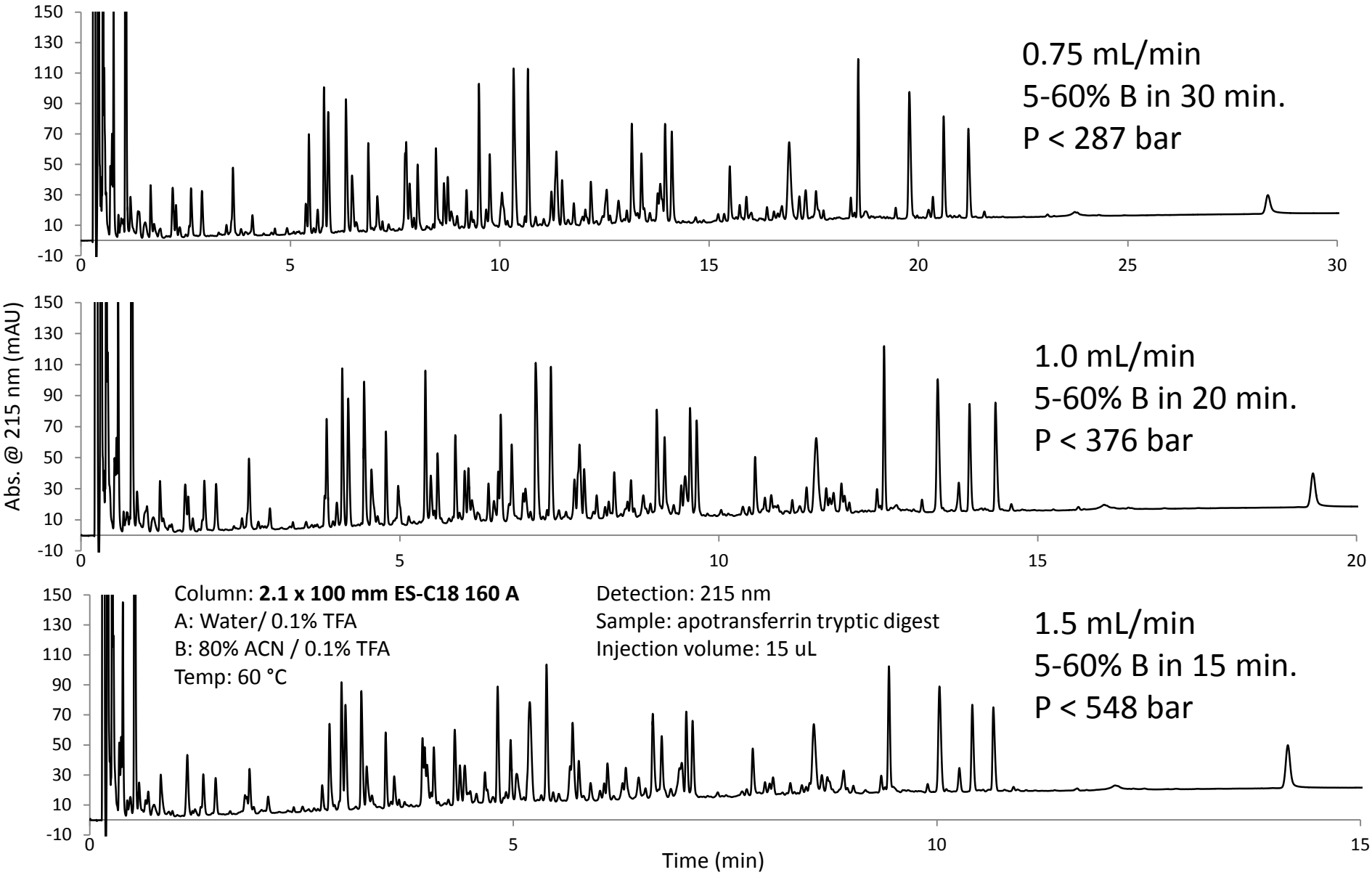
Flow rate: 4.0 mL/min
Mobile Phase A: water/0.1% TFA
Mobile Phase B: acetonitrile/0.1% TFA
Gradient: 5-60% B in 5 min

Peak Identities (in order):
1. Asp-Phe
2. Tyr-Tyr-Tyr
3. Bradykinin
4. Angiotensin II
5. Neurotensin
6. β-endorphin
7. Sauvagine
8. Mellitin



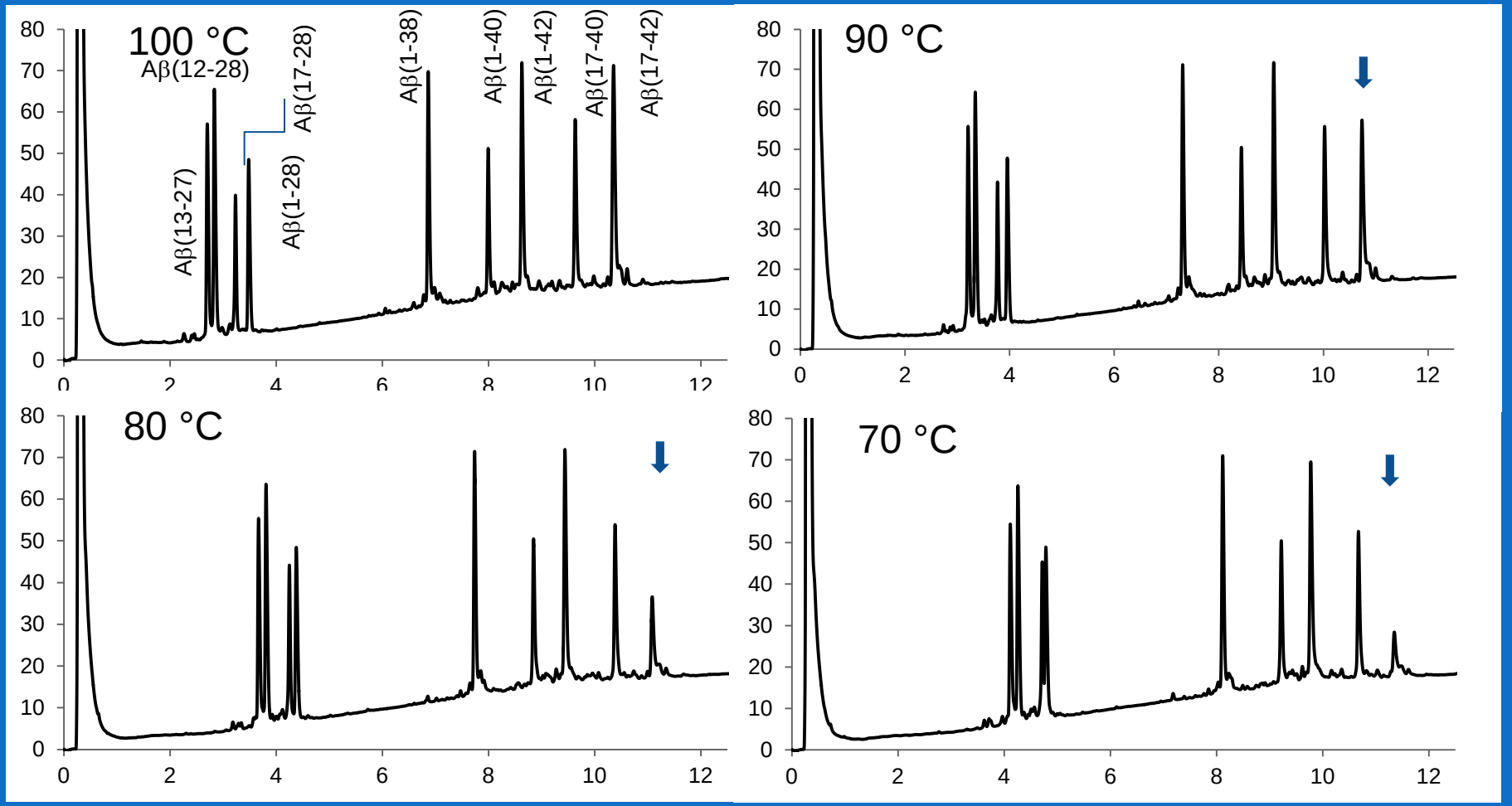


Ultra Fast High Resolution Separation of apo-Transferrin Tryptic Digest



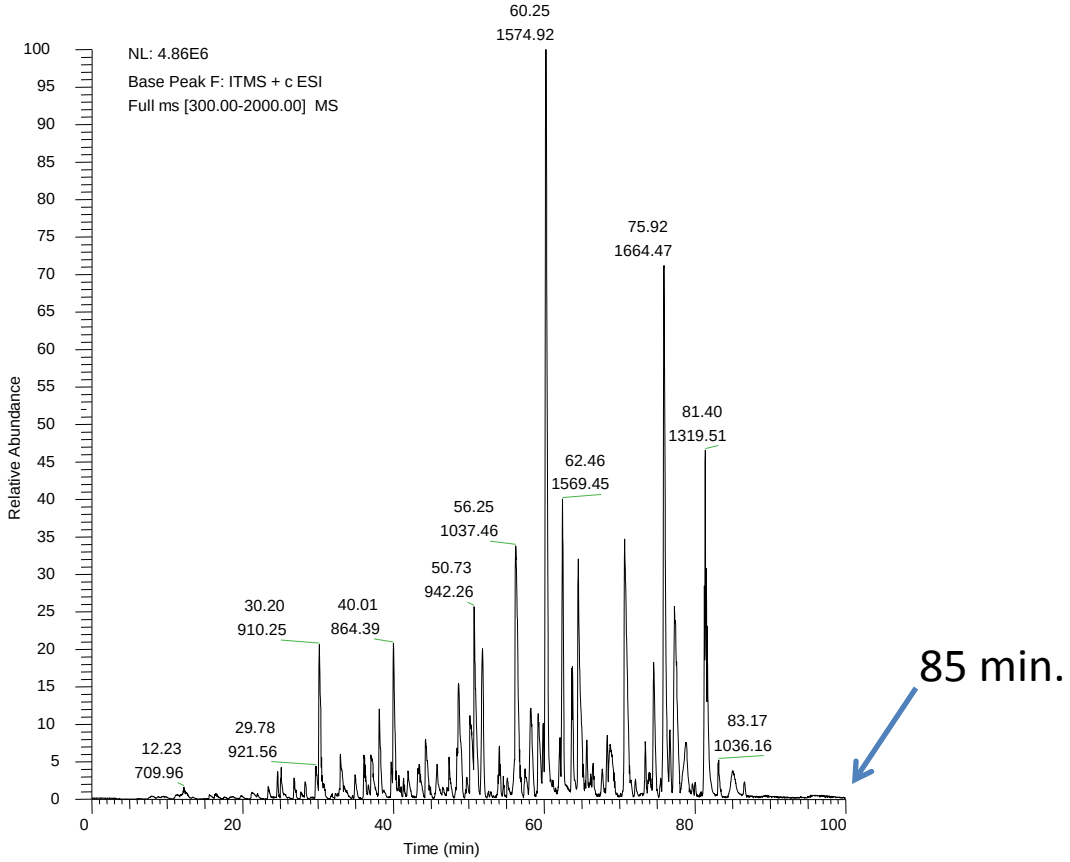
Rapid Separation at High Temperature

Column: 2.1 x 50 mm Halo Peptide ES-C18; Flow: 0.5 mL/min;
A: 0.1% TFA; B: 0.1% TFA/80% AcN; Gradient: 15-50% B in 12.5 min.;
Sample: 5 µL (250-500 ng) Aβ Peptides

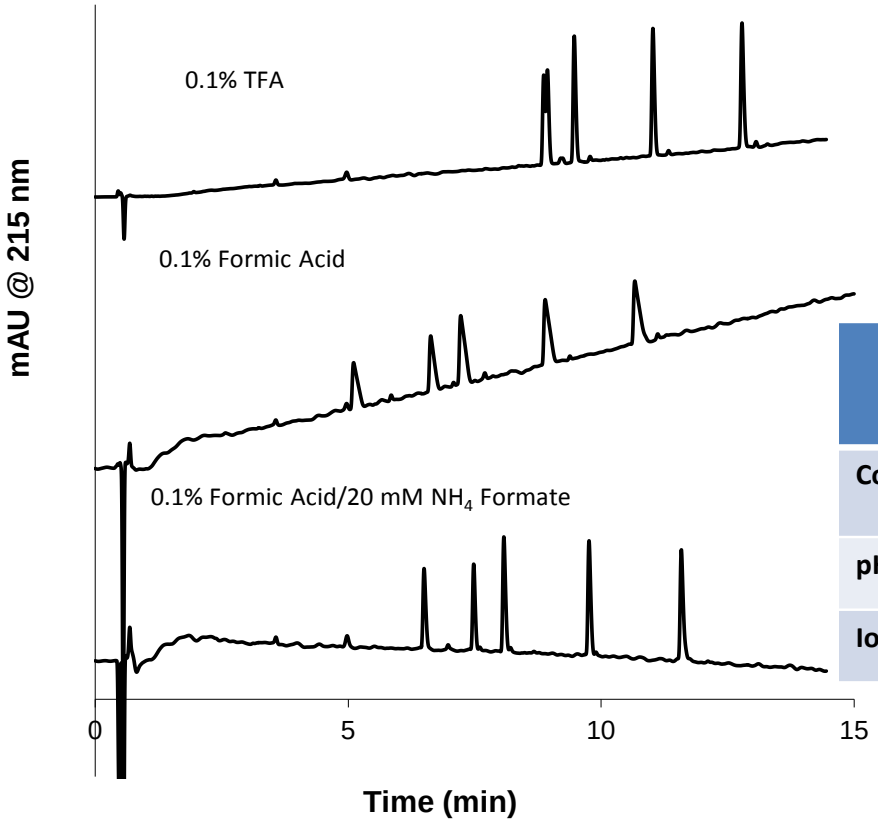


High mobile phase velocity LC/MS analysis of mixed protein digests

Column: 0.2 x 150 mm Halo Peptide ES-C18; Flow rate: 4 µL/min; Gradient: 2 - 45% B in 85 min;
A: 0.1% formic acid/water; B: acetonitrile/0.1% formic acid;
Maximum pressure: 320 bar;
Sample: mixed digest (5 pmol total of transferrin, carbonic anhydrase, and apomyoglobin).



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

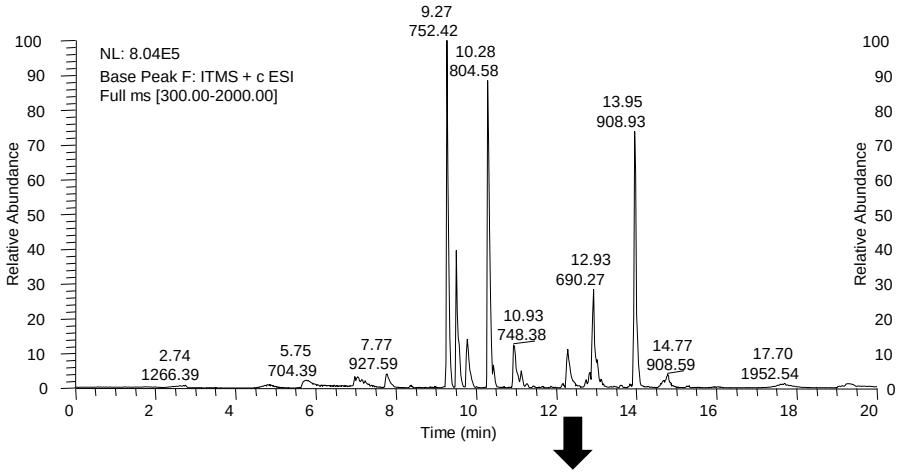
	TFA 0.1%	Formic Acid 0.1%	Ammonium Formate	AF/FA
Concentration	**0.013 M**	**0.026 M**	**0.020 M**	**20 mM AF 26 mM FA**
pH	**1.8**	**2.7**	**c. 7**	**3.3**
Ionic strength	**26 mM**	**4.4 mM**	**40 mM**	**c. 44 mM**

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.

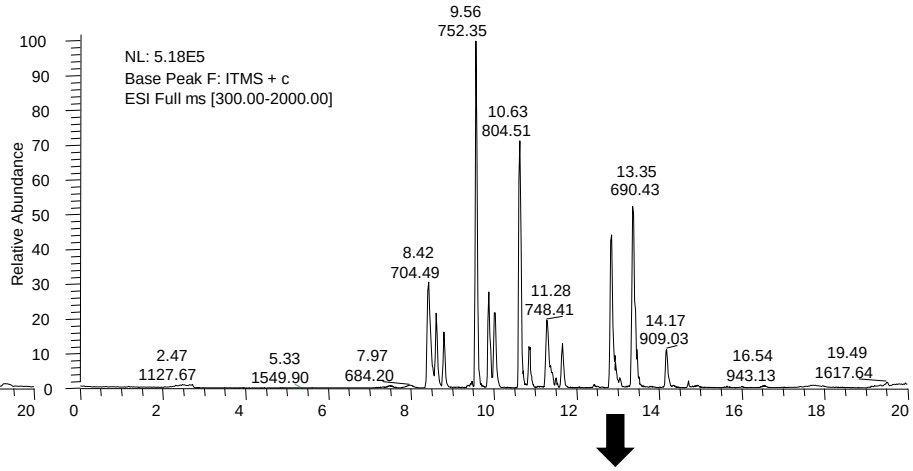
Ammonium formate as an additive for LC/MS separations

Column: 0.2 x 50 mm Halo Peptide ES-C18; Flow rate: 9 µL/min; Gradient: 2 - 45% B in 15 min; Mobile phases as shown; Sample: 2 µL (3 pmol) apomyoglobin digest.

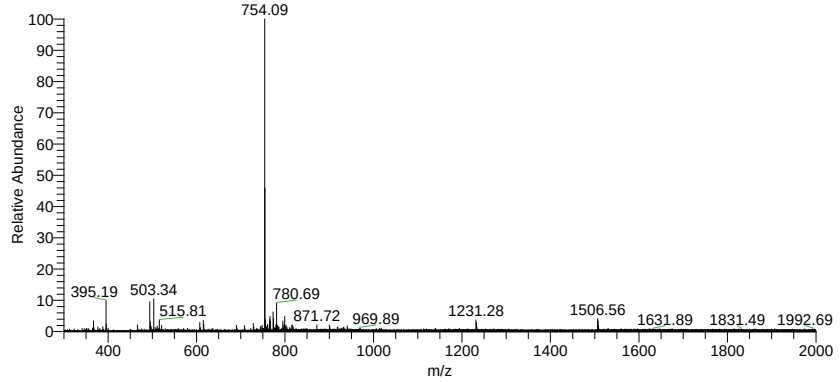
A: 0.1 % Formic Acid



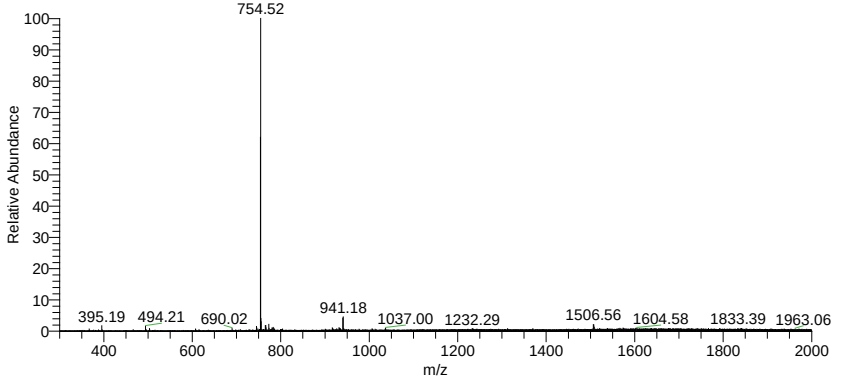
B: 0.1 % Formic Acid/10 mM Ammonium Formate



DJ_Halo_stem_ApoMyoglobin_3pmol_2_051710_100517170709 #1914-1933 RT: 12.23-12.33 AV: 5 NL: 5.78E4
F: ITMS + c ESI Full ms [300.00-2000.00]



DJ_Halo_stem_ApoMyoglobin_3pmol_AF_1_051810 #1945-1971 RT: 12.79-12.95 AV: 11 NL: 6.88E4
F: ITMS + c ESI Full ms [300.00-2000.00]



Improved Proteomic Analysis

Column: 0.2 x 150 mm Halo Peptide ES-C18; Flow: 4 µL/min
Gradient: 2 - 56% B in 85 min; Pmax - 320 bar;
A: 0.1% formic acid/10 mM AF/water; B: 80% acetonitrile/A;
Sample: 5 pmol transferrin, carbonic anhydrase, and apomyoglobin digest mixture
Detection: Thermo LTQ Ion Trap MS/Michrom ESI interface

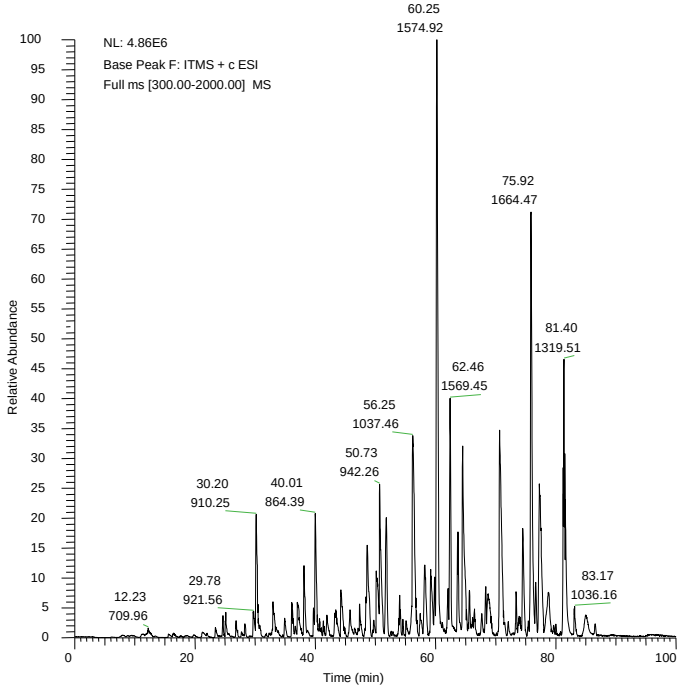


TABLE 7

Proteomic Results from Canine Prostate Carcinoma Analysis Under Various Chromatographic Conditions for Each Mobile-Phase Modifier

Column length (mm)	Flow rate (µL/min)	Experiment time (min)	Mobile-phase modifier	Protein IDs[a]	Matched MS/MS spectra	Peptide IDs[a]	Spectra/peptide ID[b]
50	9	21	0.1% FA	44	455	196	2.32
50	9	21	0.1% FA, 10 mM AF	60	697	255	2.73
150	4	140	0.1% FA	70	1142	359	3.18
150	4	140	0.1% FA, 10 mM AF	118	2028	538	3.77

[a]Results for each mobile-phase modifier generated from duplicate sample analysis with protein and peptide identifications validated using a 5% false discovery rate.
[b]Total number of database-matched MS/MS spectra, divided by the total number of peptide identifications for each condition from triplicate sample analysis.

TABLE 8

Analysis of the 61 Proteins Commonly Identified Using Both Mobile-Phase Modifier Conditions from LC-MS/MS Analysis Canine Prostate Carcinoma Using a 0.2 × 150-mm Column

Mobile-phase modifier	Average peptide IDs/protein[a]	Average spectral count/protein ID[b]	Single-spectrum protein IDs[c]
0.1% FA	6.60	20.71	3
0.1% FA, 10 mM AF	9.64	28.56	0

[a]The number of peptides identified from the 61 common identification proteins, divided by the number of common protein identifications.
[b]The total number of database-matched MS/MS spectra from the 61 common identification proteins, divided by total of common protein identifications.
[c]Protein identifications from only one single MS/MS spectra after application of a 5% false discovery rate.

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.

Product Offering Logic for P&P RP

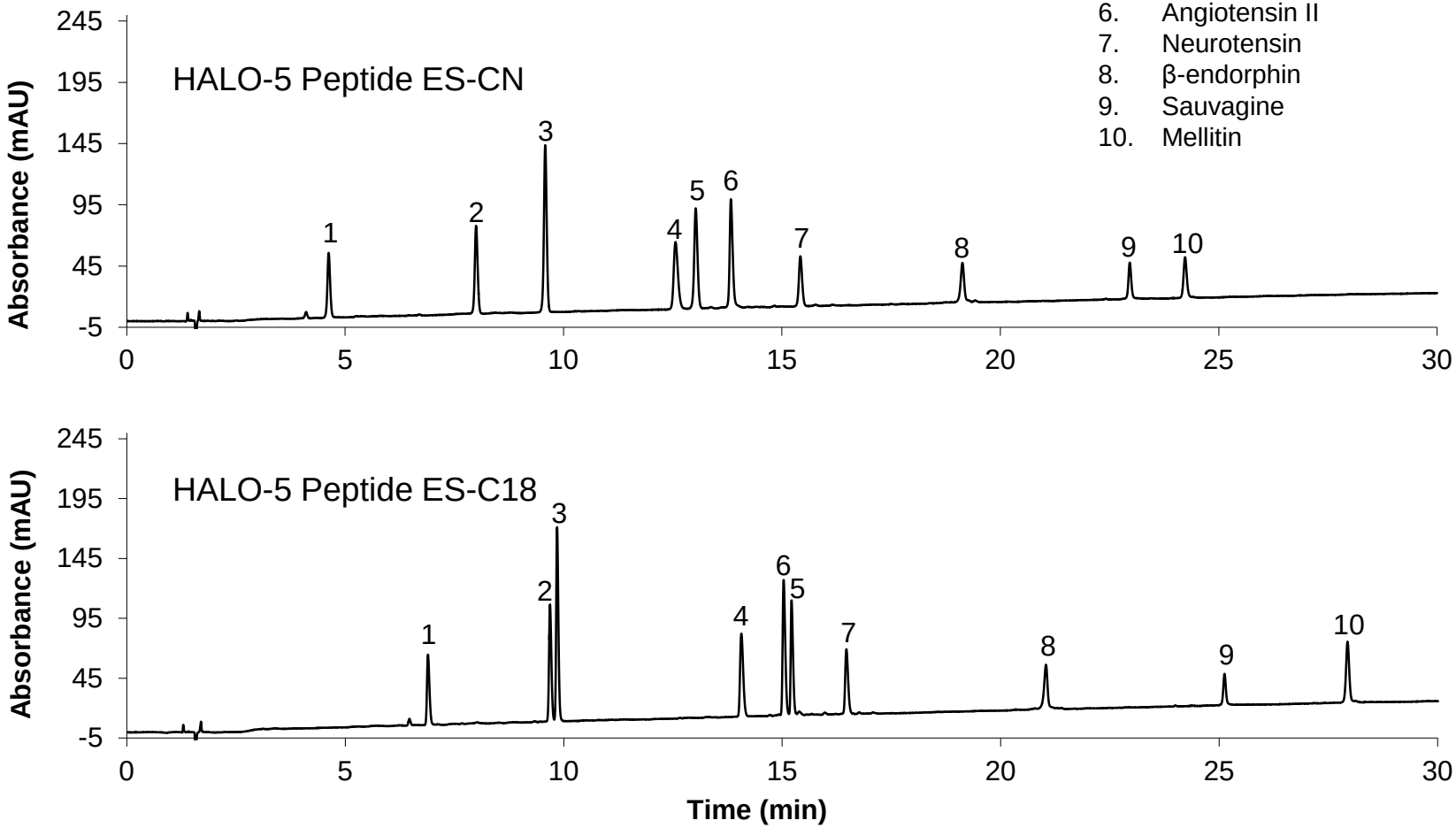
- Fused-Core Particles for highly efficient separations
- Silica Needs for Biomolecule Separations
  - 160 Å Peptide: > 1000 (10 aa) < 10000 (100 aa)
  - 400 Å Protein: > 10000 (100 aa)
  - 2.7 and 5 µm particle size
  - Similar or identical retention between particle sizes
- Bonded-Phase Needs
  - ES-C18 for Peptides; ES-CN for alternate selectivity
  - C4 for Proteins; ES-C18 for alternate selectivity
- Column Format
  - 1 cm semi prep through 75 µm capillary, 25 cm L through 20 mm

10 Peptides: ES-CN compared to ES-C18

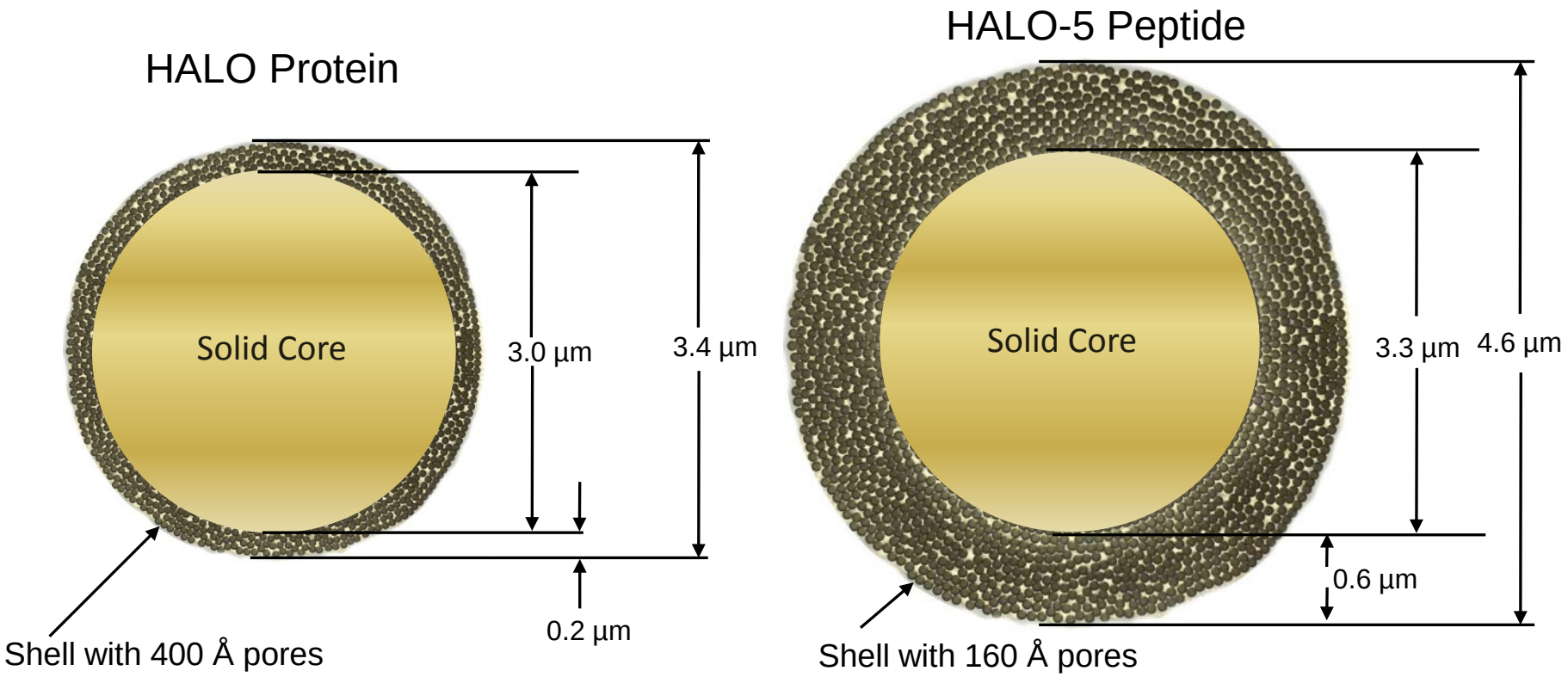
Columns: 4.6 x 150 mm
Instrument: Agilent 1100
Injection Volume: 10 µL
Detection: 215 nm
Temperature: 40 °C

Flow rate: 1.0 mL/min
Mobile Phase A: water/0.1% TFA
Mobile Phase B: ACN/0.1% TFA
Gradient: 5-50% ACN in 30 min

Peak Identities:
1. Asp-Phe
2. Angiotensin (1-7) amide
3. Tyr-Tyr-Tyr
4. Bradykinin
5. Leu-Enk
6. Angiotensin II
7. Neurotensin
8. β-endorphin
9. Sauvagine
10. Mellitin



HALO® Wide-Pore Fused-Core Particles



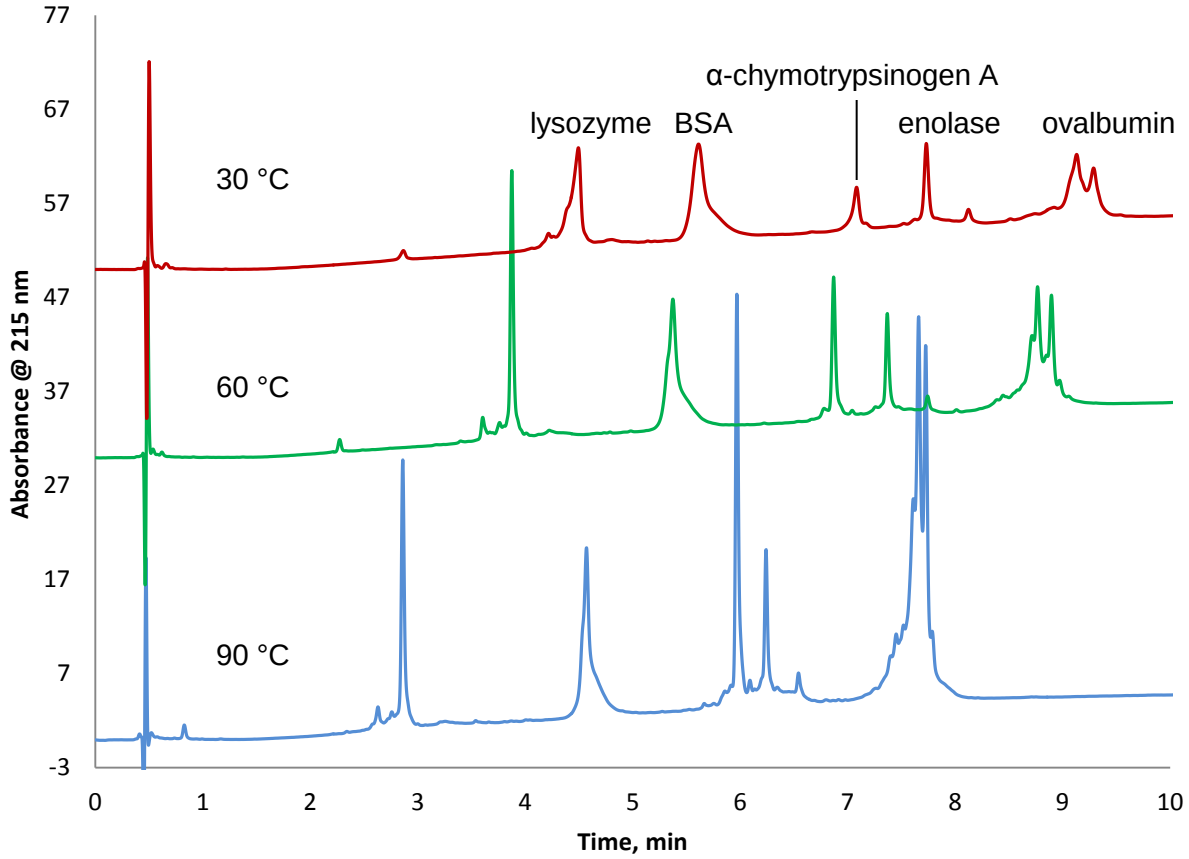
Protein Separations: Effect of Temperature

Column: 2.1 x 100 mm HALO Protein C4
Instrument: Agilent 1200 SL
Injection Volume: 2 µL
Detection: 215 nm
Temperature: as indicated

Mobile Phase A: water/0.1% TFA
Mobile Phase B: acetonitrile/0.1% TFA
Gradient: 28-58% B in 10 min.
Flow rate: 0.45 mL/min

Peak Identities (in order):

1.	Lysozyme	14.3 kDa
2.	BSA	66.4 kDa
3.	α-Chymotrypsinogen A	25.0 kDa
4.	Enolase	46.7 kDa
5.	Ovalbumin	44.0 kDa

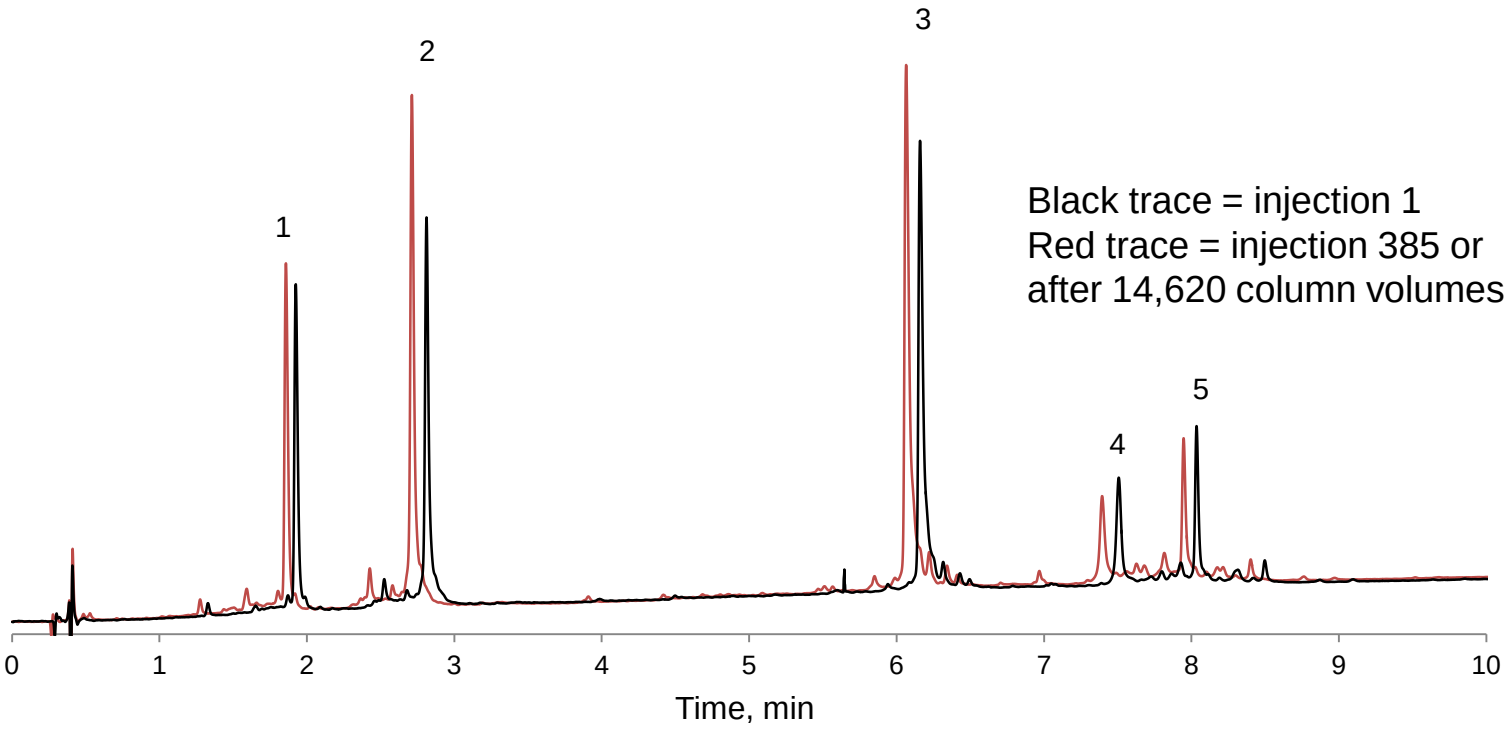


HALO Protein C4 Stability

Column: 2.1 x 100 mm
Instrument: Shimadzu Nexera
Injection Volume: 1.0 µL
Detection: 215 nm
Temperature: 90 °C

Flow rate: 0.5 mL/min
Mobile Phase A: water/0.1% TFA
Mobile Phase B: acetonitrile/0.1% TFA
Gradient: 25-40% B in 10 min

Peak identities:
1. cytochrome *c* (12.4 kDa)
2. lysozyme (14.3 kDa)
3. apomyoglobin (17 kDa)
4. catalase (250 kDa total; tetramer of ~60 kDa each)
5. enolase (93 kDa total; dimer of 46.7 kDa each)



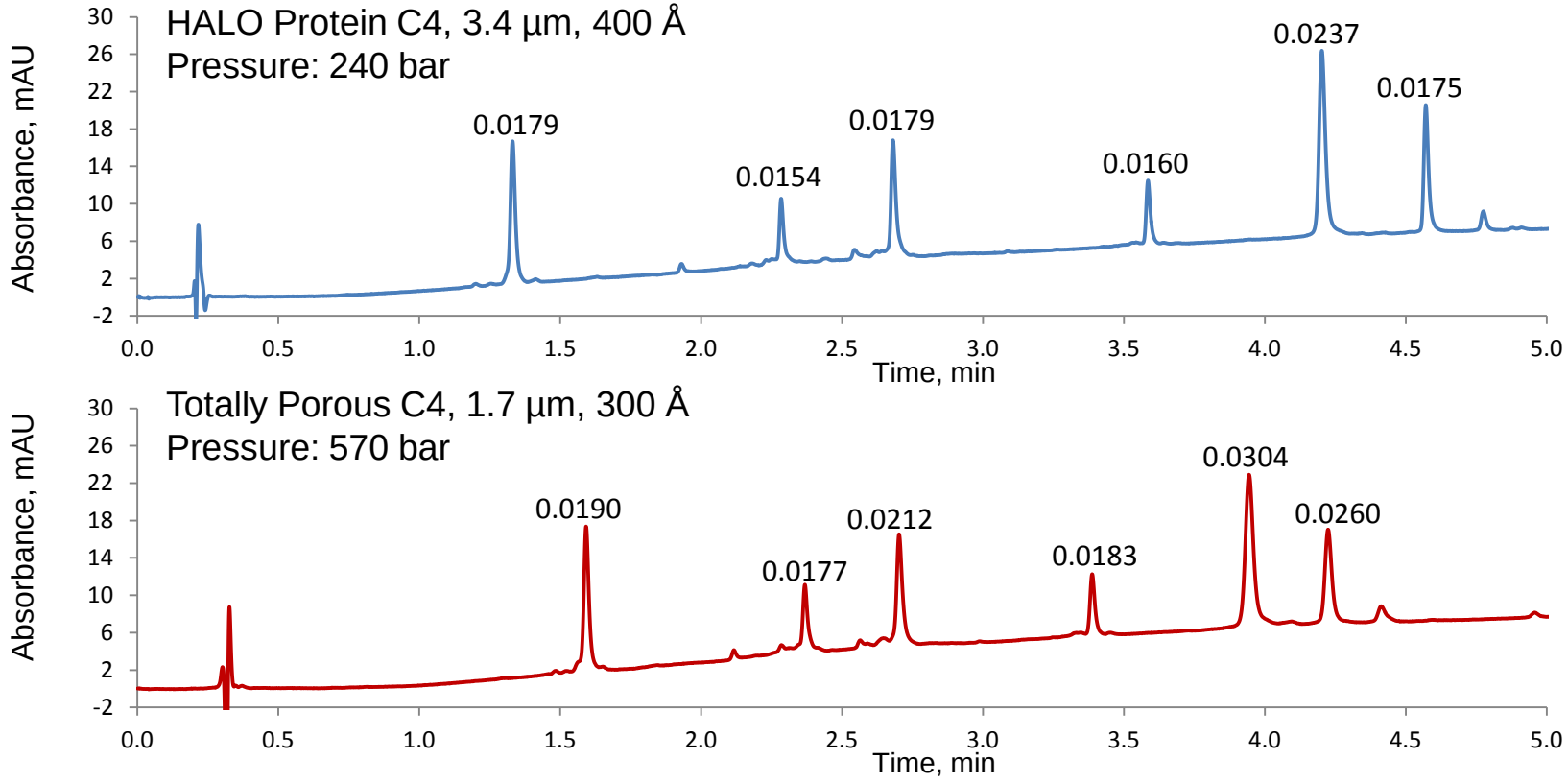
- The HALO Protein C4 bonded phase is stable up to 90 °C, showing very little loss of retention and no changes in peak shapes after almost 15,000 column volumes of mobile phase.

Protein Separations: Fused-Core compared to Totally Porous

Columns: 2.1 x 100 mm
Instrument: Agilent 1200 SL
Injection Volume: 1 µL
Detection: 215 nm
Temperature: 60 °C

Flow rate: **1.1 mL/min**
Mobile Phase A: water/0.1% TFA
Mobile Phase B: acetonitrile/0.1% TFA
Gradient: 23-52% B in 5 min

Peak Identities:
1. Ribonuclease A
2. Cytochrome *c*
3. Lysozyme
4. α-Lactalbumin
5. Catalase
6. Enolase



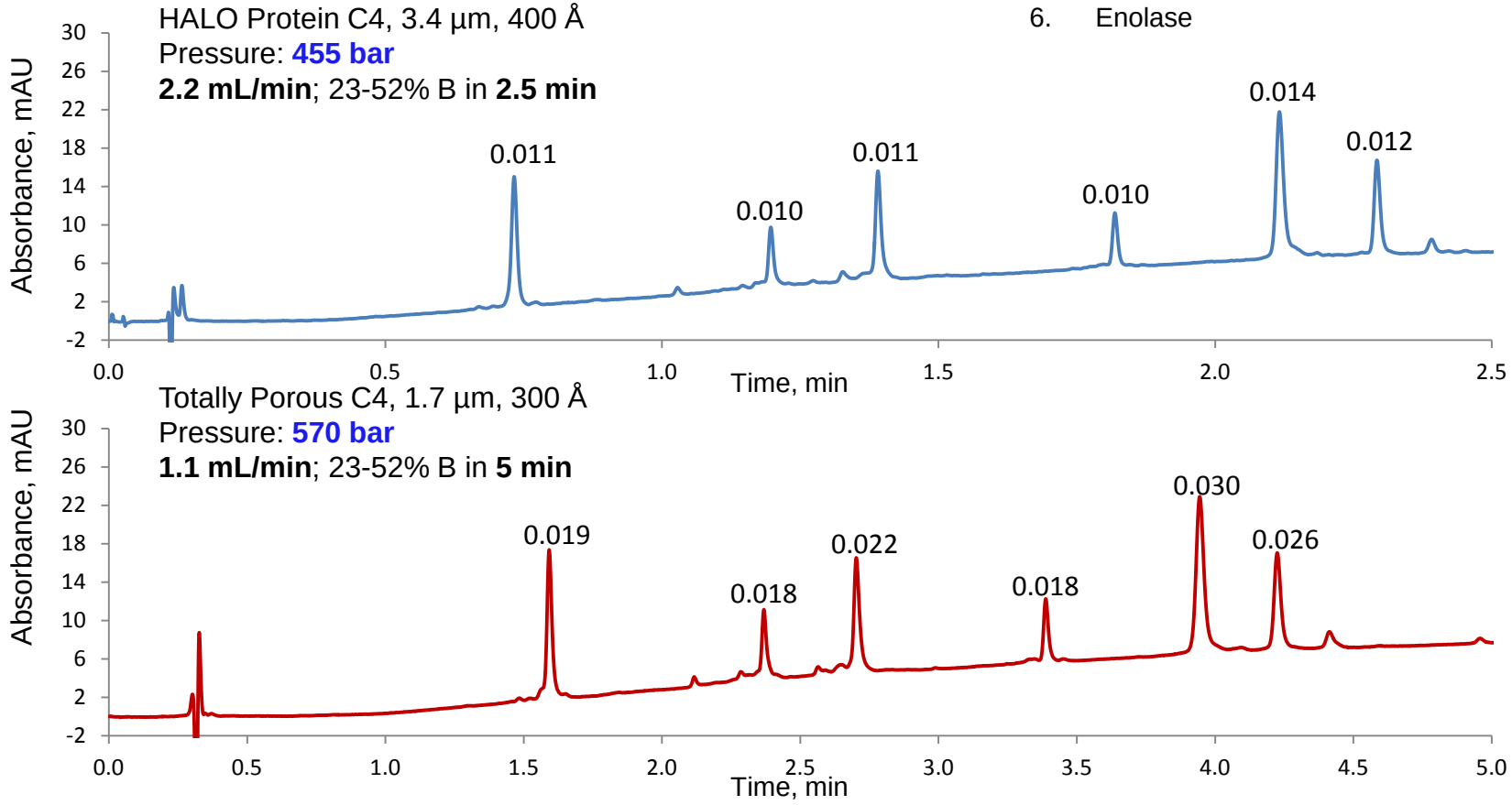
The HALO Protein column shows better performance than totally porous 1.7 micron column, at significantly lower back pressure, if measuring W½ or Peak Capacity.

Protein Separations: 3.4µm Fused-Core vs. 1.7µm Totally Porous

Column: 2.1 x 100 mm
Instrument: Agilent 1200 SL
Injection Volume: 1 µL
Detection: 215 nm
Temperature: 60 °C

Flow rate: as indicated
Mobile Phase A: water/0.1% TFA
Mobile Phase B: acetonitrile/0.1% TFA
Gradient: as indicated

Peak Identities:
1. Ribonuclease A
2. Cytochrome *c*
3. Lysozyme
4. α-Lactalbumin
5. Catalase
6. Enolase



Given the low back pressure of the HALO Protein column, the flow rate can be doubled while the gradient time is cut in half. This yields a separation in half of the time as the one run on the sub-2-µm totally porous column. Numbers above peaks are widths in minutes (double for volume compare).

Protein Recovery Studies

Protein	% Recovery
Cytochrome *c*	100 (5.8 SD)
Catalase	92 (18 SD)

- Proteins were fraction collected from a 4.6 x 100 mm HALO Protein C4 column run at 60 ^{o}C under gradient conditions with water/ACN/0.1% TFA mobile phase. Blanks were obtained by replacing the column with a union
- Lyophilized proteins were reconstituted using 3 M Urea/1% Triton X-100/0.25% acetic acid
- Protein recoveries were measured using QuantiPro™ BCA Assay Kit for 0.5-30 µg/mL protein (Sigma-Aldrich, St. Louis, MO)
- Samples were incubated at 37 ^{o}C for 100 min.
- Each sample was run in duplicate
- Absorbance values were measured at 562 nm
- HALO Protein C4 shows good recovery of proteins

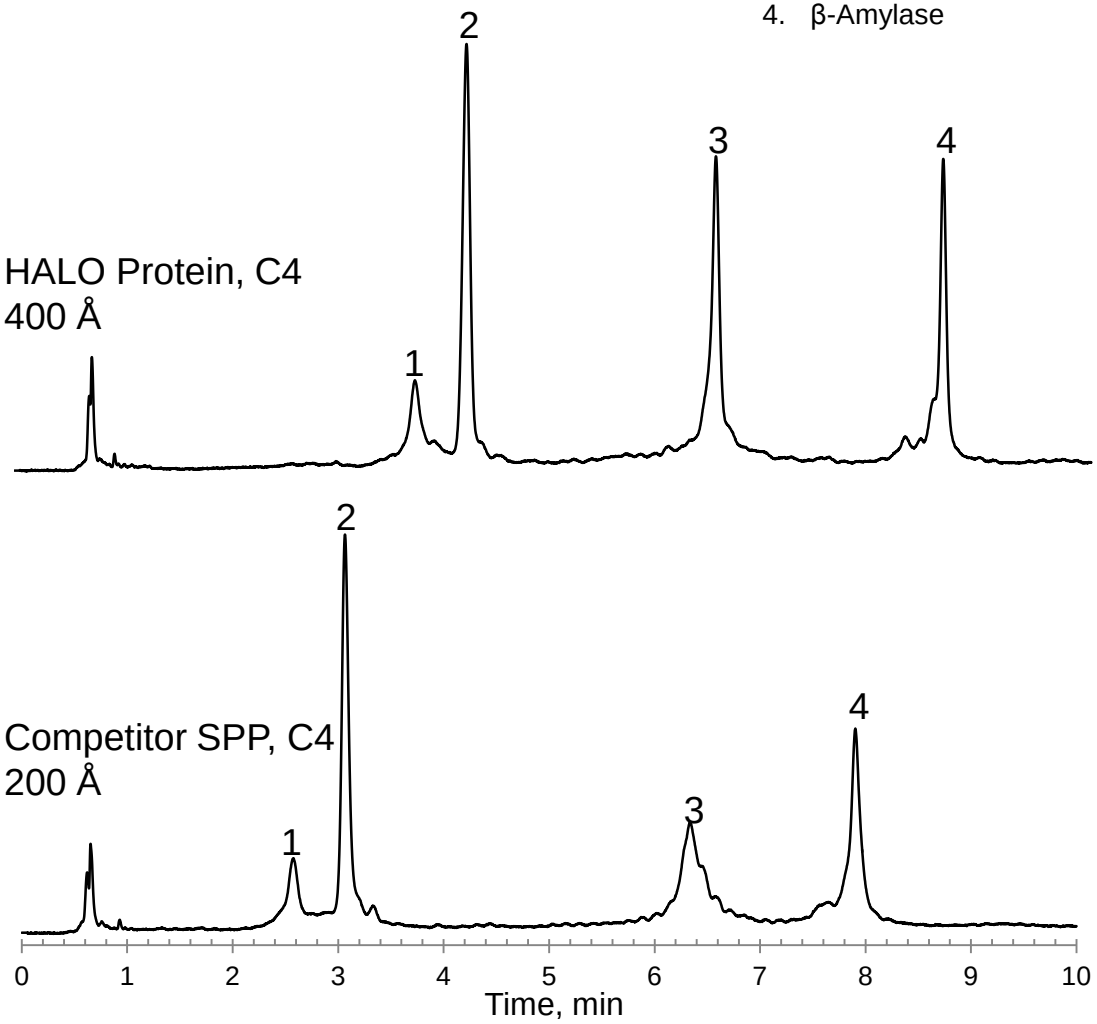
Protein Separations: Effect of Pore Size

Columns: 2.1 x 100 mm
Instrument: Shimadzu Nexera
Injection Volume: 1 µL
Detection: 280 nm
Temperature: 60 °C

Mobile Phase A: water/0.1% TFA
Mobile Phase B: 80/20 ACN/water/0.1% TFA
Gradient: 40-47% ACN in 10 min.
Flow rate: 0.3 mL/min

Peak Identities:

1.	Catalase	250 kDa [~60 kDa subunit]
2.	α-Chymotrypsinogen A	25.0 kDa
3.	β-Galactosidase	465 kDa [116 kDa subunit]
4.	β-Amylase	200 kDa [~50 kDa subunit]



- The 400 Å pores of HALO Protein enable sharp peaks for high MW biomolecules.

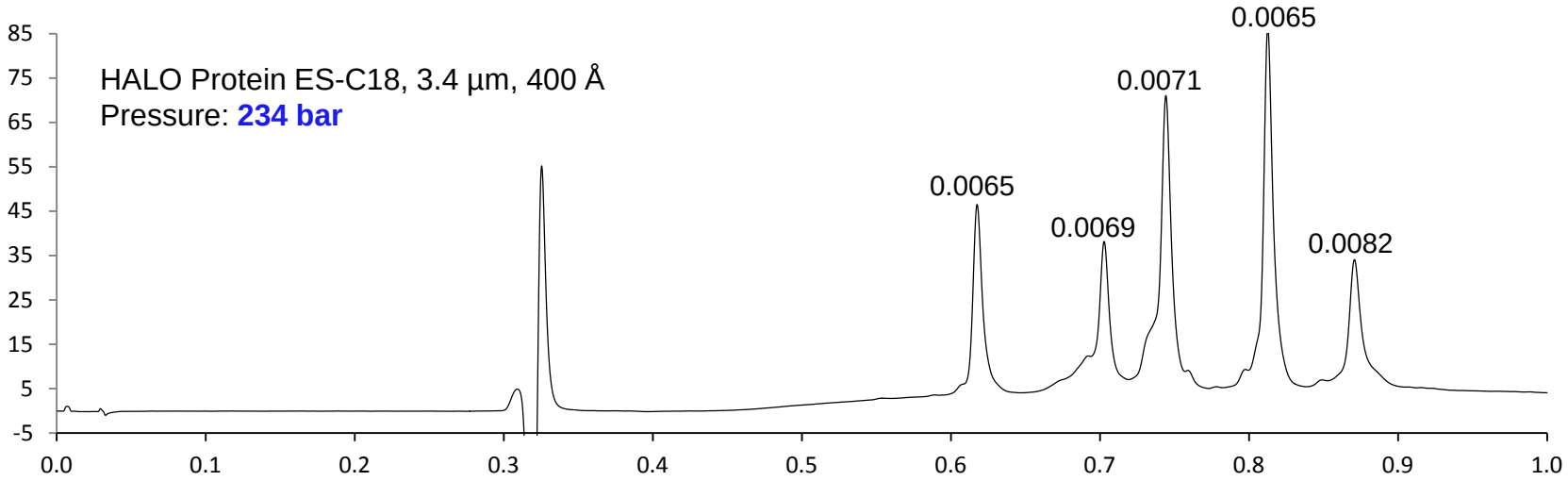
Protein Separations: 3.4µm Fused-Core vs. 3µm Totally Porous

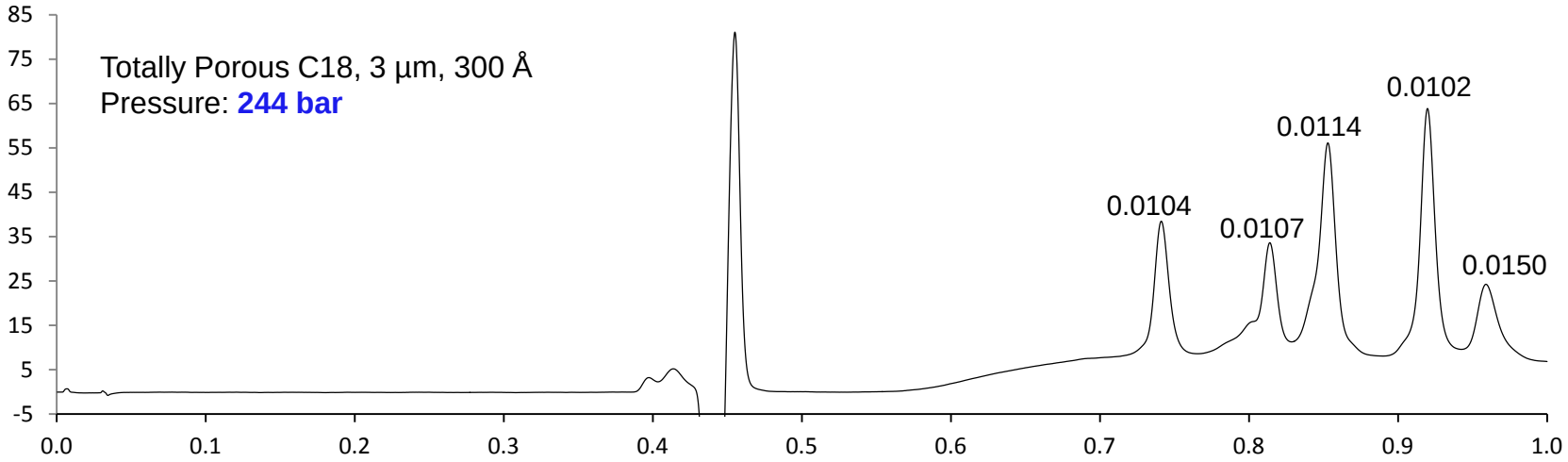
Columns: 4.6 x 100 mm
Instrument: Agilent 1200 SL
Injection Volume: 5 µL
Detection: 215 nm
Temperature: 60 °C

Flow rate: 3 mL/min
Mobile Phase A: water/0.1% TFA
Mobile Phase B: acetonitrile/0.1% TFA
Gradient: 23-85% B in 1 min
3 + 6 µL heat exchangers

Peak Identities:

1.	Ribonuclease A	13.7 kDa
2.	Cytochrome *c*	12.4 kDa
3.	Lysozyme	14.3 kDa
4.	α-Lactalbumin	14.2 kDa
5.	Catalase	250 kDa total; tetramer of ~60 kDa each





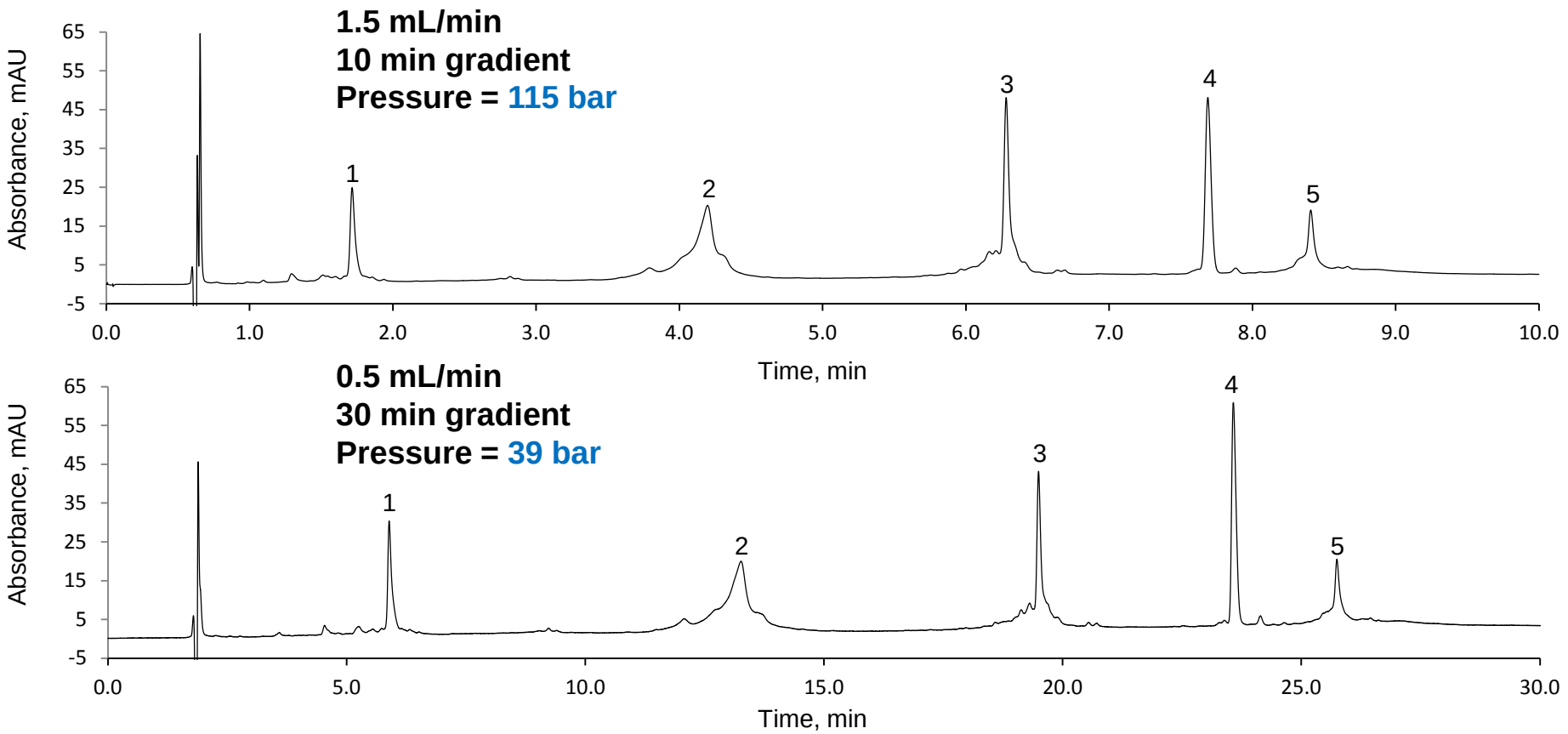
TEST CONDITIONS:

Column: 4.6 x 100 mm, HALO Protein ES-C18
Mobile Phase:
A = Water/0.1% TFA
B = Acetonitrile/0.1% TFA
Gradient: 33-50% B in 10 or 30 min
Flow Rate: as indicated
Pressure: as indicated

Temperature: 60°C
Detection: UV 215 nm, PDA
Injection Volume: 5 µL
Flow Cell: 2.5 µL semimicro
LC System: Agilent 1200 SL

Peak Identities:

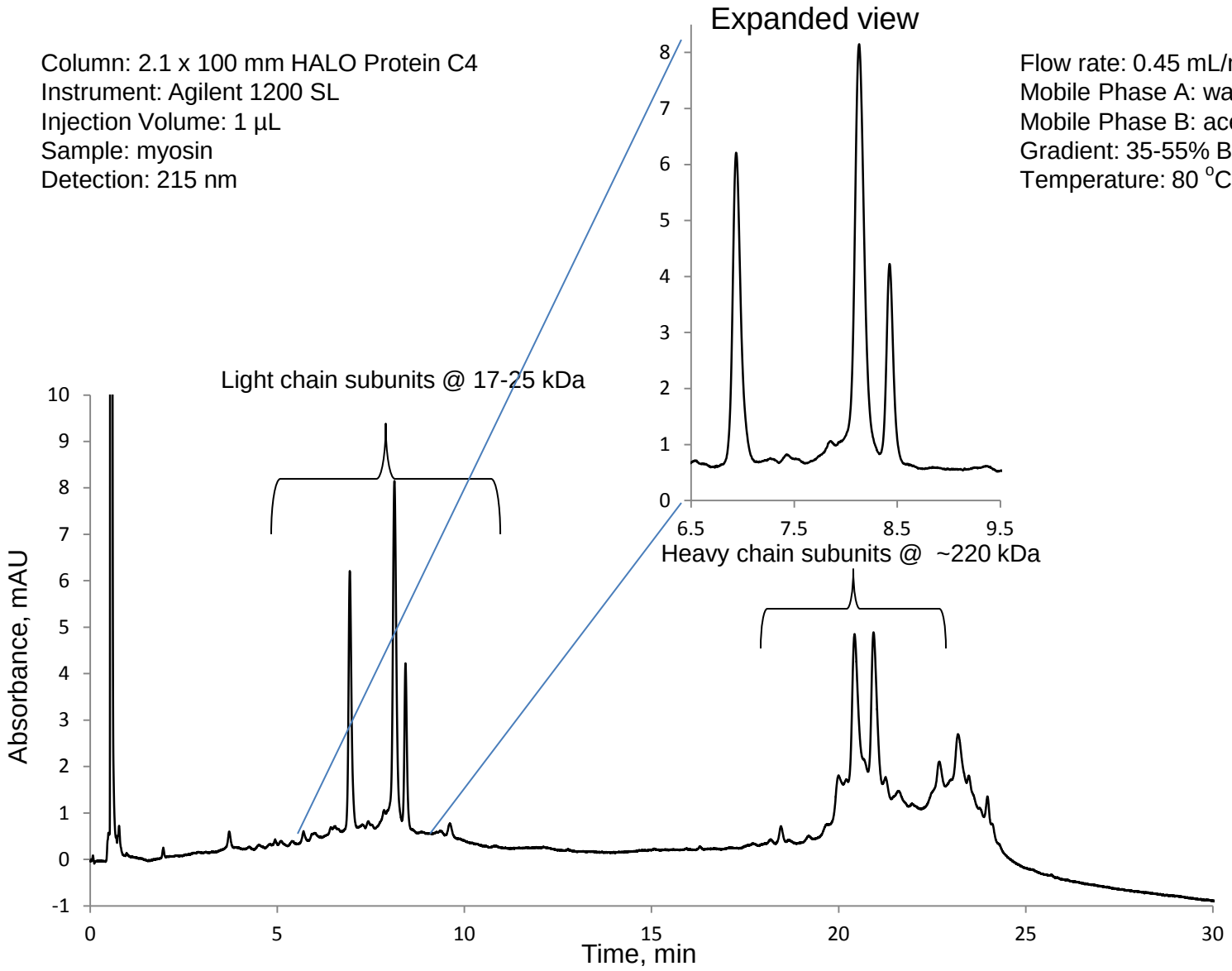
1. lysozyme 14.3 kDa
2. conalbumin 76 kDa
3. α-chymotrypsinogen A 25 kDa
4. carbonic anhydrase 29 kDa
5. β-amylase 200 kDa total; tetramer of ~50 kDa each



Large Protein Separation using 3.4 µm Fused-Core

Column: 2.1 x 100 mm HALO Protein C4
Instrument: Agilent 1200 SL
Injection Volume: 1 µL
Sample: myosin
Detection: 215 nm

Flow rate: 0.45 mL/min
Mobile Phase A: water/0.1% TFA
Mobile Phase B: acetonitrile/0.09% TFA
Gradient: 35-55% B in 30 min
Temperature: 80 °C



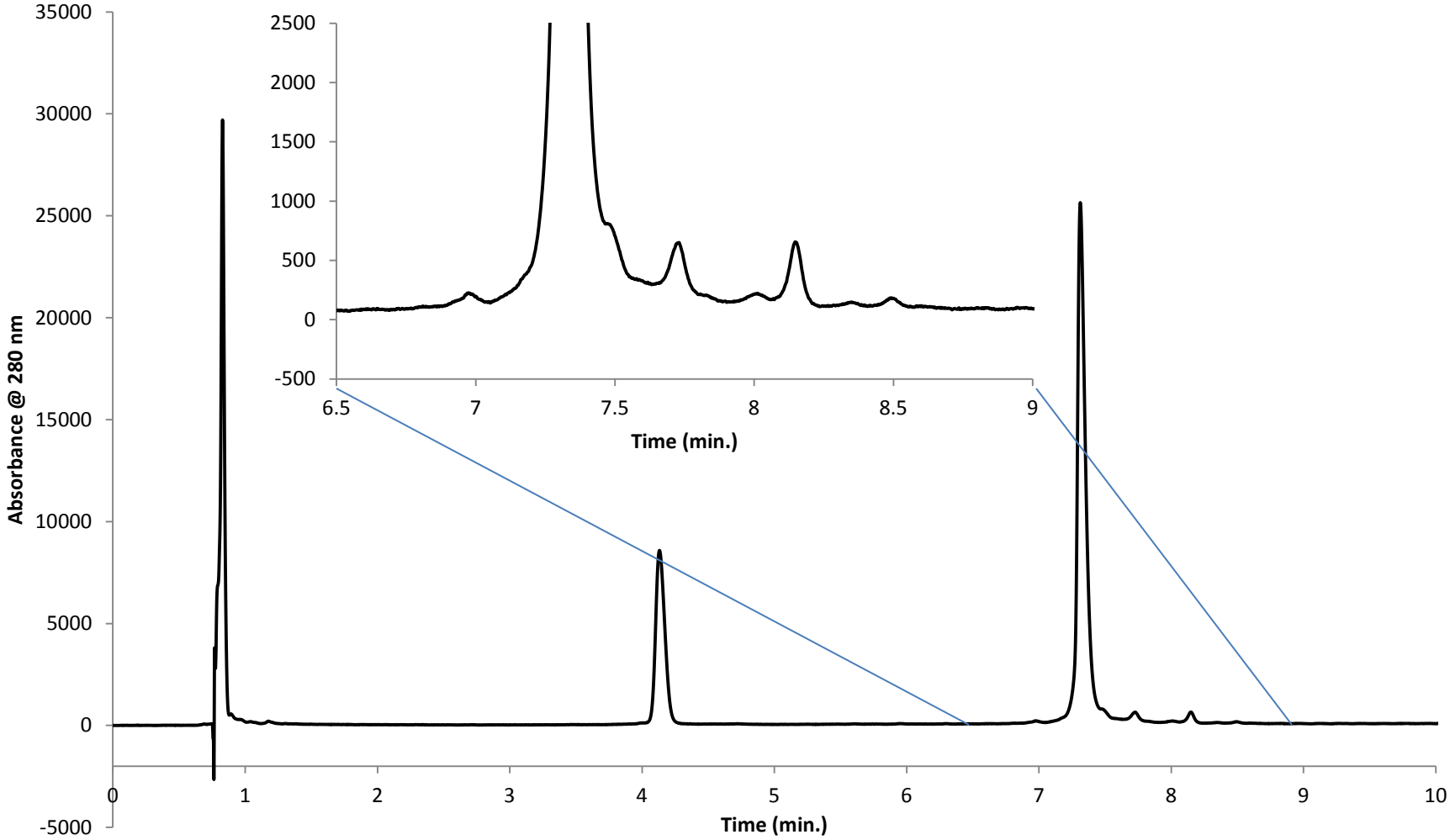
High resolution is obtained for this denatured contractile protein (purified Rabbit skeletal muscle whole myosin (Cytoskeleton, Inc.) using HALO Protein C4.

Reduced IgG2-B in TFA mAb Separation

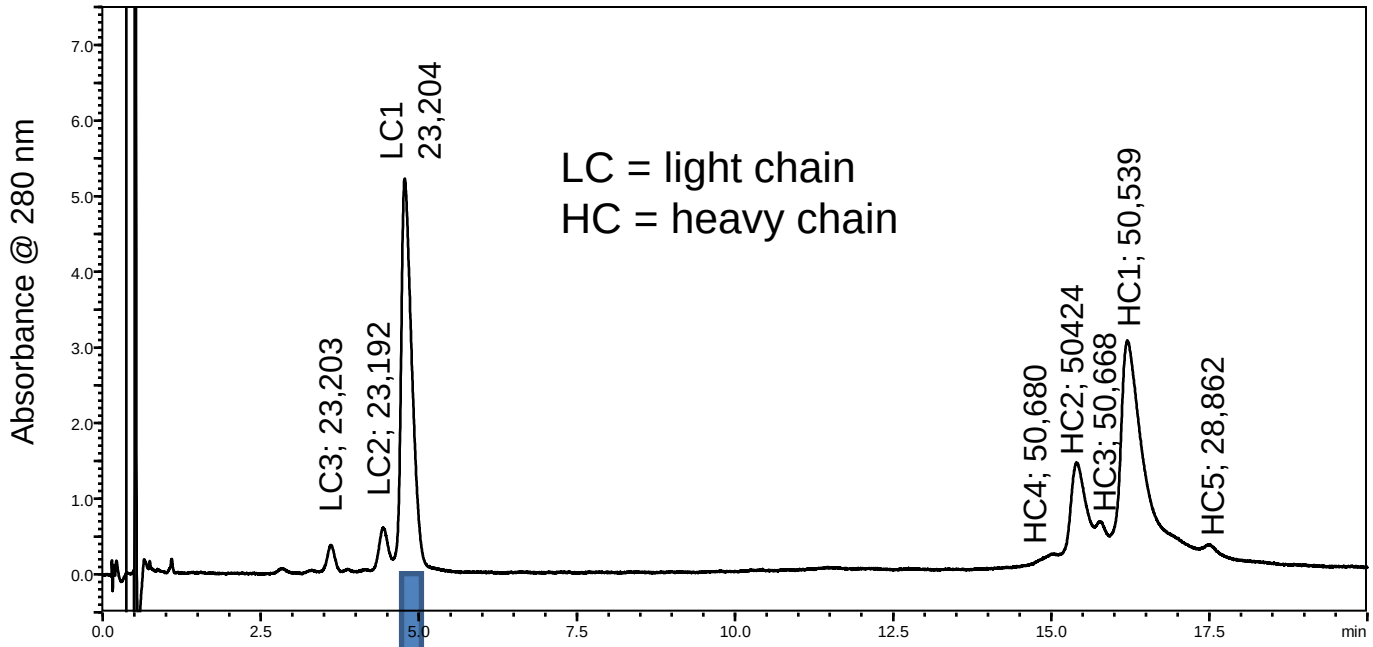
Column: 2.1 x 100 mm HALO Protein C4
Instrument: Shimadzu Nexera
Injection Volume: 1 µL
Detection: 280 nm

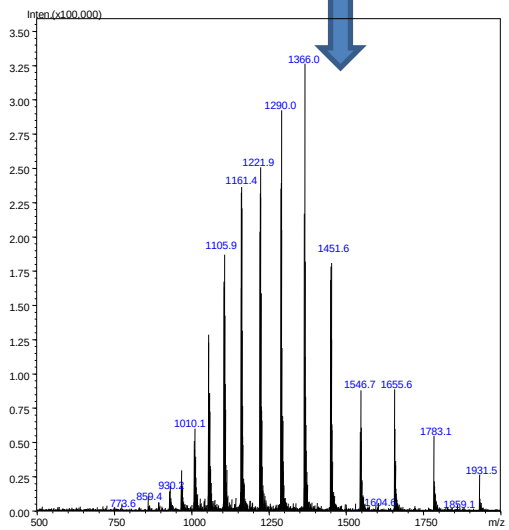
Mobile Phase A: water/0.1% TFA
Mobile Phase B: 80/20 ACN/water/0.1% TFA
Gradient: 33-40% B in 10 min.
Flow rate: 0.25 mL/min
Temperature: 80 °C

Sample: 0.5 mg/mL IgG2-B treated with 100 mM DTT in 8 M Guanidine HCl at 50 °C for 35 min.



High Resolution LC/MS: Analysis of IgG1 mAb Polypeptide Chains Using HALO Protein C4 (Formic Acid/Formate)





Sample Preparation

IgGs were reduced and alkylated by sequential treatment with 10 mM DTT, 15 mM iodoacetamide, then quenched with an additional 10 mM DTT, all in 6 M guanidine HCl/20 mM Tris-HCl buffer at pH 7.8. Reduced and alkylated IgG solutions were buffer exchanged into 0.1% TFA using VivaSpin (Sartorius Stedim Biotech, Goettigen, Germany) centrifugal concentrators with 5 kDa cut-off HY polymeric membranes. The reduced and alkylated IgGs were adjusted to 2 mg/mL protein in 0.1% TFA and stored at -25 ^{o}C until use.

Column: 2.1 mm ID x 100 mm HALO Protein C4
Flow rate: 0.4 mL/min.
A: 0.5 % formic acid with 20 mM Ammonium Formate
B: 45% AcN/45% IPA/ 0.5 % formic acid with 20 mM Ammonium Formate
Gradient: 29-32% B in 20 min.
Temperature: 80°C
Detection: 280 nm
MS Conditions: Shimadzu LCMS-2020, ESI +4.5 kV, 2 pps, 500-2000 m/z

Masses deconvoluted using MagTran

Penta-HILIC for Biomolecule Applications

Alternative to Reversed-Phase

- HILIC separation selectivity for peptide and protein fragment separations have been shown to be very different than RP
- Suitable mobile phase modifiers are available to manipulate retention and selectivity
- New bonded phases may broaden applications
- Glycopeptide and glycan separations have adopted HILIC

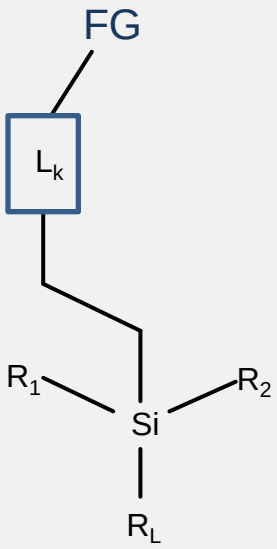
High Efficiency HILIC Materials Could be Enabling

- Many available materials do not fare well for separations of proteins and peptides
- Superficially porous particles can permit maximum throughput, rendering complex workflows more reasonable
- Target same efficiency and performance as RP (efficiency, peak shape, peak capacity)

Applications Investigated

- Peptides – synthetic S1-S5 and C1-C4 peptides (10 aa, Mant and Hodges), 11 synthetic mix, variety of tryptic digests of proteins and glycoproteins
- Glycopeptides and Glycans – tryptic and PNGase F digests of a variety of commercial proteins

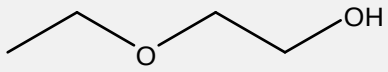
Silane Reagents for Hydrophilic Functional Groups



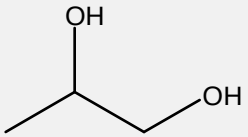
R_L = silanol reactive leaving
$R_1 = R_2$ or $R_1 \neq R_2$, $R_1 = R_L$
Eg. -Me, -OMe, -Cl, -DiiPr

Hydroxylic Functional Groups

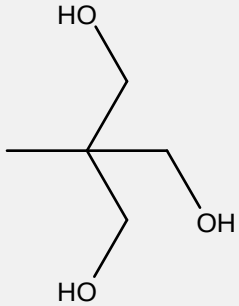
Mono-OL



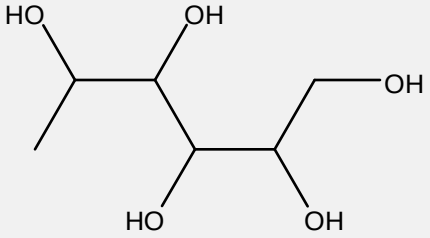
Di-OL



Tri-OL



Pentan-OL
AKA
Penta-HILIC

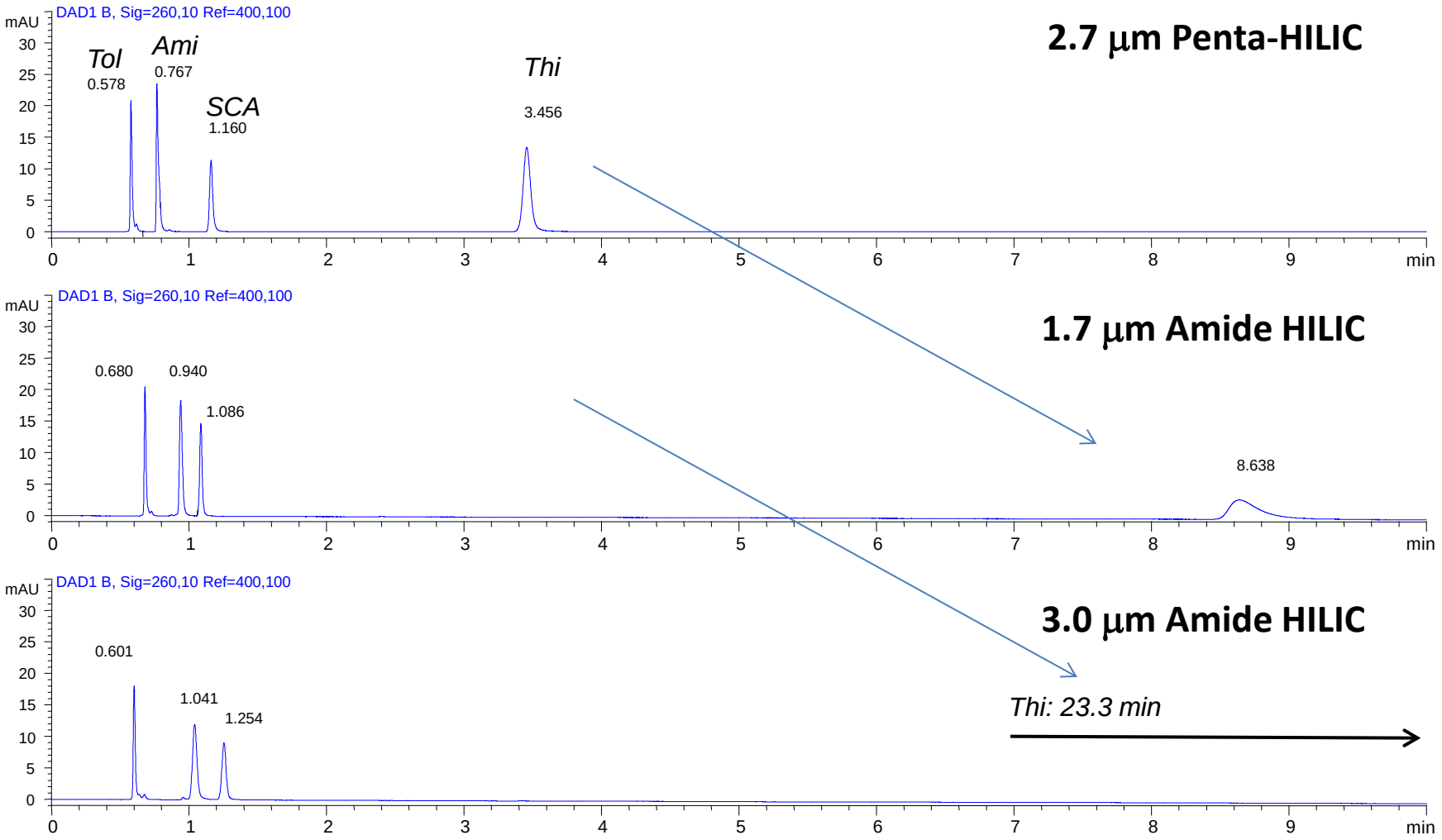


Comparative Retention of HILIC Columns

2.1 mm ID x 150 mm

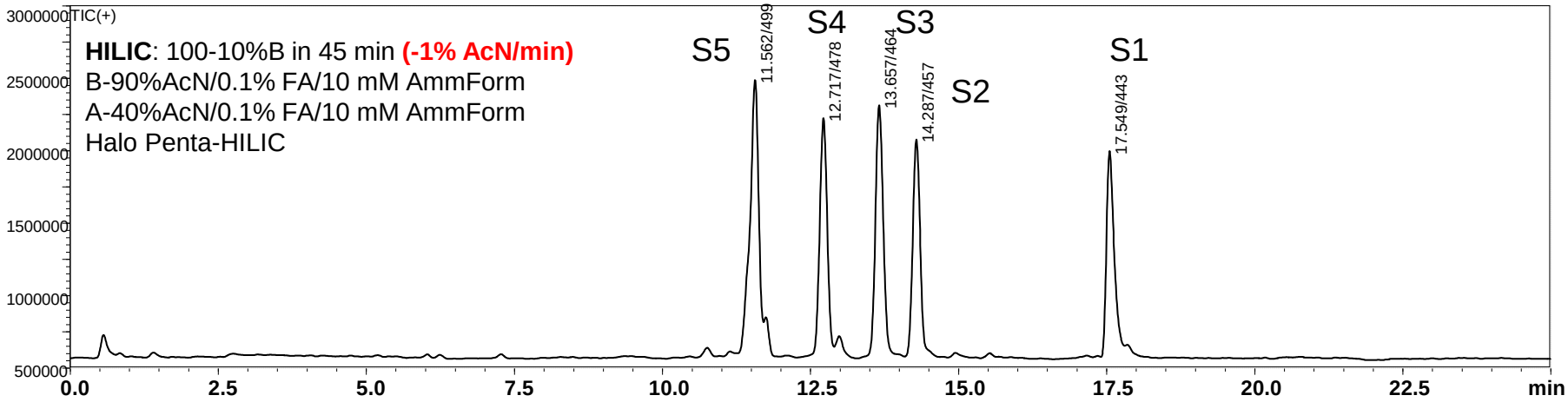
Test: 90% AcN/10 mM NH_4Form pH 3.0, 0.5 mL/min, 23 °C

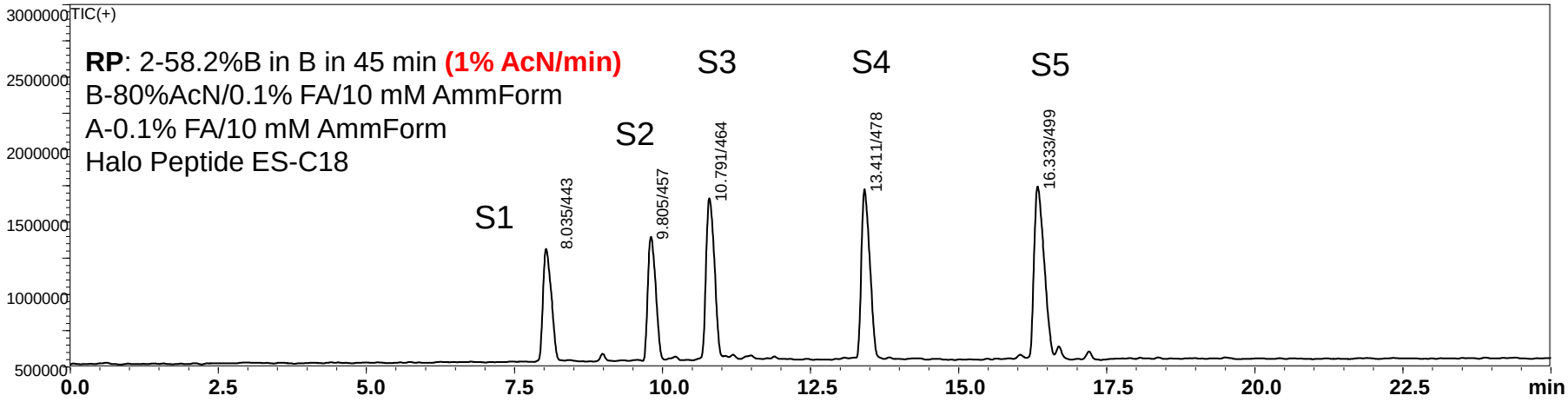
Toluene/Amitriptyline/Salycic Acid/Thiamine



Performance of RP and HILIC Columns for Peptide Separations

2.1 mm ID x 100 mm, 0.35 mL/min, 40 C, MS: SQ TIC (+ 300-2000 m/z) @ 0.35/s

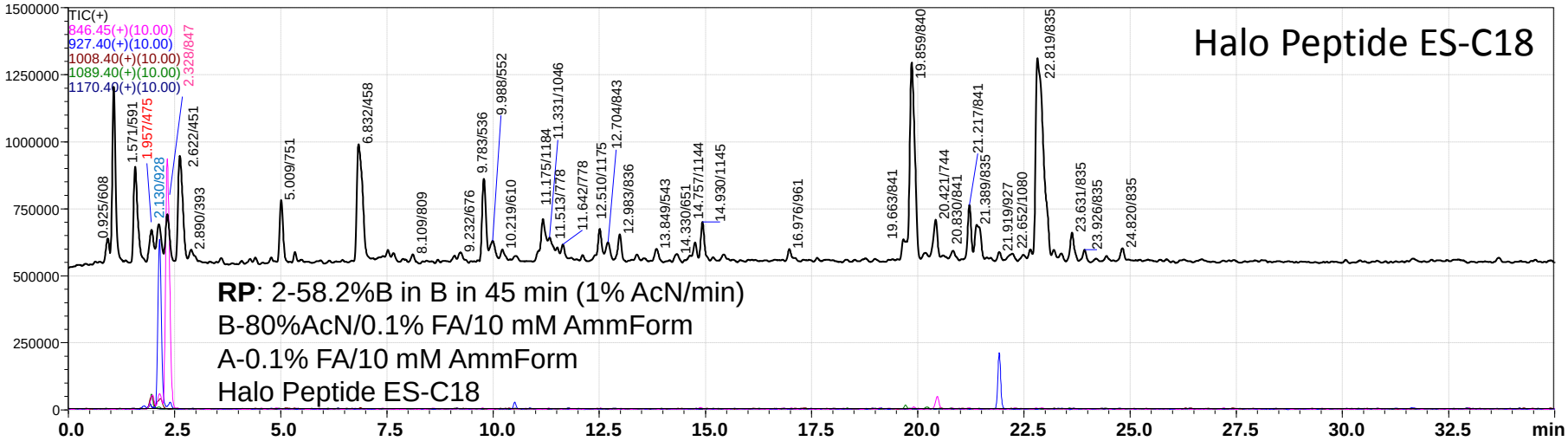


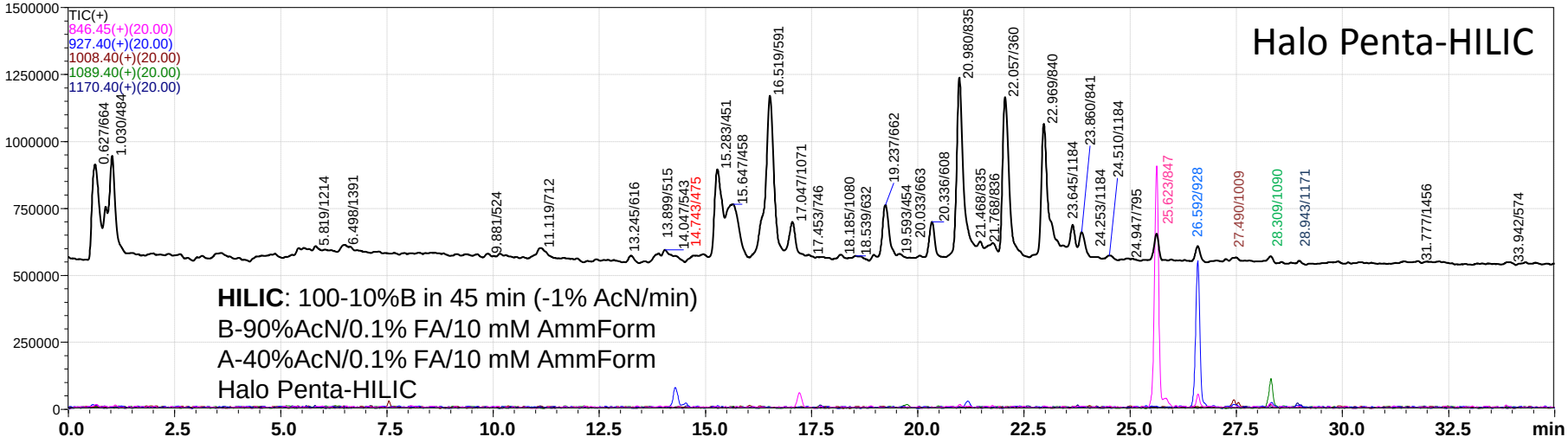


Penta-HILIC Strongly Retains Glycopeptides

2.1 mm ID x 100 mm, 0.35 mL/min, 40 C, MS: SQ TIC (+ 300-2000 m/z) @ 0.35/s

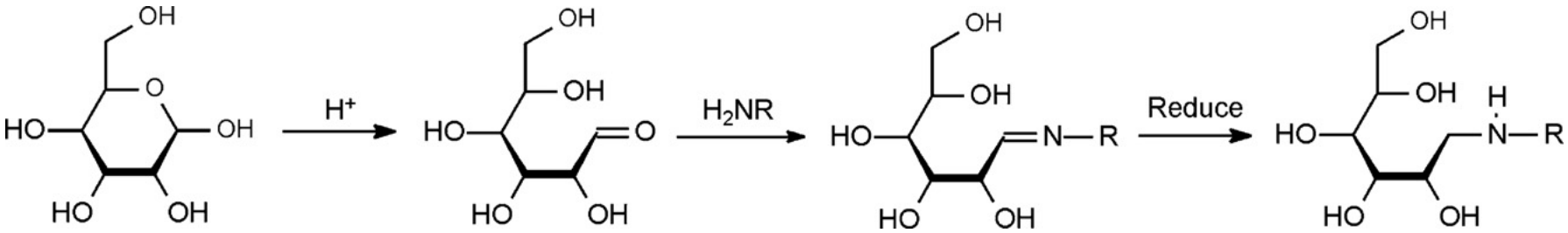
20 µg Bovine Ribonuclease B digest (CAM)





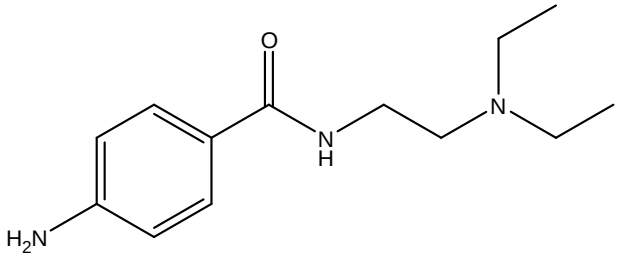
Penta-HILIC Analysis of Released and Labeled Glycans

Enzymatic deglycosylation of proteins (or peptides, etc.) using PNGase F releases glycans to yield a free reducing terminus (alditol) that is readily labeled by amines via the formation and reduction of a Schiff's base



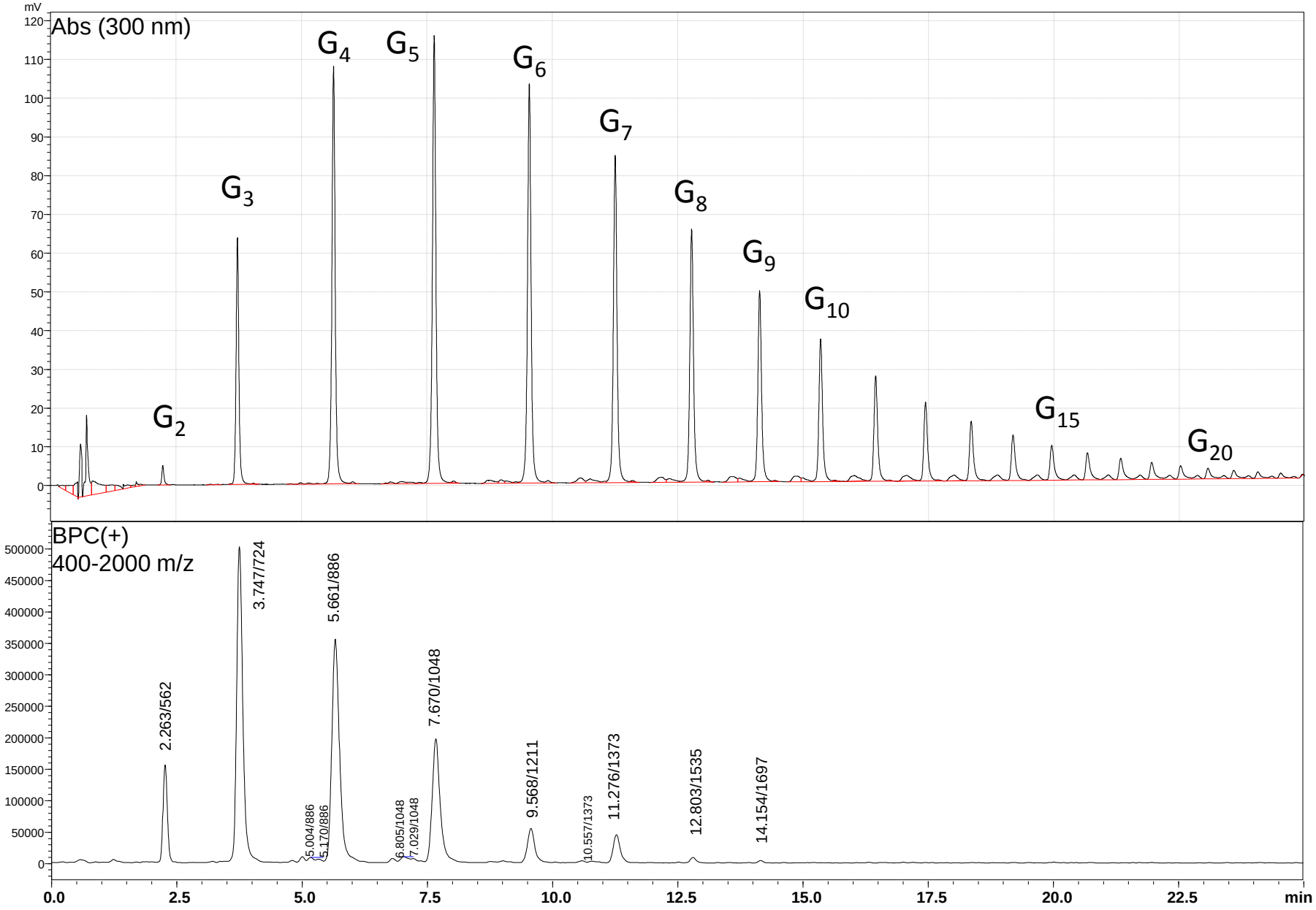
Many amines have been applied to labeling glycans, in the current work Procainamide is favored.

Standard Analysis Conditions
2.1 x 150 Penta-HILIC, 80%B to 55%B in 25 min
B:100%AcN
A: 50 mM Ammonium Formate pH 4.45 (FA titration)
0.6 mL/min, 60C
400-2000 @0.33s/0.1s each, +4.0 kV/12.5L/min, 250C DL
Absorbance Detection 300 nm



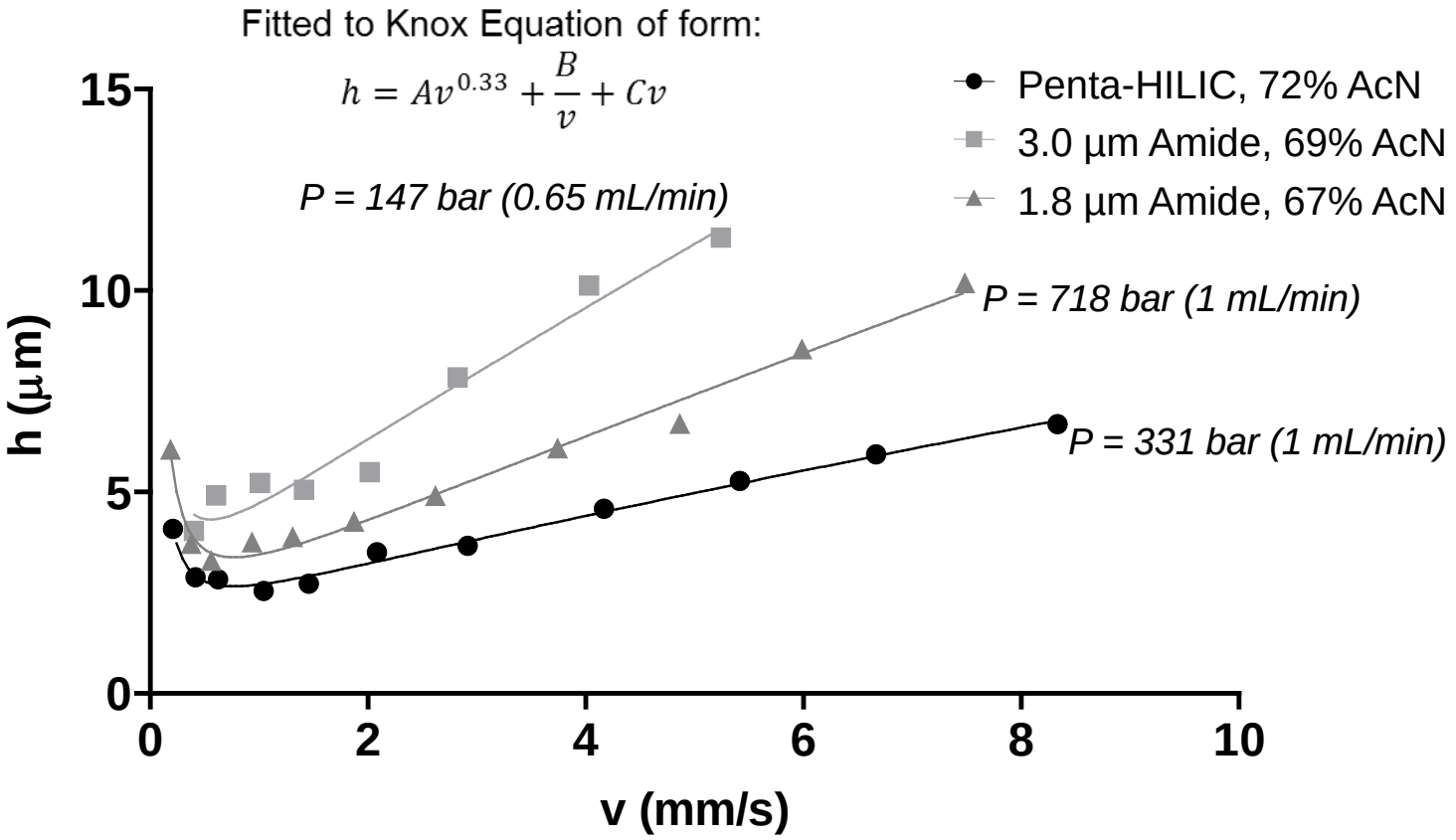
Mass: glycan + (235.325 – "O")
= Glycan + 219.32

Penta-HILIC Analysis of a Dextran Ladder

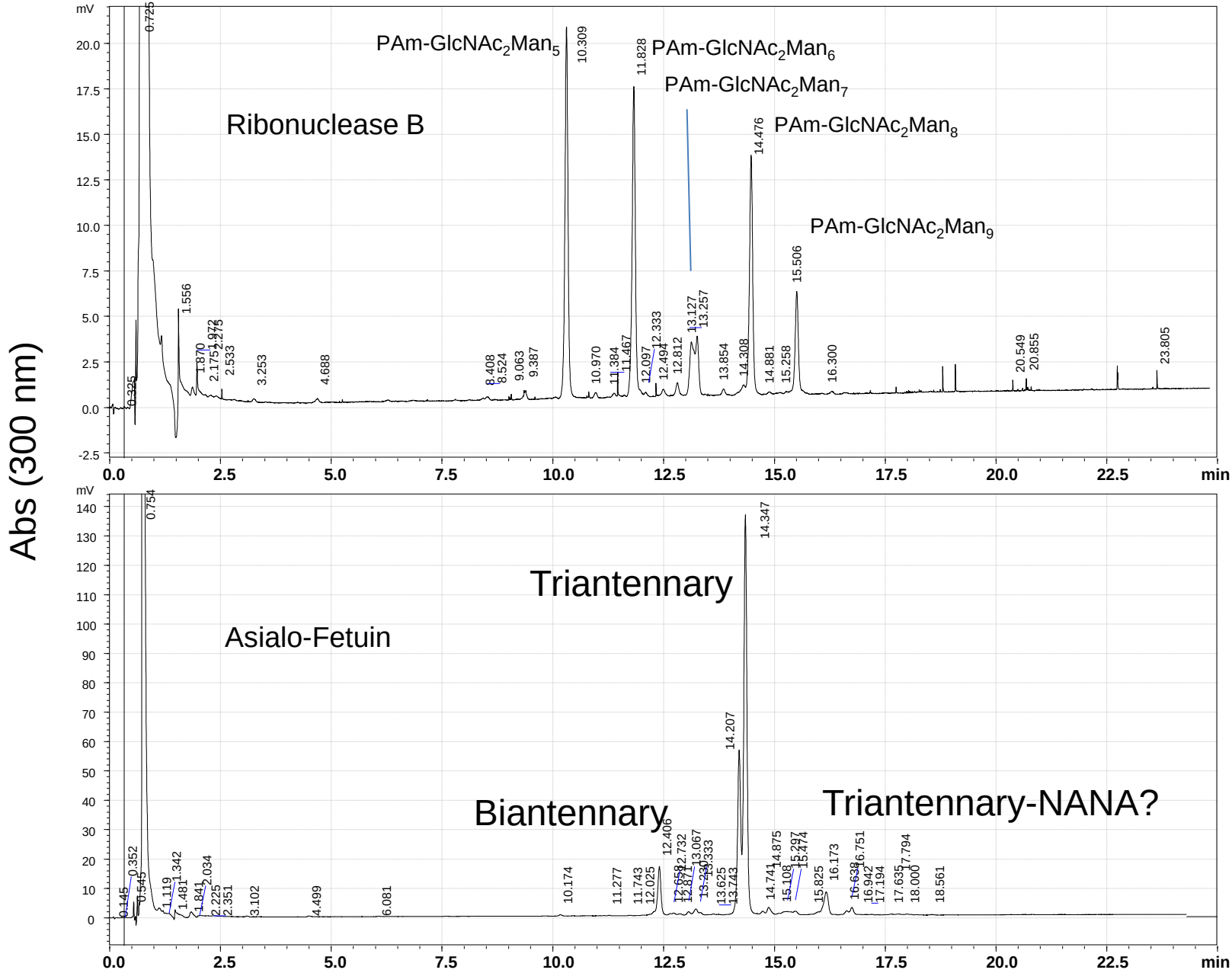


Column Efficiency Comparisons using Pam-G_5

2.1 (2.0 mm) ID x 150 mm, 60°C, k' ≈ 6,
50 mM Ammonium Formate Aqueous, pH 4.4
0.5 uL Injection (50 pmol), Abs. 300 nm

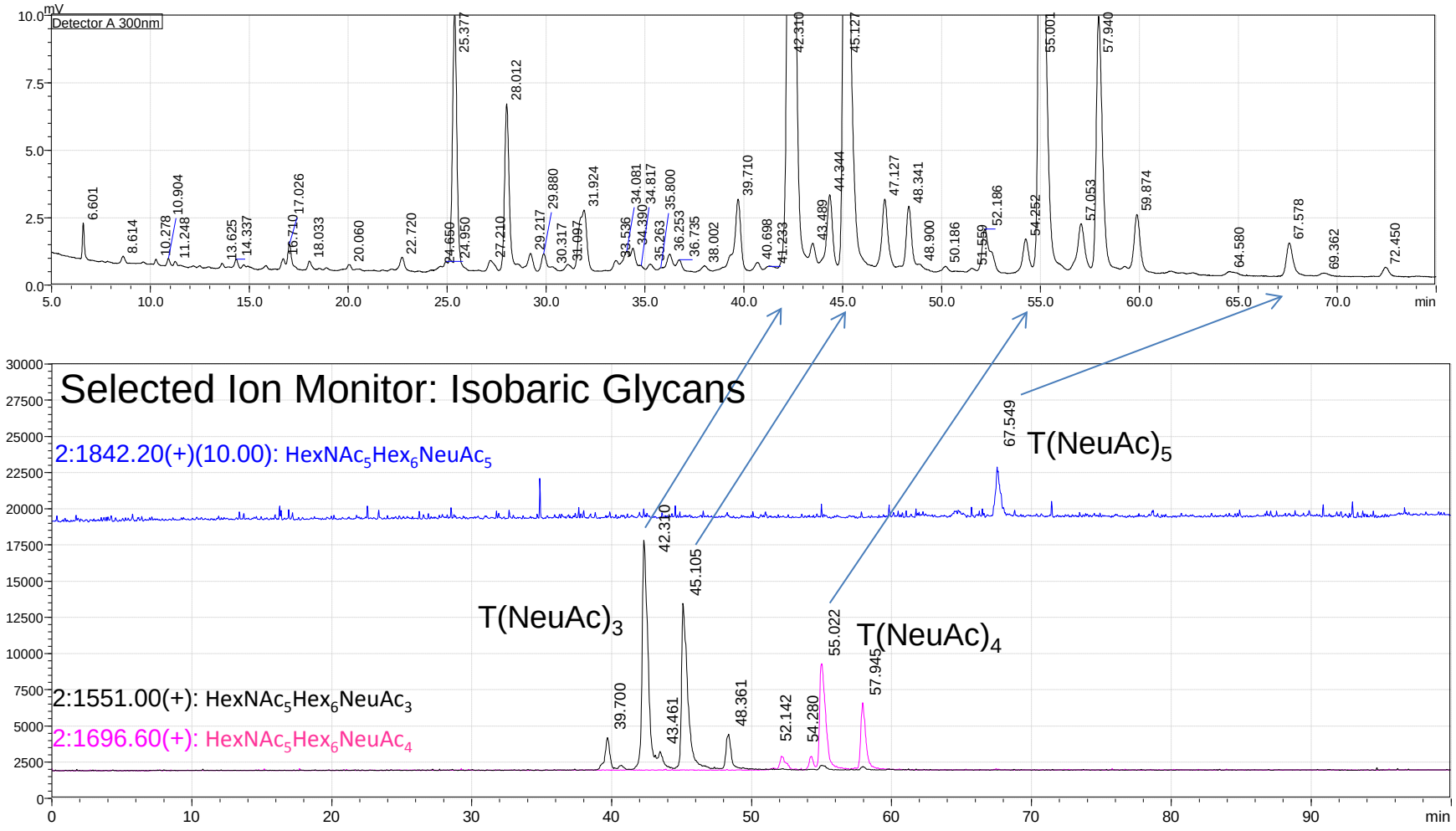


Penta-HILIC Analysis of PNGase F Released N-Glycans



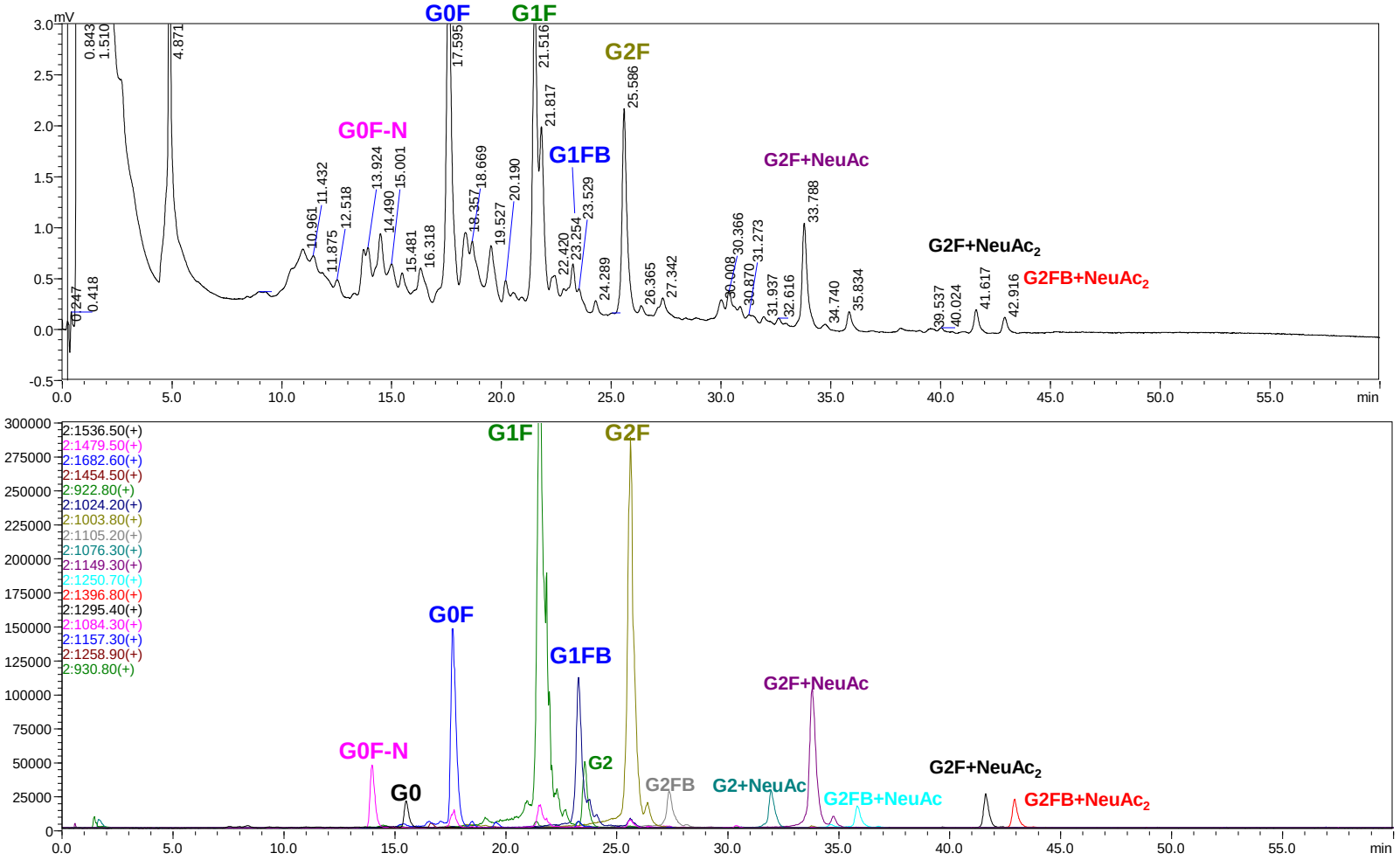
High Resolution HILIC Separations of Fetuin N-glycans: Separation of Sialic Acid Linkage Isomers

2.1 mm ID x 300 mm; 50 mM Ammonium Formate, pH 4.4, 70-55% AcN (B) in 90min., 60°C; 600 µL/min. Detection: 300 nm Abs; ESI-MS ((+) MS-2020, 4.2 kV, 400-2000 with SIM)

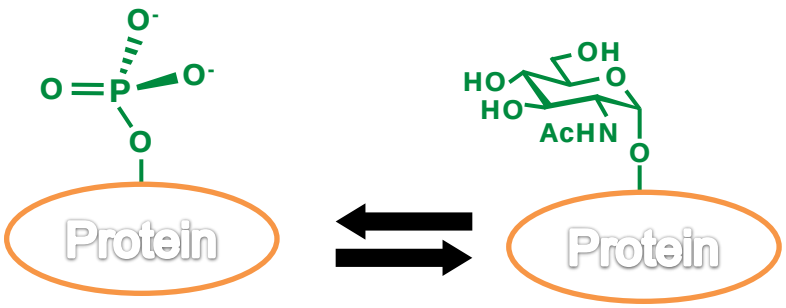


LC/MS Analysis of Human IgG Glycans using Penta-HILIC

2.1 x 150 mm Halo Penta-HILIC, 0.5 mL/min at 60°C; 77.5-60% AcN in 60 min.
Gradient conditions: B – 50 mM ammonium formate, pH 4.4, A – Acetonitrile. c. 15 ug Protein, 4 µL,
Abs (300 nm) series ESI-MS operated +4.7 kV, Scan from 500 to 2000 m/z (TIC); SIM 17 event @0.5 s

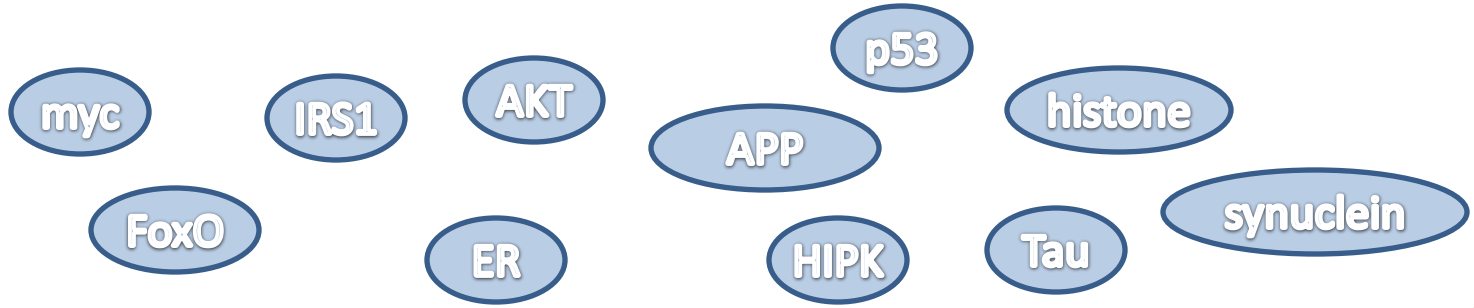


N-acetylglucosamine (O-GlcNAc)



O-GlcNAc modifies Ser and Thr. O-GlcNAc is a modifier of biological activity, in some cases, via competition for phosphorylation. Multiple sites on a particular protein can be modified by –P or –GlcNAc, close by, or far apart.

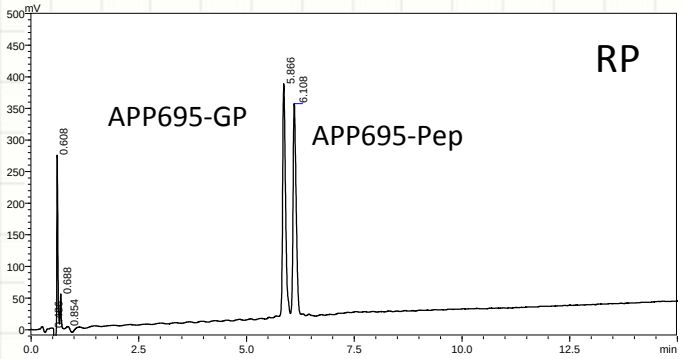
Hundreds of proteins that are implicated in the progression of diseases such as cancer, diabetes and neurodegeneration are modified by O-GlcNAc.

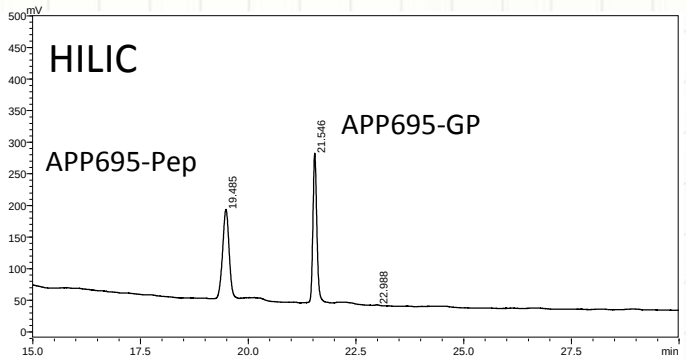


LC/MS of O-GlcNAcylated Peptides

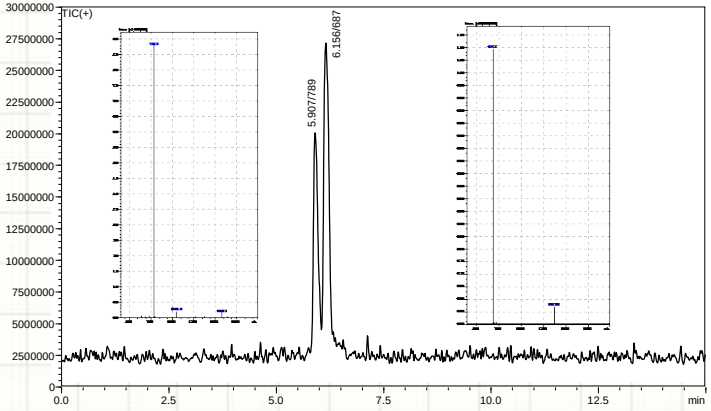
2.1 x 100 mm Halo Penta-HILIC, 0.4 mL/min at 60°C.
Gradient conditions: A – 0.1% formic acid/10 mM ammonium formate; B – 90% Acetonitrile in A.
500 pmol, MS operated +4.5 kV, with 0.45 s scan from 500 to 2000 m/z (TIC)
RP Gradient - 4% to 34% AcN/30 min (1%/min); HILIC Gradient - 90% to 60% AcN/30 min (-1%/min).

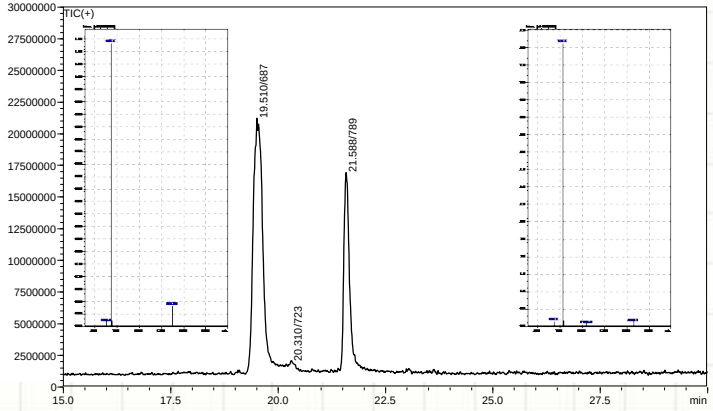
A_{210}





MS





APP695-14GPep	**VPTT(OGlcNAc)AASTPDAVDK**
APP695-14Pep	**VPTTAASTPDAVDK**

LC/MS of O-GlcNAcylated Peptides

Peptide Description	Sequence	Mass (neutral)	Rt RP (min)	Δ Rt RP (GP-P)	Rs RP	Rt HILIC (min)	Δ Rt HILIC (GP-P)	Rs HILIC
APP695-14GPep	VPTT(OGlcNAc)AASTPDAVDK	1574.8	5.87			21.55		
APP695-14Pep	VPTTAASTPDAVDK	1371.7	6.11	-0.24	1.90	19.49	2.07	9.41
MUC5AC	GTTPSPVPTTSTTSAP	1501.6	9.28			16.41		
MUC5AC-3	GTT(OGalNAc)PSPVPTTSTTSAP	1704.6	8.45	-0.83	6.88	18.68	2.27	13.40
MUC5AC-13	GTTPSPVPTTSTT(OGalNAc)SAP	1704.6	8.53	-0.75	5.82	18.51	2.10	10.72
MUC5AC3/13	GTT(OGalNAc)PSPVPTTSTT(OGalNAc)SAP	1908.1	7.76	-1.52/2	11.84	20.48	4.07/2	23.35
GP-41	Ac-CSTFRPRT(OGlcNAc)SSNAST	1758.8	7.09			18.59		
P-42	Ac-CSTFRPRTSSNAST	1555.7	7.03	0.06	0.44	17.03	1.56	11.58
GP-78	Ac-CQHPPVT(OGlcNAc)NGDTVK	1639.8	6.47			20.32		
P-84	Ac-CQHPPVTNGDTVK	1436.7	6.56	-0.10	0.66	18.72	1.61	11.23
GP-79	Ac-CKIADFGLS(OGlcNAc)KIVEHQ	1932.0	19.36			19.15		
P-85	Ac-CKIADFGLSKIVEHQ	1728.9	20.80	-1.44	8.16	17.21	1.94	14.76
GP-17s	CTLHTKAS(OGlcNAc)GMALLHQ	1854.9	13.62			17.29		
P-20s	CTLHTKASGMALLHQ	1651.8	14.23	-0.61	3.06	15.15	2.14	15.38
GP-15	Ac-CFELLPT(O-GlcNAc)PPLSP	1557.8	25.16			5.64		
P-18	Ac-CFELLPTPPLSP	1354.7	27.16	-2.00	8.88	2.71	2.93	20.11
GP-46	Ac-CRSSHYGGS(OGlcNAc)LPNVNQI	1975.9	12.48			17.32		
P-47	Ac-CRSSHYGGSLPNVNQI	1772.8	12.96	-0.48	3.83	15.43	1.89	13.91
GP-51	Ac-CSALNRTS(OGlcNAc)SDSALHT	1806.8	9.08			17.23		
P-52	Ac-CSALNRTSSDSALHT	1603.7	9.55	-0.47	3.85	15.55	1.69	12.42
GP-16	Ac-CKIPGVS(OGlcNAc)TPQTL	1487.7	16.41			13.27		
P-19	Ac-CKIPGVSTPQTL	1284.6	16.98	-0.58	3.74	10.59	2.68	21.63
GP-2-p53	Ac-CQLWVDS(OGlcNAc)TPPPG	1543.7	16.43			12.72		
P-3-p53	Ac-CQLWVDSTPPPG	1340.6	17.66	-1.23	7.23	10.41	2.31	10.28
GP-17r	Ac-CLHTKAS(OGlcNAc)GMALL	1488.7	16.21			10.59		
P-20r	Ac-CLHTKASGMALL	1285.6	16.98	-0.77	2.79	7.45	3.14	24.73
Average			13.01	-0.73	4.93	15.29	2.17	15.21
Standard Deviation			5.95	0.54	3.32	4.74	0.47	5.13
% RSD			45.7	74.3	67.3	31.0	21.8	33.7

HILIC shows better Resolution, Lower Variability in Resolution

Conclusions

- Fused-core silica packing materials have proven high utility for biomolecule separations, without the obligation of very high pressure operation.
- Fused-Core RP materials with enlarged pore sizes (400 and160 Å) have particular utility for protein S/F analyses, are highly robust, and allow ***fast*** peptide and protein separations.
- New polar bonded phase Fused-core columns exhibit high utility for HILIC separations, and particular application uses in glycobiology/protein modifications.

Acknowledgements

Thank you for your Attention!

- Neurology Research-
  - Dr. Doug Walker, Sun Health Research Institute, Sun City, AZ
- Protein S/F, Proteomics and Glycomics-
  - Prof. Ron Orlando, and Students, CCRC, University of GA.
  - Prof. Bob Hodges and Colin Mant, U. Colorado
- NIH/SBIR for Financial Support