Bioseparations with 3.4- and 5-Micron Wide-Pore Superficially Porous Particles

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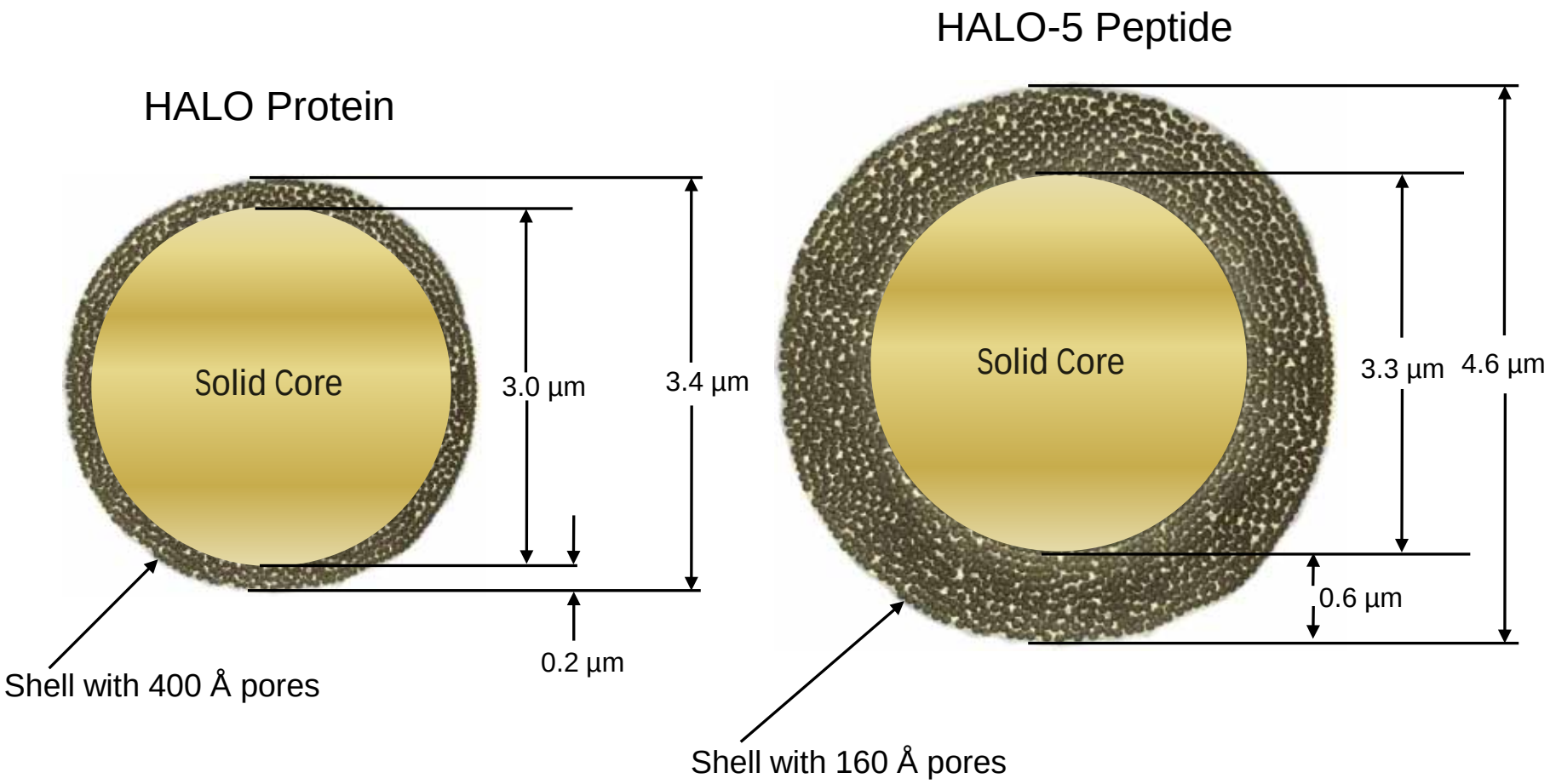
Objective

Columns packed with modern superficially porous (core-shell) particles (SPP) exhibit extraordinarily high performance for small-molecule separations compared to columns packed with totally porous particles of the same size. Many published reports by independent researchers verify that 2.5 – 2.7 micron SPP particles with small pores (ca. 70 – 90 Å) can produce columns with the efficiency of sub-2-micron particle columns with nearly half the back pressure of the smaller particles. The success of the SPP particles with small molecules has generated interest in the development of SPP particles with larger pore sizes for the separation of bigger molecules. The short diffusion paths provided by the thin porous shells on SPP particles are more advantageous for the separation of high molecular weight molecules that diffuse slowly compared to smaller molecules. SPP particles are now available in a variety of pore sizes from 120 Å to 400 Å for biomolecule separations. Recent introductions of nominally 3.4-micron and 5-micron larger pore SPP particles have increased the range of utility for the superficially porous particle technology. Columns of these larger-particle wide-pore SPP are demonstrated in this poster to exhibit significant performance advantages over totally porous particles of similar and smaller particle size. This report also provides examples of 3.4 and 5-micron SPP columns with different bonded phases to further demonstrate the performance and advantages for larger particle wide-pore SPP.

Physical Characteristics of Fused-Core Particles

Fused-Core Particle	Particle Size (µm)	Pore Size (Å)	BET Surface Area (m^2/g)	Shell Thickness (µm)	Separation Utility
Halo	2.7	90	135	0.5	Small molecules < 5000 MW
HALO-5	4.6	90	90	0.6	Small molecules < 5000 MW
Halo Peptide	2.7	160	80	0.5	Peptides < 15 kDa
HALO-5 Peptide	4.6	160	60	0.6	Peptides < 15 kDa
HALO Protein	3.4	400	15	0.2	Proteins < 400 kDa

HALO® Wide-Pore Fused-Core Particles



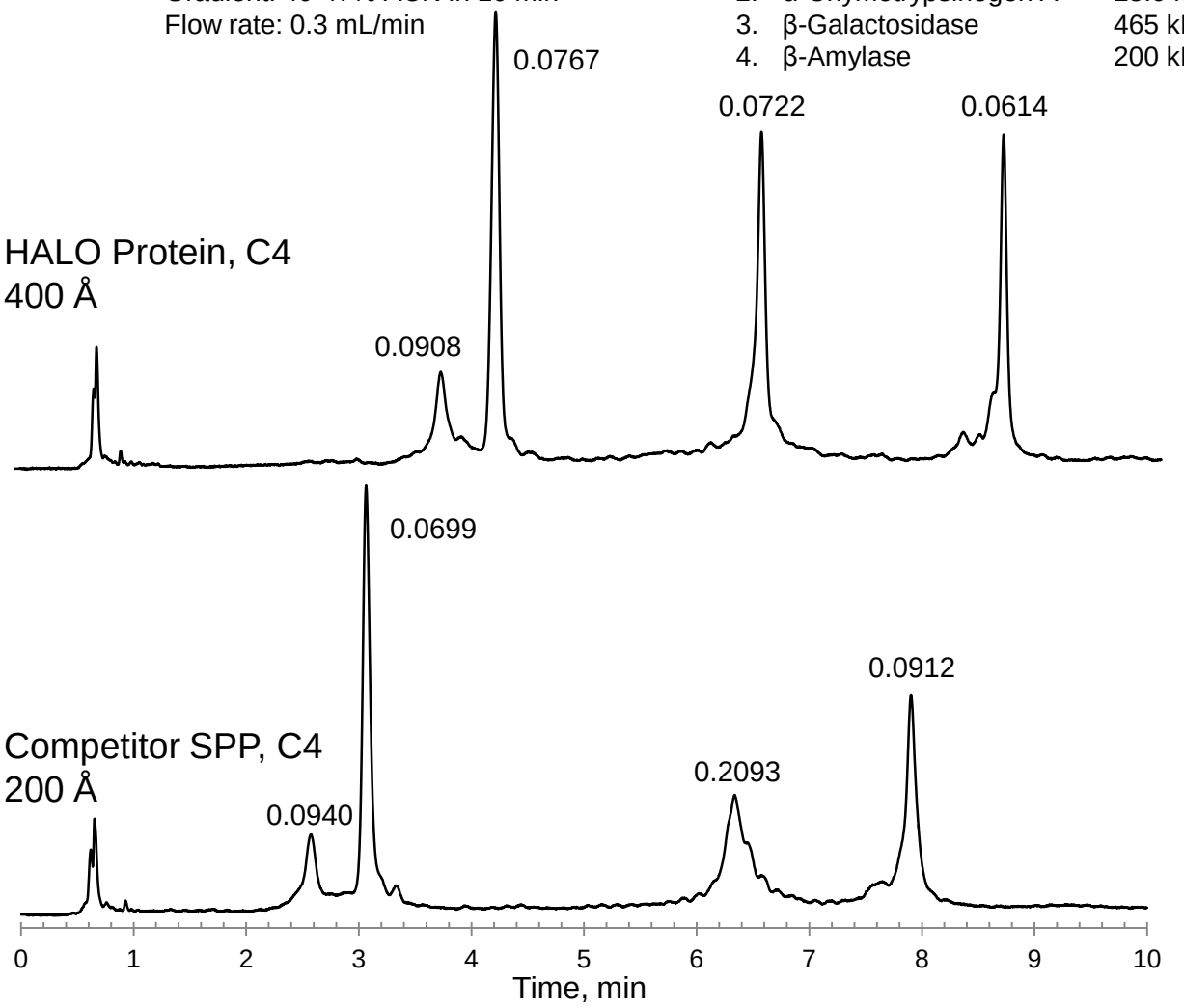
Protein Separations: Effect of Pore Size

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

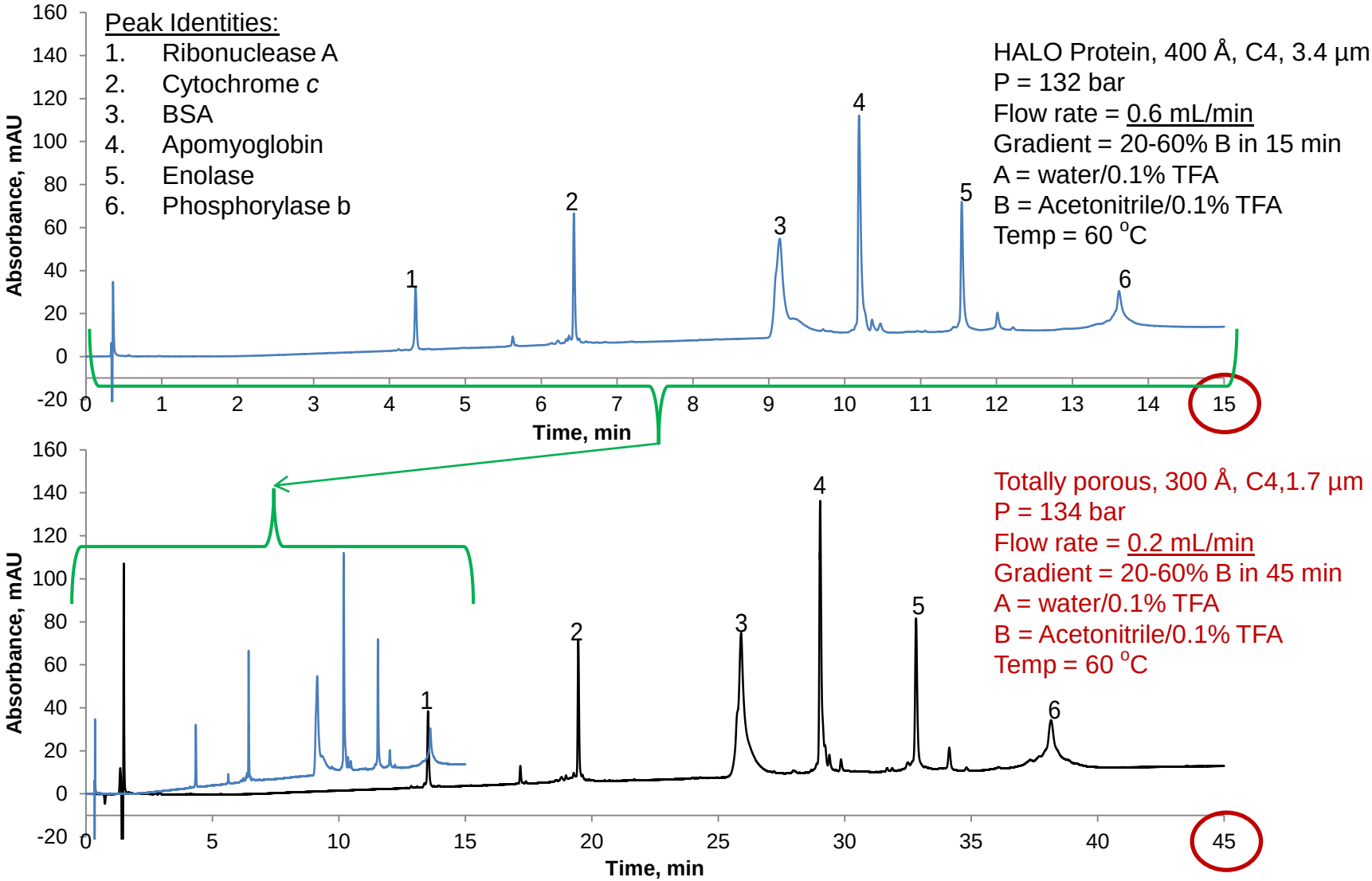
Peak Identities (in order):

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]



- Peak widths in minutes provided above each peak.
- The 400 Å pores of HALO Protein enable sharp peaks for high MW biomolecules.

Protein Separations: Fused-Core compared to Totally Porous



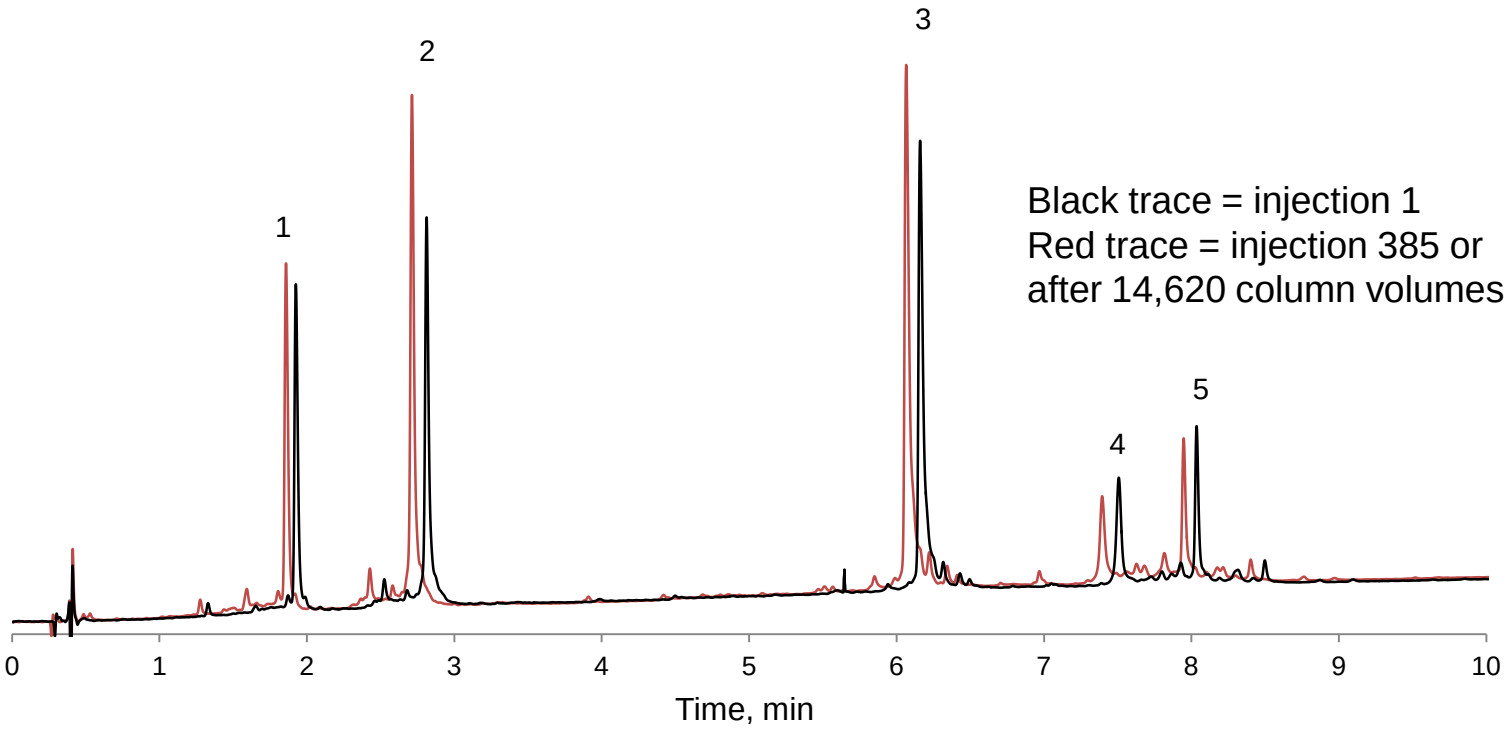
- Separation is 3 times faster at the same back pressure on the HALO Protein column compared to the same sample run on a sub-2-µm totally porous particle column

HALO Protein C4: Stability

Column: 2.1 x 100 mm
Instrument: Shimadzu Nexera
Injection Volume: 1.0 µL
Detection: 215 nm
Temperature: 90 ^{o}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)



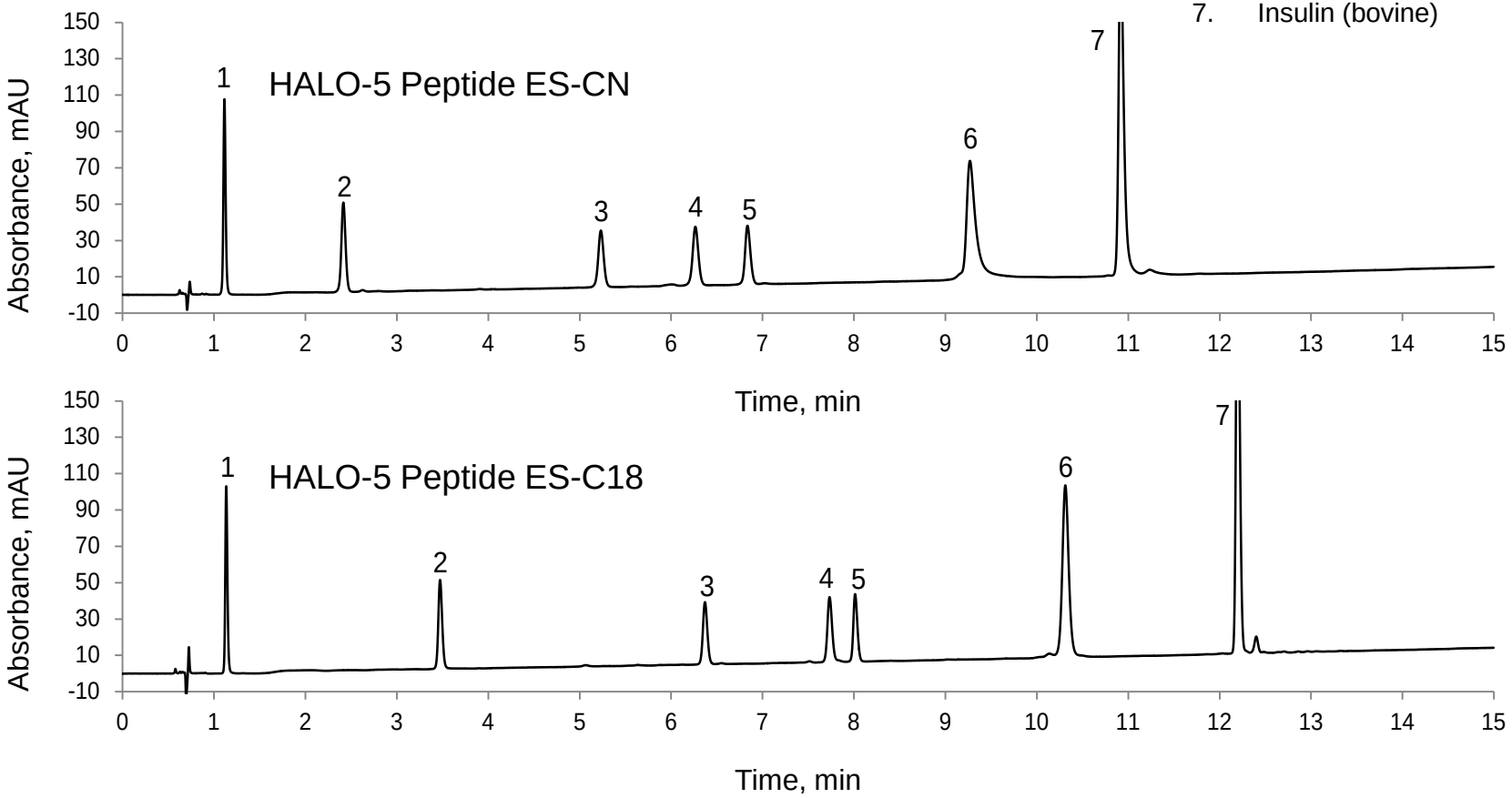
- The HALO Protein C4 bonded phase is stable up to 90 ^{o}C, showing very little loss of retention.

HALO-5 Peptide: Comparison of Bonded Phases

Columns: 4.6 x 100 mm
Instrument: Agilent 1100
Injection Volume: 5 µL
Detection: 220 nm
Temperature: 30 °C

Flow rate: 1.5 mL/min
Mobile Phase A: 10/90 acetonitrile/water/0.1% TFA
Mobile Phase B: 70/30 acetonitrile/water/0.1% TFA
Gradient: 0-50% B in 15 min

Peak Identities:
1. Gly-Tyr
2. Val-Tyr-Val
3. Methionine Enkephalin
4. Angiotensin II
5. Leucine Enkephalin
6. Ribonuclease A (bovine)
7. Insulin (bovine)

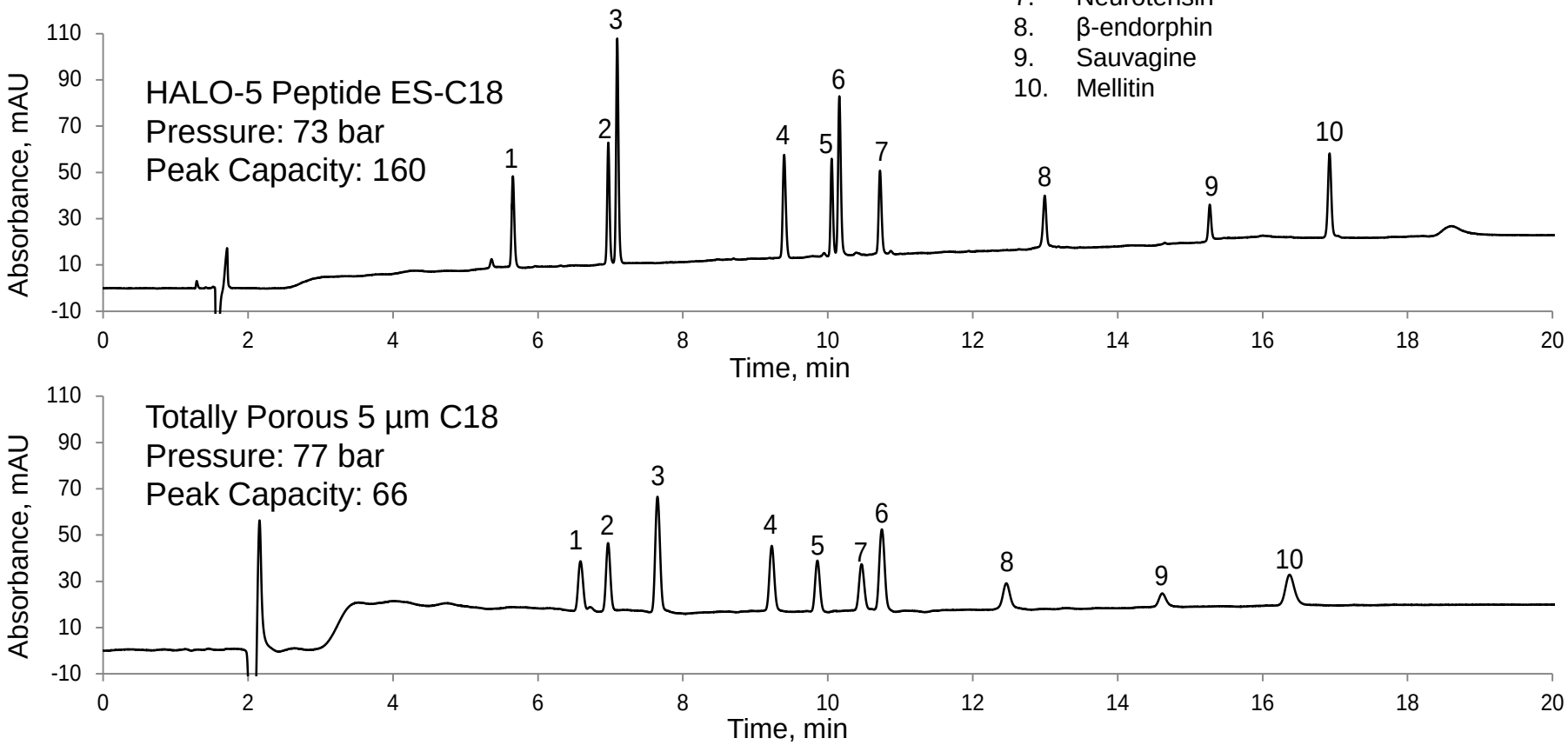


Peptide Separations: Fused-Core compared to Totally Porous

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: acetonitrile/0.1% TFA
Gradient: 5-60% B in 20 min

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

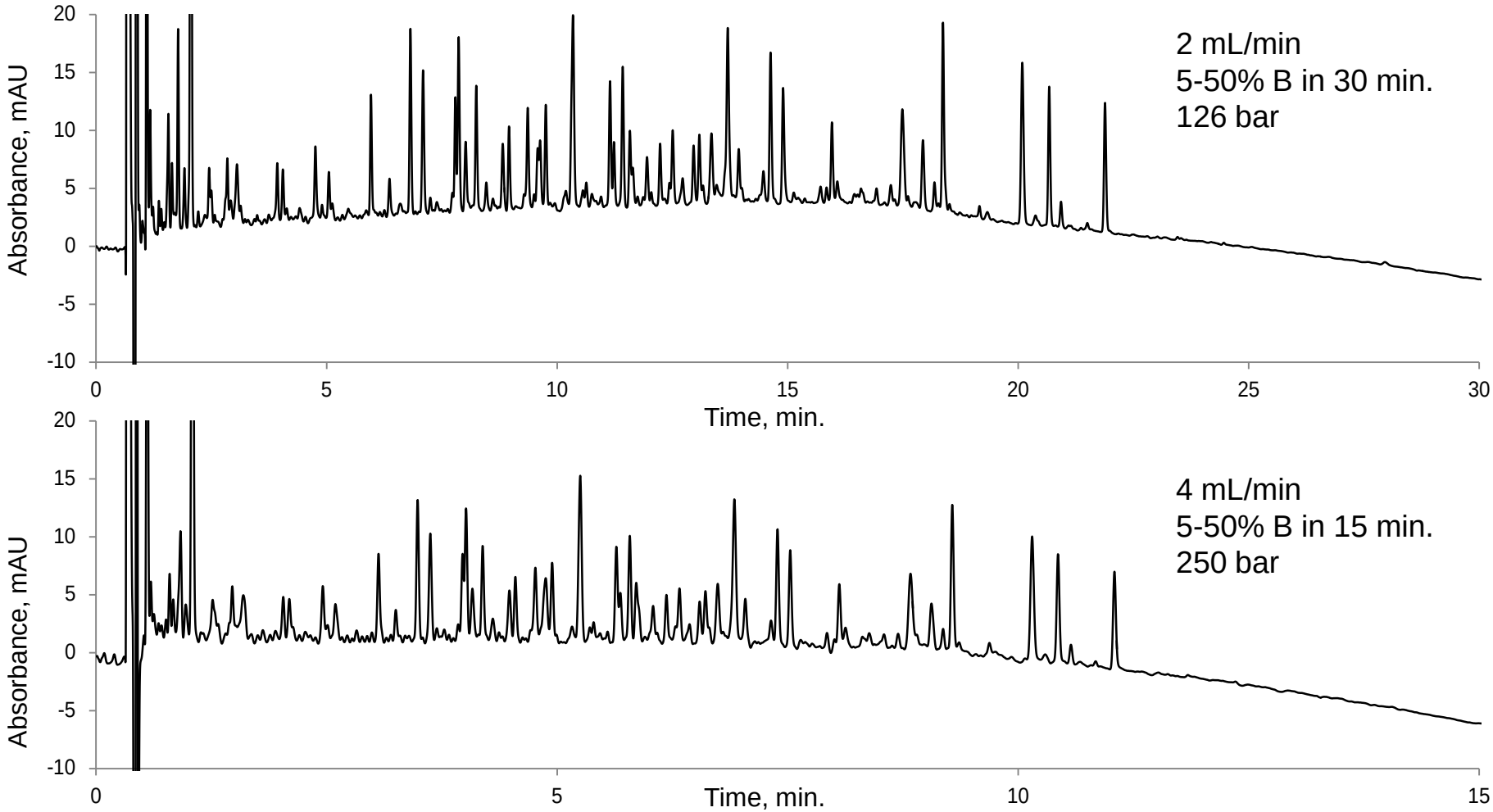


- The Fused-core column with its very narrow peak shapes has more than double the peak capacity of the totally porous 5-micron column.

HALO-5 Peptide ES-C18: Tryptic Digest

Column: 4.6 x 150 mm
Mobile Phase A: water/0.1% TFA
Mobile Phase B: acetonitrile/0.1% TFA
Gradient: as indicated

Detection: 215 nm
Sample: apotransferrin tryptic digest
Injection volume: 15 µL
Temperature: 60 °C



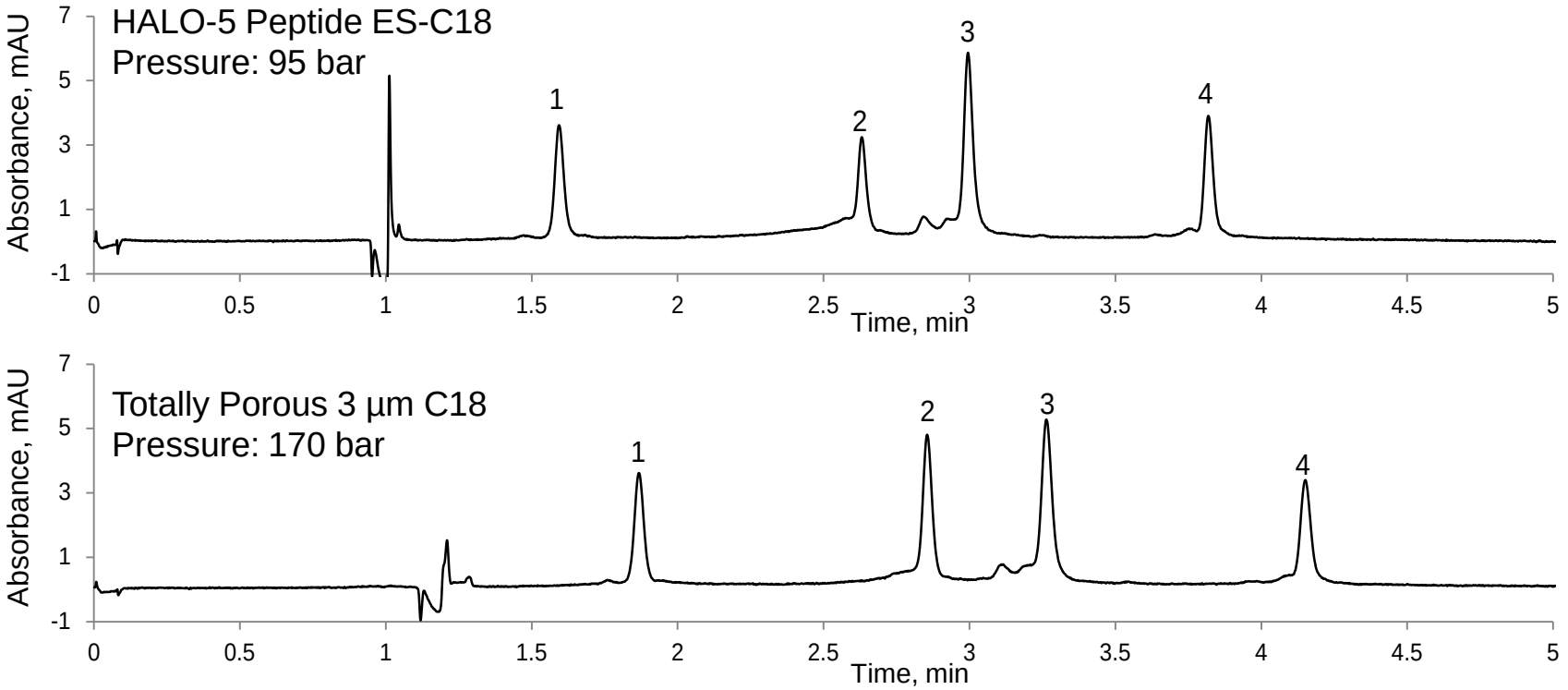
- High resolution is maintained for this tryptic digest using the 5-micron Fused-core column while doubling the flow rate.

Small Protein Separations: Fused-Core compared to Totally Porous

Columns: 4.6 x 150 mm
Instrument: Agilent 1200 SL
Injection Volume: 15 µL
Detection: 280 nm
Temperature: 60 ^{o}C

Flow rate: 1.5 mL/min
Mobile Phase A: water/0.1% TFA
Mobile Phase B: acetonitrile/0.1% TFA
Gradient: 28-55% B in 5 min

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



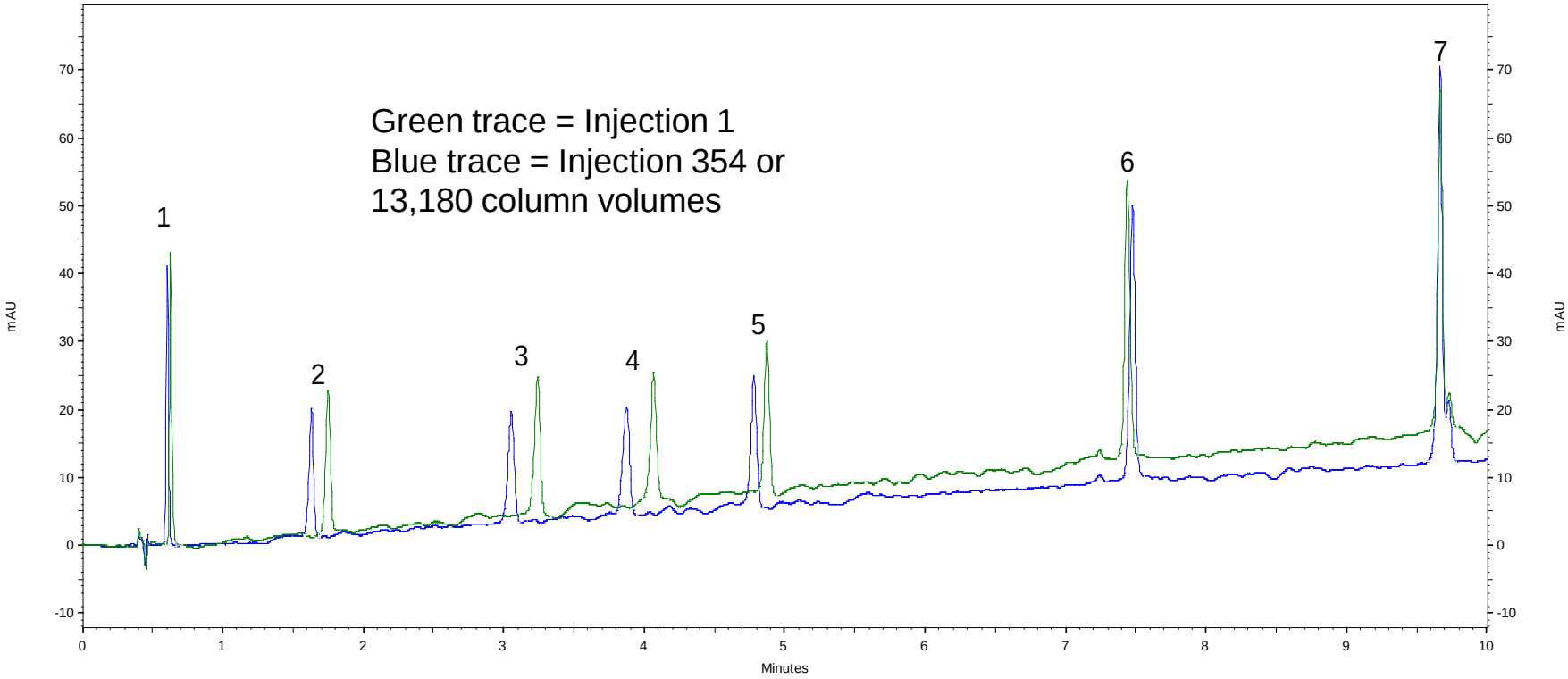
- The Fused-core 5-micron column shows equivalent performance to the totally porous 3-micron column, but at significantly lower back pressure. Lower pressures lead to longer column life and more reproducible separations.

HALO-5 Peptide ES-CN: Stability

Column: 2.1 x 100 mm
Instrument: Perkin Elmer FX-15
Injection Volume: 1.0 µL
Detection: 215 nm
Temperature: 90 ^{o}C

Flow rate: 0.5 mL/min
Mobile Phase A: water/0.1% TFA
Mobile Phase B: 70/30 acetonitrile/water/0.1% TFA
Gradient: 5-45% B in 10 min

Peak Identities:
1. Gly-Tyr
2. Val-Tyr-Val
3. Methionine Enkephalin
4. Angiotensin II
5. Leucine Enkephalin
6. Ribonuclease A (bovine)
7. Insulin (bovine)



- The HALO-5 Peptide ES-CN bonded phase demonstrates superior stability at high temperature and low pH.

Conclusions

- The different bonded phases that are available on the larger particle size Fused-core particles enable biomolecule analyses to be optimized, depending on the requirements for the separation.
- The bonded phases on the 3.4 and 5-micron Fused-core particles are stable at very high temperature (90 ^{o}C) and at low pH.
- Both the 3.4 and 5-micron Fused-core particles show superior performance compared to totally porous particles, whether the metric is peak capacity or back pressure or time of analysis.
- The low back pressure of the 5-micron Fused-core particles enables faster flow rates to be used for separations of tryptic digests while maintaining resolution.

Acknowledgment

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