

# Thermo Scientific Accucore HPLC Columns

# Core Enhanced Technology

## Accucore HPLC Column Range

Founded on state-of-the-art Core Enhanced Technology™ and utilizing vast experience in phase bonding and packing, Thermo Scientific™ Accucore™ HPLC columns provide a unique chromatography solution to enhance laboratory workflow and efficiency. Available in a wide range of stationary phase selectivities and compatible with almost any instrument, these columns provide an excellent return on investment.

### Accucore HPLC Columns

Containing solid core particles, which are engineered to a diameter of 2.6  $\mu\text{m}$  and a very narrow particle size distribution; Accucore HPLC columns allow high speed, high resolution separation, with back pressures significantly lower than those associated with UHPLC.

### Accucore HPLC Columns for Biomolecules

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

### Accucore XL HPLC Columns

Using 4  $\mu\text{m}$  solid core particles, Accucore XL HPLC columns allow users of conventional HPLC methods to enjoy performance far beyond that of columns packed with 5  $\mu\text{m}$ , 4  $\mu\text{m}$  or even 3  $\mu\text{m}$  fully porous particles.

### The key components of Core Enhanced Technology

#### Solid Core Particles

With a solid central core and porous outer layer, these particles generate high speed, high resolution separations without excessive backpressure

#### Tight Control of Particle Diameter

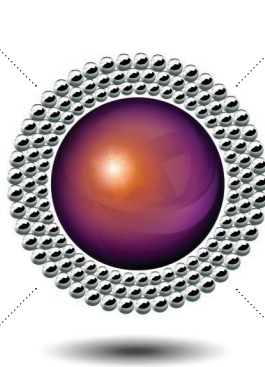
Enhanced selection process keeps particle size distribution to a minimum and produces high efficiency columns

#### Automated Packing Process

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

#### Advanced Bonding Technology

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



### Accucore HPLC Columns

- Rugged and reproducible 2.6  $\mu\text{m}$  solid core particles
- Fast separations with superb resolution
- Low backpressures

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### Accucore HPLC Columns for Biomolecules

- 150 Å pore size solid core particles for fast biomolecule separations
- Superb resolution at low backpressures
- Exceptionally rugged analytical and nano scale columns

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### Accucore XL HPLC Columns

- 4  $\mu\text{m}$  solid core particles for all users
- Same system, same method, better results
- Robust, fast and easy to use

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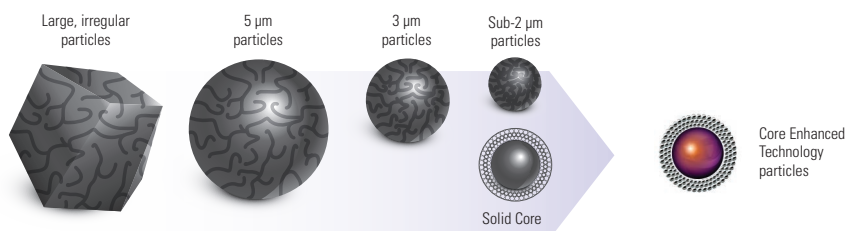
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# Particle Evolution

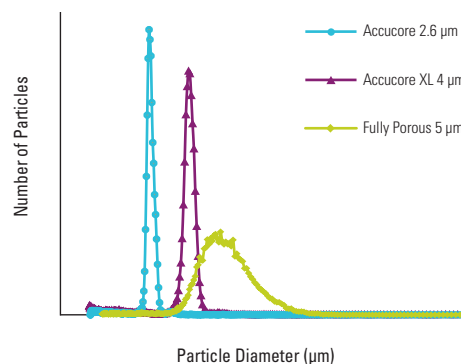
In the search for ever faster and better separations the size and shape of column packing materials has evolved in the decades since the invention of HPLC.

Packing materials have changed from large pellicular particles via smaller totally porous particles to spherical particles with diameters of less than 2  $\mu\text{m}$ .

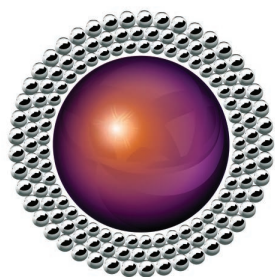
Our Core Enhanced Technology has changed things again. These particles are not totally porous, but rather have a solid core and a porous outer layer.



Material	Accucore 2.6 $\mu\text{m}$	Accucore XL 4 $\mu\text{m}$	Fully Porous
Average Particle Size Distribution ( $D_{90}/D_{10}$ )	1.12	1.15	~ 1.5

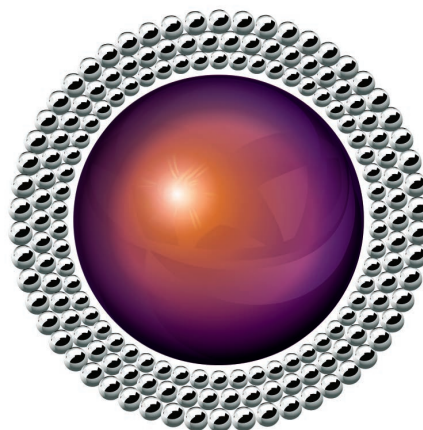


Accucore 2.6  $\mu\text{m}$  solid core particle



Porous layer depth = 0.5  $\mu\text{m}$

Accucore XL 4  $\mu\text{m}$  solid core particle



Porous layer depth = 0.6  $\mu\text{m}$

# Why Core Enhanced Technology Works

The factors that affect chromatographic efficiency are resistance to mass transfer, longitudinal diffusion and eddy diffusion, the C, B and A terms respectively from the van Deemter equation.

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

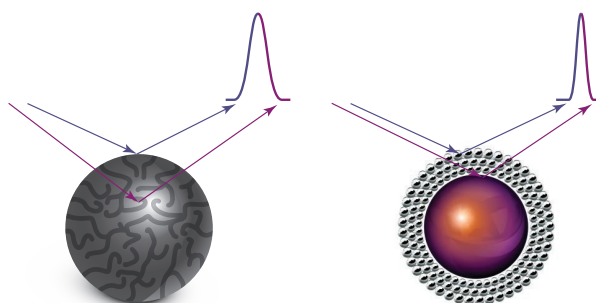
$H$  Height equivalent to theoretical plate (column length/efficiency)

$A$  Eddy diffusion

$B$  Longitudinal diffusion

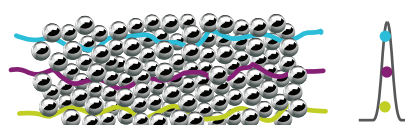
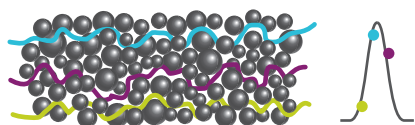
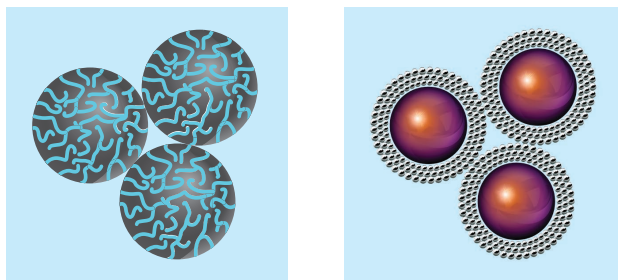
$C$  Resistance to Mass Transfer

$u$  Mobile phase linear velocity



Resistance to mass transfer is minimized by the solid core design of Core Enhanced Technology particles as the diffusional path of analytes is limited by the depth of the outer porous layer. The effect of this minimization is most noticeable for larger molecules.

The solid core design of the particles reduces the amount of mobile phase in the column resulting in a reduced void volume and less longitudinal diffusion. This effect can be seen in the lower  $t_0$  values obtained with Accucore HPLC columns compared to columns of the same dimensions packed with fully porous materials.



The tight control of Core Enhanced Technology particle diameter and automated packing process used for Accucore HPLC 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)

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

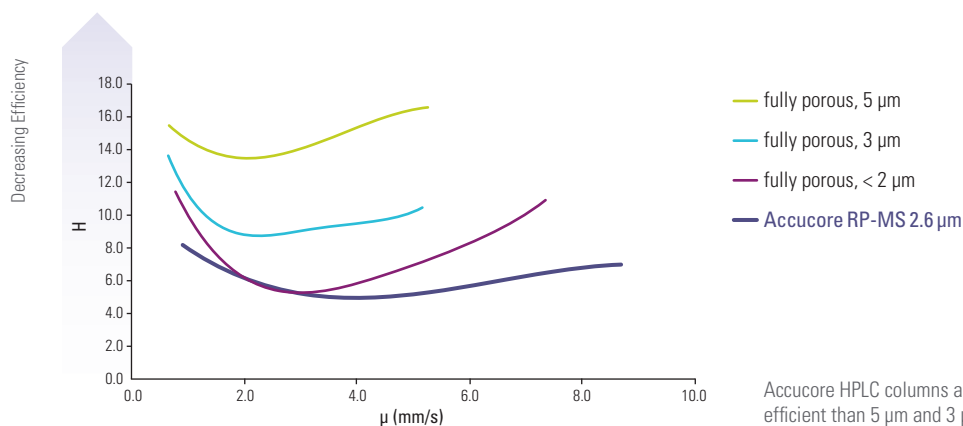
This equation above shows how backpressure is related to particle diameter.

2.6  $\mu\text{m}$  solid core particles generate backpressures lower than sub 2  $\mu\text{m}$  fully porous particles.

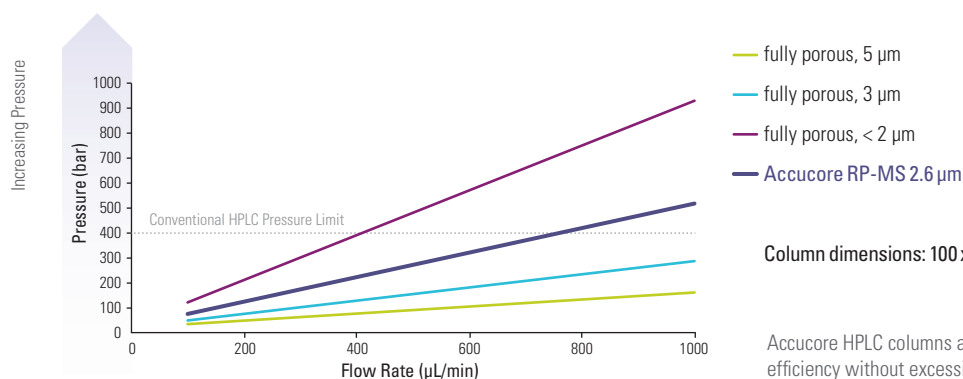
4  $\mu\text{m}$  solid core particles generate backpressures slightly higher than 5  $\mu\text{m}$  fully porous particles.

## Core Enhanced Technology Effect

The plots below show how the efficiency and backpressure of Accucore HPLC columns compare to columns packed with traditional totally porous 5  $\mu\text{m}$ , 3  $\mu\text{m}$  and < 2  $\mu\text{m}$  particles.



Accucore HPLC columns are more efficient than 5  $\mu\text{m}$  and 3  $\mu\text{m}$  columns.



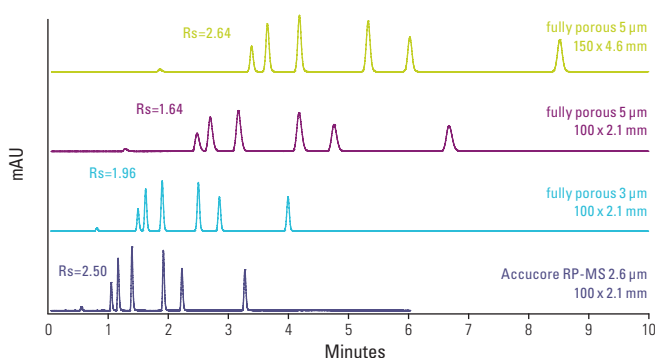
Column dimensions: 100 x 2.1 mm

Accucore HPLC columns achieve this efficiency without excessive backpressure.



## Faster than 5 $\mu\text{m}$ and 3 $\mu\text{m}$

Using Accucore HPLC columns excellent separations can be achieved in shorter times. The examples on this page show how by increasing flow rates while maintaining efficiency, and therefore resolution, the time taken to separate a mixture can be reduced by a factor of 3 and solvent costs can be reduced by 7-times!



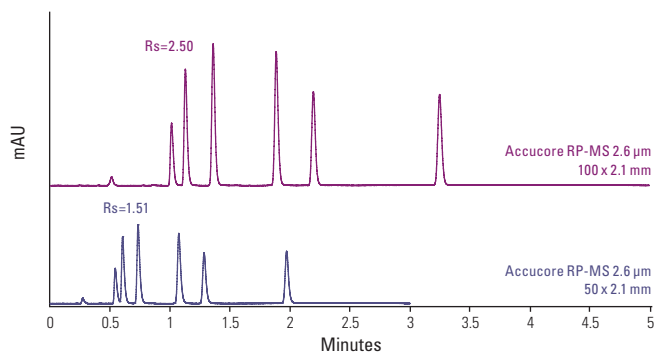
	Accucore RP-MS 2.6 $\mu\text{m}$ , 100 x 2.1 mm	fully porous 3 $\mu\text{m}$ , 100 x 2.1 mm	fully porous 5 $\mu\text{m}$ , 100 x 2.1 mm	fully porous 5 $\mu\text{m}$ , 150 x 4.6 mm
Resolution (critical pair)	2.50	1.96	1.64	2.64
Run time (min) including gradient re-equilibration	6.00	7.00	11.50	17.00

Mobile phase A:	water
Mobile phase B:	acetonitrile
Gradient:	Accucore RP-MS 2.6 $\mu\text{m}$ 100 x 2.1 mm = 35–60 % B in 3.5 minutes fully porous 3 $\mu\text{m}$ 100 x 2.1 mm = 35–60 % B in 4.0 minutes fully porous 5 $\mu\text{m}$ 100 x 2.1 mm = 35–60 % B in 6.7 minutes fully porous 5 $\mu\text{m}$ 150 x 4.6 mm = 35–60 % B in 10.0 minutes
Flow:	Accucore RP-MS 2.6 $\mu\text{m}$ 100 x 2.1 mm = 400 $\mu\text{L}/\text{min}$ fully porous 3 $\mu\text{m}$ 100 x 2.1 mm = 350 $\mu\text{L}/\text{min}$ fully porous 5 $\mu\text{m}$ 100 x 2.1 mm = 210 $\mu\text{L}/\text{min}$ fully porous 5 $\mu\text{m}$ 150 x 4.6 mm = 1000 $\mu\text{L}/\text{min}$
Temperature:	30 $^{\circ}\text{C}$
Injection:	1 $\mu\text{L}$ (fully porous 5 $\mu\text{m}$ 150 x 4.6 mm = 5 $\mu\text{L}$ )
Detection:	UV at 247 nm (0.1 s rise time, 20 Hz)
Analytes:	1. Tebuthiuron 2. Metoxuron 3. Monuron 4. Chlorotoluron 5. Diuron 6. Linuron

*Reducing analysis time and solvent costs results in higher throughput and lower cost per analysis.*

# Short Columns for Even Faster Separations

The separating power of Accucore HPLC columns means that by using shorter column dimensions acceptable resolution can be maintained, with even greater increases in throughput and reduction in costs.



Mobile phase A:	water
Mobile phase B:	acetonitrile
Gradient:	Accucore RP-MS 2.6 µm 50 x 2.1 mm = 35–60 % B in 1.8 minutes Accucore RP-MS 2.6 µm 100 x 2.1 mm = 35–60 % B in 3.5 minutes
Flow:	400 µL/min

## Analysis Time and Solvent Savings

	Accucore RP-MS 2.6 µm, 50 x 2.1 mm	Accucore RP-MS 2.6 µm, 100 x 2.1 mm
Resolution (critical pair)	1.51	2.50
Run time (min) including gradient re-equilibration	3.00	6.00



*A 50 mm column gives acceptable separation with a doubling of productivity and halving of solvent costs.*

# Higher Peak Capacity than 5 µm or 3 µm

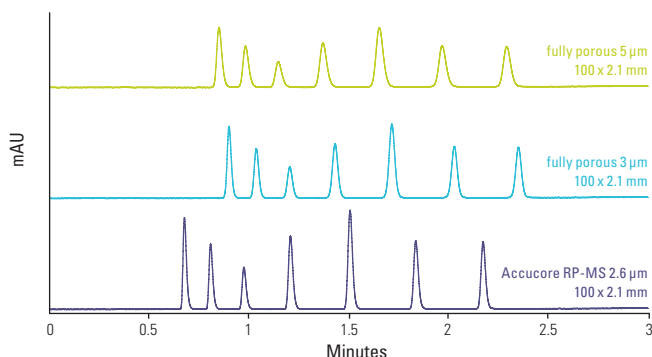
As an alternative to speeding up analysis the high resolution offered by Accucore HPLC columns can also be used to improve complex separations through an increase in peak capacity.

$n_c$  Peak capacity

$t_g$  Gradient time

$\bar{w}$  Peak width (10% height)

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



Mobile phase A: water

Mobile phase B: acetonitrile

Gradient: 65–95 % B in 2.1 minutes  
95 % B for 0.4 minutes

Flow: 400 µL/min

Temperature: 40 °C

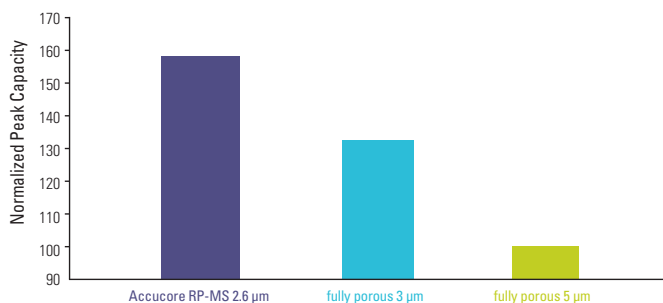
Injection: 1 µL

Detection: UV at 247 nm (0.1 s rise time, 20 Hz)

Analytes:

1. Acetophenone
2. Propiophenone
3. Butyrophenone
4. Valerophenone
5. Hexanophenone
6. Heptanophenone
7. Octanophenone

## Peak Capacity Comparison



Accucore RP-MS 2.6 µm 158

fully porous 3 µm 132

fully porous 5 µm 100

*The higher the peak capacity the more analytes can be identified.*

## More Sensitive than 5 µm or 3 µm

According to the formula shown below, the sharper, taller peaks obtained with Accucore HPLC columns result in a higher signal to noise ratio (S/N) and therefore better sensitivity.

$c_{max}$  Concentration at peak apex

$N$  Efficiency

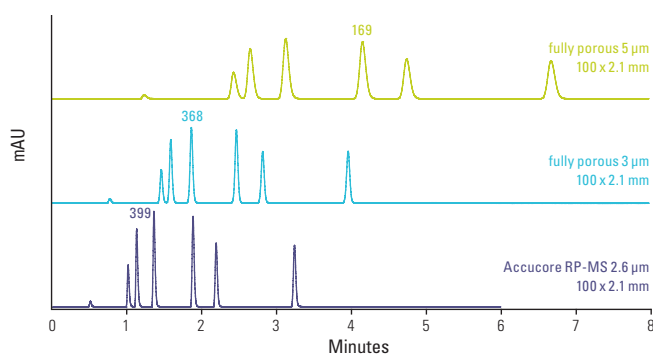
$V_i$  Injection volume

$L$  Column length

$d_c$  Column internal diameter

$k'$  Capacity factor

$$c_{max} \propto \frac{\sqrt{N} V_i}{L d_c^2 (1 + k')}$$



Mobile phase A:	water
Mobile phase B:	acetonitrile
Gradient:	<b>Accucore RP-MS 2.6 µm</b> 100 x 2.1 mm = 35–60 % B in 3.5 minutes <b>fully porous 3 µm</b> 100 x 2.1 mm = 35–60 % B in 4.0 minutes <b>fully porous 5 µm</b> 100 x 2.1 mm = 35–60 % B in 6.7 minutes
Flow:	<b>Accucore RP-MS 2.6 µm</b> 100 x 2.1 mm = 400 µL/min <b>fully porous 3 µm</b> 100 x 2.1 mm = 350 µL/min <b>fully porous 5 µm</b> 100 x 2.1 mm = 210 µL/min
Temperature:	30 °C
Injection:	1 µL
Detection:	UV at 247 nm (0.1 s rise time, 20 Hz)
Analytes:	1. Tebuthiuron 2. Metoxuron 3. Monuron 4. Chlorotoluron 5. Diuron 6. Linuron

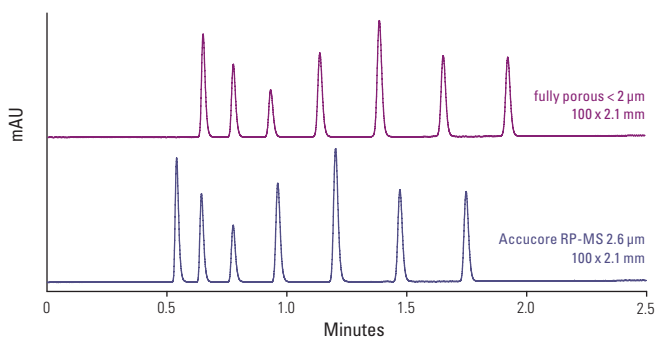
### Sensitivity

Column	S/N (6-sigma) for Monuron	Increase in Sensitivity
Accucore 2.6 µm, 100 x 2.1 mm	399	136 %
fully porous 3 µm, 100 x 2.1 mm	368	117 %
fully porous 5 µm, 100 x 2.1 mm	169	—

*Better sensitivity allows reliable detection and determination of small peaks, for example low level impurities.*

# Equivalent Performance to Sub-2 $\mu\text{m}$ with Lower Pressure

With solid core design, tight particle size distribution and uniform packed bed Accucore HPLC columns have broadly equivalent performance to sub-2  $\mu\text{m}$  columns and yet generate only a fraction of the backpressure.



Mobile phase A:	water
Mobile phase B:	acetonitrile
Gradient:	65–95 % B in 1.7 minutes 95 % B for 0.3 minutes
Flow:	500 $\mu\text{L}/\text{min}$
Temperature:	40 $^{\circ}\text{C}$
Injection:	1 $\mu\text{L}$
Detection:	UV at 247 nm (0.1 s rise time, 20 Hz)
Analytes:	1. Acetophenone 2. Propiophenone 3. Butyrophenone 4. Valerophenone 5. Hexanophenone 6. Heptanophenone 7. Octanophenone

## Pressure

	Accucore RP-MS 2.6 $\mu\text{m}$ , 100 x 2.1 mm	Fully Porous < 2 $\mu\text{m}$ , 100 x 2.1 mm
Resolution (critical pair)	3.72	4.20
Run time (min)	3.50	3.50
Maximum pressure (bar)	171	338

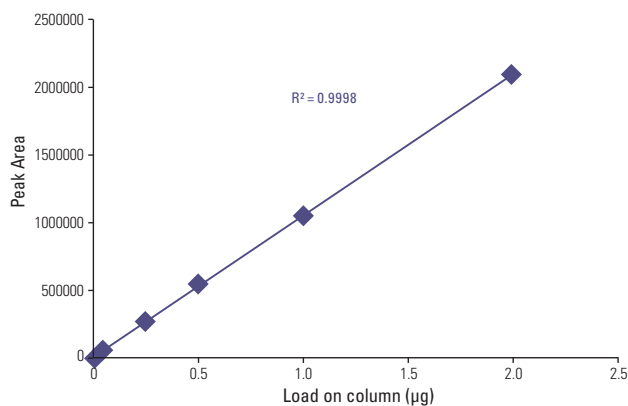


*Lower backpressure eliminates the requirement for UHPLC systems with maximum pressure ratings >600 bar. If a UHPLC system is used then the lower backpressure reduces wear on the instrument.*

# Loading Capacity

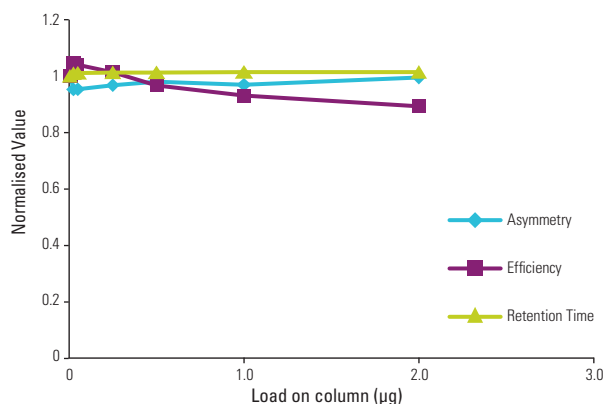
With tightly packed beds and high bonded phase coverage Accucore HPLC columns have loading capacities that allow a wide range of analyte concentrations to be determined.

The example below shows minimal change in retention and peak shape with increasing analyte concentration.



Column:	Accucore RP-MS 100 x 2.1 mm
Mobile phase:	68:32 (v/v) water/methanol
Flow:	1.0 mL/min
Temperature:	40 °C
Injection:	1 µL
Detection:	UV at 254 nm

Concentration (ng/µL)	Load on Column (µg)
5	0.005
25	0.025
50	0.050
250	0.250
500	0.500
1000	1.000
2000	2.000



# Simple Method Transfer

Fast HPLC is often performed using lower volume columns.

A few simple steps are required to transfer a method to a lower volume Accucore HPLC column.

## Method Transfer Tool

A convenient method transfer tool is available at the Chromatography Resource Center  
[www.thermoscientific.com/crc](http://www.thermoscientific.com/crc)

- **Adjust Flow Rate**

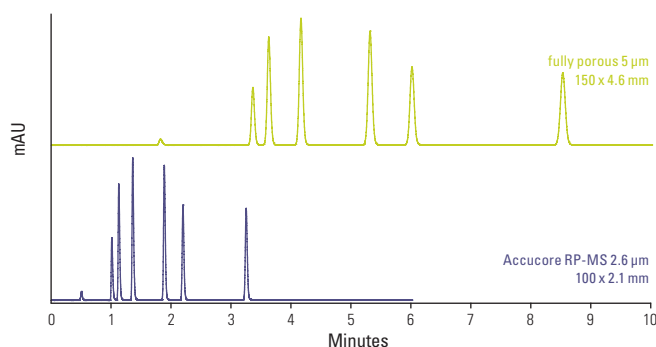
Keep linear velocity constant between original and new method, taking into account particle size and geometry

- **Adjust Injection Volume**

Keep the ratio of injection volume to column volume constant

- **Adjust Gradient Profile**

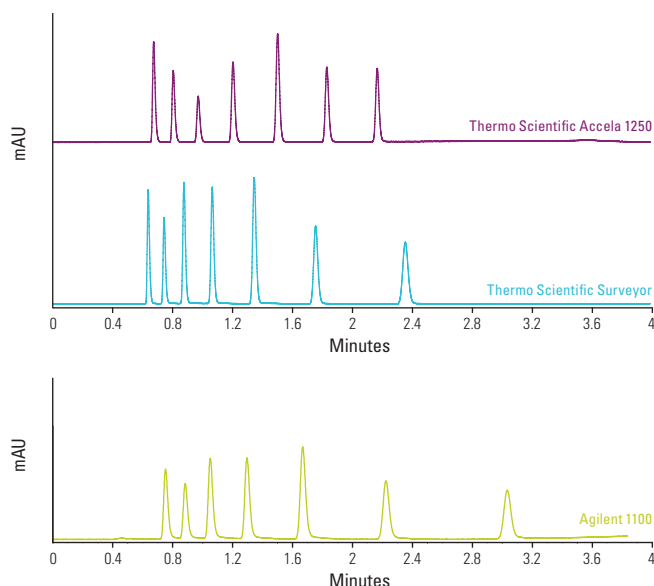
Keep the number of column volumes constant for each gradient segment



Mobile phase A:	water
Mobile phase B:	acetonitrile
Gradient:	Accucore RP-MS 2.6 $\mu\text{m}$ 100 x 2.1 mm = 35–60 % B in 3.5 minutes fully porous 5 $\mu\text{m}$ 150 x 4.6 mm = 35–60 % B in 10.0 minutes
Flow:	Accucore RP-MS 2.6 $\mu\text{m}$ 100 x 2.1 mm = 400 $\mu\text{L}/\text{min}$ fully porous 5 $\mu\text{m}$ 150 x 4.6 mm = 1000 $\mu\text{L}/\text{min}$
Injection:	Accucore RP-MS 2.6 $\mu\text{m}$ 100 x 2.1 mm = 1 $\mu\text{L}$ fully porous 5 $\mu\text{m}$ 150 x 4.6 mm = 5 $\mu\text{L}$
Temperature:	30 $^{\circ}\text{C}$
Detection:	UV at 247 nm (0.1 s rise time, 20 Hz)
Analytes:	1. Tebuthiuron 2. Metoxuron 3. Monuron 4. Chlorotoluron 5. Diuron 6. Linuron

# UHPLC System Not Required

The low backpressures generated associated with Core Enhanced Technology mean that Accucore HPLC columns can be used with both UHPLC and HPLC systems.



Column:	Accucore RP-MS 2.6 $\mu$ m, 100 x 2.1 mm
Mobile phase A:	water
Mobile phase B:	acetonitrile
Gradient:	65–95 % B in 2.1 minutes 95 % B for 0.4 minute
Flow:	400 $\mu$ L/min
Temperature:	40 °C
Injection:	1 $\mu$ L
Detection:	UV at 247 nm (0.1s rise time, 20 Hz)
Analytes:	Phenones 1. Acetophenone 2. Propiophenone 3. Butyrophenone 4. Valerophenone 5. Hexanophenone 6. Heptanophenone 7. Octanophenone

## System Comparison

The UHPLC system gives the best performance and any HPLC system can benefit from the faster, high resolution separations offered by Accucore HPLC columns. The higher resolution observed with the Surveyor is caused by the gradient delay.

	Accela 1250	Surveyor	Agilent 1100
Run Time (min)	2.5	3.0	3.5
Average Peak Width at 50 % height (min)	0.02	0.02	0.04
Average Resolution (USP)	6.15	6.53	5.33



*In order to get the best out of Accucore HPLC columns the system should be optimized for high efficiency separations.*

**See Instrument Optimization on page 13.**

# Instrument Optimization

Accucore HPLC columns produce very narrow peaks. In order to preserve this efficiency the HPLC system should be optimized to reduce any potential causes of peak broadening.

Potential causes of peak broadening are:

## Extra-column band broadening

The following equation for extra-column broadening shows that it is important to limit injection volume, minimize flow cell volume and make sure that short, narrow ID tubing is used.

$K$  Constant  $r_c$  Tubing radius  
 $V_{inj}$  Injection volume  $l_c$  Tubing length  
 $V_{cell}$  Flow cell volume  $D_m$  Diffusion coefficient in mobile phase  
 $F$  Flow rate

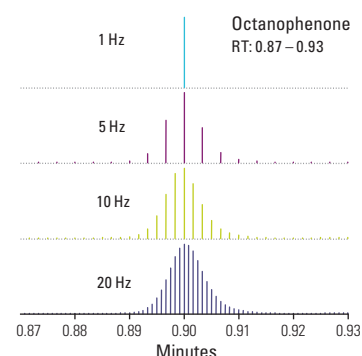
$$\sigma_{ext}^2 = \left( K_{inj} \frac{V_{inj}^2}{12} \right) + \left( K_{cell} \frac{V_{cell}^2}{12} + \pi^2 F^2 \right) + \left( \frac{r_c^4 l_c F}{7.6 D_m} \right)$$

## Slow detector response

The detector time constant or sampling rate must be optimized for narrow peaks. If this is not done then losses in intensity and increases in peak width are seen.

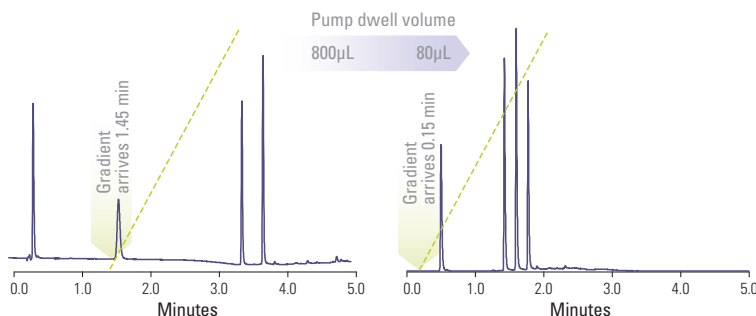
	Data point*	Peak width 4σ (s)	Peak area	Peak height (mAu)
1 Hz	2	2.04	246330	107.4
5 Hz	6	0.96	57244	118.4
10 Hz	10	0.87	55750	114.5
20 Hz	18	0.87	55319	115.4

\* Number of data points are collected over 4σ



## Fast gradients

For fast gradients it is also important to minimize the pump dwell volume to ensure that the gradient reaches the column as quickly as possible.



Column:	fully porous < 2 μm, 50 x 2.1 mm
Mobile phase A:	water + 0.1% formic acid
Mobile phase B:	acetonitrile + 0.1 % formic acid
Gradient:	5–100 % B in 2 minutes
Flow:	550 μL/min
Temperature:	25 °C
Injection:	0.5 μL
Detection:	UV at 270 nm (2 μL flow cell)
Tubing column-detector:	0.005" ID
Analytes:	1. Sulphaguanidine 2. Sulphamerazine 3. Sulphamonomethoxine 4. Sulphaquinoxaline

# Reproducible Chromatography

The advanced bonding technology and automated packing process used for Accucore HPLC columns results in exceptionally reproducible chromatography.

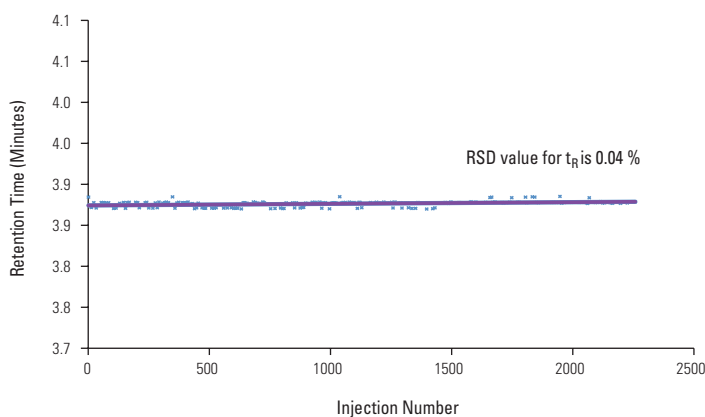
## Batch-to-Batch Reproducibility

Accucore C18, 2.6 $\mu$ m				
Batch No	HR/10	HS	SS	HBC
11541	2.31	1.77	1.39	0.20
11551	2.38	1.77	1.40	0.21
11547	2.33	1.77	1.37	0.20
11589	2.36	1.77	1.41	0.20
11645	2.34	1.77	1.38	0.20
11610	2.34	1.78	1.41	0.21
Mean	2.34	1.77	1.39	0.21
% RSD	1%	0%	1%	1%

*Phase characterization values on six different batches of material show excellent reproducibility.*

## Run-to-Run Reproducibility

### Rosuvastatin Retention



*Over 2400 injections with very stable retention times.*

Column:	Accucore C18, 50 x 2.1 mm (analytical)
Mobile phase A:	0.1% formic acid in water
Mobile phase B:	0.1% formic acid in acetonitrile
Gradient:	0 % B for 0.5 minutes 0–100 % B in 2.0 minutes 100 % B for 2.0 minutes 100–0 % B in 0.5 minutes
Flow:	600 $\mu$ L/min

# Long Lasting Columns

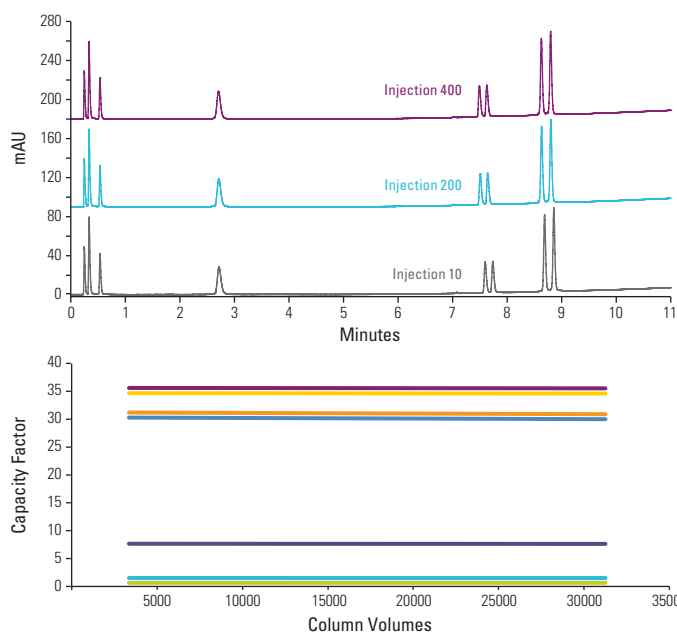
Chromatographers today demand long lifetimes from the columns they use.

## Mechanical Stability and Stable Bonded Phase

The highly uniform packed bed in Accucore HPLC columns is created by the use of tightly controlled particle size and automated packing process and has excellent mechanical stability.

The advanced bonding technology used for Accucore HPLC columns creates robust bonded phases that are highly resistant to the effects of pH and temperature.

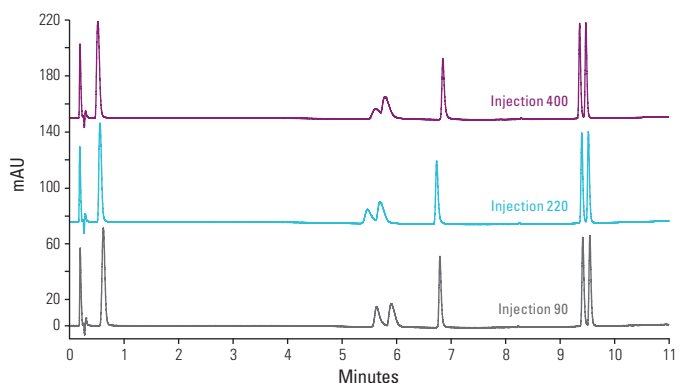
## Accucore HPLC columns show excellent stability at pH <2



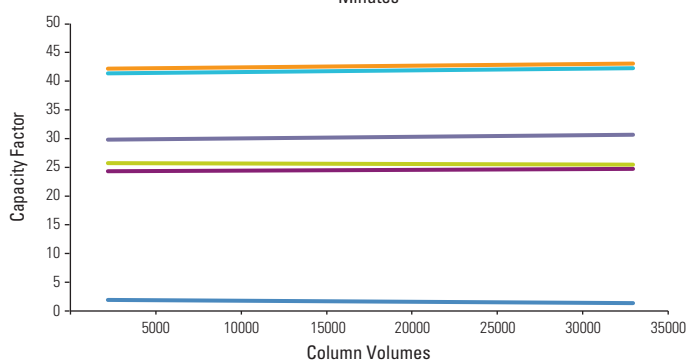
Column:	Accucore C18 2.6 $\mu$ m, 100 x 2.1 mm
Mobile phase A:	water + 0.1 % trifluoroacetic acid
Mobile phase B:	methanol + 0.1 % trifluoroacetic acid
Gradient:	25 % B for 0.75 minutes 25–100 % B in 9.25 minutes 100 % B for 2.00 minutes 100–25 % B in 0.20 minutes 25 % B for 4.80 minutes
Flow:	400 $\mu$ L/min
Temperature:	30 $^{\circ}$ C
Injection:	1 $\mu$ L
Detection:	UV at 254 nm (0.1s rise time, 20 Hz)
Analytes:	1. Uracil ( $t_p$ ) 2. Acetaminophen 3. p-Hydroxybenzoic acid 4. O-Hydroxybenzoic acid 5. Amitriptyline 6. Nortriptyline 7. Di-isopropyl phthalate 8. Di-n-propyl phthalate

*Accucore HPLC columns are robust and long lasting.*

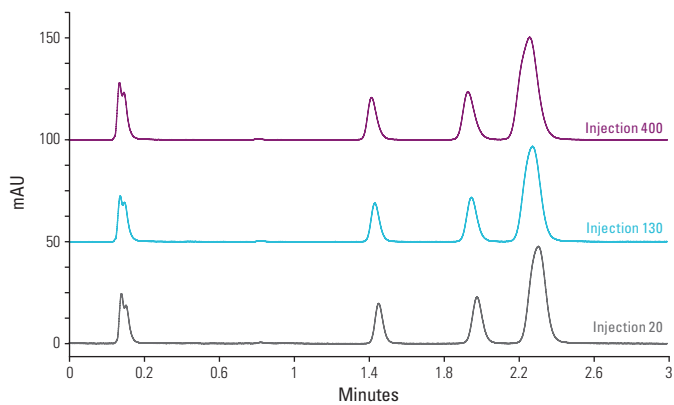
## Accucore HPLC columns are also stable at pH >10



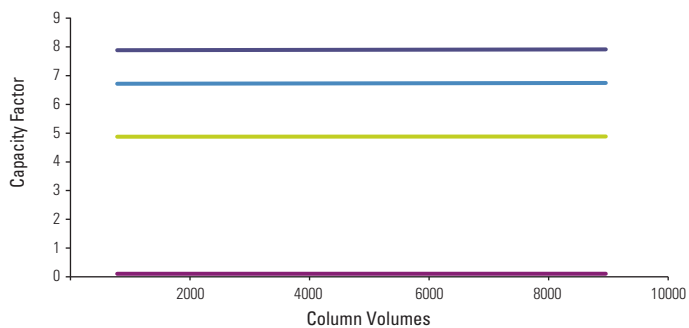
Column:	Accucore C18 2.6 $\mu$ m, 100 x 2.1 mm
Mobile phase A:	water + 0.1 % ammonia
Mobile phase B:	methanol + 0.1 % ammonia
Gradient:	15 % B for 1.0 minutes 15–100 % B in 7.0 minutes 100 % B for 3.00 minutes 100–15 % B in 0.20 minutes 15 % B for 4.80 minutes
Flow:	400 $\mu$ L/min
Temperature:	30 $^{\circ}$ C
Injection:	1 $\mu$ L
Detection:	UV at 254 nm (0.1 s rise time, 20 Hz)
Analytes:	1. Uracil ( $t_R$ ) 2. 4-Chlorocinnamic acid 3. Procainamide 4. 4-Pentylbenzoic Acid 5. N-Acetylprocainamide 6. Di-isopropyl phthalate 7. Di-n-propyl phthalate



## And also stable at elevated temperature







Column:	Accucore C18 2.6 $\mu$ m, 100 x 2.1 mm
Mobile phase:	35:65 (v/v) water/methanol
Flow:	400 $\mu$ L/min
Temperature:	70 $^{\circ}$ C
Injection:	1.5 $\mu$ L
Detection:	UV at 254 nm (0.1 s rise time, 20 Hz)
Analytes:	1. Theophylline ( $t_R$ )/Caffeine 2. Phenol 3. Butylbenzene 4. o-Terphenyl 5. Pentylbenzene/Triphenylene






# Phase Characterization

Accucore phases are characterized using three tests based on the Tanaka testing protocols<sup>1</sup>. This detailed phase characterization allows the retentivity, selectivity and secondary interactions demonstrated by HPLC packing materials under specified conditions to be objectively compared.



## T1: Hydrophobic Interactions

			Parameter	Term
	HR	Hydrophobic Retention	Retention of compounds based on their hydrophobicity	$k'$
	HS	Hydrophobic Selectivity	Separation of compounds that have similar structure, but differ slightly in hydrophobicity	$\alpha$
	SS	Steric Selectivity	Separation of compounds that have similar structure, but differ in shape	$\alpha$
	HBC	Hydrogen Bonding Capacity	Separation related to degree of end capping	$\alpha$

## T2: Secondary Interactions Under Neutral pH

			Parameter	Term
	BA	Base Activity	Peak shape for basic analytes resulting from total silanol activity (all dissociated at pH 7.6)	$t_r$
	C	Chelation	Peak shapes for chelating analytes resulting from silica metal content	$t_r$
	IEX(7.6)	Ion Exchange Capacity (pH 7.6)	Separation between basic and neutral compounds resulting from total silanol activity (all dissociated at pH 7.6)	$\alpha$

## T3: Secondary Interactions Under Acidic pH

			Parameter	Term
	AI	Acid Interaction	Interactions resulting in poor peak shape for acidic analytes	$t_r$
	IEX(2.7)	Ion Exchange Capacity (pH 2.7)	Separation between basic and neutral compounds resulting from acidic silanol activity	$\alpha$

The results of the phase characterizations are shown in the radar plots used in this guide.

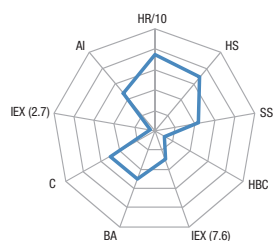
1. K. Kimata, K. Iwaguchi, S. Onishi, K. Jinno, R. Eksteen, K. Hosoya, M. Arki, N. Tanaka, J. Chromatogr. Sci. 27 (1989) 721

# Optimum Selectivity

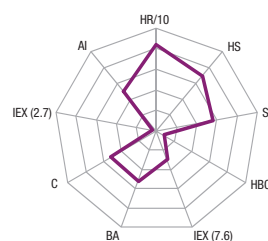
Accucore based on 2.6  $\mu\text{m}$  particles is available in fourteen different phases to provide an unrivalled range of selectivities.

Each of the bonded phases is manufactured using advanced bonding technology and is characterized using a testing regime based on the Tanaka Tests. See page 17 for further details of these tests.

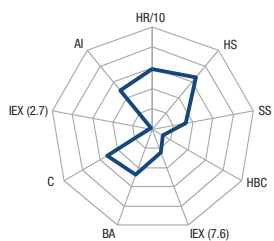
The radar plots below show the results of the characterisation and allow for quick and easy comparison of the phase selectivities.



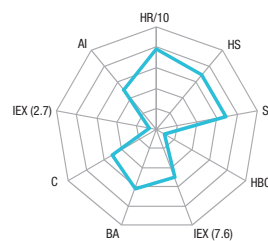
**Accucore RP-MS**  
Optimized for MS detection, excellent combination of speed and quality of separation



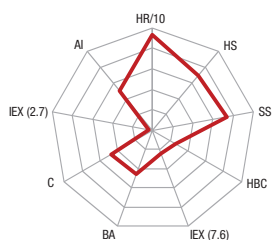
**Accucore C18**  
Optimum retention for non-polar analytes



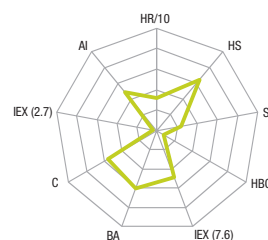
**Accucore C8**  
Lower hydrophobicity than C18 recommended for analytes with moderate hydrophobicity



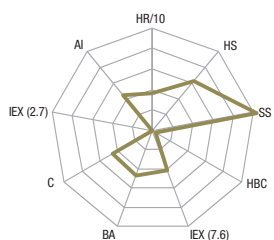
**Accucore aQ**  
Compatible with 100% aqueous mobile phases, special selectivity for polar analytes



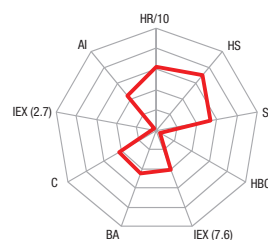
**Accucore Polar Premium**  
Rugged amide embedded C18 phase that offers complementary selectivity to conventional C18



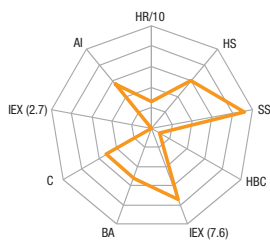
**Accucore Phenyl-Hexyl**  
Unique selectivity for aromatic and moderately polar analytes



**Accucore Phenyl-X**  
Unique reversed-phase shape selectivity with high aromatic selectivity



**Accucore C30**  
High shape selectivity for hydrophobic, long chain, structurally related isomers



#### Accucore PFP

Alternative selectivity to C18, particularly for halogenated analytes

## HILIC

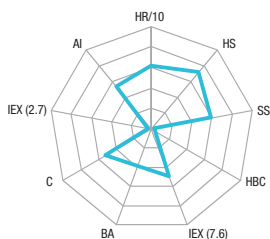
#### Accucore HILIC

Enhanced Retention of polar and hydrophilic analytes

## HILIC

#### Accucore Urea-HILIC

Unique HILIC selectivity and low ion exchange activity



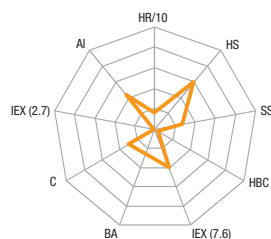
#### Accucore 150-C18

Phase characteristics are designed for the separation of peptides

## HILIC

#### Accucore 150-Amide-HILIC

Designed for the separation of hydrophilic biomolecules in HILIC mode. An excellent choice for glycan separations

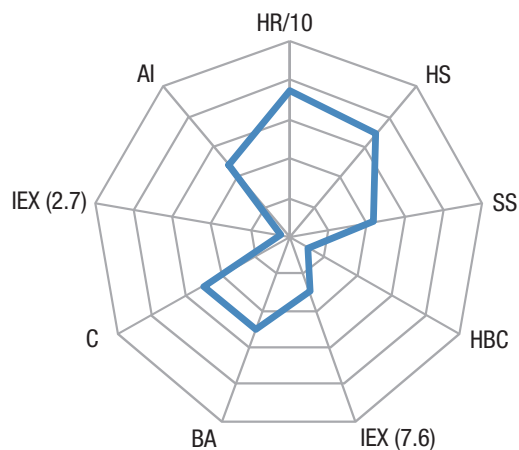
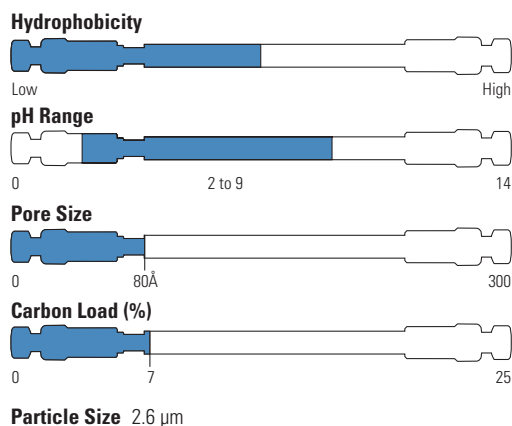


#### Accucore 150-C4

Lower hydrophobicity for optimal retention of proteins and larger peptides



# Accucore RP-MS



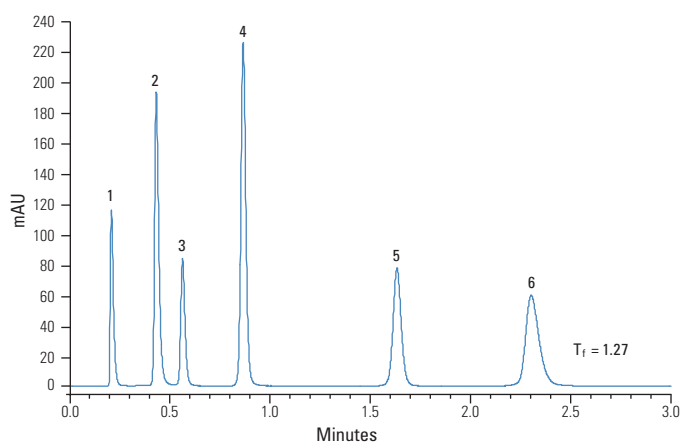
- Optimized for MS detection
- Excellent peak shapes
- Excellent combination of speed and efficiency

Accucore RP-MS uses an optimized alkyl chain length for more effective coverage of the silica surface. This coverage results in a significant reduction in non-hydrophobic interactions and thus highly efficient peaks with very low tailing.

RP-MS offers slightly lower retention than C18 and this combined with high efficiencies and low peak tailing make this the phase of choice for use with MS detection.

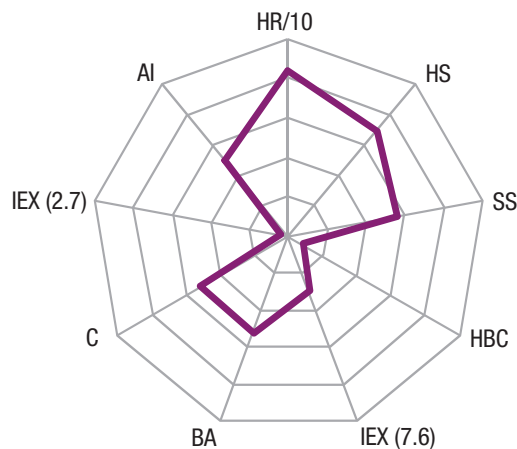
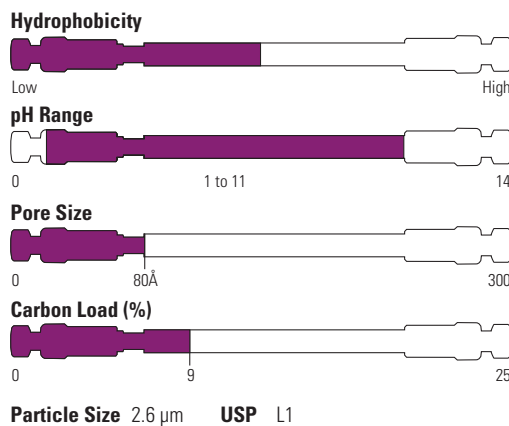
The selectivity offered by Accucore RP-MS matches that of C18 columns.

## Bases



Column:	Accucore RP-MS 2.6 µm, 50 mm x 2.1 mm
Mobile phase:	65:35 (v/v) methanol/25mM potassium phosphate pH 7.0
Flow:	500 µL/min
Temperature:	30 °C
Injection:	1 µL
Detection:	UV at 215 nm
Backpressure:	232 bar
Analytes:	1. Uracil ( $t_r$ ) 2. Propranolol 3. Butylparaben 4. Naphthalene 5. Acenaphthene 6. Amitriptyline

# Accucore C18

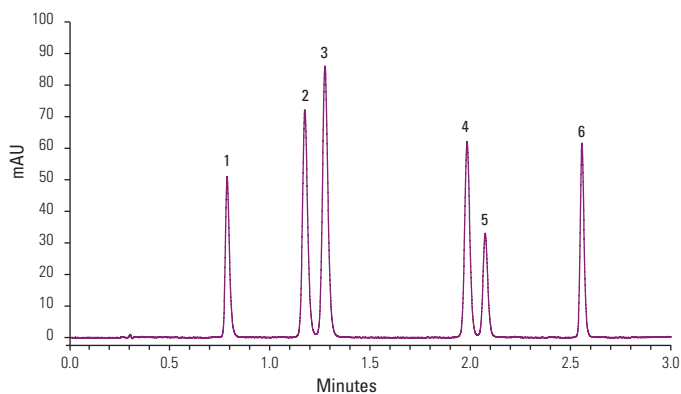


- Optimum retention of non-polar compounds
- Hydrophobic interaction mechanism
- Separates a broad range of analytes

The carbon loading of Accucore C18 phase provides high retention of non-polar analytes via a predominantly hydrophobic interaction mechanism.

The highly retentive nature of Accucore C18 phase means that it can be used to separate a broad range of analytes.

## Triazines



Column: Accucore C18 2.6 µm, 50 mm x 2.1 mm

Mobile phase A: water

Mobile phase B: acetonitrile

Gradient: 35 % B for 1.0 minute  
35–70 % B in 1.5 minutes

Flow: 600 µL/min

Temperature: 25 °C

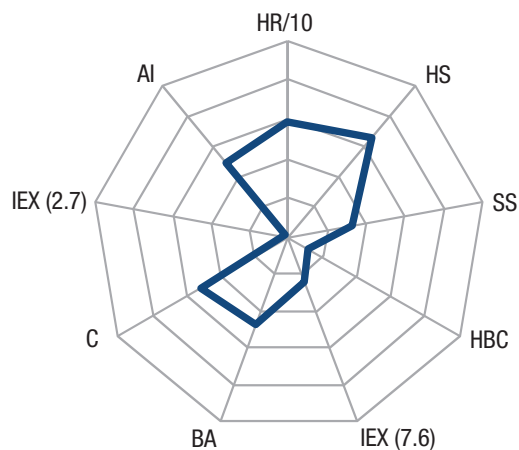
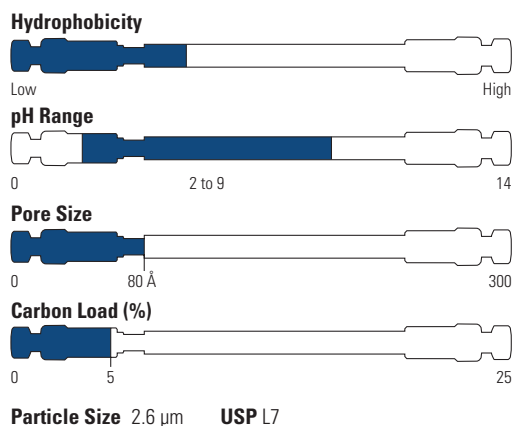
Injection: 2 µL

Detection: UV at 280 nm

Backpressure: 298 bar

Analytes: 1. Simazine  
2. Simetryn  
3. Atrazine  
4. Ametryn  
5. Propazine  
6. Prometryn

# Accucore C8

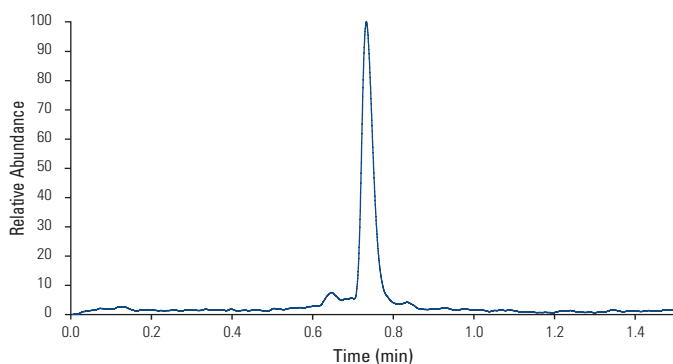


- Lower hydrophobic retention
- Complementary steric selectivity to C18
- Low levels of secondary interactions
- Recommended for moderately polar analytes

Accucore C8 HPLC columns offer lower hydrophobic retention than columns packed with longer alkyl chain length material, such as C18, and are therefore recommended for analytes with medium hydrophobicity or when a less hydrophobic phase provides optimum retention.

The low levels of secondary interactions demonstrated in the phase characterization are the result of excellent bonded phase coverage and allow users of Accucore C8 HPLC columns to benefit from excellent peak shapes.

## Testosterone

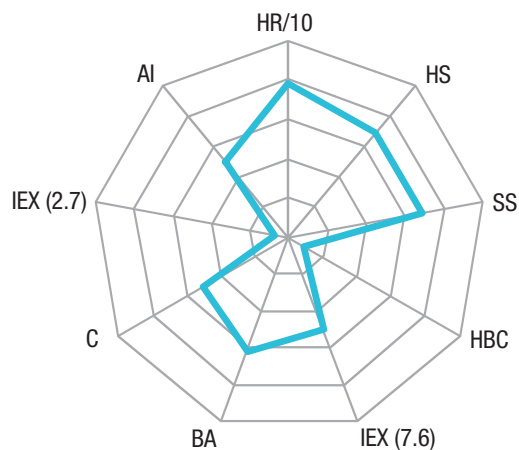
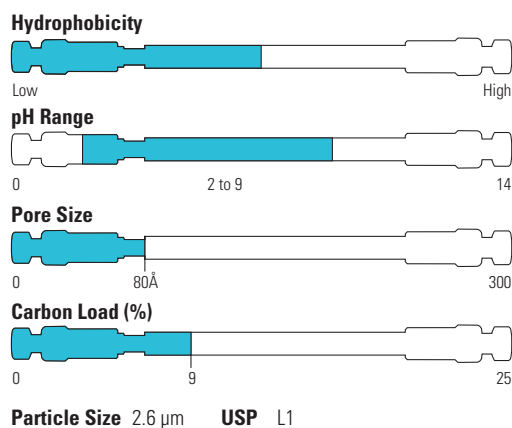


Column:	Accucore C8 2.6 µm, 50 x 2.1 mm
Mobile phase A:	water + 0.1% formic acid
Mobile phase B:	acetonitrile + 0.1% formic acid
Gradient:	5–95 % B in 0.8 minutes
Flow:	1500 µL/min
Temperature:	60 °C
Injection:	5 µL
Detection:	ESI-MS/MS

Retention time ( $t_R$ , /min)	0.73
%RSD $t_R$	0.22
%RSD Area	3.01

Data from six injections.

# Accucore aQ

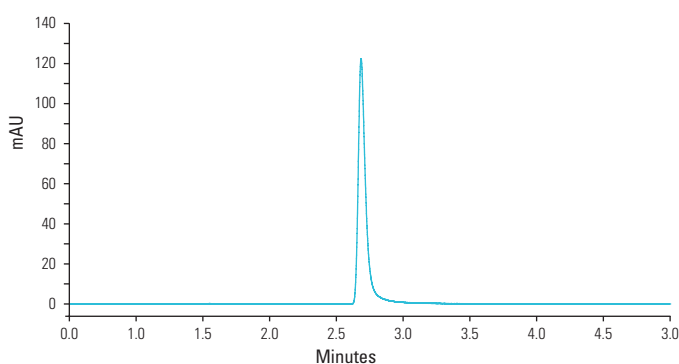


- Retention and resolution of polar analytes
- Polar endcapped C18 stationary phase for alternative selectivity
- Ideal for highly aqueous mobile phases

The polar functional group used to endcap Accucore aQ phase provides an additional controlled interaction mechanism by which polar compounds can be retained and resolved, making Accucore aQ phase ideal for the quantitative analysis of trace levels of polar analytes.

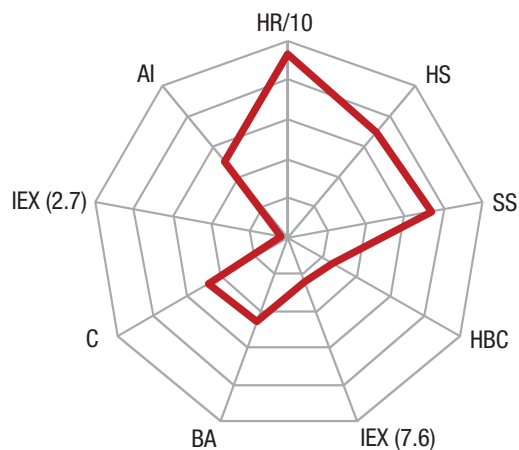
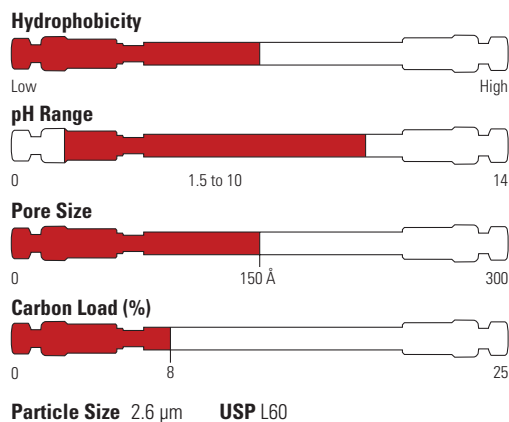
The wettability of reversed phase media can be increased by the introduction of polar functional groups. The polar endcapping of Accucore aQ media also makes it usable in 100% aqueous mobile phases without the risk of loss of performance or poor stability.

## Lamivudine (USP)



Column:	Accucore aQ 2.6 $\mu\text{m}$ , 50 mm x 2.1 mm
Mobile phase:	95:5 (v/v) ammonium acetate, pH 3.80/methanol
Flow:	200 $\mu\text{L}/\text{min}$
Temperature:	35 $^{\circ}\text{C}$
Injection:	1 $\mu\text{L}$
Detection:	UV at 277 nm
Analyte:	Lamivudine
%RSD $t_r$	0.00
%RSD Peak area	1.72
(%RSD calculated from 6 replicate injections)	
USP acceptance criteria: % RSD ( $t_r$ , Peak Area) <2.0	

# Accucore Polar Premium

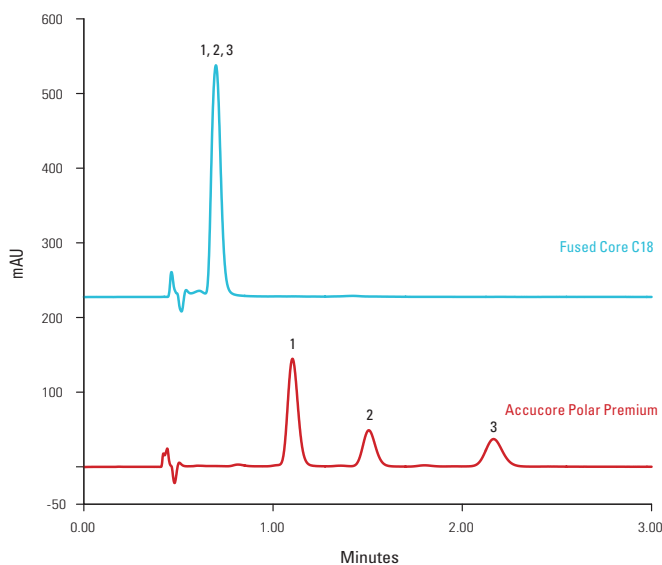


- Rugged amide-embedded C18 phase
- Selectivity complementary to conventional C18 phases
- Stable over a wide pH range and compatible with 100% aqueous mobile phase

Accucore Polar Premium is an exceptionally rugged polar embedded reverse phase material that offers high efficiency, wider operating pH range and unique selectivity complementary to standard C18 phases.

The specially designed bonded phase is stable from pH 1.5 to 10.5 and will not undergo phase collapse in 100% aqueous mobile phase.

## Curcuminoids (Turmeric)



**Columns:** Accucore Polar Premium 2.6 µm, 100 x 3.0 mm  
Fused Core C18, 100 x 3.0 mm

**Mobile phase:** methanol : 10 mM phosphoric acid, 80 : 20

**Flow:** 800 µL/min

**Temperature:** 40 °C

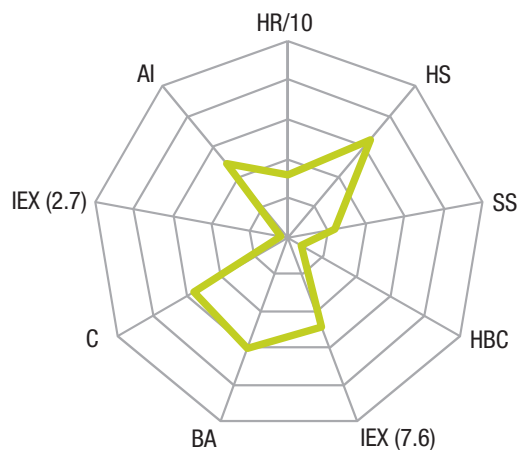
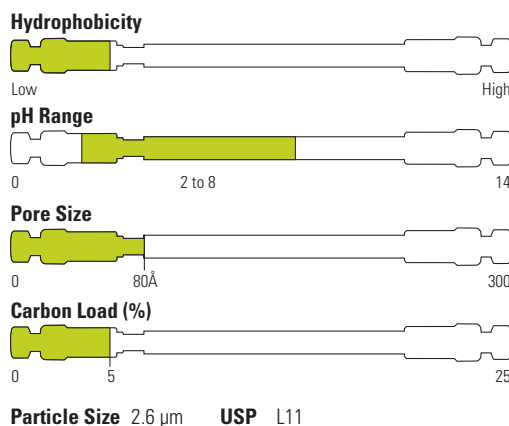
**Injection:** 6 µL

**Detection:** UV at 428 nm

**Analytes:** 1. Curcumin  
2. Desmethoxycurcumin  
3. Bis-desmethoxycurcumin

The Accucore Polar Premium HPLC column provides desirable selectivity that resolves the major and minor component under simple isocratic conditions in less than three minutes, while the C18 columns fail to separate these components.

# Accucore Phenyl-Hexyl

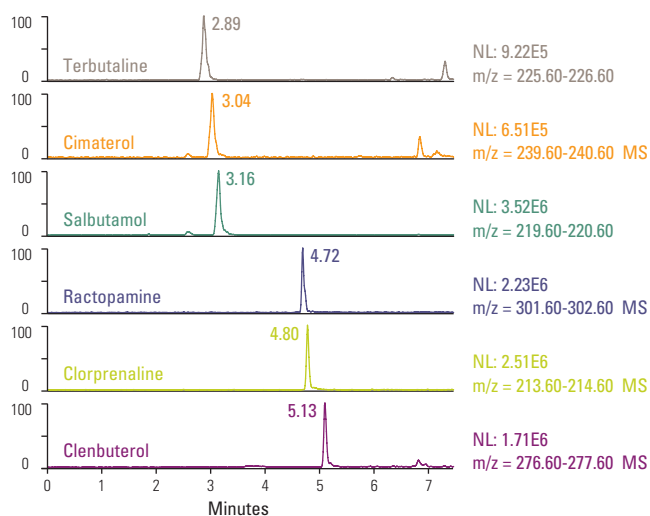


- Mixed-mode selectivity for aromatic and moderately polar analytes
- Enhanced Pi-pi interactions with aromatics
- Moderate hydrophobicity

The C6 chain in Accucore Phenyl-Hexyl phase exhibits classical RP retention and selectivity, while the phenyl ring can add special selectivity by interacting with polar groups within the solutes. This results in a mixed-mode separation mechanism. The reduced hydrophobicity of this phase makes it ideal for the separation of very non-polar compounds.

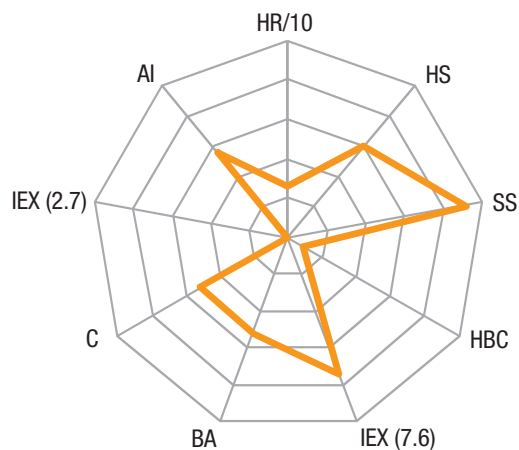
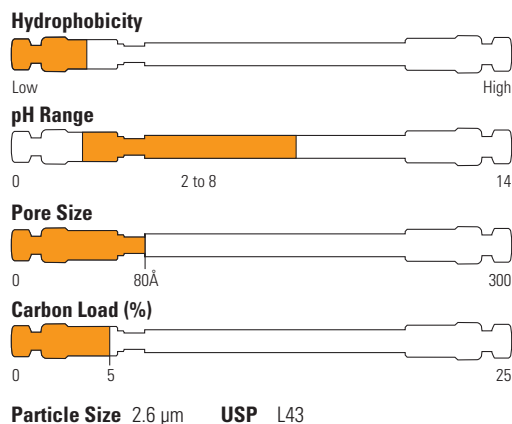
Phenyl-Hexyl phase should be selected for complex samples where some peaks are well resolved on a conventional alkyl phases, but are not well resolved on a conventional phenyl phase. While other peaks are well resolved on a phenyl phase, but not well resolved on a conventional alkyl phase.

## Beta-agonists



Column:	Accucore Phenyl-Hexyl 2.6 µm, 100 x 2.1 mm
Mobile phase A:	ammonium acetate 5 mM, pH 4
Mobile phase B:	acetonitrile
Gradient:	5 % B for 1 minute 5–100 % B in 9 minutes
Flow:	250 µL/min
Temperature:	40 °C
Injection:	1 µL
Detection:	+ESI-MS (45 °C, 4.5 kV, 60 V, scan 150 – 350)
Backpressure:	120 bar

## Accucore PFP

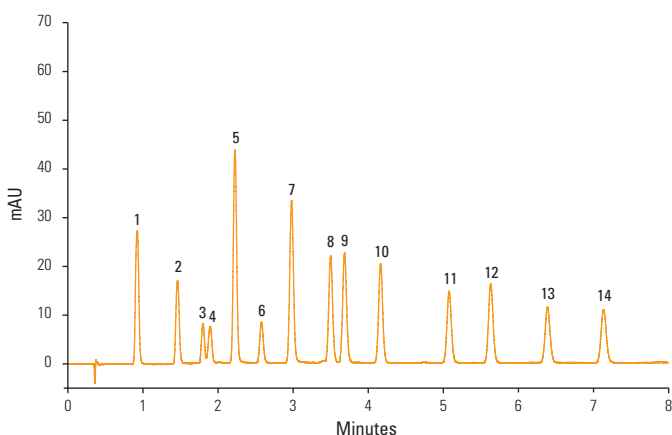


- Alternative selectivity to C18
- Extra retention for halogenated species
- Unique selectivity for non-halogenated polar compounds

Introduction of fluorine groups into the Accucore PFP (pentafluorophenyl) stationary phase causes significant changes in solute-stationary phase interactions. This can lead to extra retention and selectivity for positional isomers of halogenated compounds.

PFP Columns are also well suited to the selective analysis of non-halogenated compounds, in particular polar compounds containing hydroxyl, carboxyl, nitro, or other polar groups. High selectivity is often most apparent when the functional groups are located on an aromatic or other rigid ring system.

### Positional Isomers



Column: Accucore PFP 2.6 µm, 50 x 2.1 mm

Mobile phase A: water + 0.1 % formic acid

Mobile phase B: acetonitrile + 0.1 % formic acid

Gradient: 15–30 % B in 7 minutes

Flow: 600 µL/min

Temperature: 50 °C

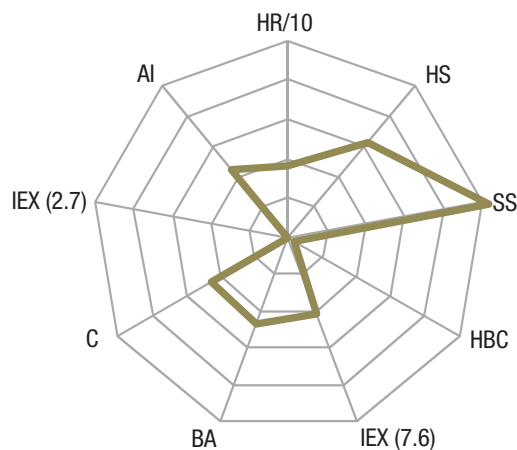
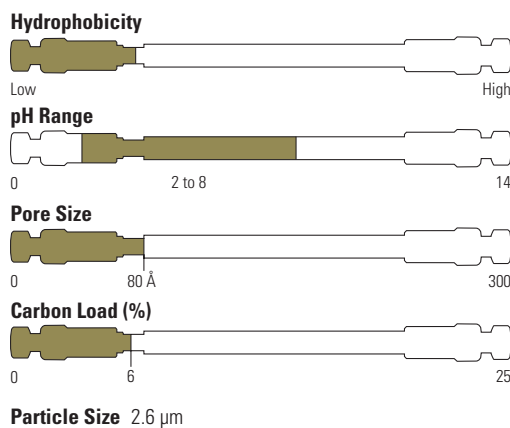
Injection: 2 µL

Detection: UV at 270 nm

Analytes:

1. 3,4-Dimethoxyphenol
2. 2,6-Dimethoxyphenol
3. 2,6-Difluorophenol
4. 3,5-Dimethoxyphenol
5. 2,4-Difluorophenol
6. 2,3-Difluorophenol
7. 3,4-Difluorophenol
8. 3,5-Dimethylphenol
9. 2,6-Dimethylphenol
10. 2,6-Dichlorophenol
11. 4-Chloro-3-Methylphenol
12. 4-Chloro-2-Methylphenol
13. 3,4-Dichlorophenol
14. 3,5-Dichlorophenol

# Accucore Phenyl-X



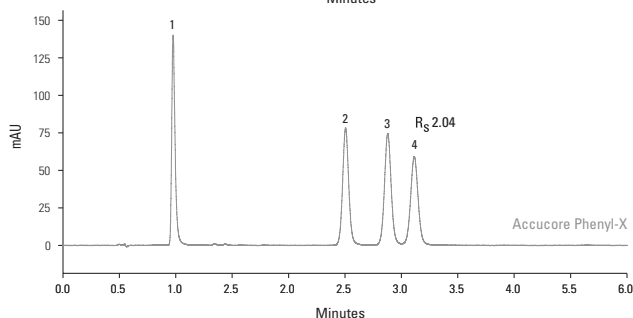
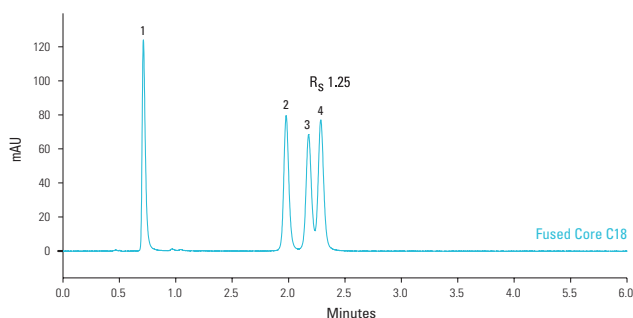
- Unique reversed-phase shape selectivity
- Enhanced selectivity for aromatic compounds
- Compatible with highly aqueous mobile phases
- Robust, high-efficiency, low column bleed

The proprietary Accucore Phenyl-X alkyl aromatic bonded phase provides a unique selectivity when compared to other reversed phase materials such as C18 or Phenyl.

Phenyl-X exhibits particularly high aromatic selectivity.

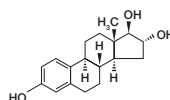
The advanced design of the bonded phase makes it compatible with highly aqueous mobile phases and robust, demonstrating very low bleed.

## Estrogens

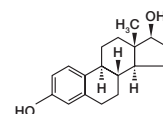


Columns:	Accucore Phenyl-X 2.6 µm, 100 x 2.1 mm Fused Core C18, 100 x 2.1 mm
Mobile phase:	15:40:45 (v/v/v) acetonitrile: methanol : water
Flow:	400 µL/min
Temperature:	40 °C
Injection:	1 µL
Detection:	UV at 220 nm
Wash solvent:	Same as mobile phase

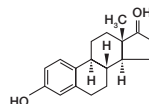
1. Estriol (E3)



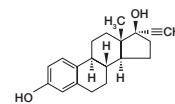
2. Estradiol (E2)



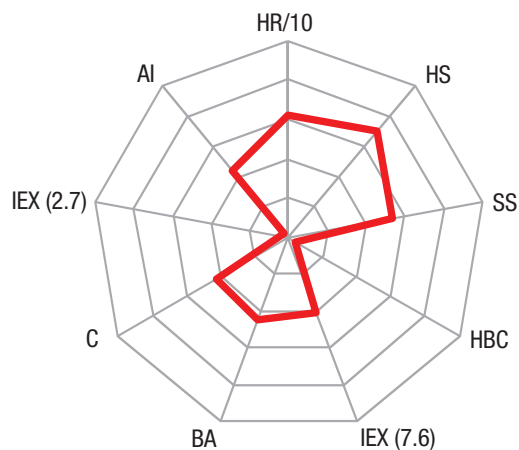
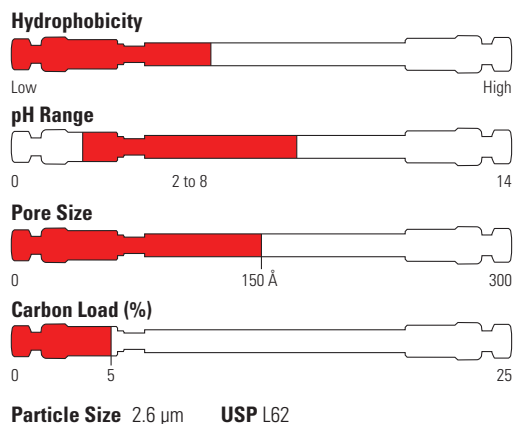
3. Estrone (E1)



4. Ethynylestradiol



## Accucore C30

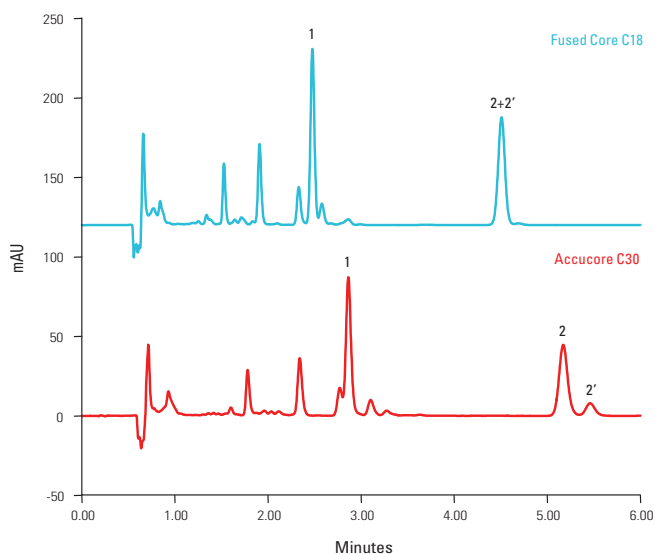


- Ideal for separation of hydrophobic, long alkyl chain compounds
- High shape selectivity for structurally related isomers
- Excellent aqueous-compatibility

Accucore C30 offers high shape selectivity for hydrophobic, long chain, structurally related isomers, for example carotenoids and steroids. This is a different form of shape selectivity from that measured in the SS phase characterisation test.

It is also an excellent alternative to normal-phase columns for lipid analysis. The optimized bonding density of the long alkyl chains facilitated by a wider pore diameter particle result in a phase that is stable even in highly aqueous mobile phases.

### Vitamin K isomers

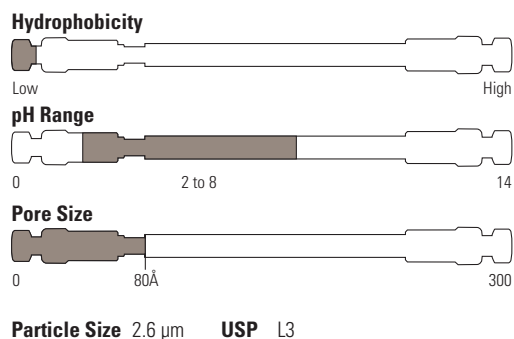


<b>Columns:</b>	Accucore C30 2.6 µm, 100 x 3.0 mm Fused Core C18, 100 x 3.0 mm
<b>Mobile phase:</b>	methanol: 2 mM ammonium acetate, 98:2
<b>Flow:</b>	650 µL/min
<b>Temperature:</b>	20 °C
<b>Injection:</b>	5 µL
<b>Detection:</b>	UV at 250 nm

Accucore C30 shows better separation for vitamin K1 isomers than the C18 column.

Chromatogram showing the separation of Vitamin K compounds  
1-Vitamin K2, 2'-Vitamin K1 (trans isomer), 2'-Vitamin K1 (cis isomer)

# Accucore HILIC



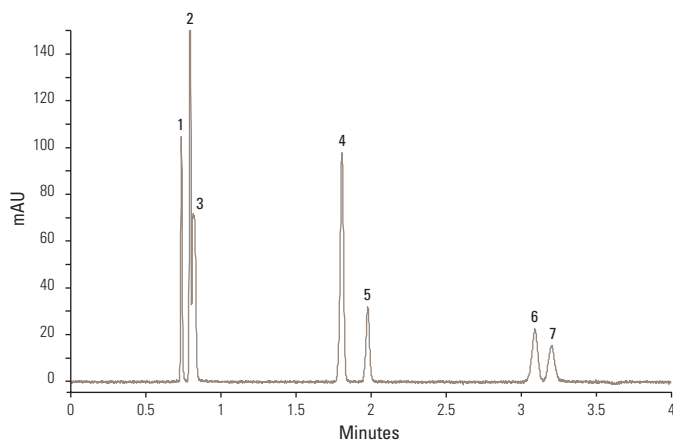
## HILIC

- Enhanced retention of polar and hydrophilic analytes
- Alternative selectivity to C18 without ion-pair or derivatization

Analyte properties that govern retention with Accucore HILIC phase are acidity/basicity, which determines hydrogen bonding, and polarizability which determines dipole-dipole interactions.

The highly organic mobile phases used with Accucore HILIC phase ensure efficient desolvation in ESI MS detection, which in turn leads to improved sensitivity.

### Catecholamines



Column: Accucore HILIC 2.6  $\mu\text{m}$ , 150 x 4.6 mm

Mobile phase: 85:15 (v/v) acetonitrile/100mM ammonium formate, pH 3.2

Flow: 2000  $\mu\text{L}/\text{min}$

Temperature: 40  $^{\circ}\text{C}$

Injection: 5  $\mu\text{L}$

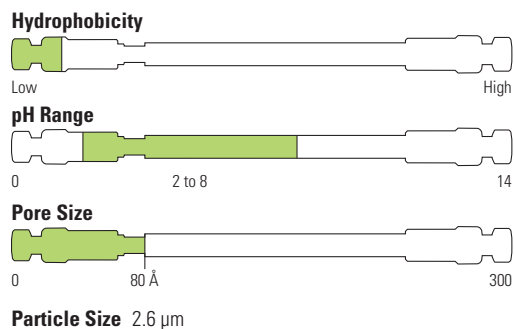
Detection: UV at 280 nm

Backpressure: 157 bar

Analytes:

1. Catechol
2. 5-HIAA
3. DOPAC
4. Serotonin
5. L-tyrosine
6. Dopamine
7. L-DOPA

# Accucore Urea-HILIC



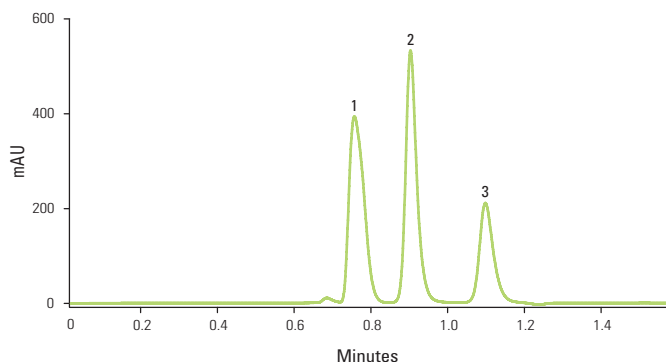
## HILIC

- Bonded hydrophilic stationary phase
- Unique selectivity compared to other HILIC phases
- Low ion exchange activity

Accucore Urea-HILIC has an alternative selectivity and lower ion exchange activity than other HILIC phases.

In HILIC mode the separation occurs through two mechanisms. The primary mechanism a partitioning effect due to the enriched water layer around the polar or charged substrate material. The secondary mechanism involves interaction between the analyte and the active surface moiety. The bonded hydrophilic stationary phase provides retention of broad range of polar analytes using up to 20% aqueous mobile phase.

### Analgesic compounds



Column: Accucore Urea-HILIC 2.6 μm, 100 x 2.1 mm

Mobile phase: composition 10:80:10, A : B : C

A: water

B: acetonitrile

C: 100 mM ammonium acetate adjusted to pH 4.9

Flow: 300 μL/min

Run time: 2 minutes

Temperature: 35 °C

Injection: 2 μL into 10 μL partial loop mode.

Injection wash solvent: water:acetonitrile 20:80

Detection: UV at 230 nm

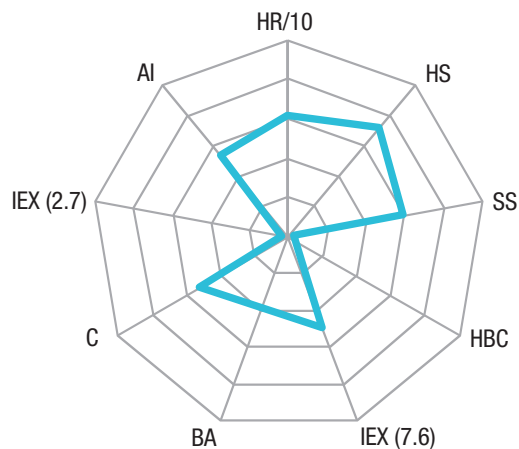
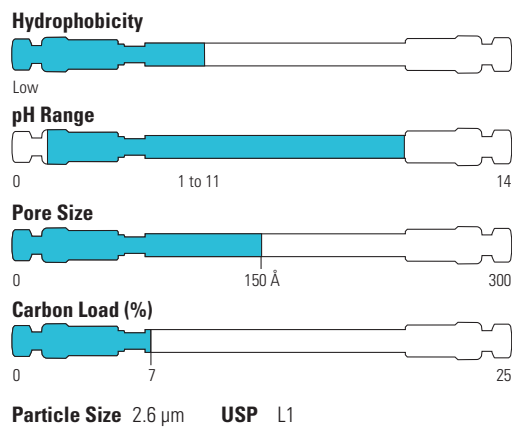
Backpressure: 71 bar

	Acetaminophen		Salicylic acid			Aspirin		
	$t_R$	$A_s$	$t_R$	$A_s$	$R_s$	$t_R$	$A_s$	$R_s$
Mean	0.760	1.474	0.908	1.303	2.359	1.100	1.318	3.264
CV %	0.00	1.17	0.48	0.92	0.49	0.00	0.63	0.48

Data from eight replicate analyses of a mixture of acetaminophen, salicylic acid and aspirin

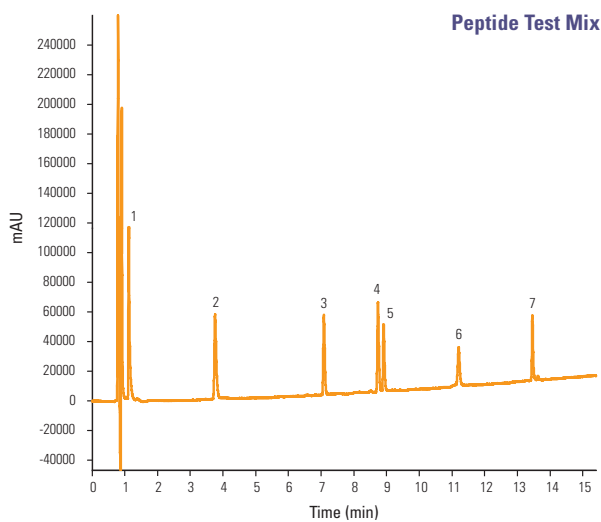
Retention time ( $t_R$ ), peak asymmetry ( $A_s$ ), peak resolution ( $R_s$ )

# Accucore 150-C18



- Designed for the separation of peptides
- Outstanding resolution
- 150 Å pore diameter material

## Peptide Separations



Column: Accucore 150-C18 2.6 µm, 100 x 2.1 mm

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 in 15.0 minutes  
50 % B for 2.0 minutes  
50–0 % B in 0.1 minutes  
0 % B for 5.0 minutes

Flow: 300 µL/min

Temperature: 35 °C

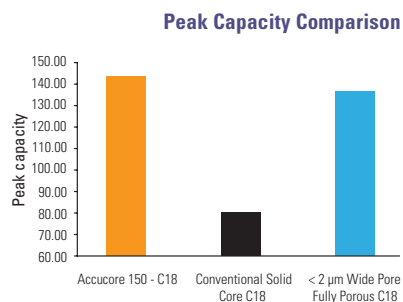
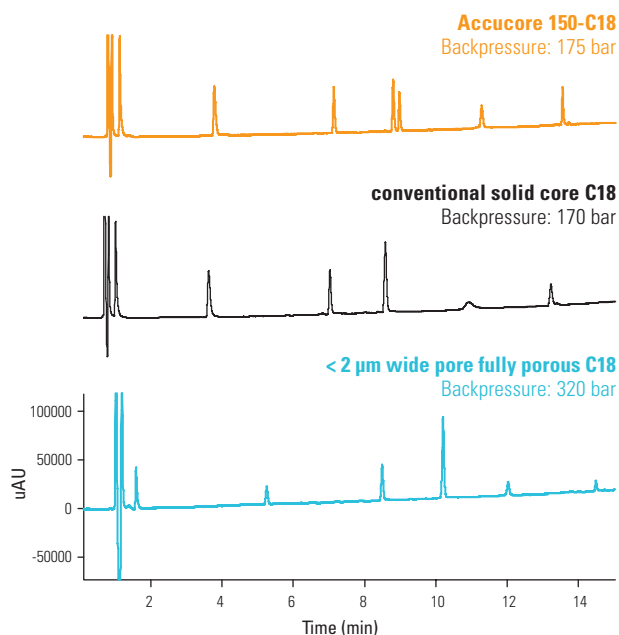
Injection: 5 µL

Detection: UV at 220 nm

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



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

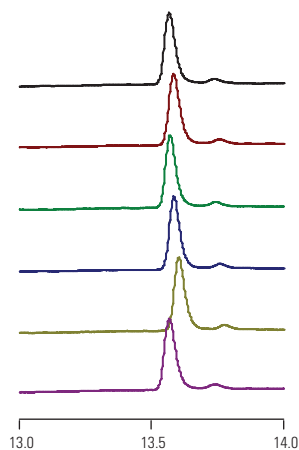
$n_c$  Peak capacity

$t_g$  Gradient time

$\bar{w}$  Average peak width 10% height

## Reproducible Separations

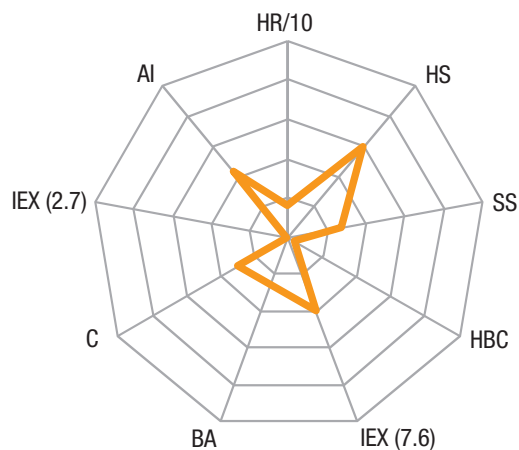
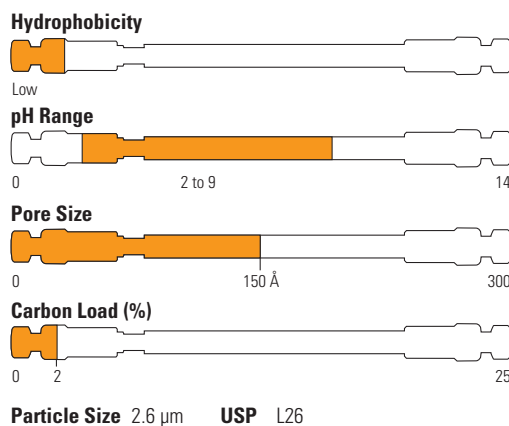
Precision of retention times is critical for reliable analysis. The Accucore 150-C18 column exhibits excellent retention time reproducibility.



Protein	Mean Retention Time (min)	% RSD
Insulin	13.58	0.11

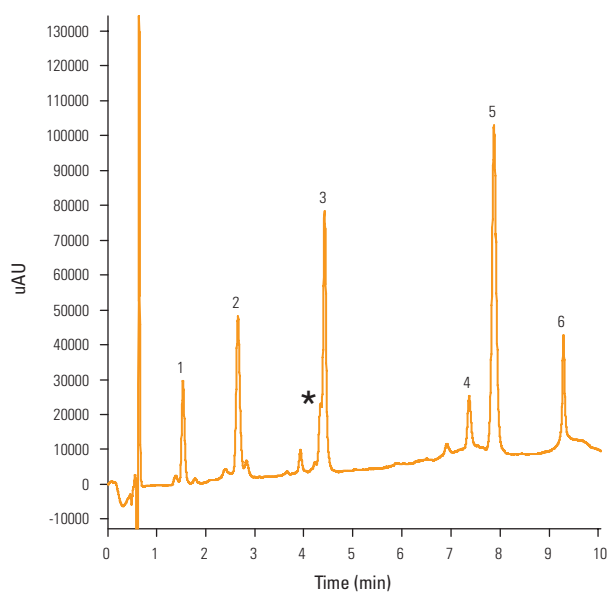
**Data from six injections.**

## Accucore 150-C4



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

## Intact Protein Separation



**Column:** Accucore 150-C4 2.6 µm, 100 x 2.1 mm

**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 in 8.0 minutes  
 30–95 % B in 2.0 minutes  
 95 % B for 1.0 minute  
 95–0 % B in 0.1 minutes  
 0 % B for 4.0 minutes

**Flow:** 400 µL/min

**Temperature:** 40 °C

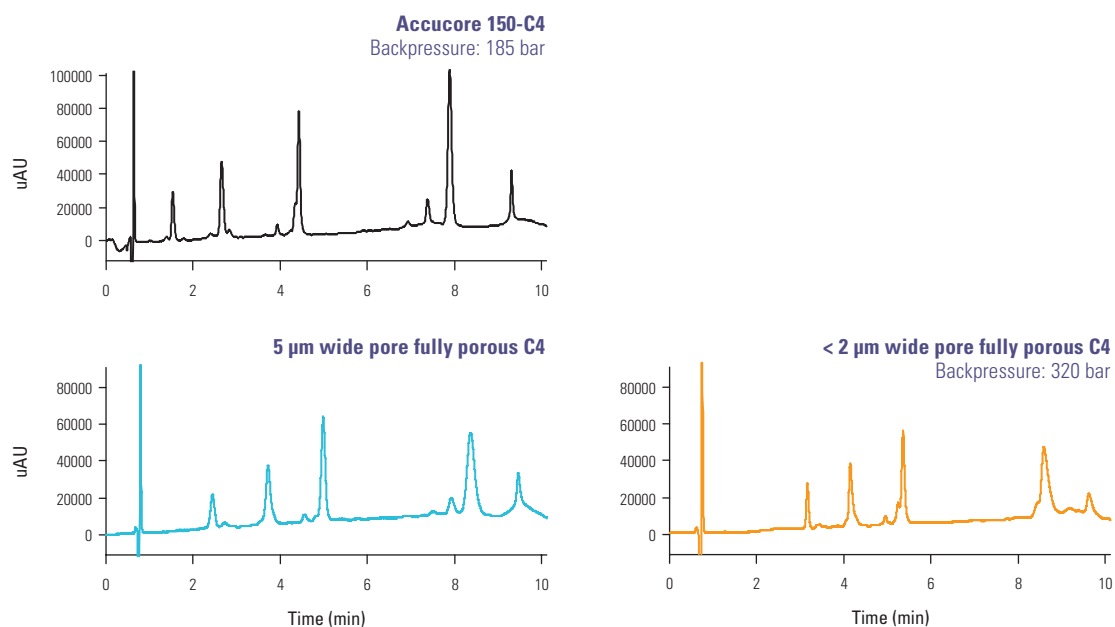
**Injection:** 2 µL of 10 pmol/µL solution

**Detection:** UV (214 and 280 nm)

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

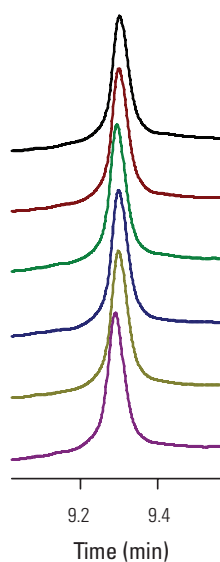
## Excellent Resolution

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

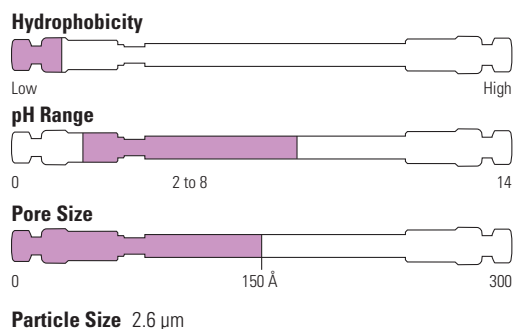
The Accucore 150-C4 column exhibits excellent peak shape and retention time reproducibility.



Protein	Mean Retention Time (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.

# Accucore 150-Amide-HILIC



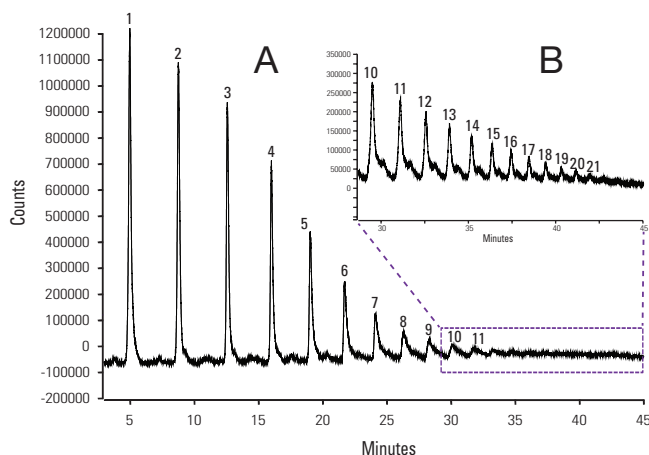
## HILIC

- Amide phase bonded onto 150 Å pore diameter solid core particles
- High retention of a broad range of hydrophilic analytes in HILIC mode
- Recommended for hydrophilic biomolecules such as glycans

Accucore 150-Amide-HILIC is designed for the separation of hydrophilic biomolecules in HILIC mode.

The amide bonded phases provide strong hydrogen bonding interaction between the stationary phase and the analytes, resulting in unique selectivity compared to other HILIC phases. Combined with larger pore size of the solid core particles, Accucore 150-Amide-HILIC is well suited for separating a variety of hydrophilic molecules, including carbohydrates and peptides. As a result the Accucore 150-Amide-HILIC is an excellent choice for glycan separations.

## Glycan Ladder



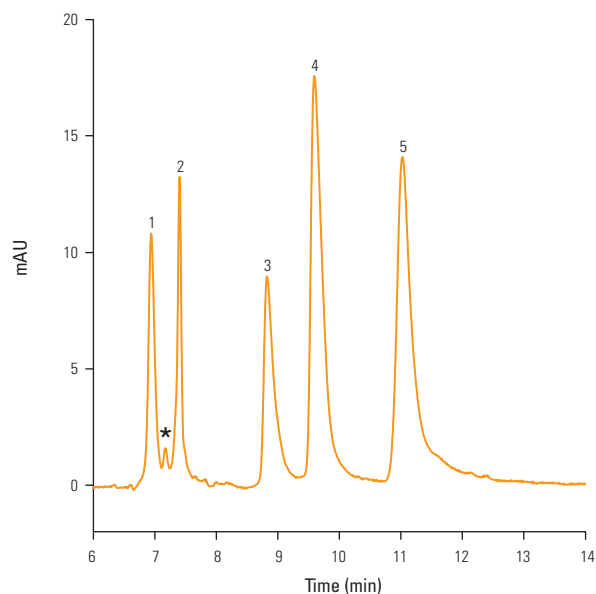
(A) 2 μL injection of sample, where 11 glycans were separated.

(B) 5 μL injection of sample, zoomed-in to the later part of the gradient rise. A further 10 glycans were detected.

Column:	Accucore 150-Amide-HILIC, 2.6 μm, 100 x 2.1 mm
Mobile phase A:	acetonitrile
Mobile phase B:	50 mM ammonium formate, pH 4.5
Gradient:	20–50 % B in 40.0 minutes 50 % B for 5.0 minutes 50–20 % B in 0.5 minutes 50 % B for 4.5 minutes
Flow:	500 μL/min
Backpressure at starting conditions:	110 bar
Run time:	50 minutes
Temperature:	60 °C
Injection:	2 μL to 5 μL of sample.
Injection wash solvent:	80:20 (v/v) acetonitrile:water.
Fluorescence detector acquisition parameters:	330 nm excitation wavelength; 420 nm emission wavelength; acquisition start after 3 min from gradient start.

# nanoLC Column Separations

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



Column: Accucore 150-C4, 2.6  $\mu$ m, 150mm x 75  $\mu$ m

Mobile phase A: 0.1 % formic acid in water

Mobile phase B: 0.1 % formic acid in acetonitrile

Gradient: 0–30 % B in 1 minute  
30–60 % B in 10 minutes  
60–95 % B in 1 minute  
95 % B for 3 minutes

Flow: 300 nL/min

Temperature: 40 °C

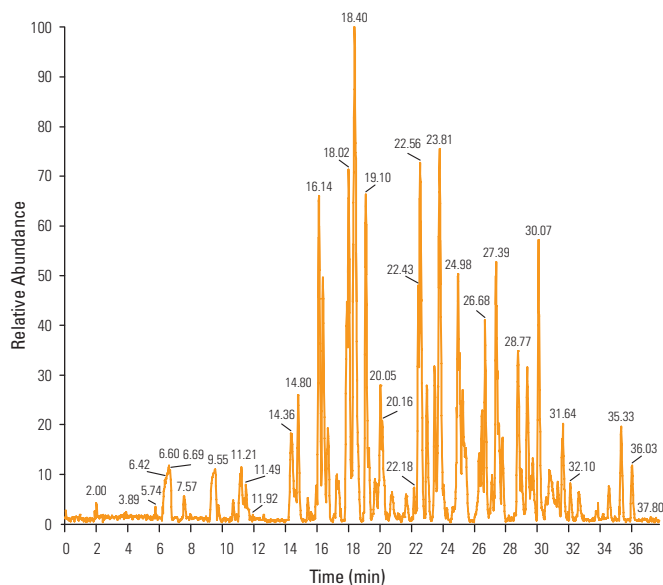
Backpressure: 204 bar

Injection: 0.25  $\mu$ L of 2 pmol/ $\mu$ L solution

Detection: UV at 214 nm

Peak Number	Retention Time (min)	Protein	kDa
1	6.89	Cytochrome C	12
2	7.34	Insulin	6
3	8.77	Myoglobin	18
4	9.57	Carbonic anhydrase	30
5	11.02	Ovalbumin	45
*		Carbonic anhydrase impurity	

Base peak 50 fmol loading of BSA digest



Column: Accucore 150-C18, 2.6  $\mu$ m, 150mm x 75  $\mu$ m

Mobile phase A: 0.1 % formic acid in water

Mobile phase B: 90 % acetonitrile in water

Gradient: 4–40 % B in 30 minutes  
40–95 % B in 2 minutes  
95 % B for 2 minutes

Flow: 300 nL/min

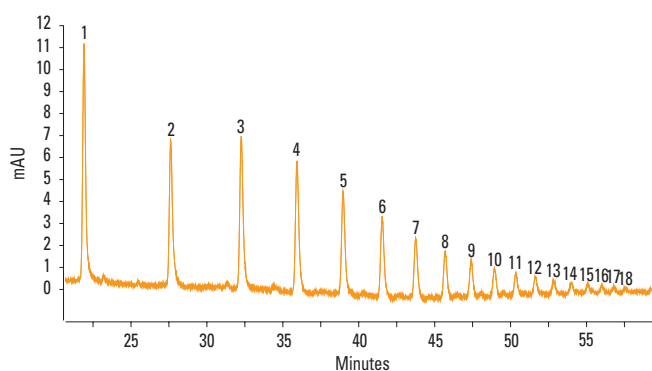
Temperature: Ambient

Backpressure: 198 bar (100 % A)

Injection: direct on-column loading of 1  $\mu$ L of BSA digest,  
50 fmol/ $\mu$ L in water + 0.1% formic acid

Detection: Thermo Scientific LTQ Orbitrap™ XL Mass  
Spectrometer coupled with a Proxeon  
Nano Spray Flex Ion Source

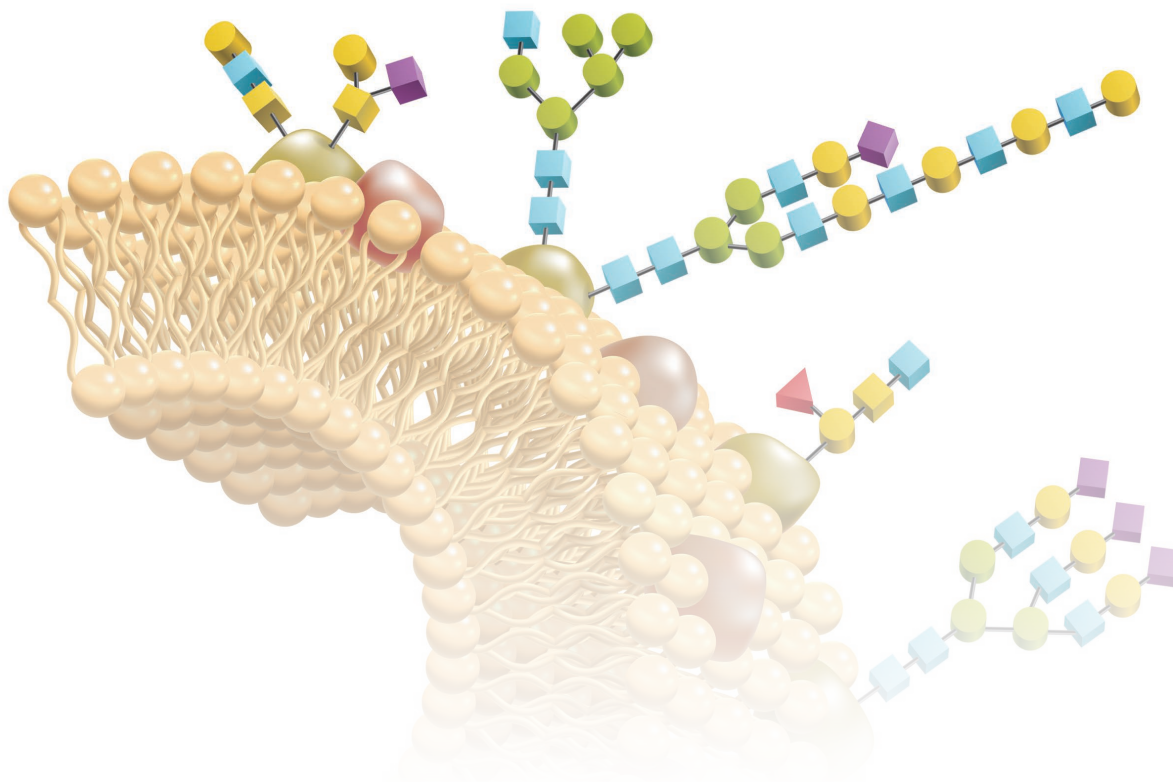
## 2-AB labelled dextran ladder



Column:	Accucore 150-Amide-HILIC 2.6 $\mu\text{m}$ , 150 mm x 75 $\mu\text{m}$
Mobile phase A:	98:2 (v/v) acetonitrile: water
Mobile phase B:	2:98 (v/v) acetonitrile: water
Gradient:	0–50 %B in 50 minutes 50 % B for 8 minutes
Flow:	200 nL/min
Temperature:	40 °C
Backpressure:	60 bar (100% A)
Sample Pick-up:	0.5 $\mu\text{L}$ at 20 $\mu\text{L}/\text{min}$
Sample Loading:	1 $\mu\text{L}$ at 280 bar
Detection:	UV (240 and 330 nm)

The separation is achieved using simple aqueous HILIC gradient with no pH adjustment.

At least 18 homopolymers were clearly identified. Excellent resolution factors were found, with average  $R_s$  values of 10.22 for the first 5 peaks, 4.30 for peaks 6-10 and 2.91 for peaks 10-18.



# Accucore HPLC Column Formats

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

Format	Column ID	Optimum Flow Rate	Optimum Injection Volume	Backpressure Rating	Temperature Rating
Nano	75 µm	300 nL/min	1 µL*	800 bar	70 °C
Narrowbore	2.1 mm	400 µL/min	1 µL	1000 bar	70 °C
Analytical	3.0 mm	800 µL/min	3 µL	1000 bar	70 °C
Analytical	4.6 mm	1800 µL/min	5 µL	1000 bar	70 °C

\* with trap column

## Analytical and Narrowbore 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.



## Thermo Scientific 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.

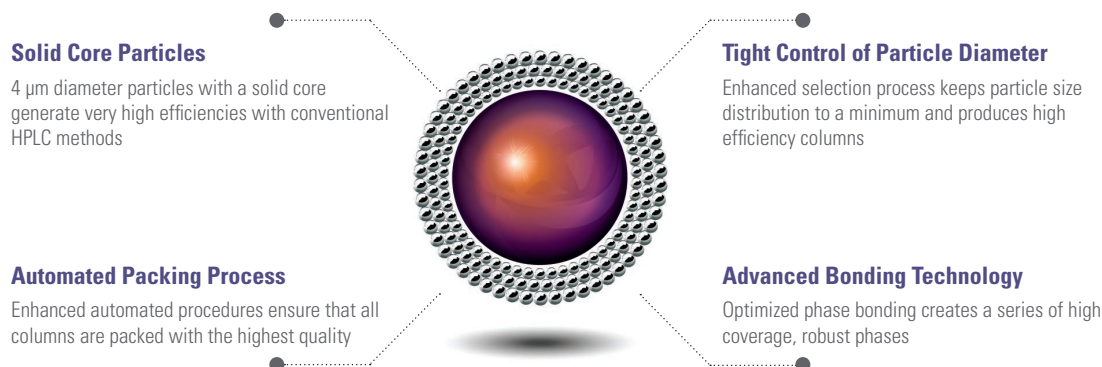
Accucore nanoViper columns are available in 150 and 500 mm lengths for ultra-high peak capacity.



## Accucore XL HPLC Columns

Based on Core Enhanced Technology using 4  $\mu\text{m}$  solid core particles, Accucore XL HPLC columns allow users of conventional HPLC methods to enjoy performance far beyond that of columns packed with 5  $\mu\text{m}$ , 4  $\mu\text{m}$  or even 3  $\mu\text{m}$  fully porous particles. Very high separation efficiencies using standard HPLC instruments and conditions provide increased peak resolution and lower limits of detection. An ultra-stable packed bed results in exceptionally robust columns that demonstrate excellent retention and response reproducibility.

The key components of Core Enhanced Technology are:



Features and benefits of Accucore XL HPLC columns:

- **Compatible with conventional HPLC methods**
- **High resolution**
- **Sharp, tall peak shape**
- **Reproducible chromatography**
- **Long column lifetime**
- **No need to change methods or invest in new equipment**
- **Separate difficult to resolve peaks**
- **Lower limits of detection – detect trace levels of analytes**
- **Confidence in your results**
- **Use columns for longer**

## Accucore XL HPLC columns

Ultimate core performance for conventional HPLC Methods

## Adjusting Conventional HPLC Methods

For users of conventional HPLC methods working in regulated environments there may be regulatory issues to consider when changing columns in order to realise the improvements offered by newer technologies. For example USP (United States Pharmacopeia) General Chapter <621> Chromatography-System Suitability describes the maximum adjustments that can be made to an analysis so that a method still fulfils the requirements of the system suitability test.

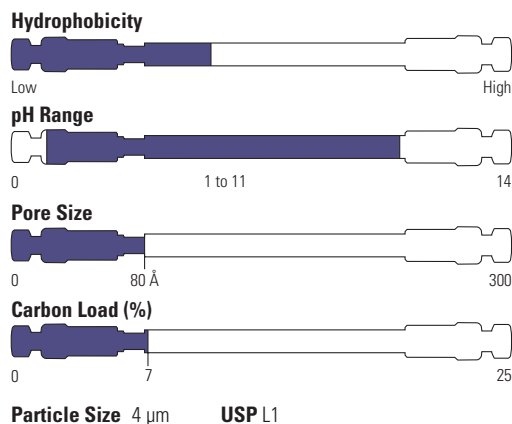
Column Parameter	Allowed Change
Column length	± 70%
Column internal diameter	± 25%
Particle size	Reduction of up to 50%; no increase

Method Parameter	Allowed Change
Flow rate	± 50%
Injection volume	System suitability testing (SST) criteria must be met
Column temperature	± 10%
Mobile phase pH	± 0.2
UV wavelength	No changes outside manufacturer specifications
Concentration of salts in buffer	± 10%
Composition of mobile phase	Minor component adjustment ± 30% or ± 10% absolute, whichever is smaller

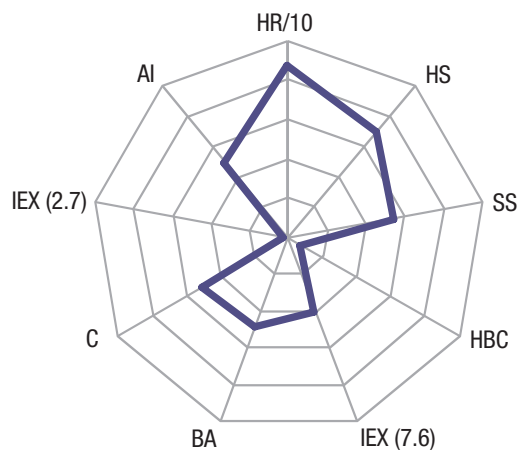
Transferring a method from a column packed with a 5 µm fully porous material to an Accucore XL 4 µm HPLC column requires no changes to method parameters and involves only a 20% reduction in particle size—thus meeting the above requirements.



## Accucore XL C18

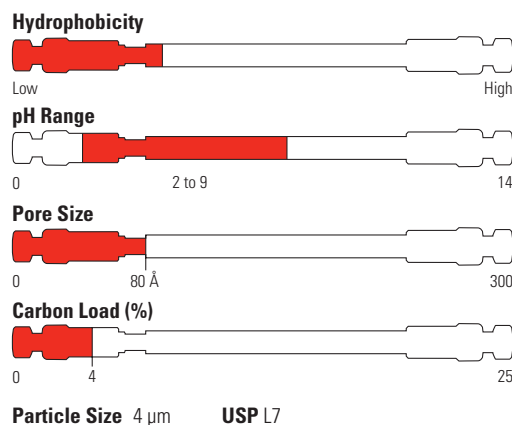


- Optimum retention of non-polar compounds
- Hydrophobic interaction mechanism
- Separates a broad range of analytes

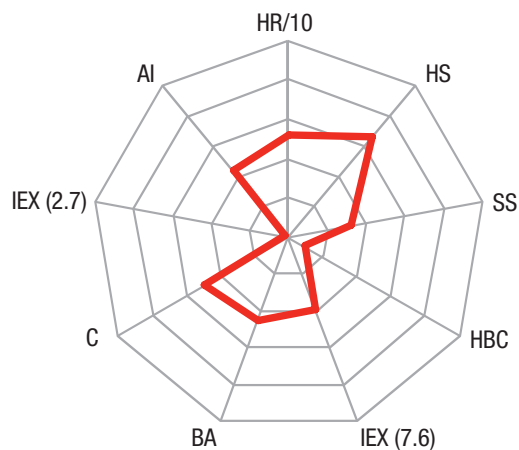


The carbon loading of Accucore XL C18 provides high retention of non-polar analytes via a predominantly hydrophobic interaction mechanism. The highly retentive nature of the phase means that it can be used to separate a broad range of analytes.

## Accucore XL C8



- Similar selectivity to C18 with lower retention
- Recommended for analytes with moderate hydrophobicity



Accucore XL C8 offers lower hydrophobic retention than columns packed with longer alkyl chain length material, such as C18. It is then therefore recommended for analytes with moderate hydrophobicity, or when a less hydrophobic phase provides optimum retention.

## Column Formats

Accucore XL HPLC columns are offered in analytical and micro formats. Optimum conditions and ratings are shown in the table below.

Column ID	Optimum Flow Rate	Optimum Injection Volume	Backpressure Rating	Temperature Rating
2.1 mm	0.3 mL/min	2 µL	600 bar	70 °C
3.0 mm	0.6 mL/min	5 µL	600 bar	70 °C
4.6 mm	1.3 mL/min	10 µL	600 bar	70 °C

### Analytical and Narrowbore 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 600 bar.



### Guard Cartridges

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



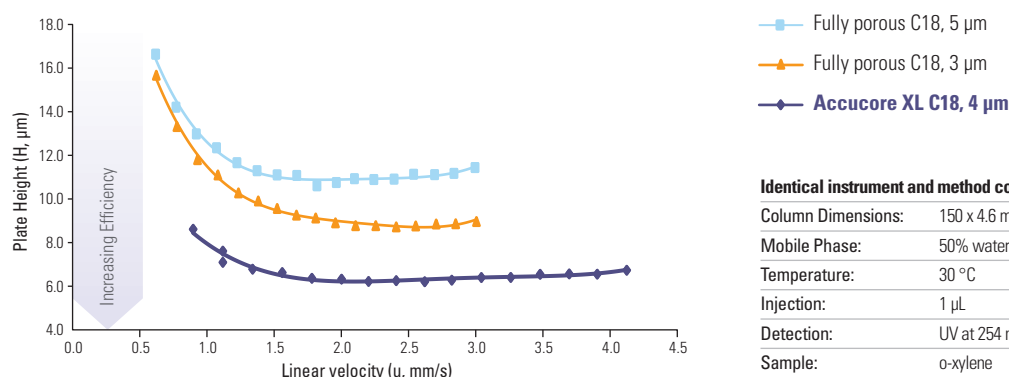
# 4 $\mu\text{m}$ Solid Core Particles for all Users

The 4  $\mu\text{m}$  solid core particles used in Accucore XL HPLC columns have been specifically designed to get the optimum chromatographic performance from conventional HPLC instruments.

- Very high efficiencies
- Little decrease in efficiency as flow rate is increased
- Moderate backpressures

## Efficiency

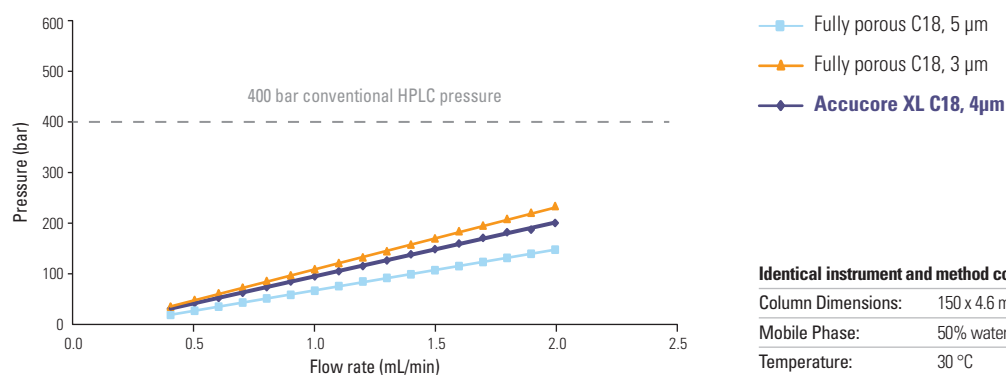
Accucore XL HPLC columns generate higher efficiencies than columns packed with 5  $\mu\text{m}$  and 3  $\mu\text{m}$  fully porous material—as shown in the van Deemter curve below.



- 75% higher efficiency than 5  $\mu\text{m}$  fully porous
- 50% higher efficiency than 3  $\mu\text{m}$  fully porous

## Backpressure

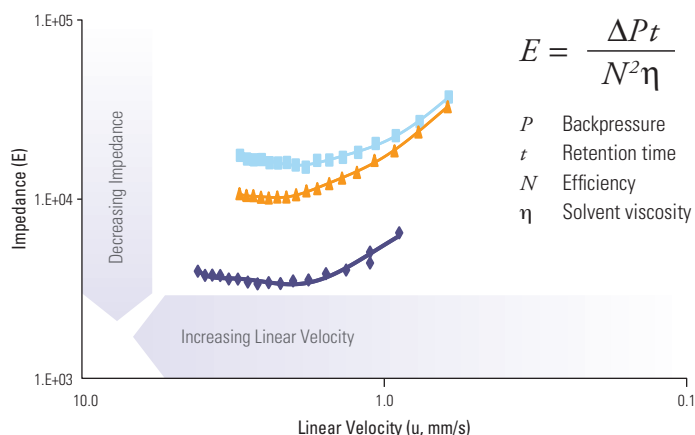
Accucore XL HPLC columns generate reasonable backpressures, moderately higher than fully porous 5  $\mu\text{m}$  and lower than fully porous 3  $\mu\text{m}$ , that are compatible with conventional HPLC instruments.



- Backpressures between those generated by 3  $\mu\text{m}$  and 5  $\mu\text{m}$  fully porous
- Within conventional HPLC instrumentation pressure limit—even at high flow rates

## Impedance

Impedance (E) combines retention time, efficiency and backpressure in a single term. Lower impedance values indicate fast and higher efficiency separations performed at lower backpressures.



- Fully porous C18, 5 µm
- ▲— Fully porous C18, 3 µm
- ◆— **Accucore XL C18, 4 µm**

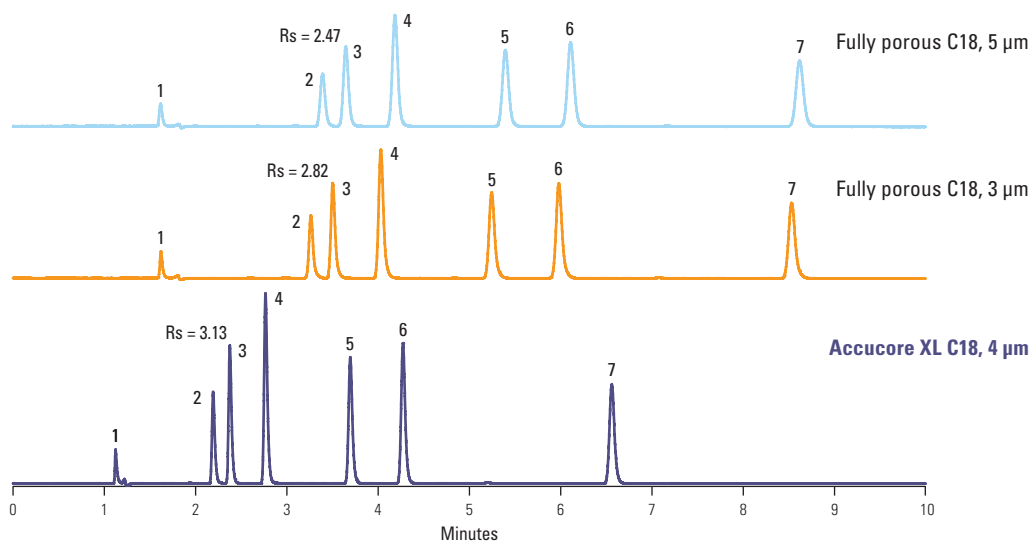
### Identical instrument and method conditions for all columns

Column Dimensions:	150 x 4.6 mm
Mobile Phase:	50% water : 50% acetonitrile
Temperature:	30 °C
Injection:	1 µL
Detection:	UV at 254 nm (0.1 s rise time, 20 Hz)
Sample:	o-xylene

- **78% lower impedance than 5 µm fully porous**
- **67% lower impedance than 3 µm fully porous**

## Resolution

The high chromatographic efficiencies offered by Accucore XL HPLC columns represent tall, narrow peaks. This provides significant advantages in terms of better peak separations (resolution) and lower limits of detection.



- **27% higher resolution than 5 µm fully porous**
- **11% higher resolution than 3 µm fully porous**

### Identical instrument and method conditions for all columns

Column Dimensions:	150 x 4.6 mm
Mobile Phase A:	water
Mobile Phase B:	acetonitrile
Gradient:	35–60% B in 10 minutes
Flow:	1.0 mL/min
Temperature:	30 °C
Injection:	5 µL
Detection:	UV at 247 nm (0.1 s rise time, 20 Hz)
Analytes:	1. Uracil ( $t_r$ ) 2. Tebuthiuron 3. Metoxuron 4. Monuron 5. Chlorotoluron 6. Diuron 7. Linuron

## Sensitivity

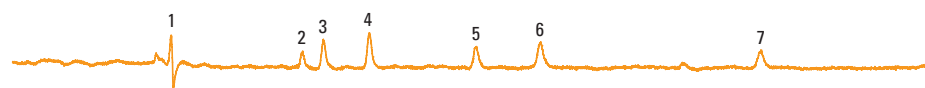
$\overline{S/N} = 4.86$

Fully porous C18, 5  $\mu\text{m}$



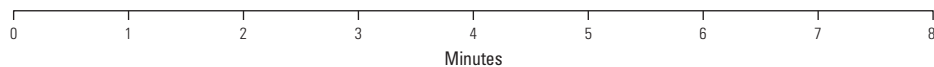
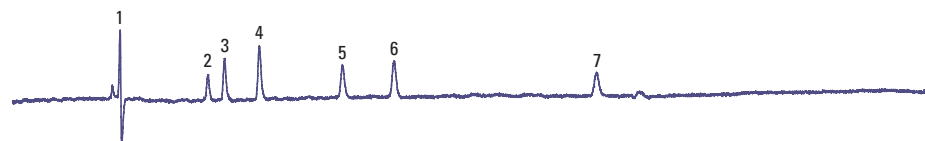
$\overline{S/N} = 5.31$

Fully porous C18, 3  $\mu\text{m}$



$\overline{S/N} = 10.61$

Accucore XL C18, 4  $\mu\text{m}$



Column	Amount on Column	Average S/N	Limit of Detection (based on $S/N = 3$ )
Fully porous C18, 5 $\mu\text{m}$	1 ng	4.86	0.62 ng
Fully porous C18, 3 $\mu\text{m}$	1 ng	5.31	0.56 ng
Accucore XL C18, 4 $\mu\text{m}$	1 ng	10.61	0.28 ng

### Identical instrument and method conditions for all columns

Column Dimensions:	150 x 4.6 mm
Mobile Phase A:	water
Mobile Phase B:	acetonitrile
Gradient:	35–60% B in 7.5 minutes
Flow:	1.3 mL/min
Temperature:	30 °C
Injection:	1 $\mu\text{L}$
Detection:	UV at 247 nm (0.1 s rise time, 20 Hz)

Analytes:

1. Uracil ( $t_r$ )
2. Tebuthiuron
3. Metoxuron
4. Monuron
5. Chlorotoluron
6. Diuron
7. Linuron (each at 1 ng/ $\mu\text{L}$ )

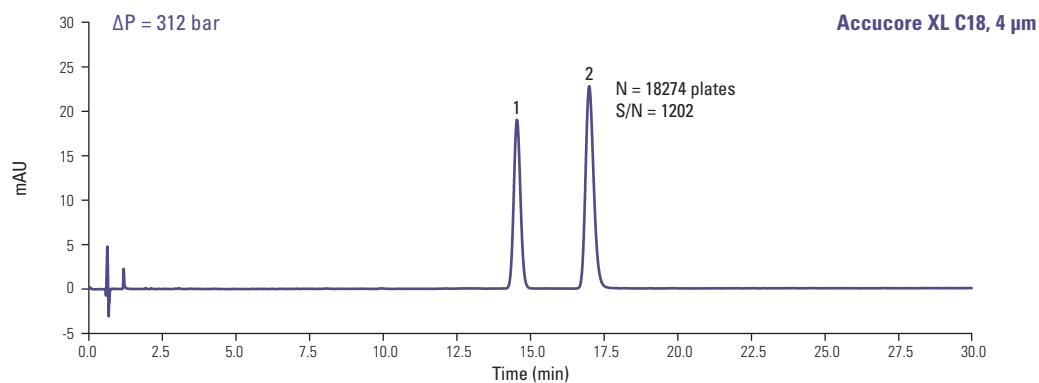
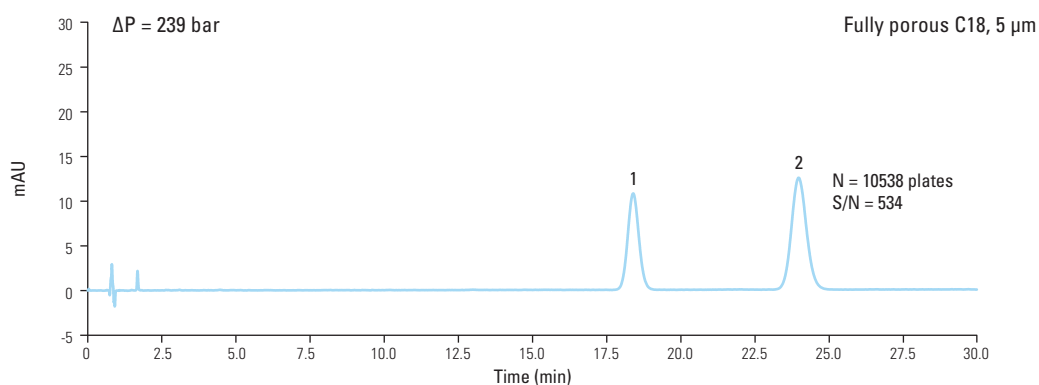
- 120% more sensitive than 5  $\mu\text{m}$  fully porous
- 100% more sensitive than 3  $\mu\text{m}$  fully porous



# Same System, Same Method, Better Results

The following applications show the improvements in performance that Accucore XL HPLC columns offer without any changes in instrument configuration or method conditions.

## Ibuprofen and Valerophenone (USP)



### Analytes

1. Valerophenone
2. Ibuprofen

Columns: Accucore XL C18 4  $\mu m$ , 150 x 4.6 mm  
Fully porous C18 5  $\mu m$ , 150 x 4.6 mm

Mobile phase: 66.3:33.7 (v/v) water with phosphoric acid, pH 2.5:methanol

Flow: 2 mL/min

Temperature: 30 °C

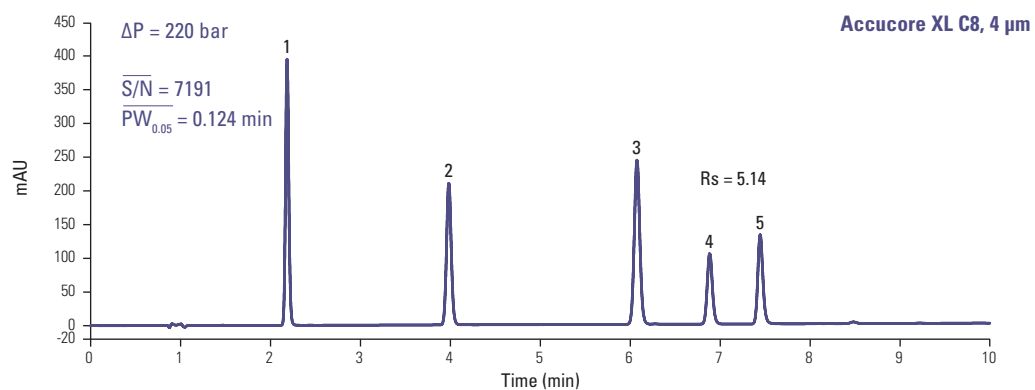
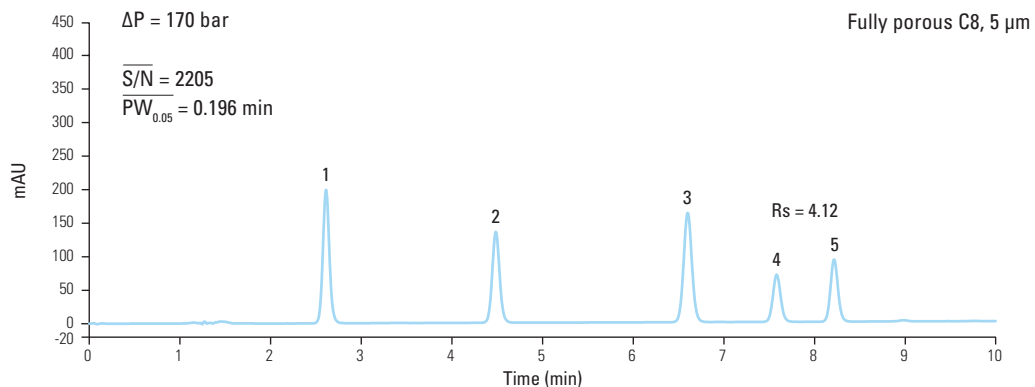
Injection: 5  $\mu L$

Detection: UV at 214 nm



- 73% higher efficiency
- 125% higher sensitivity

# Endocrine Disruptors



## Analytes

1. Desethyl Atrazine
2. Simazine
3. Atrazine
4. Diuron
5. Bisphenol A

Columns: Accucore XL C8 4  $\mu m$ , 150 x 4.6 mm  
 Fully porous C8 5  $\mu m$ , 150 x 4.6 mm

Mobile phase A: water

Mobile phase B: acetonitrile

Gradient: 25–70 % B in 20.0 minutes  
 70–75 % B in 0.1 minutes  
 75–25 % B in 4.9 minutes

Flow: 1.5 mL/min

Temperature: 25 °C

Injection volume: 5  $\mu L$

Detection: UV at 220 nm



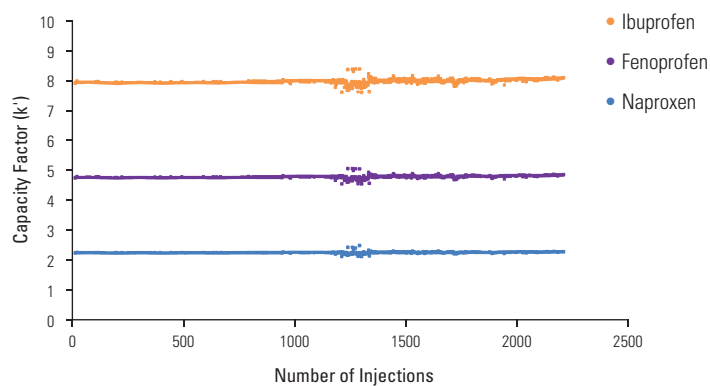
- 31% better resolution of critical pair
- 37% narrower peaks
- 226% higher sensitivity

# Robust, Fast and Easy to Use

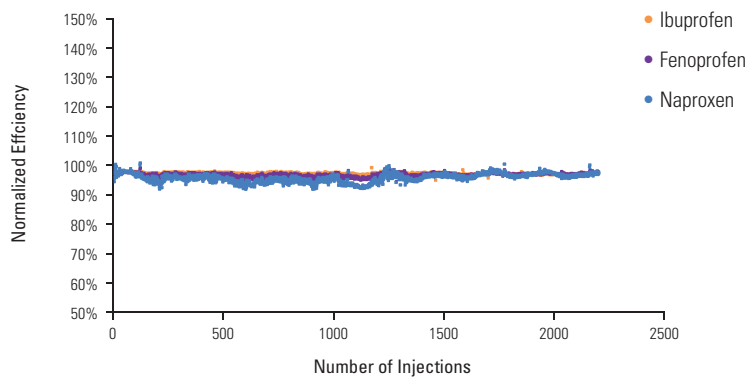
## Robustness

Accucore XL HPLC columns are extremely robust offering excellent performance over extended use.

### Stability–Retention



### Stability–Efficiency



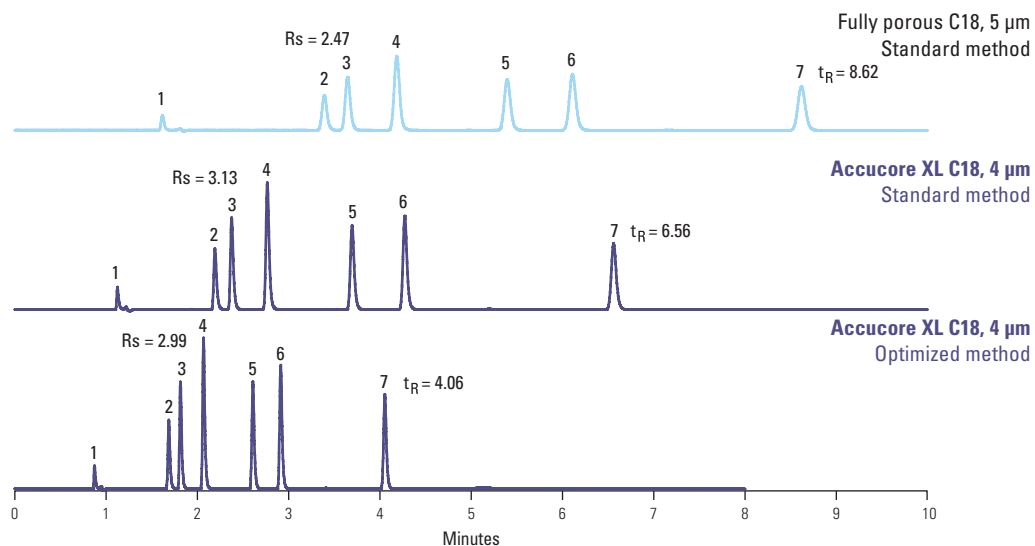
Column:	Accucore XL C8 4 $\mu$ m, 50 x 2.1 mm
Mobile Phase:	40:60 acetonitrile:20 mM ammonium formate pH3
Flow:	0.3 mL/min
Temperature:	30 °C
Injection:	2 $\mu$ L
Detection:	UV at 233 nm
Analytes:	Non-Steroidal Anti Inflammatory Drugs (NSAIDs) ibuprofen, fenoprofen, naproxen



*Stable retention and efficiency over thousands of injections.*

## Productivity

In addition to using established conventional methods, the high efficiencies offered by Accucore XL HPLC columns, across a wide range of flow rates, allow methods to be optimized to reduce run times and increase productivity.



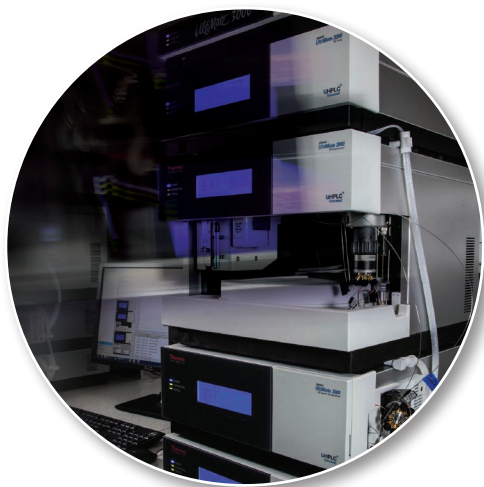
Column	Method	$t_R$ of last peak	Reduction in time
Fully porous C18, 5 $\mu$ m	Standard	8.62 min	0%
Accucore XL C18, 4 $\mu$ m	Standard	6.56 min	24%
Accucore XL C18, 4 $\mu$ m	Optimized	4.06 min	53%

Column Dimensions: 150 x 4.6 mm ID

Mobile Phase A: water

Mobile Phase B: acetonitrile

	Standard Method	Optimized Method
Gradient:	35–60% B in 10 minutes	35–60% B in 4 minutes
Flow:	1.0 mL/min	1.3 mL/min
Temperature:	30 °C	
Injection:	5 $\mu$ L	
Detection:	UV at 247 nm (0.1 s rise time, 20 Hz)	
Analytes:	1. Uracil ( $t_R$ ) 2. Tebuthiuron 3. Metoxuron 4. Monuron 5. Chlorotoluron 6. Diuron 7. Linuron	



*Run time reduced by over a third with an improvement in performance.*

# Ordering Information

## Accucore HPLC Columns

Description	Particle Size	Length (mm)	2.1 mm ID	3.0 mm ID	4.6 mm ID
<b>Accucore C18</b>	<b>2.6 µm</b>	30	17126-032130	17126-033030	17126-034630
		50	17126-052130	17126-053030	17126-054630
		100	17126-102130	17126-103030	17126-104630
		150	17126-152130	17126-153030	17126-154630
<b>Accucore RP-MS</b>	<b>2.6 µm</b>	30	17626-032130	17626-033030	17626-034630
		50	17626-052130	17626-053030	17626-054630
		100	17626-102130	17626-103030	17626-104630
		150	17626-152130	17626-153030	17626-154630
<b>Accucore C8</b>	<b>2.6 µm</b>	30	17226-032130	17226-033030	17226-034630
		50	17226-052130	17226-053030	17226-054630
		100	17226-102130	17226-103030	17226-104630
		150	17226-152130	17226-153030	17226-154630
<b>Accucore aQ</b>	<b>2.6 µm</b>	30	17326-032130	17326-033030	17326-034630
		50	17326-052130	17326-053030	17326-054630
		100	17326-102130	17326-103030	17326-104630
		150	17326-152130	17326-153030	17326-154630
<b>Accucore Polar Premium</b>	<b>2.6 µm</b>	50	28026-052130	28026-053030	28026-054630
		100	28026-102130	28026-103030	28026-104630
		150	28026-152130	28026-153030	28026-154630
		250	28026-252130	—	—
<b>Accucore Phenyl-Hexyl</b>	<b>2.6 µm</b>	30	17926-032130	17926-033030	17926-034630
		50	17926-052130	17926-053030	17926-054630
		100	17926-102130	17926-103030	17926-104630
		150	17926-152130	17926-153030	17926-154630
<b>Accucore PFP</b>	<b>2.6 µm</b>	30	17426-032130	17426-033030	17426-034630
		50	17426-052130	17426-053030	17426-054630
		100	17426-102130	17426-103030	17426-104630
		150	17426-152130	17426-153030	17426-154630
<b>Accucore Phenyl-X</b>	<b>2.6 µm</b>	50	27926-052130	27926-053030	27926-054630
		100	27926-102130	27926-103030	27926-104630
		150	27926-152130	27926-153030	27926-154630
		250	27926-252130	—	—
<b>Accucore C30</b>	<b>2.6 µm</b>	50	27826-052130	27826-053030	27826-054630
		100	27826-102130	27826-103030	27826-104630
		150	27826-152130	27826-153030	27826-154630
		250	27826-252130	—	—
<b>Accucore HILIC</b>	<b>2.6 µm</b>	30	17526-032130	17526-033030	17526-034630
		50	17526-052130	17526-053030	17526-054630
		100	17526-102130	17526-103030	17526-104630
		150	17526-152130	17526-153030	17526-154630
<b>Accucore Urea-HILIC</b>	<b>2.6 µm</b>	50	27726-052130	27726-053030	27726-054630
		100	27726-102130	27726-103030	27726-104630
		150	27726-152130	27726-153030	27726-154630
		250	27726-252130	—	—

## Accucore Defender Guard Cartridges (4/pk)

Description	Particle Size	Length (mm)	2.1 mm ID	3.0 mm ID	4.6 mm ID
<b>Accucore C18</b>	<b>2.6 µm</b>	10	17126-012105	17126-013005	17126-014005
<b>Accucore RP-MS</b>	<b>2.6 µm</b>	10	17626-012105	17626-013005	17626-014005
<b>Accucore C8</b>	<b>2.6 µm</b>	10	17226-012105	17226-013005	17226-014005
<b>Accucore aQ</b>	<b>2.6 µm</b>	10	17326-012105	17326-013005	17326-014005
<b>Accucore Polar Premium</b>	<b>2.6 µm</b>	10	28026-012105	—	—
<b>Accucore Phenyl-Hexyl</b>	<b>2.6 µm</b>	10	17926-012105	17926-013005	17926-014005
<b>Accucore PFP</b>	<b>2.6 µm</b>	10	17426-012105	17426-013005	17426-014005
<b>Accucore Phenyl-X</b>	<b>2.6 µm</b>	10	27926-012105	—	—
<b>Accucore C30</b>	<b>2.6 µm</b>	10	27826-012105	—	—
<b>Accucore HILIC</b>	<b>2.6 µm</b>	10	17526-012105	17526-013005	17526-014005
<b>Accucore Urea-HILIC</b>	<b>2.6 µm</b>	10	27726-012105	—	—

## Accucore HPLC Columns for Biomolecules

Description	Particle Size	Length (mm)	2.1 mm ID	3.0 mm ID	4.6 mm ID
<b>Accucore 150-C18</b>	<b>2.6 µm</b>	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
<b>Accucore 150-C4</b>	<b>2.6 µm</b>	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
<b>Accucore 150-Amide-HILIC 2.6 µm</b>	<b>2.6 µm</b>	50	16726-052130	16726-053030	16726-054630
		100	16726-102130	16726-103030	16726-104630
		150	16726-152130	16726-153030	16726-154630
		250	16726-252130	—	—

## Accucore nanoViper Columns

Description	Particle Size	Length (mm)	75 µm ID
<b>Accucore 150-C18</b>	<b>2.6 µm</b>	150	16126-157569
		500	16126-507569
<b>Accucore 150-C4</b>	<b>2.6 µm</b>	150	16526-157569
		500	16526-507569
<b>Accucore 150-Amide-HILIC 2.6 µm</b>	<b>2.6 µm</b>	150	16726-157569

## Accucore for Biomolecules Defender Guard Cartridges (4/pk)

Description	Particle Size	Length (mm)	2.1 mm ID	3.0 mm ID	4.6 mm ID
<b>Accucore 150-C18</b>	<b>2.6 µm</b>	10	16126-012105	16126-013005	16126-014005
<b>Accucore 150-C4</b>	<b>2.6 µm</b>	10	16526-012105	16526-013005	16526-014005
<b>Accucore 150-Amide-HILIC 2.6 µm</b>	<b>2.6 µm</b>	10	16726-012105	—	—

## Accucore XL HPLC Columns

Description	Particle Size	Length (mm)	2.1 mm ID	3.0 mm ID	4.6 mm ID
<b>Accucore XL C18</b>	<b>4 µm</b>	50	74104-052130	74104-053030	74104-054630
		100	74104-102130	74104-103030	74104-104630
		150	74104-152130	74104-153030	74104-154630
		250	74104-252130	74104-253030	74104-254630
<b>Accucore XL C8</b>	<b>4 µm</b>	50	74204-052130	74204-053030	74204-054630
		100	74204-102130	74204-103030	74204-104630
		150	74204-152130	74204-153030	74204-154630
		250	74204-252130	74204-253030	74204-254630

## Accucore XL Guard Cartridges (4/pk)

Description	Particle Size	Length (mm)	2.1 mm ID	3.0 mm ID	4.6 mm ID
<b>Accucore XL C18</b>	<b>4 µm</b>	10	74104-012101	74104-013001	74104-014001
<b>Accucore XL C8</b>	<b>4 µm</b>	10	74204-012101	74204-013001	74204-014001

## UNIGUARD Direct-Connection Guard Cartridge Holders

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



# Accucore Kits

For validation of the performance of Accucore HPLC columns or verification of the optimum selectivity for user's separations.

## Accucore Validation Kit

Validate the reproducibility of Accucore. Contains 3 Accucore C18 HPLC columns.

Description	Particle Size	Length (mm)	2.1 mm ID
Accucore Validation Kit	2.6 µm	50	17126-052130-3V
		100	17126-102130-3V
		150	17126-152130-3V

## Accucore Narrow Selectivity Kit

Verify optimum selectivity over a narrow range. Contains 1 each of Accucore C18, RP-MS and aQ HPLC columns.

Description	Particle Size	Length (mm)	2.1 mm ID
Accucore Narrow Selectivity Kit	2.6 µm	50	17X26-052130-3VA
		100	17X26-102130-3VA
		150	17X26-152130-3VA

## Accucore Wide Selectivity Kit

Verify selectivity over a wide range. Contains 1 each of Accucore C18, Phenyl-Hexyl and PFP columns.

Description	Particle Size	Length (mm)	2.1 mm ID
Accucore Wide Selectivity Kit	2.6 µm	50	17X26-052130-3VB
		100	17X26-102130-3VB
		150	17X26-152130-3VB

## Accucore Polar Selectivity Kit

Verify selectivity for polar analytes. Contains 1 each of Accucore aQ, PFP and HILIC HPLC columns.

Description	Particle Size	Length (mm)	2.1 mm ID
Accucore Polar Selectivity Kit	2.6 µm	50	17X26-052130-3VC
		100	17X26-102130-3VC
		150	17X26-152130-3VC

# 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.

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

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