



Thermo Scientific Accucore HPLC Columns  
for Biomolecule Separations

Ultimate Core Performance  
**to Maximize Your Investment**

# Core Enhanced Technology – for Biomolecules

The range of Accucore™ HPLC columns packed with 150 Å pore diameter particles allow biomolecule separations to benefit from the superb resolution and high speed enabled by the Core Enhanced Technology™ that Accucore is based on.

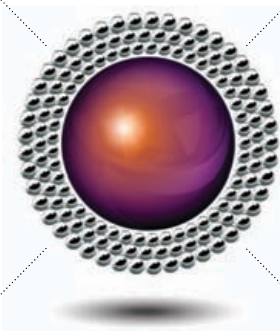
The key components of Core Enhanced Technology are:

### Solid Core Particles

2.6 µm diameter particles with a solid core generate high speed, high resolution separations without excessive backpressure

### Automated Packing Process

Enhanced automated procedures ensure that all columns are packed with the highest quality



### Tight Control of Particle Diameter

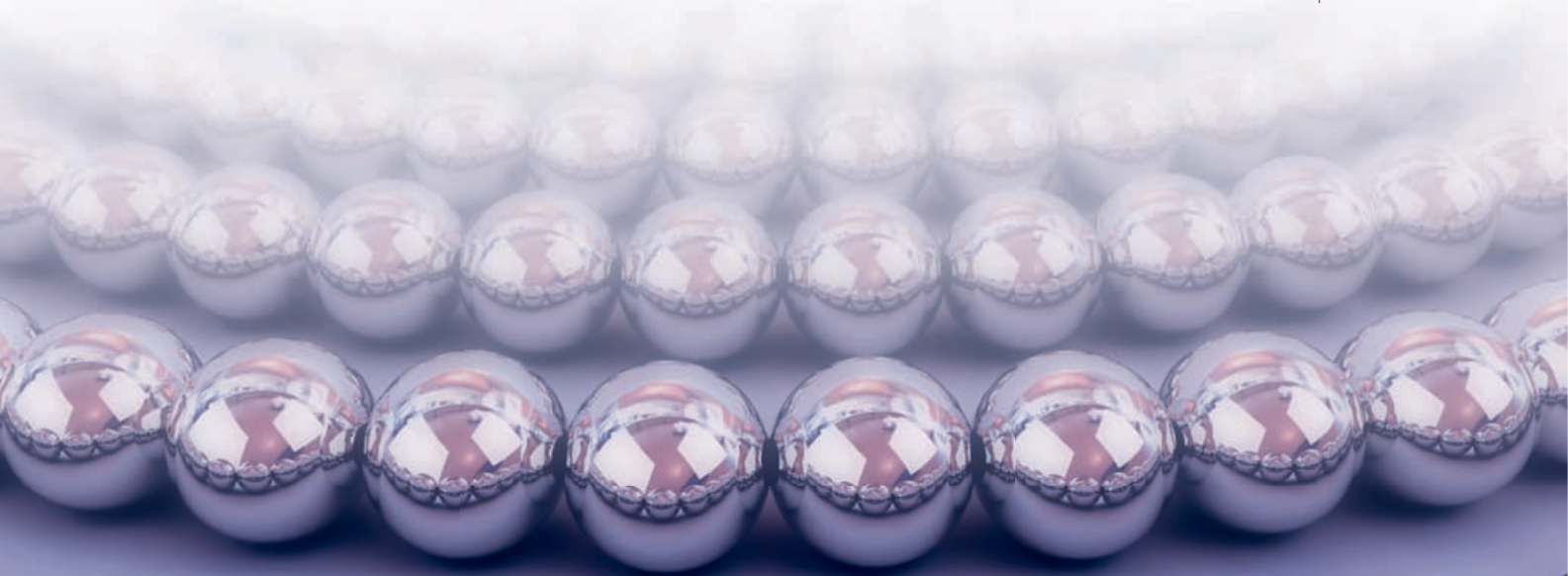
Enhanced selection process keeps particle size distribution to a minimum and produces high efficiency columns

### Advanced Bonding Technology

Optimized phase bonding creates a series of high coverage, robust phases

	150 Å Solid Core Particles	Tight Control of Particle Diameter	Advanced Bonding Technology	Automated Packing Process
Fast Separations	•	•	•	•
High Peak Capacity	•	•	•	•
Increased Sensitivity	•	•	•	•
Lower Pressure	•	•	•	•
Good Loading Capacity		•	•	•
Reproducible Chromatography		•	•	•
Long Lifetime	•	•	•	•
Peptide and Protein Analysis	•		•	

The table above shows how the components of Core Enhanced Technology with 150 Å pore diameter particles contribute to the outstanding features of Accucore HPLC columns for biomolecule separations.



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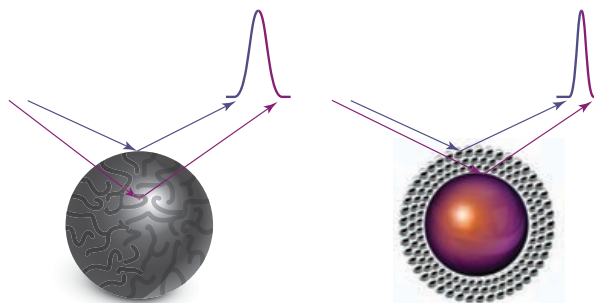


# Why Core Enhanced Technology Works

Two factors that strongly affect chromatographic efficiency are mass transfer and eddy diffusion, the C and A terms respectively from the Van Deemter equation.

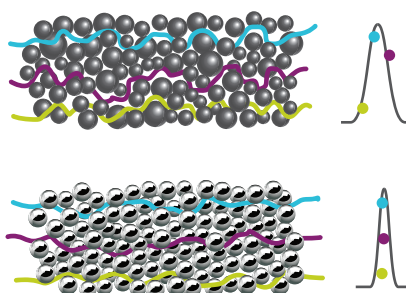
$$H = A + \frac{B}{u} + Cu$$

- H* Height equivalent to theoretical plate (column length/efficiency)
- A* Eddy diffusion
- B* Longitudinal diffusion
- C* Resistance to mass transfer
- u* Mobile phase linear velocity



Resistance to mass transfer is minimized by the solid core design of Accucore particles as the diffusional path of analytes is limited by the depth of the outer porous layer.

Material	Accucore 2.6 $\mu\text{m}$
Particle Size Distribution (D90/10)	1.15



The tight control of particle diameter and automated packing process used for Accucore columns result in a tight, highly uniform packed bed that minimizes eddy diffusion.

## Lower Backpressure

- L* Column length (cm)
- $\eta$  Mobile phase viscosity (cP)
- F* Flow rate (mL/min)
- $d_p^2$  Particle diameter ( $\mu\text{m}$ )
- $d_c^2$  Column diameter (cm)

$$\Delta P \sim \frac{250L\eta F}{d_p^2 d_c^2}$$

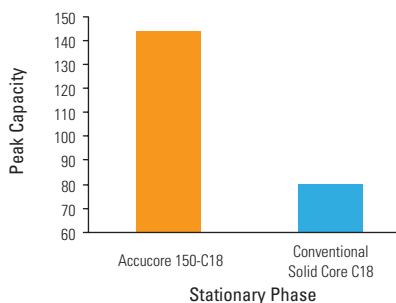
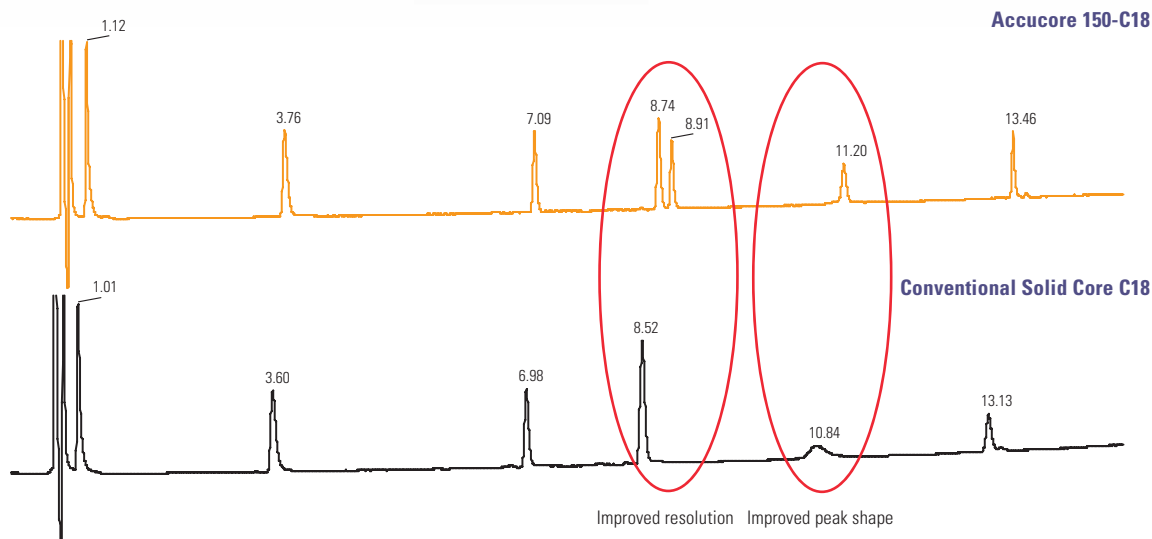
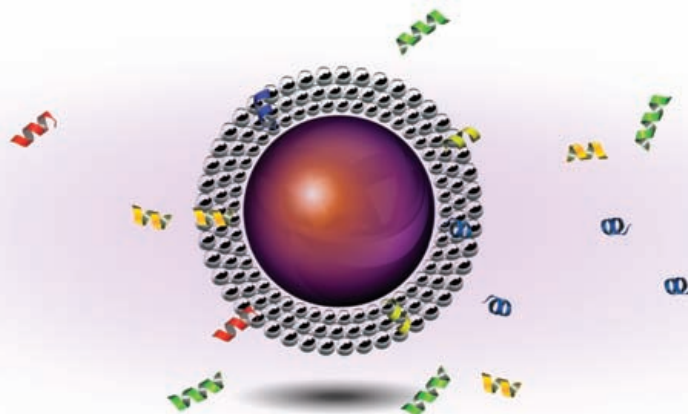
The equation above shows how backpressure is related to particle diameter.

The tightly controlled 2.6  $\mu\text{m}$  diameter of Accucore particles also results in lower backpressures than typically seen with sub-2  $\mu\text{m}$  materials.

# 150 Å Pore Diameter Particles

The 150 Å pore diameter solid core particles used in the Accucore 150-C18 and 150-C4 columns are designed specifically to provide the optimum combination of retention and resolution for peptides and proteins.

The chromatograms below show the improved resolution and peak shapes achieved with Accucore 150-C18 compared to a C18 solid core phase with an 80 Å pore diameter.



### Peak Capacity Comparison

Sharper peaks result in a significantly higher peak capacity for 150-C18 compared to the conventional solid core C18 column.

## Column Formats

Accucore HPLC columns for biomolecules are offered in both analytical, micro and nano formats. Optimum conditions and backpressure ratings are shown in the table below.

Format	Column ID	Optimum Flow Rate	Optimum Injection Volume	Backpressure Rating	Temperature Rating
Nano	75 $\mu\text{m}$	300 nL/min	1 $\mu\text{L}$	800 bar	70 $^{\circ}\text{C}$
Micro	2.1 mm	400 $\mu\text{L}/\text{min}$	1 $\mu\text{L}$	1000 bar	70 $^{\circ}\text{C}$
Analytical	3.0 mm	800 $\mu\text{L}/\text{min}$	3 $\mu\text{L}$	1000 bar	70 $^{\circ}\text{C}$
Analytical	4.6 mm	1800 $\mu\text{L}/\text{min}$	5 $\mu\text{L}$	1000 bar	70 $^{\circ}\text{C}$

### Analytical and Micro Columns

Accucore HPLC columns are packed into our high pressure hardware. These stainless steel columns are engineered to the highest quality and have a pressure rating of 1000 bar.



### Thermo Scientific Defender Guard Cartridges

Guard columns are designed to protect your column from particulates introduced from the matrix or instrument and from any strongly retained components in the injected sample.

Defender™ Guard Cartridges have been designed specifically to work with high speed, high efficiency separations.



### nanoViper Columns















The nanoViper fingertight connection system for nanoLC connections eliminates the assembly of PEEK sleeve connections. It is preassembled and fingertight to maximize ease-of-use. The nanoViper fitting is capable of withstanding pressures up to a 1000 bar and is compatible with third party valves and unions.

Accucore nanoViper columns are available in 150 and 500 mm lengths – for ultra-high peak capacity.

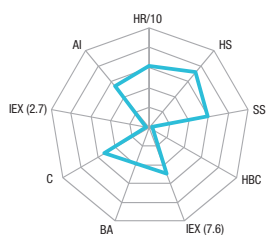


# Biomolecule Phases

Like all other Accucore products, the 150 Å pore size particles and the phases based on them are fully characterized using the suite of tests shown below.

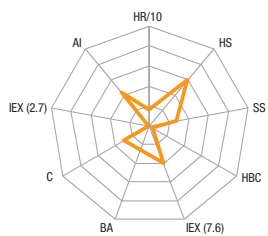
Silica Support Properties	Bonded Phase Properties	Hydrophobic Interactions	Secondary Interactions	Acidic Interactions
Surface Area 	Carbon load 	Hydrophobic retention  HR	Base activity  BA	Acid interaction  AI
Pore Size 		Hydrophobic selectivity  HS	Chelation  C	Ion exchange capacity (pH 2.7)  IEX(2.7)
Particle Size 		Steric selectivity  SS	Ion exchange capacity (pH 7.6)  IEX(7.6)	
Particle Size Distribution 		Hydrogen bonding capacity  HBC		

Details, including the phase characterization, are given below for the Accucore biomolecule phases.



## Accucore 150-C18

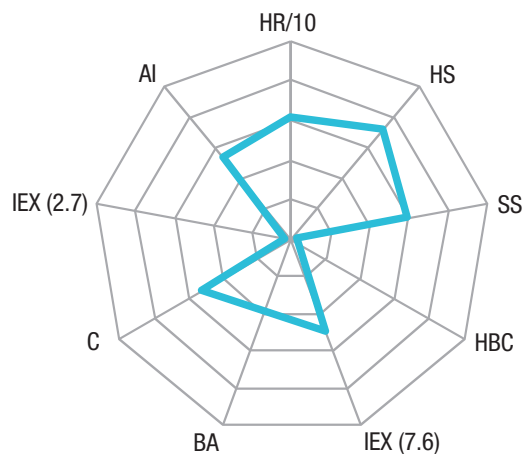
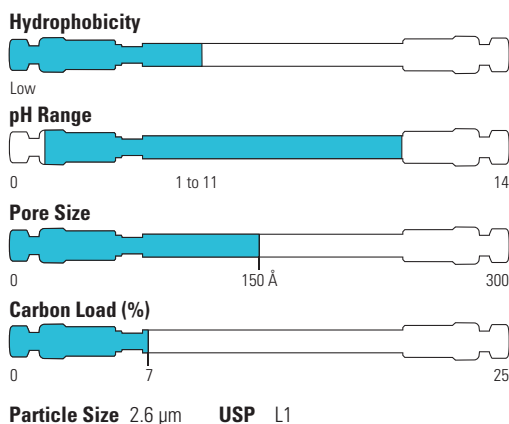
Phase characteristics are designed for the separation of peptides



## Accucore 150-C4

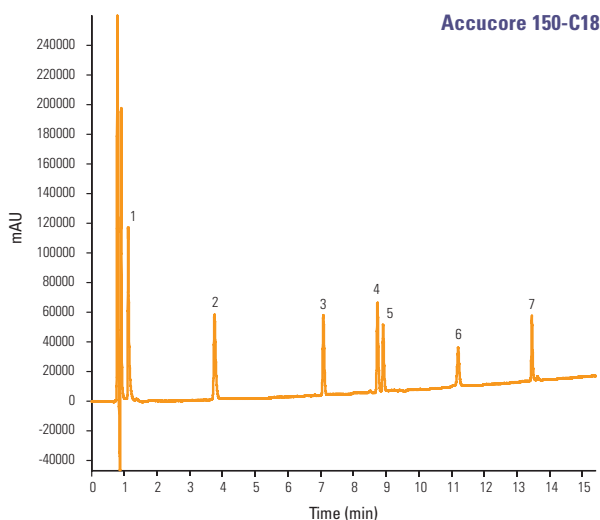
Lower hydrophobic retention, optimal retention of proteins and larger peptides

# Accucore 150-C18



- Designed for the separation of peptides
- Outstanding resolution

## Peptide Separations



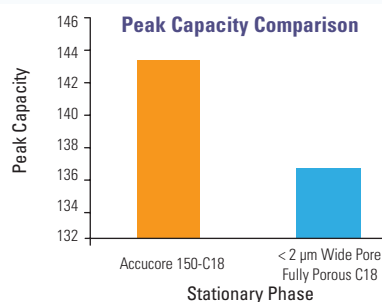
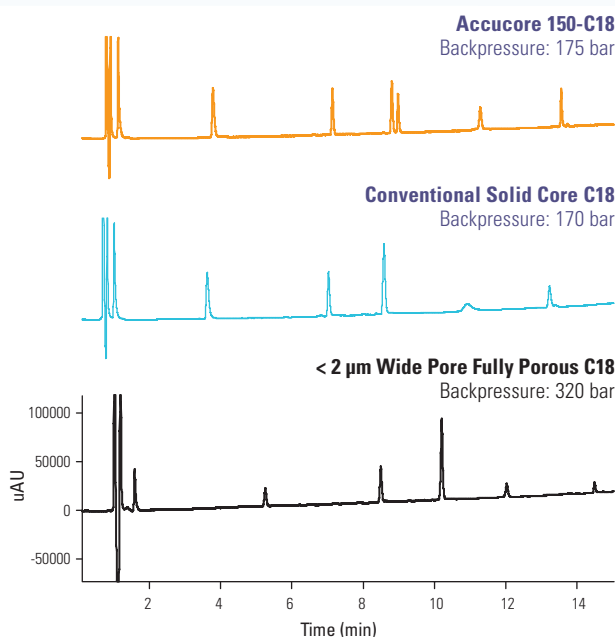
**Mobile phase A:** 0.1 % TFA in 10:90 acetonitrile:water  
**Mobile phase B:** 0.1 % TFA in 70:30 acetonitrile:water  
**Gradient:** 0-50 % B over 15 min; hold for 2 min; drop to 0 % in 0.1 min; hold at 0 % B for 5 min  
**Flow:** 300 µL/min  
**Temperature:** 35 °C  
**Column:** Accucore 150-C18 2.6 µm, 100 x 2.1 mm  
**Injection:** 5 µL  
**Detection:** UV (220 nm)

Peak Number	t <sub>r</sub> /min	Peptide	MW	Concentration (µg/mL)
1	1.12	Glycine-Tyrosine	238.24 Da	2.0
2	3.76	Valine-Tyrosine-Valine	379.45 Da	17.0
3	7.09	Met-Enkephalin	573.66 Da	21.0
4	8.74	Angiotensin III	931.09 Da	15.0
5	8.91	Leu-Enkephalin	569.65 Da	21.0
6	11.20	Ribonuclease A	~ 13700 Da	42.5
7	13.46	Insulin	5733.49 Da	30.0



## High Peak Capacity

Higher peak capacities facilitate increased peptide identifications. Accucore 150-C18 provides much narrower peak widths, therefore significantly higher peak capacity than a column packed with < 2 μm wide pore fully porous C18.



$$n_c = 1 + \left( \frac{t_g}{w} \right)$$

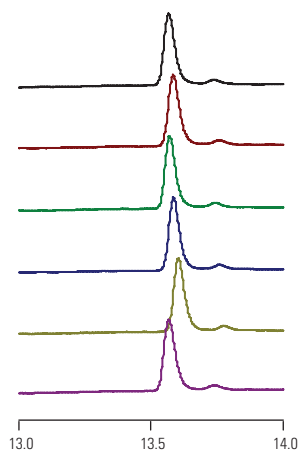
$n_c$  Peak capacity

$t_g$  Gradient time

$w$  Average peak width 10% height

## Reproducible Separations

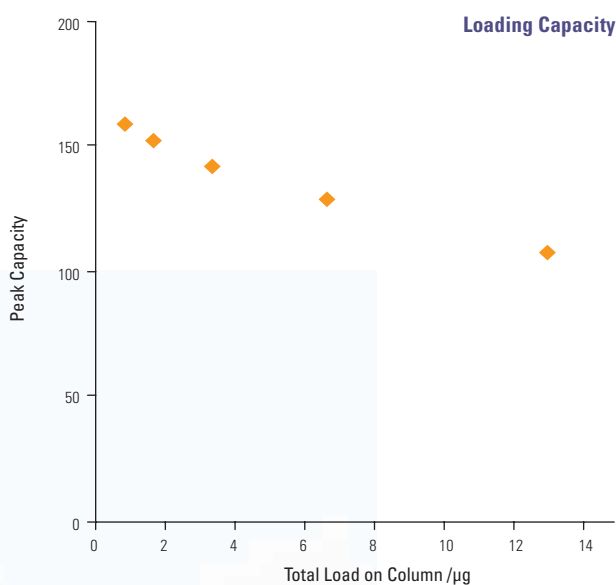
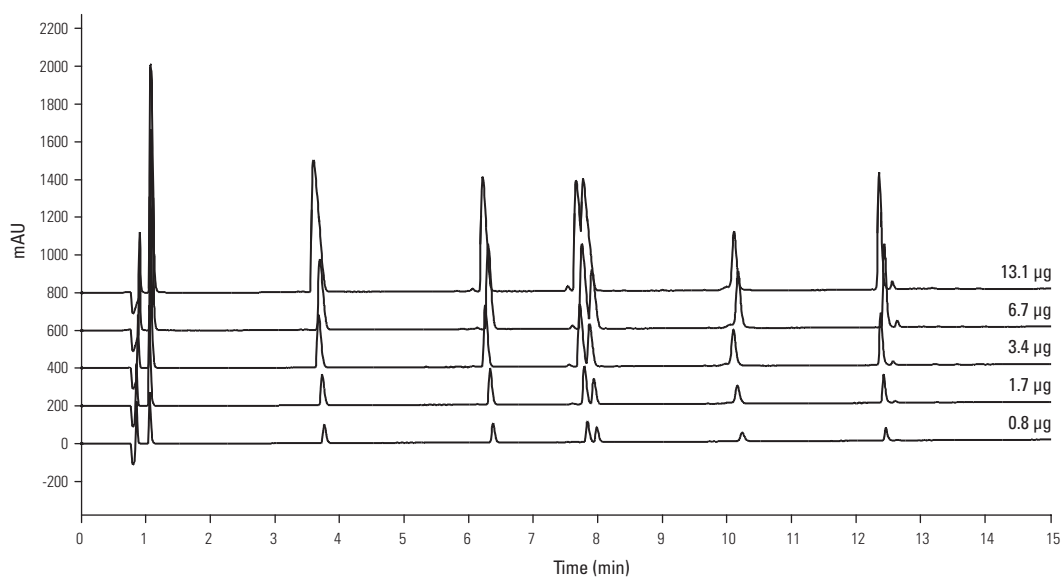
Precision of retention times is critical for reliable analysis. The Accucore 150-C18 column exhibits excellent retention time reproducibility.



Peak Number	Peptide	$t_r$ (min)	% RSD
7	Insulin	13.58	0.11

## Excellent Loading Capacity

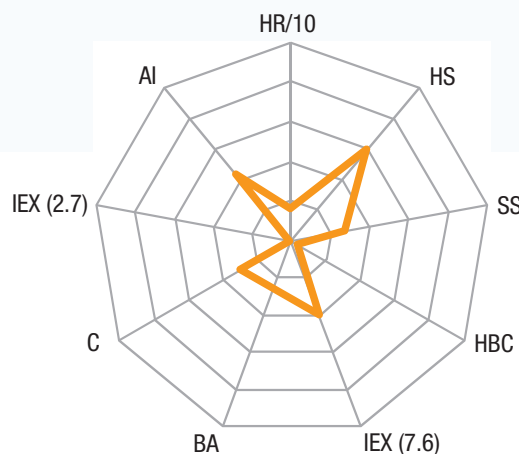
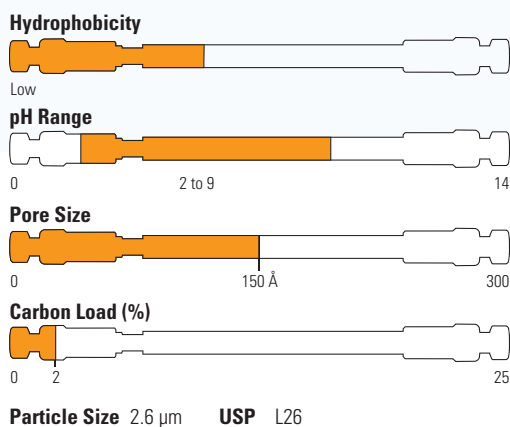
The data below demonstrates the impact on peak capacity of increasing the load of peptides on the Accucore 150-C18 column. Only for loads exceeding 2  $\mu\text{g}$  on the column are significant decreases in peak capacity observed.



Total Load on Column ( $\mu\text{g}$ )	Peak Capacity
0.8	159.24
1.7	152.75
3.4	142.31
6.7	128.87
13.1	107.76

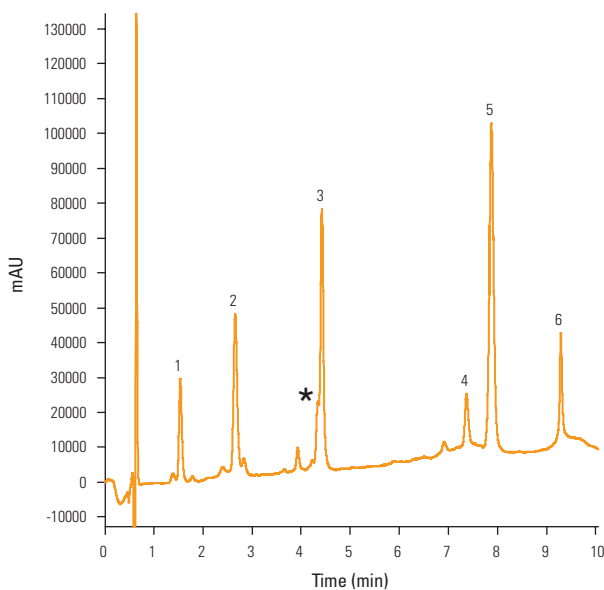


# Accucore 150-C4



- Significantly lower hydrophobic retention than C18
- Ideal for retention of proteins and larger peptides

## Intact Protein Separation

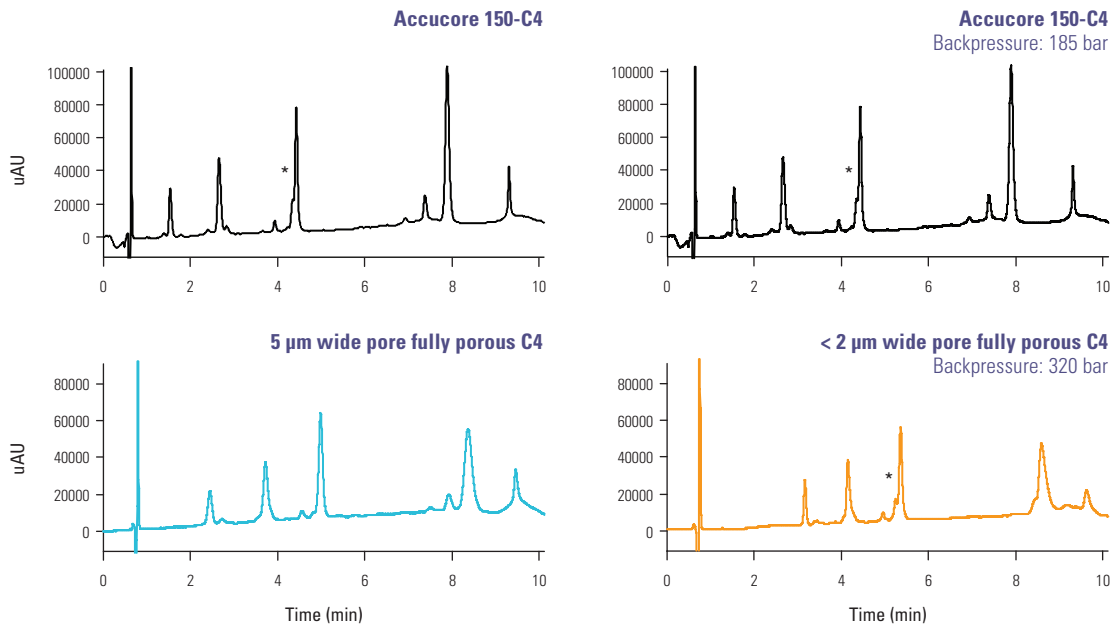


**Mobile phase A:** 0.1 % TFA in 30:70 acetonitrile:water  
**Mobile phase B:** 0.1 % TFA in 98:2 acetonitrile:water  
**Gradient:** 0-30 % B over 8 mins  
 30-95 % B over 2 mins  
 95 % B hold for 1 min  
 0 % B hold for 4 mins  
**Flow:** 400 µL/min  
**Temperature:** 40 °C  
**Column:** Accucore 150-C4 2.6 µm, 100 x 2.1 mm  
**Injection:** 2 µL 10 pmol/µL solution  
**Detection:** UV (214 and 280 nm)

Peak Number	t <sub>r</sub> /min	Protein	MW	Concentration (µg/mL)
1	1.54	Insulin	6 kDa	40
2	2.66	Cytochrome C	12 kDa	80
3	4.42	Lysozyme	14 kDa	100
4	7.38	Myoglobin	18 kDa	120
5	7.88	Carbonic anhydrase	30 kDa	200
6	7.88	Ovalbumin	45 kDa	300
*		Carbonic anhydrase impurity		

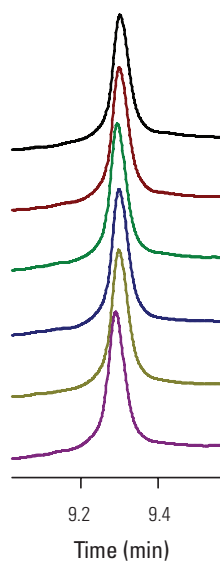
## Excellent Resolution

Accucore 150-C4 provides significantly sharper and higher peaks than a column packed with 5  $\mu\text{m}$  wide pore fully porous C4 – thus offering better resolution and sensitivity. The Accucore 150-C4 also performs better than a column packed with < 2  $\mu\text{m}$  wide pore fully porous C4 and generates only a fraction of the backpressure.



## Reproducible Results

The Accucore 150-C4 column exhibits excellent peak shape and retention time reproducibility.

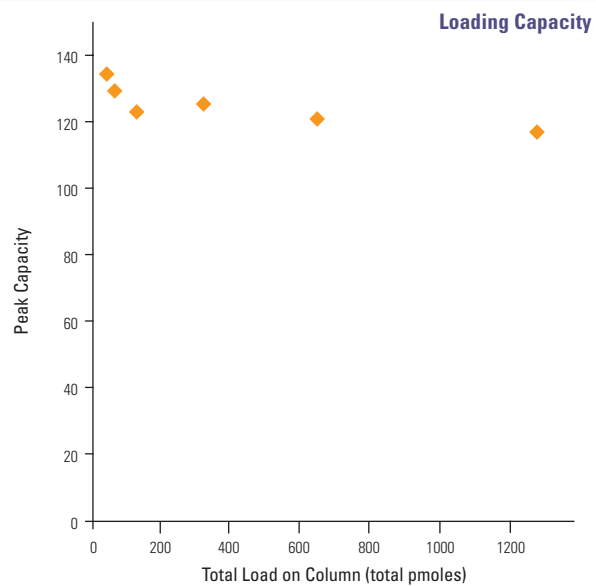
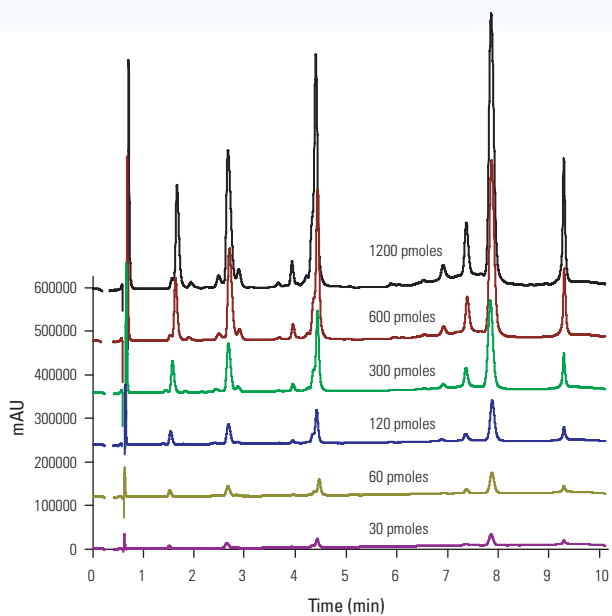


Protein	Mean $t_r$ / min	% RSD	Mean peak width at half height / min	Mean asymmetry
Ovalbumin (45 kDa)	9.30	0.06	0.05	1.14

Data From Six Injections

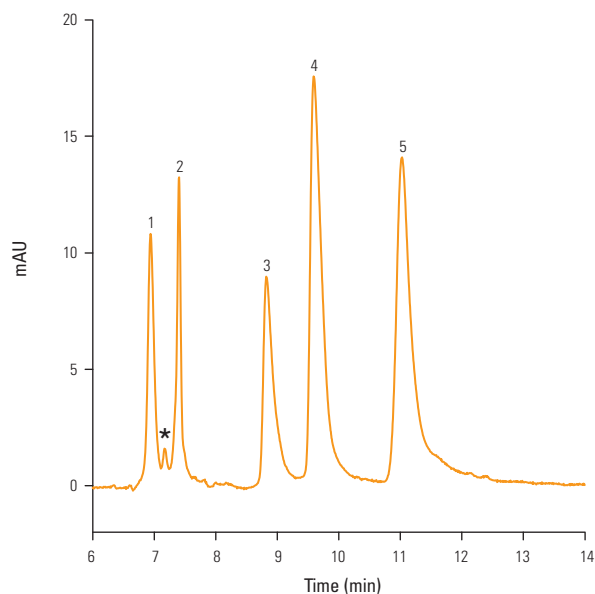
## Proven Loading Capacity

As the load on the Accucore 150-C4 column increases up to 1200 pmoles (25  $\mu$ g) peak shapes remain very sharp as shown by the peak capacity plot.



# nanoViper Column Separations

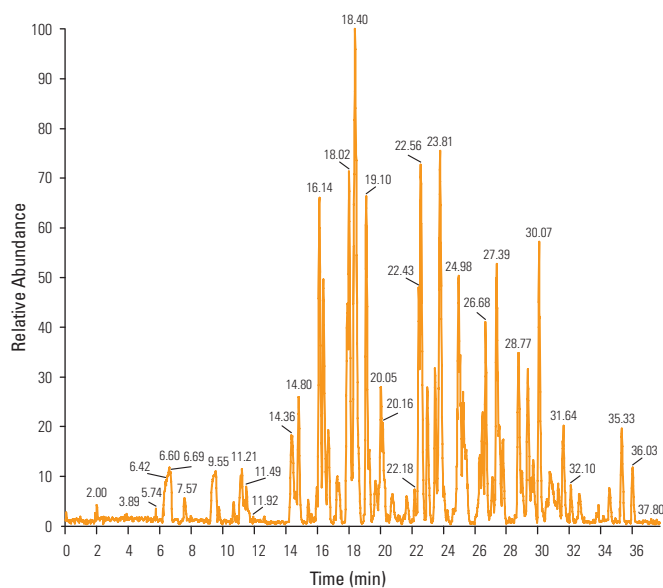
Protein separation using formic acid as an MS compatible mobile phase additive



<b>Mobile phase A:</b>	0.1 % formic acid in water
<b>Mobile phase B:</b>	0.1 % formic acid in acetonitrile
<b>Gradient:</b>	0-30 % B over 1 min 30-60 % B over 10 mins 60-95 % B over 1 min 95 % B hold for 3 mins
<b>Flow:</b>	300 nL/min
<b>Temperature:</b>	40 °C
<b>Column:</b>	Accucore 150-C4, 2.6 µm, 150 x 75 µm
<b>Backpressure:</b>	204 bar (100 % A)
<b>Injection:</b>	0.25 µL 2 pmol/µL solution
<b>Detection:</b>	UV (214 nm)

Peak Number	t <sub>R</sub> /min	Protein
1	6.89	Cytochrome C (12 kDa)
2	7.34	Insulin (6 kDa)
3	8.77	Myoglobin (18 kDa)
4	9.57	Carbonic anhydrase (30 kDa)
5	11.02	Ovalbumin (45 kDa)
*		Carbonic anhydrase impurity

Base peak 50 fmol loading of BSA digest



<b>Mobile phase A:</b>	0.1 % formic acid in water
<b>Mobile phase B:</b>	90 % acetonitrile in water
<b>Gradient:</b>	4-40 % B over 30 mins 40-95 % B over 2 mins 95 % B hold for 2 mins
<b>Flow:</b>	300 nL/min
<b>Temperature:</b>	not controlled
<b>Column:</b>	Accucore 150-C18, 2.6 µm, 150 x 75 µm
<b>Backpressure:</b>	198 bar (100 % A)
<b>Injection:</b>	Direct on-column loading of 1 µL of BSA digest, 50 fmol/µL in water + 0.1% formic acid
<b>Detection:</b>	Thermo Scientific LTQ Orbitrap XL Mass Spectrometer coupled with a Proxeon Nano Spray Flex Ion Source

# Ordering Information

## Accucore HPLC Columns

Description	Particle Size	Length (mm)	2.1 mm ID	3.0 mm ID	4.6 mm ID
Accucore 150-C18	2.6 µm	30	16126-032130	16126-033030	16126-034630
		50	16126-052130	16126-053030	16126-054630
		100	16126-102130	16126-103030	16126-104630
		150	16126-152130	16126-153030	16126-154630
Accucore 150-C4	2.6 µm	30	16526-032130	16526-033030	16526-034630
		50	16526-052130	16526-053030	16526-054630
		100	16526-102130	16526-103030	16526-104630
		150	16526-152130	16526-153030	16526-154630

## Accucore Defender Guard Columns (4/pk)

Description	Particle Size	Length (mm)	2.1 mm ID	3.0 mm ID	4.6 mm ID
Accucore 150-C18	2.6 µm	10	16126-012105	16126-013005	16126-014005
Accucore 150-C4	2.6 µm	10	16526-012105	16526-013005	16526-014005

## UNIGUARD Direct-Connection Guard Cartridge Holders

Description	2.1 mm ID	3.0 mm ID	4.6 mm ID
UNIGUARD Drop-In Guard Cartridge Holder	852-00	852-00	850-00
Standard Replacement Tip	850-RT	850-RT	850-RT

## Accucore nanoViper Columns

Description	Particle Size	Length (mm)	75 µm ID
Accucore 150-C18	2.6 µm	150	16126-157569
		500	16126-507569
Accucore 150-C4	2.6 µm	150	16526-157569
		500	16526-507569

### Ultimate Core Performance

## to Maximize Your Investment

- 150 Å pore size solid core particles for fast biomolecule separations
- Superb resolution of peptides and intact proteins at low backpressures
- Exceptionally rugged and easy to use analytical and nano scale columns

# Resources

## for Chromatographers

### Thermo Scientific Chromatography Columns and Consumables Catalog

This extensive catalog offers 600 pages of proven chromatography tools and product selection guides. Available online, with a robust search tool and optimized for your iPad®.

Visit [www.thermoscientific.com/catalog](http://www.thermoscientific.com/catalog)



### Chromatography Resource Center

Our web-based resource center provides technical support, applications, technical tips and literature to help move your separations forward.

Visit [www.thermoscientific.com/crc](http://www.thermoscientific.com/crc)



For more information visit: [www.thermoscientific.com/accucore](http://www.thermoscientific.com/accucore)

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