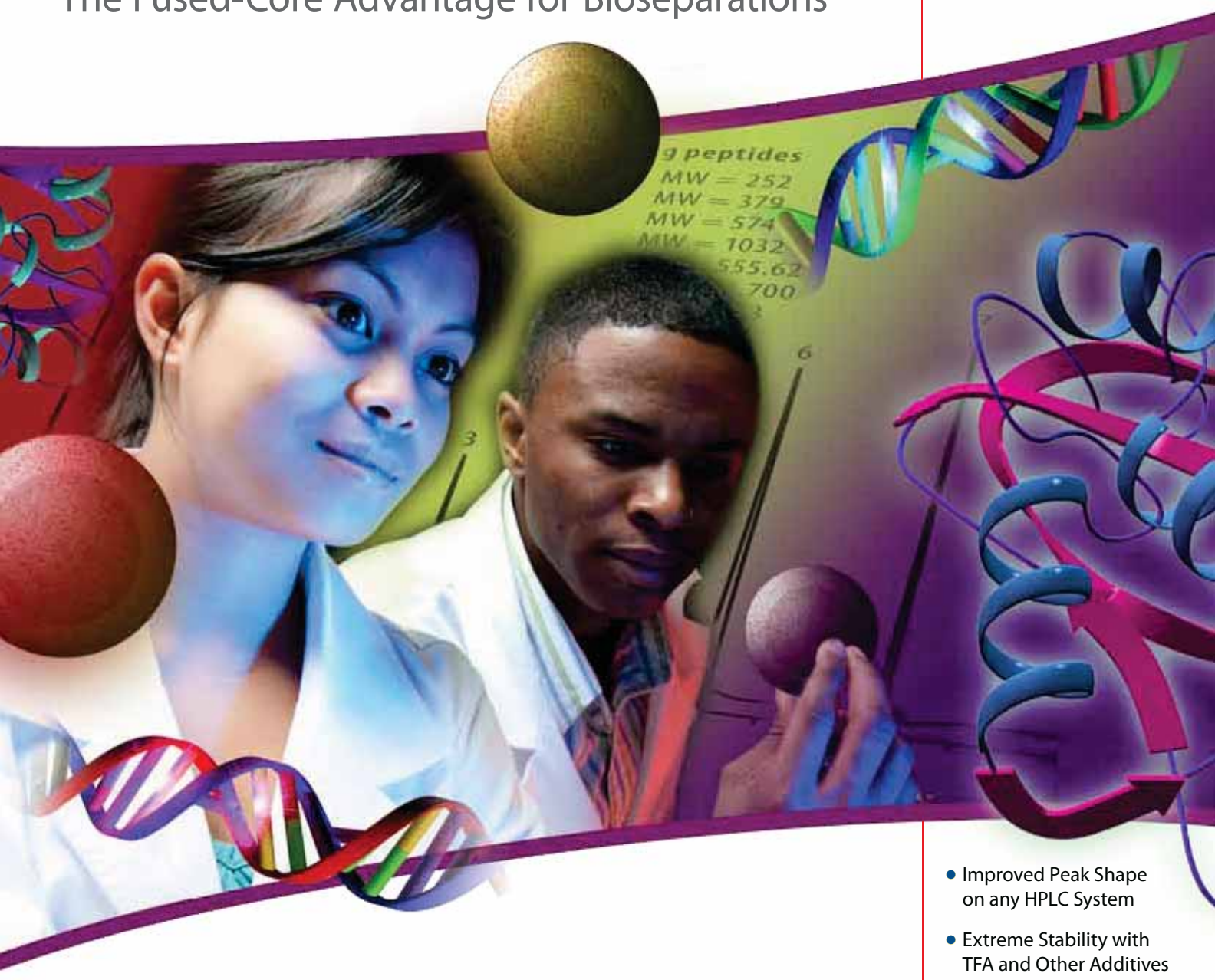
Ascentis Express Peptide ES-C18 HPLC Columns

The Fused-Core Advantage for Bioseparations

SUPELCO Analytical

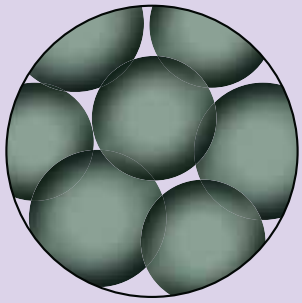


- Improved Peak Shape on any HPLC System
- Extreme Stability with TFA and Other Additives
- Rugged HPLC Column Design

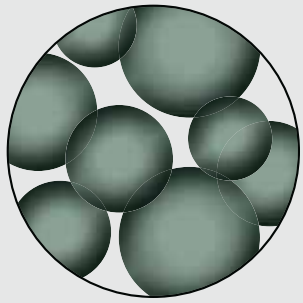
The Fused-Core Advantage

Narrow Particle Size Distribution

Fused-Core Particles

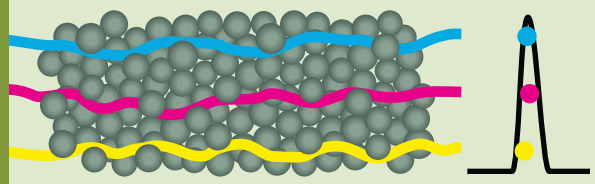


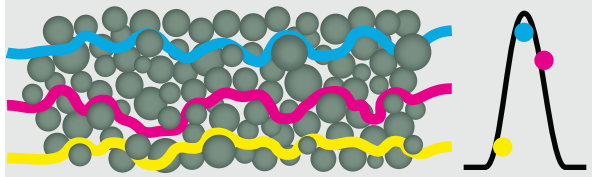
Traditional Porous Particles



The innovative manufacturing process for Fused-Core particles produces a very narrow particle size distribution. A narrow particle size distribution allows for the use of large porosity frits that resist clogging, resulting in a **more rugged column**. Traditional porous particles are not manufactured in a way to yield extremely narrow particle size distributions.

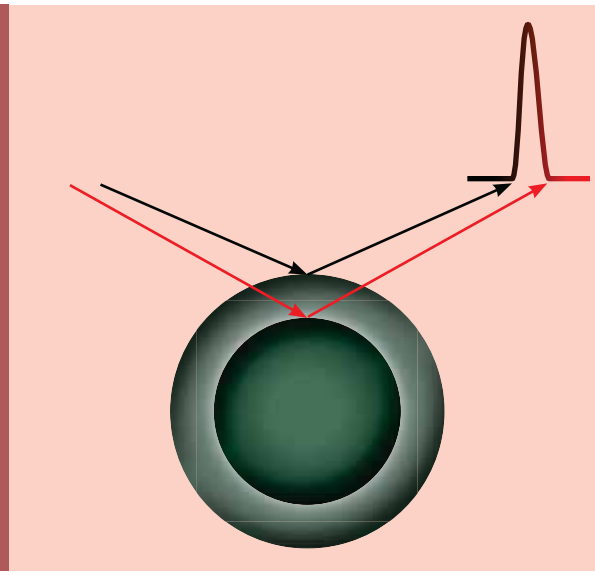
More Consistent Bed

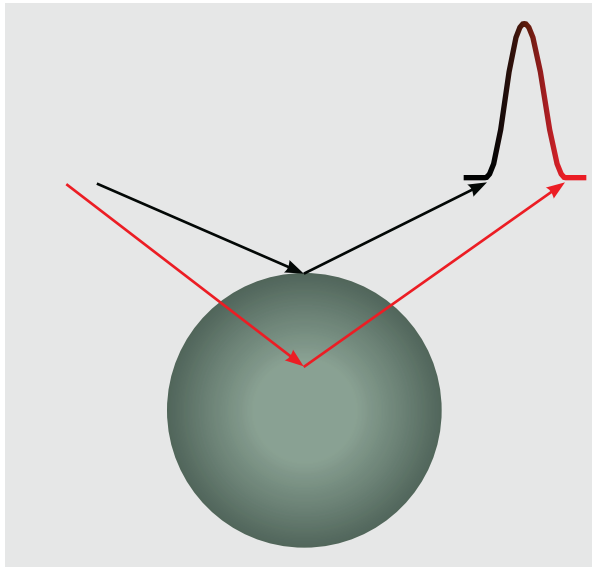




The "A" term in the van Deemter equation accounts for the effects of inhomogeneities in the packed bed of an HPLC column. Narrow particle size distributions form a more consistent packed bed and a consistent path length, **minimizing analyte diffusion** through the column. This eddy diffusion is effectively independent of mobile phase velocity.

Shorter Diffusion Path





The short diffusion path of the Fused-Core particle **yields sharper peaks** than traditional porous particle columns. The minimized resistance to mass transfer, the "C" term in the van Deemter equation, of the Fused-Core particle provides sharper peaks than traditional porous particles. The short diffusion path also **permits the use of higher flow rates** without peak broadening.

A Breakthrough in Bioseparations Performance

Ascentis Express Peptide ES-C18

Ascentis® Express Peptide ES-C18 is a high-speed, high-performance liquid chromatography column based on a 160 Å Fused-Core® particle design. The Fused-Core particle provides a thin porous shell of high-purity silica surrounding a solid silica core. This particle design exhibits very high column efficiency for high MW solutes (up to 20 kDa) due to the shallow diffusion paths in the 0.5-micron thick porous shell and the small overall particle size of 2.7 microns.

Table 1. Specifications for Ascentis Express Peptide ES-C18

Silica	High Purity Type B
Phase	Sterically protected C18
pH range	1 – 9
Temperature	100 °C
Average pore diameter	160 Å
Surface area, nitrogen	80 sq.m/g
Pore volume	0.30 mL/g
Particle density	1.3 g/cc

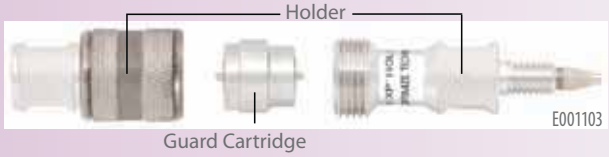
Ordering Information

Ascentis Express Peptide ES-C18 Columns

		Length (cm)					Guard Cartridges
		3	5	7.5	10	15	Pk. of 3
Analytical (mm I.D.)	2.1	53299-U	53301-U	53304-U	53306-U	53307-U	53536-U
	3.0	53308-U	53311-U	53312-U	53313-U	53314-U	53537-U
	4.6	53316-U	53318-U	53323-U	53324-U	53328-U	53542-U
Capillary (µm I.D.)	75	—	53543-U	—	—	53549-U	—
	100	—	53544-U	—	—	53552-U	—
	200	—	53545-U	—	—	53553-U	—
	300	—	53546-U	—	—	53552-U	—
	500	—	53547-U	—	—	53558-U	—
	1000	—	53548-U	—	—	53561-U	—

Ascentis Express Cartridge Holder

Description	Cat. No.
Universal Guard Holder	
Holder w/EXP Titanium Hybrid Ferrule (cartridge not included)	53500-U



Applications

Ascentis Express Peptide ES-C18 columns utilize a steric-protected C18 bonded phase with extremely high resistance to acid-catalyzed hydrolysis of the siloxane bond that attaches the C18 chain to the surface. Thus, the combination of low pH and elevated temperature operation of the column is well tolerated. Peptide separations are efficiently conducted using low pH mobile phase modifiers, often at 0.1% concentration. Most popularly employing trifluoroacetic acid (TFA), and the related perfluorocarboxylic acids, pentafluoropropionic acid (PFPA) and heptafluorobutyric acid (HFBA). Additional opportunities for UV detection at low pH operation is with mineral acids such as phosphoric acid (1-20 mM). For MS detection 0.1% formic acid is most commonly employed (sometimes acetic acid), but significant benefit to peak shape can be realized with 0.1% formic acid adjusted to pH 3.5 (with ammonium hydroxide, for instance), especially with basic peptides. This is likely due to greater availability of formate anion for ion pairing.

Pharmaceutical Peptides

Many peptides have been investigated as therapeutic pharmaceutical drugs and are active vasodilators, vasoconstrictors, hormones, and neuropeptides. Using reversed-phase HPLC, it is possible to solve the tasks of identification, purity monitoring, and quantitative analysis in many cases, including those where the application of other methods is impossible.

Synthetic Peptides

The difficulty with synthetic peptides involves the production of many "deletion variants". A deletion may occur at any point in the peptides synthesis and so several versions of the peptide are produced which are absent one, two or three amino acids from the desired product. This makes for an interesting chromatographic problem because the resultant peptide mix contains peptides that are very similar in structure.

Peptide Mapping

Protein analysis and characterization has become more crucial due to many biopharmaceutical advances. Peptide mapping via LC-MS is one such technique that is commonly used today. A typical procedure involves the preparation of a tryptic digest from the protein, with subsequent characterization using reversed-phase, gradient HPLC separation followed by mass spectral analysis and database search.

Difficult Separations on Ascentis Express Peptide ES-C18

Figure 1. Basic Peptides

column: Ascentis Express Peptide ES-C18, 10 cm x 2.1 mm I.D. (53306-U)
mobile phase A: 0.1% (v/v) additive in water
mobile phase B: 25:75, 0.4% (v/v) additive in water:acetonitrile
additive: formic acid, pH 3.5 (adjusted with ammonium hydroxide)
gradient: initial = 15% B, slope = 2% MeCN / column volume
flow rate: 0.3 mL/min
temp.: 35 °C
det.: ESI(+)-TOF
injection: 1 µL
sample: 5 mg/L peptide 1 & 3, 1 mg/L peptide 2, 15 mg/L peptide 4

Peptide probes (listed in order of elution):

1.	ac-GGGLGGAGGLKG	monoisotopic mass: 941.5
2.	ac-KYGLGGAGGLKG	monoisotopic mass: 1118.6
3.	ac-GGAVKALKGLKG	monoisotopic mass: 1139.7
4.	ac-KYALKALKGLKG	monoisotopic mass: 1330.8

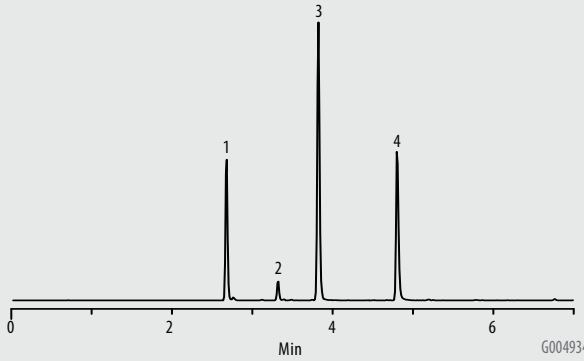


Figure 2. Peptide Test Mix

column: Ascentis Express Peptide ES-C18, 10 cm x 4.6 mm I.D. (53324-U)
mobile phase A: 0.1% (w/v) TFA in 90:10 water:acetonitrile
mobile phase B: 0.095% (w/v) TFA in 25:75 water:acetonitrile
gradient: initial = 0% B to 50% B in 15 min.
flow rate: 1.5 mL/min
temp.: 30 °C
det.: UV at 220 nm
injection: 5 µL

The test mix employed contains the following peptides

1.	Gly-Tyr	MW = 252
2.	Val-Tyr-Val	MW = 379
3.	Met Enkephalin	MW = 574
4.	Angiotensin II	MW = 1032
5.	Leu-Enkephalin	MW = 555
6.	Ribonuclease	MW = 13,700
7.	Bovine Insulin	MW = 5733

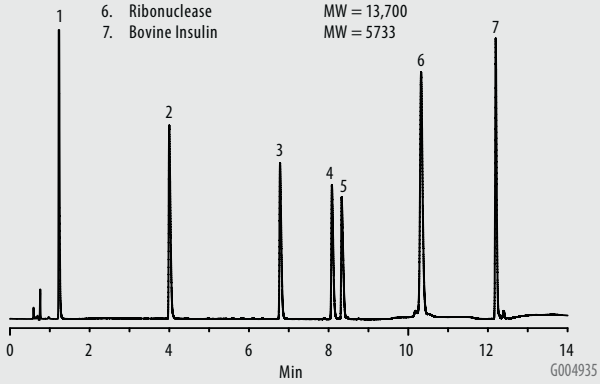


Figure 3. Carbonic Anhydrase Tryptic Digest

column: Ascentis Express Peptide ES-C18, 10 cm x 4.6 mm I.D. (53324-U)
mobile phase A: 0.1% (w/v) TFA in water
mobile phase B: 0.1% TFA (w/v) in 40:60 water:acetonitrile
gradient: initial = 3% B to 100% B in 53 min.
flow rate: 1.0 mL/min
temp.: 30 °C
det.: UV at 215 nm
injection: 20 µL

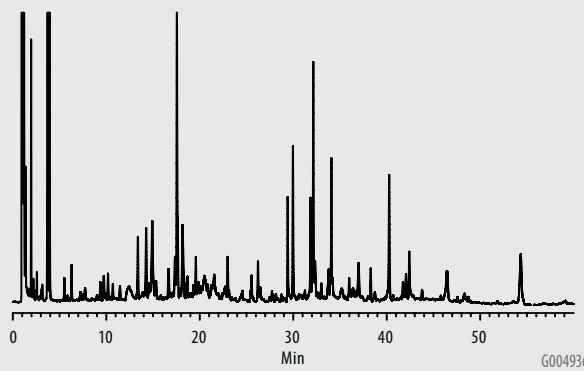
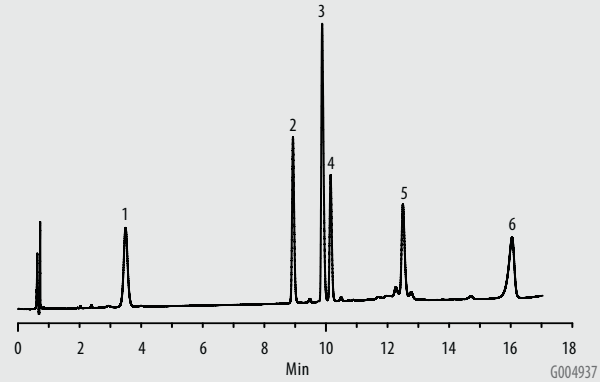


Figure 4. Small Proteins

column: Ascentis Express Peptide ES-C18, 10 cm x 4.6 mm I.D. (53324-U)
mobile phase A: 0.1% (w/v) TFA in 90:10 water:acetonitrile
mobile phase B: 0.095% (w/v) TFA in 25:75 water:acetonitrile
gradient: initial = 25% B to 40% B in 15 min.; then to 60% B at 20 min.
flow rate: 1.5 mL/min
temp.: 30 °C
det.: UV at 220 nm
injection: 4 µL

1.	Ribonuclease	MW = 13,700
2.	Porcine Insulin	MW = 5,780
3.	Bovine Insulin	MW = 5,730
4.	Human Insulin	MW = 5,800
5.	Cytochrom C	MW = 12,327
6.	Lysozyme	MW = 14,700



Competitor Comparison

Basic Peptides

Columns run under equivalent conditions of gradient slope (Δ % MeCN per column volume).

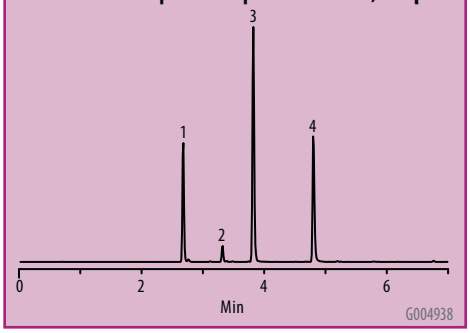
column: C18, 10 cm x 2.1 mm I.D.
mobile phase A: 0.1% additive in water
mobile phase B: 25:75, (0.4 % additive):acetonitrile
additive: formic acid, pH 3.5 (adjusted with ammonium hydroxide)
gradient: initial = 15% B, slope = 2% MeCN / column volume
flow rate: 0.3 mL/min
temp.: 35 °C
det.: ESI(+)-TOF
injection: 1 µL
sample: 5 mg/L peptide 1 & 3, 1 mg/L peptide 2, 15 mg/L peptide 4

Peptide probes (listed in order of elution):

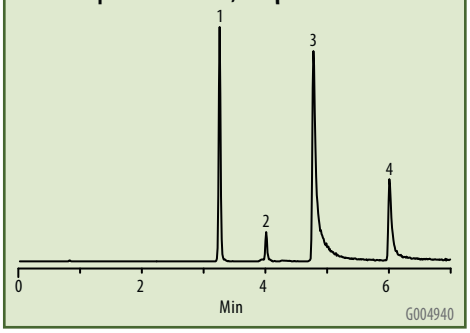
1. ac-GGGLGGAGGLKG Monoisotopic mass: 941.5
2. ac-KYGLGGAGGLKG Monoisotopic mass: 1118.6
3. ac-GGAVKALKGLKG Monoisotopic mass: 1139.7
4. ac-KYALKALKGLKG Monoisotopic mass: 1330.8

Peptide probes increase in basicity and hydrophobicity.

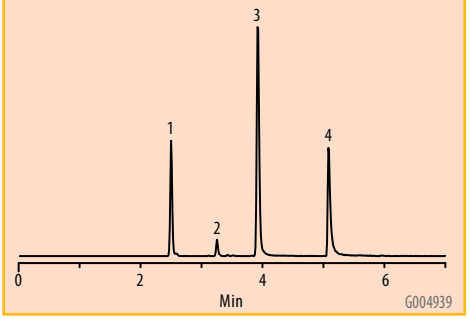
A. Ascentis Express Peptide ES-C18, 2.7µm



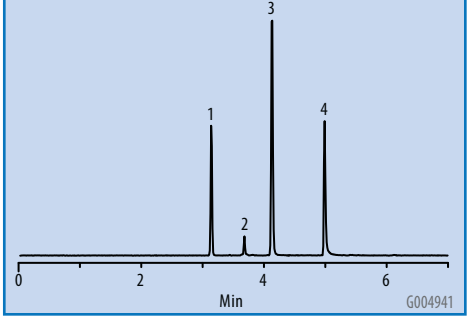
C. Competitor V C18, 1.5µm



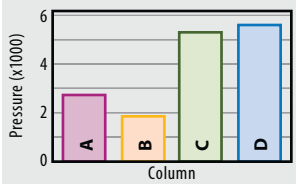
B. Competitor Z C18, 3.5µm



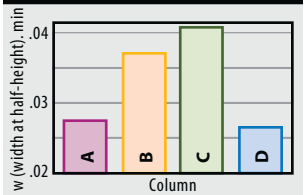
D. Competitor W C18, 1.7µm



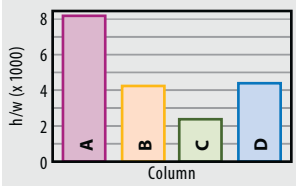
Column Backpressure



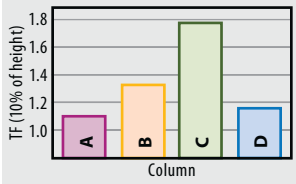
Avg Peak Width of Basic Peptides



Avg h/w of Basic Peptides



Avg Tailing Factor of Basic Peptides



Experimental Setup: Columns and Elution Gradients

Column	Pore µm	(Å)	ID (mm)	L (cm)	CV (mL)*	Grad slope start %B	[Δ % MeCN / CV]
A. Ascentis Express Peptide ES-C18	2.7	160	2.1	10	0.190	15	2
B. Competitor Z C18	3.5	300	2.1	10	0.216	15	2
C. Competitor V C18	1.5	120	2.0	10	0.205	15	2
D. Competitor W C18	1.7	130	2.1	10	0.186	15	2

* CV determined as follows: t_0 = r.t. of unretained component. d_0 = r.t. of unretained component with ZDV union in place of column. **CV** = (t_0 - d_0) x mL/min

Some Quantitative Comparisons

Column	Peak 1			Peak 2			Peak 3			Peak 4		
	$w_{½}$	$h/w_{½}$	$TF_{0.1}$	$w_{½}$	$h/w_{½}$	$TF_{0.1}$	$w_{½}$	$h/w_{½}$	$TF_{0.1}$	$w_{½}$	$h/w_{½}$	$TF_{0.1}$
A. Ascentis Express Peptide ES-C18, 2.7 µm	0.0258	9725	1.20	0.0224	1520	1.00	0.0344	11321	1.00	0.0267	9706	1.30
B. Competitor Z C18, 3.5 µm	0.0344	4082	1.14	0.0344	572	1.25	0.0387	8267	1.08	0.0387	3871	1.74
C. Competitor V C18, 1.5 µm	0.0344	4418	1.03	0.0344	548	1.06	0.0516	3044	2.69	0.0430	1281	2.38
D. Competitor W C18, 1.7 µm	0.0258	7568	1.10	0.0241	1203	1.20	0.0241	1942	1.00	0.0301	6684	1.30

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