Thermo Scientific
Accucore HPLC Columns
Technical Guide

Ultimate Core Performance
to Maximize Your Investment

Thermo
SCIENTIFIC

Core Enhanced Technology

To meet modern requirements for outstanding performance free from constraints on the type of instruments that can be used we have developed Accucore™ HPLC columns using an exciting new concept.

The key components of Core Enhanced Technology

Solid Core Particles

2.6μm diameter particles with a solid core generate high speed, high resolution separations without excessive backpressure
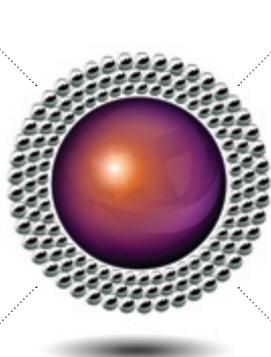
Tight Control of Particle Diameter

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

	Solid Core Particles	Tight Control of Particle Diameter	Advanced Bonding Technology	Automated Packing Process
Fast Separations	•	•	•	•
High Peak Capacity	•	•	•	•
Increased Sensitivity	•	•	•	•
Lower Pressure	•	•		•
Loading Capacity		•	•	•
Reproducible Chromatography		•	•	•
Long Lifetime	•	•	•	•
Wide Selectivity			•	

The table above shows how the components of Core Enhanced Technology contribute to the outstanding features of Accucore HPLC columns.

Table of Contents

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 2μm.

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.

Large, irregular particles   5μm particles   3μm particles   Sub-2μm particles

Solid Core

Core Enhanced Technology particles
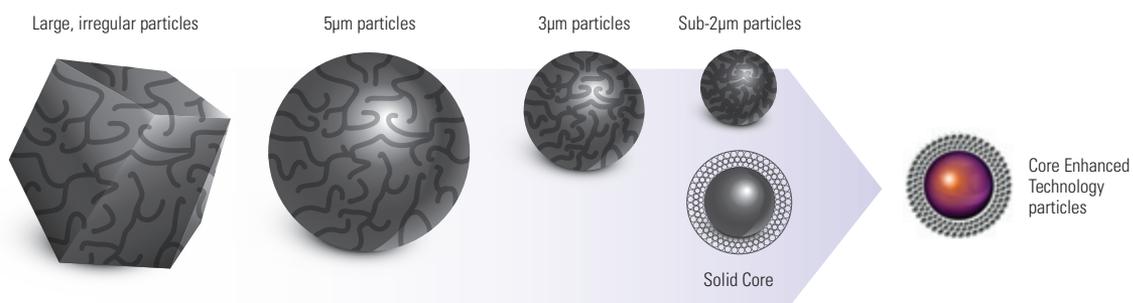
Core Enhanced Technology

Core enhanced technology offers the advantages associated with sub-2μm packing materials – fast, high-resolution separations – without the high backpressures required to work with the smaller particles.

Theory of Fast HPLC

The Van Deemter curves and kinetic plot below show the impact of packing material particle diameter and type on chromatographic efficiency.

The highest efficiencies and lowest rate of efficiency loss with increasing flow rate are seen with the smallest particle diameter and the Core Enhanced Technology.

— fully porous, 5μm
— fully porous, 3μm
— fully porous, 1.9μm
— Core Enhanced Technology 2.6μm
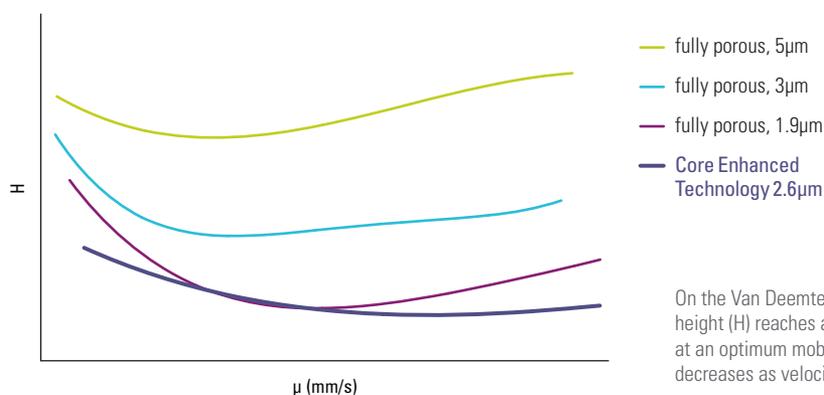
On the Van Deemter curve it can be seen that plate height (H) reaches a minimum (maximum efficiency) at an optimum mobile phase velocity and then decreases as velocity increases.

— totally porous, 5μm
— totally porous, 3μm
— totally porous, 1.9μm
— Core Enhanced Technology 2.6μm
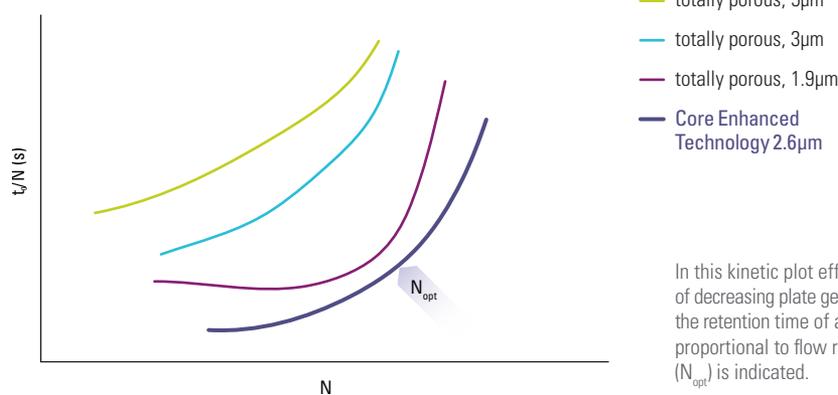
In this kinetic plot efficiency (N) decreases as function of decreasing plate generation time (t_0/N) – where t_0 is the retention time of an unretained peak and inversely proportional to flow rate. The optimal performance (N_{opt}) is indicated.

Resolution

The general chromatographic resolution equation shows that resolution is directly proportional to the square root of efficiency. This means that with small diameter and core enhanced technology relatively high flow rates can be used, whilst maintaining the high efficiencies required for good separations.
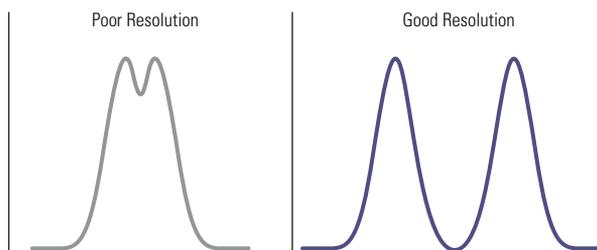
α Selectivity
N Efficiency
k' Capacity factor

$$R_s = \frac{1}{4} \frac{(\alpha - 1)}{\alpha} \sqrt{N} \frac{k'}{1 + k'}$$

Poor Resolution

Good Resolution

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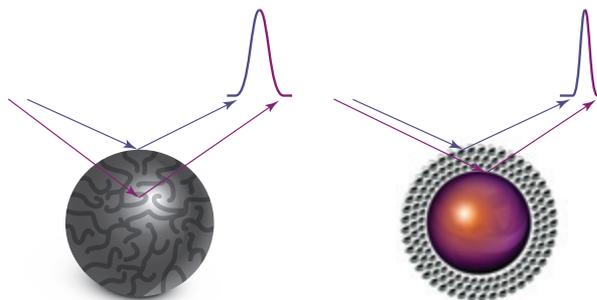
Resistance to mass transfer is minimized by the solid core design of Core Enhanced Technology particles as the diffusional path of analytes is limited by the depth of the outer porous layer.

Material	Accucore 2.6µm
Average Particle Size Distribution (D90/10)	1.12
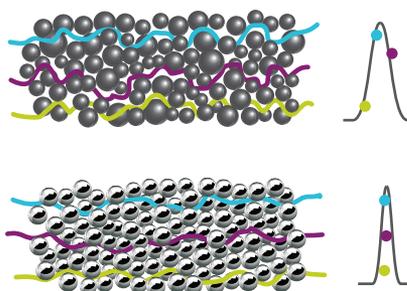
The tight control of Core Enhanced Technology particle diameter and automated packing process used for Accucore HPLC columns result in a tight, highly uniform packed bed that minimizes eddy diffusion.

Lower Backpressure

L Column length (cm)

η Mobile phase viscosity (cP)

F Flow rate (mL/min)

d_p^2 Particle diameter (µm)
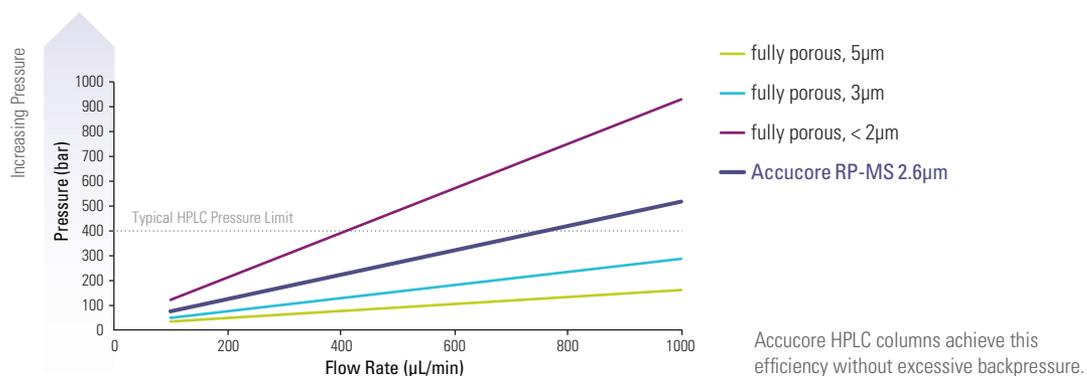
d_c^2 Column diameter (cm)

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

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

The tightly controlled 2.6µm diameter of Accucore particles also results in lower backpressures than typically seen with sub-2µm materials.

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 5μm, 3μm and 1.9μm particles.

— fully porous, 5μm
— fully porous, 3μm
— fully porous, < 2μm
— Accucore RP-MS 2.6μm

Accucore HPLC columns are more efficient than 5μm and 3μm columns.

— fully porous, 5μm
— fully porous, 3μm
— fully porous, < 2μm
— Accucore RP-MS 2.6μm

Accucore HPLC columns achieve this efficiency without excessive backpressure.
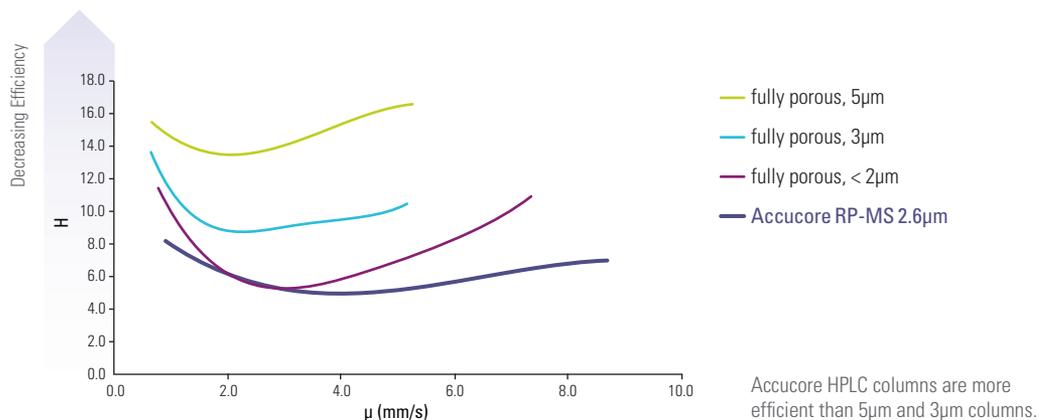
Faster than 5µm and 3µm

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!

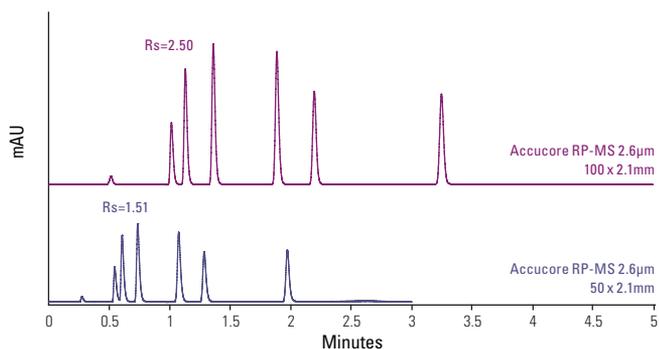|		
Mobile phase:	A – Water; B – Acetonitrile	
Gradient:	Accucore RP-MS 2.6µm 100 x 2.1mm = 35 – 60% B in 3.5 minutes fully porous 3µm 100 x 2.1mm = 35 – 60% B in 4.0 minutes fully porous 5µm 100 x 2.1mm = 35 – 60% B in 6.7 minutes fully porous 5µm 150 x 4.6mm = 35 – 60% B in 10.0 minutes	
Flow rate:	Accucore RP-MS 2.6µm 100 x 2.1mm = 400µL/min fully porous 3µm 100 x 2.1mm = 350µL/min fully porous 5µm 100 x 2.1mm = 210µ/min fully porous 5µm 150 x 4.6mm = 1000µL/min	
Injection volume:	1µL (fully porous 5µm 150 x 4.6mm = 5µL)	
Temperature:	30°C	
Detection:	UV at 247nm (0.1s rise time, 20Hz)	
Analytes:	1. Tebuthiuron 2. Metoxuron 3. Monuron 4. Chlorotoluron 5. Diuron 6. Linuron	

	Accucore RP-MS 2.6µm, 100 x 2.1mm	Fully Porous 3µm, 100 x 2.1mm	Fully Porous 5µm, 100 x 2.1mm	Fully Porous 5µm, 150 x 4.6mm
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

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; B – Acetonitrile
Gradient:	Accucore RP-MS 2.6µm 50 x 2.1mm = 35 – 60% B in 1.8 minutes Accucore RP-MS 2.6µm 100 x 2.1mm = 35 – 60% B in 3.5 minutes
Flow rate:	400µL/min

Analysis Time and Solvent Savings

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

A 50mm column gives acceptable separation with a doubling of productivity and halving of solvent costs.
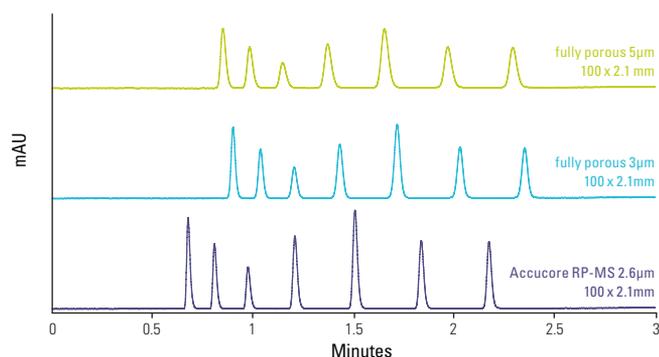
Higher Peak Capacity than 5µm or 3µm

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.

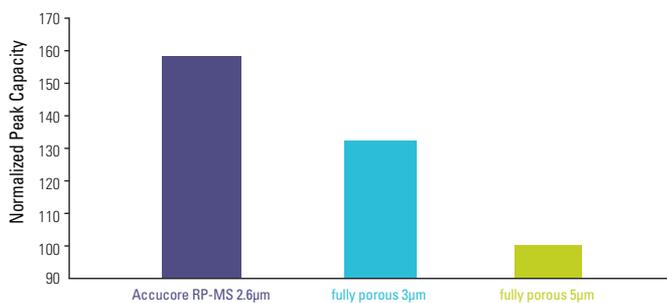n_c Peak capacity

t_g Gradient time

W Peak width (10% height)

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

Mobile phase:	A – Water; B – Acetonitrile
Gradient:	65 – 95% B in 2.1 minutes, 95% B for 0.4 minute
Flow rate:	400µL/min
Injection volume:	1µL
Temperature:	40°C
Detection:	UV at 247nm (0.1s rise time, 20Hz)
Analytes:	1. Acetophenone 2. Propiophenone 3. Butyrophenone 4. Valerophenone 5. Hexanophenone 6. Heptanophenone 7. Octanophenone

Peak Capacity Comparison

Accucore RP-MS 2.6µm	158
fully porous 3µm	132
fully porous 5µm	100

The higher the peak capacity the more analytes can be identified within a run.

More Sensitive than 5µm or 3µm

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.

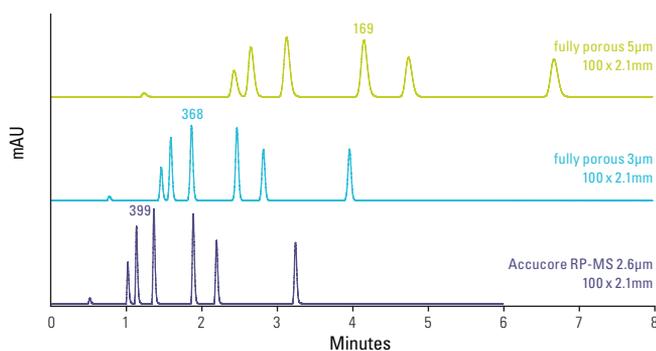c_{max} Concentration at peak apex

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; B – Acetonitrile
Gradient:	Accucore RP-MS 2.6µm 100 x 2.1mm = 35 – 60% B in 3.5 minutes fully porous 3µm 100 x 2.1mm = 35 – 60% B in 4.0 minutes fully porous 5µm 100 x 2.1mm = 35 – 60% B in 6.7 minutes
Flow rate:	Accucore RP-MS 2.6µm 100 x 2.1mm = 400µL/min fully porous 3µm 100 x 2.1mm = 350µL/min fully porous 5µm 100 x 2.1mm = 210µL/min
Injection volume:	1µL
Temperature:	30°C
Detection:	UV at 247nm (0.1s rise time, 20Hz)
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 2.6µm, 100 x 2.1mm	399	136%
fully porous 3µm, 100 x 2.1mm	368	117%
fully porous 5µm, 100 x 2.1mm	169	–

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

Equivalent Performance to Sub-2μm with Lower Pressure

With solid core design, tight particle size distribution and uniform packed bed Accucore HPLC columns have broadly equivalent performance to sub-2μm columns and yet generate only a fraction of the backpressure.
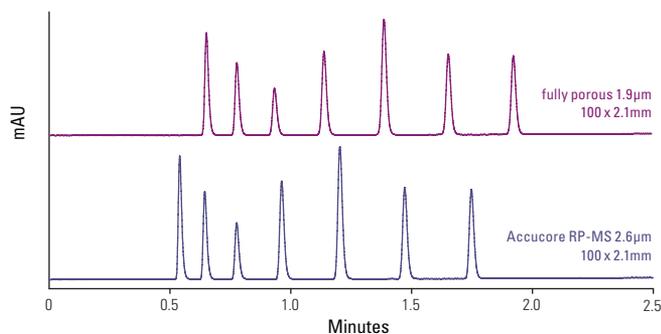
| Mobile phase:	A – Water; B – Acetonitrile
Gradient:	65 – 95% B in 1.7 minutes 95% B for 0.3 minute
Flow rate:	500μL/min
Injection volume:	1μL
Temperature:	40°C
Detection:	UV at 247nm (0.1s rise time, 20Hz)
Analytes:	1. Acetophenone 2. Propiophenone 3. Butyrophenone 4. Valerophenone 5. Hexanophenone 6. Heptanophenone 7. Octanophenone

Pressure

	Accucore RP-MS 2.6μm, 100 x 2.1mm	Fully Porous < 2μm, 100 x 2.1mm
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.

$R^2 = 0.9998$
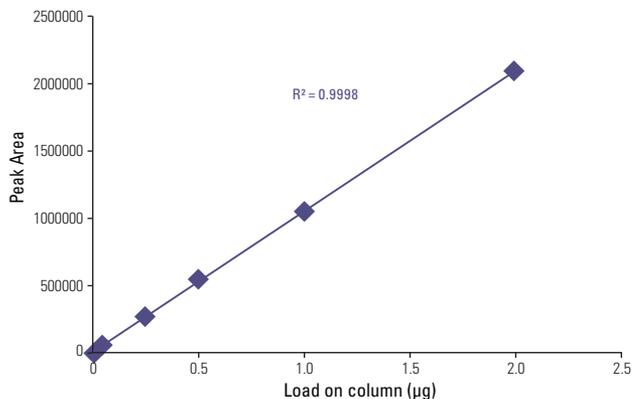
Column:	Accucore RP-MS 100 x 2.1mm
Mobile phase:	68:32 (v/v) Water/Methanol
Flow rate:	1.0mL/min
Temperature:	40° C
Detection:	254nm
Injection volume:	1µL

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

◆ Asymmetry
■ Efficiency
▲ Retention Time
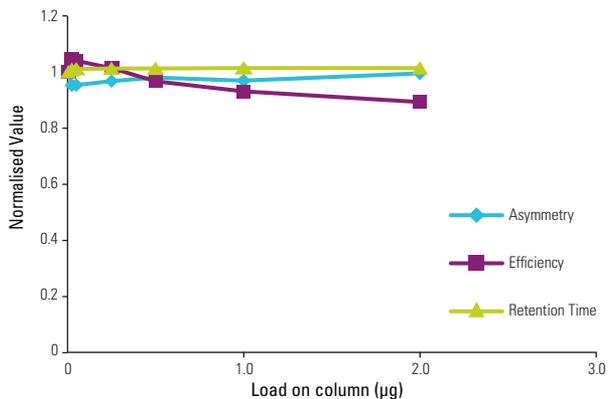
Simple Method Transfer

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
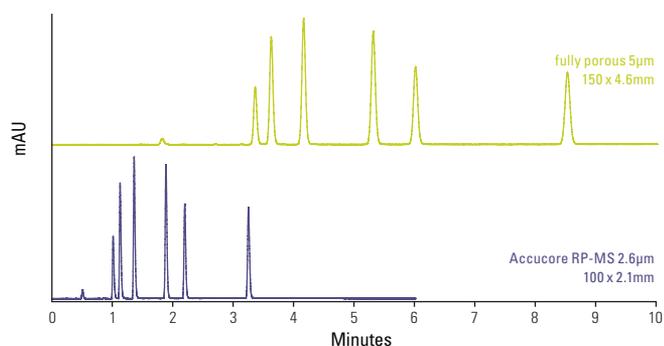www.thermoscientific.com/crc

- **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
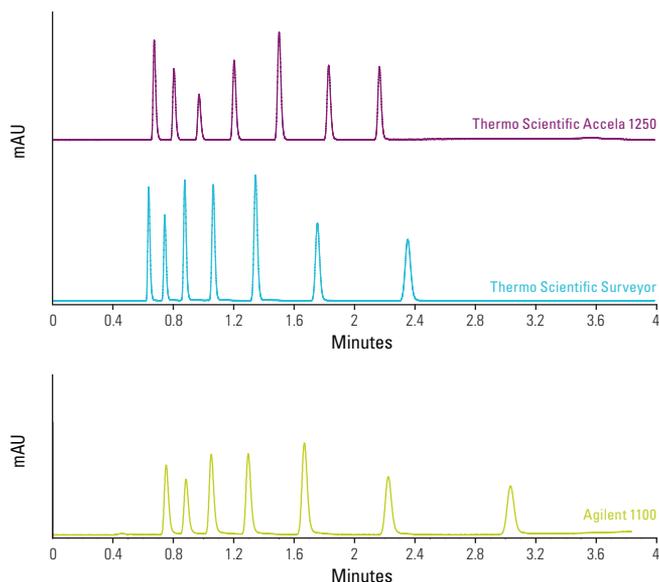
- **Adjust Gradient Profile**
  Keep the number of column volumes constant for each gradient segment

Mobile phase:	A – Water; B – Acetonitrile
Gradient:	Accucore RP-MS 2.6µm 100 x 2.1mm = 35 – 60% B in 3.5 minutes fully porous 5µm 150 x 4.6mm = 35 – 60% B in 10.0 minutes
Flow rate:	Accucore RP-MS 2.6µm 100 x 2.1mm = 400µL/min fully porous 5µm 150 x 4.6mm = 1000µL/min
Injection volume:	Accucore RP-MS 2.6µm 100 x 2.1mm = 1µL fully porous 5µm 150 x 4.6mm = 5µL
Temperature:	30°C
Detection:	UV at 247nm (0.1s rise time, 20Hz)
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 2.6µm, 100 x 2.1mm
Mobile phase:	A – Water; B – Acetonitrile
Gradient:	65 – 95% B in 2.1 minutes 95% B for 0.4 minute
Flow rate:	400µL/min
Injection volume:	1µL
Temperature:	40°C
Detection:	UV at 247nm (0.1s rise time, 20Hz)
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 14.

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

V_{inj} Injection volume

V_{cell} Flow cell volume

F Flow rate

r_c Tubing radius

l_c Tubing length

D_m Diffusion coefficient in mobile phase

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

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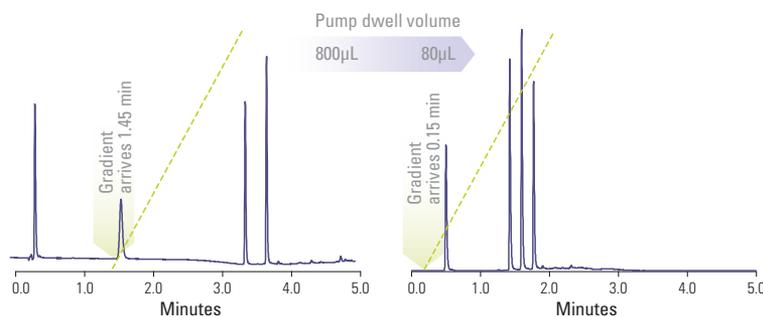
	Data point*	Peak width 4σ (s)	Peak Area	Peak height (mAu)
1Hz	2	2.04	246330	107.4
5Hz	6	0.96	57244	118.4
10Hz	10	0.87	55750	114.5
20Hz	18	0.87	55319	115.4

* Number of data points are collected over 4σ
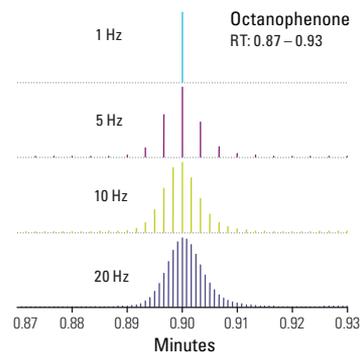
Fast Gradients

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 < 2µm, 50 x 2.1mm
Mobile phase:	A – Water + 0.1% Formic Acid B – Acetonitrile + 0.1% Formic Acid
Gradient:	5 to 100% B in 2 min
Flow rate:	0.55mL/min
Temperature:	25°C
Detection:	UV@ 270nm (2µL flow cell)
Tubing column – detector: 0.005" ID	
Injection volume: 0.5µL	
Analytes:	1. Sulphaguanidine 2. Sulphamerazine 3. Sulphamonomethoxine 4. Sulphaquinoxaline

Reproducible Chromatography

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

Packing Material Analysis

Every batch of Accucore packing material manufactured is analyzed, compared with specifications and issued with a certificate of analysis.

Material Properties and Analysis Based on Tanaka Tests

Silica Support Properties	Bonded Phase Properties	Hydrophobic Interactions	Secondary Interactions	Acidic Interactions	HILIC Interactions
Surface Area	Carbon load	Hydrophobic retention (HR)	Base activity (BA)	Acid interaction (AI)	HILIC retention & selectivity
Pore Size		Hydrophobic selectivity (HS)	Chelation (C)	Ion exchange capacity pH 2.6 (IEX(2.6))	
Particle Size		Steric selectivity (SS)	Ion exchange capacity (pH 7.6) (IEX(7.6))		
Particle Size Distribution		Hydrogen bonding capacity (HBC)			

Column Testing

Every Accucore HPLC column is also tested after packing and again compared to specifications and issued with a certificate.

Retention time	Capacity factor	Efficiency	Asymmetry	Backpressure

This material and column testing is evidence that every Accucore HPLC column will give reproducible chromatography, batch after batch, column after column.
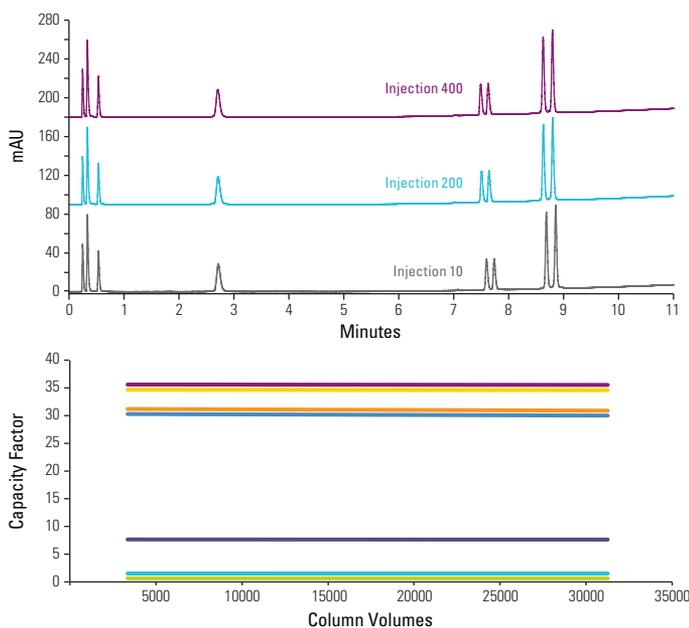
Long Lasting Columns

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

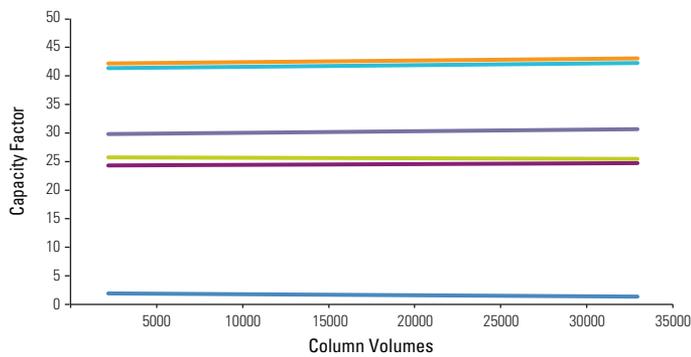| Column:	Accucore C18 2.6μm, 100 x 2.1mm	
Mobile phase:	A – Water + 0.1% Trifluoroacetic Acid B – Methanol + 0.1% Trifluoroacetic Acid	
Gradient:	Time (min)	% B
	0	25
	0.75	25
	10.00	100
	12.00	100
	12.20	25
	17.00	25
Flow rate:	400μL/min	
Injection volume:	1μL	
Temperature:	30°C	
Detection:	UV at 254nm (0.1s rise time, 20Hz)	
Order of elution:	1. Uracil (t_0)	
	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 2.6µm, 100 x 2.1mm	
Mobile phase:	A – Water + 0.1% Ammonia B – Methanol + 0.1% Ammonia	
Gradient:	Time (min)	% B
	0	15
	1.00	15
	8.00	100
	11.00	100
	11.20	15
	16.00	15
Flow rate:	400µL/min	
Injection volume:	1µL	
Temperature:	30°C	
Detection:	UV at 254nm (0.1s rise time, 20Hz)	
Order of elution:	1. Uracil (t_0) 2. 4-Chlorocinnamic acid 3. Procainamite 4. 4-Pentylbenzoic Acid 5. N-Acetylprocainamide 6. Di-isopropyl phthalate 7. Di-n-propyl phthalate	

And also stable at elevated temperature

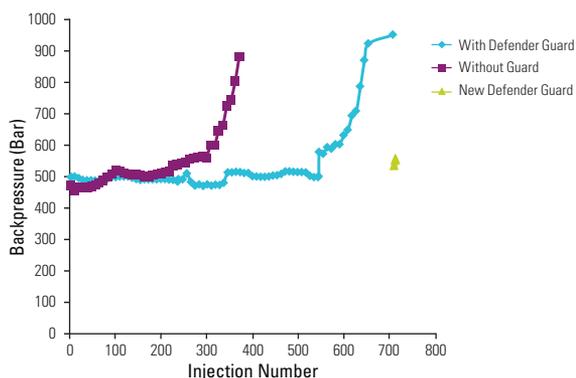| Column:	Accucore C18 2.6µm, 100 x 2.1mm
Mobile phase:	35:65 (v/v) Water/Methanol
Flow rate:	400µL/min
Injection volume:	1.5µL
Temperature:	70°C
Detection:	UV at 254nm (0.1s rise time, 20Hz)
Order of elution:	1. Theophylline/Caffeine (t_0) 2. Phenol 3. Butylbenzene 4. o-Terphenyl 5. Pentylbenzene/Triphenylene
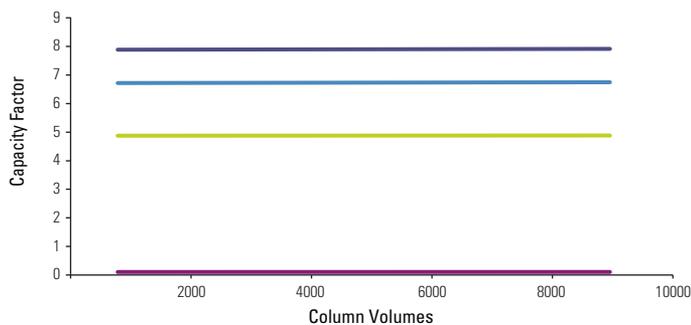
Column Protection

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.

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

Backpressure v Injection Number

Accucore C18 2.6μm, 100mm x 2.1mm
10mm x 2.1mm Defender Guard Cartridge

Mobile:	35:65 (v/v) Acetonitrile/Water
Flow:	550μL/min
Temperature:	40°C
Detection:	UV at 254nm
Injection volume:	1μL

Protein crash sample: rat plasma diluted 1 in 4 with Acetonitrile

1 injection test mix followed by 9 protein crash injections

Defender Guard Cartridges extend the lifetime of your Accucore HPLC column.
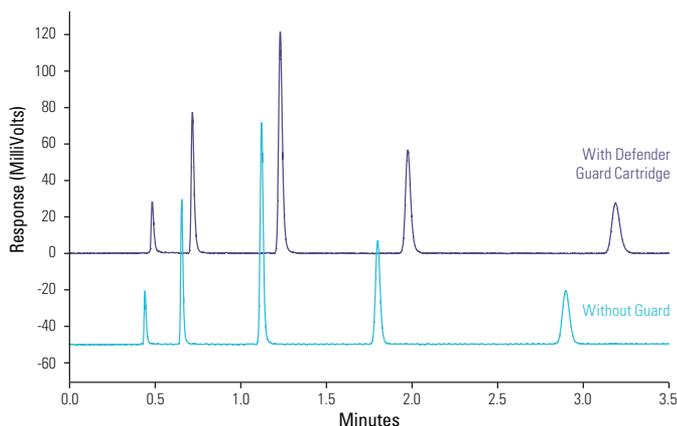
Accucore C18 2.6μm, 100mm x 2.1mm
10mm x 2.1mm Defender Guard Cartridge

Mobile phase:	50:50 (v/v) Acetonitrile/Water
Flow rate:	400μL/min
Temperature:	30°C
Detection:	UV at 254nm
Injection volume:	1μL
Analytes:	1. Theophylline
	2. p-Nitroaniline
	3. Methyl Benzoate
	4. Phenetole
	5. o-Xylene

Parameter	With Defender Guard	Without Defender Guard
Selectivity	1.81	1.81
Efficiency (N/m)	185672	213350
Tailing	1.18	1.07
Retention Time (minutes)	3.19	2.90
Backpressure (bar)	280	256

Defender Guard Cartridges have minimal effect on column performance.
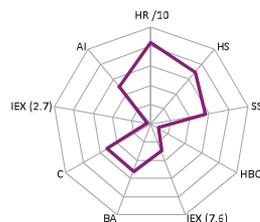
Optimum Selectivity

Accucore HPLC columns are available in a series of phases to provide a wide 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 15 for further details of these tests.

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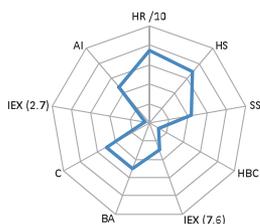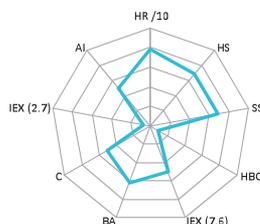

Accucore RP-MS

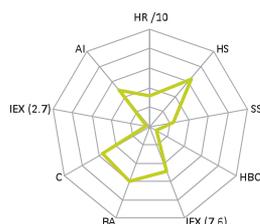Optimized for MS detection, excellent combination of speed and quality of separation

Accucore C18

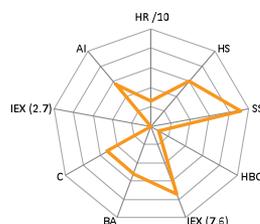Optimum retention for non-polar analytes

Accucore aQ

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

Phenyl-Hexyl

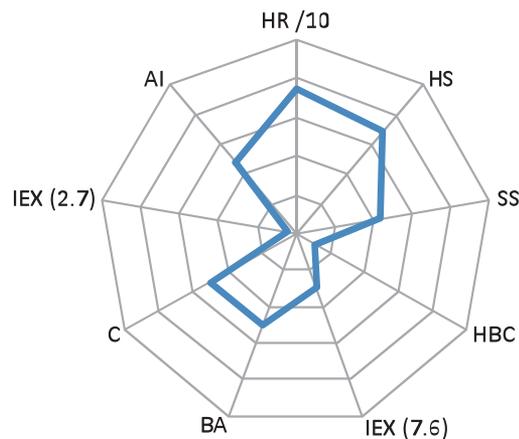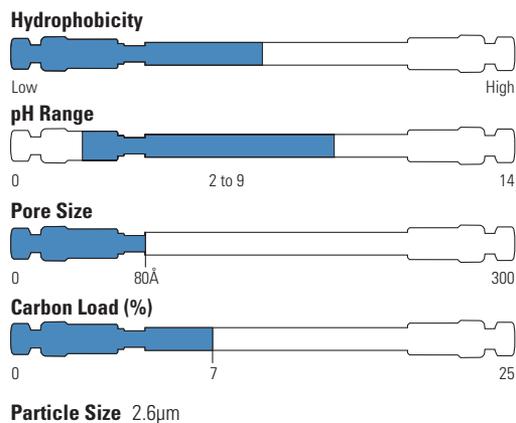Unique selectivity for aromatic and moderately polar analytes

Accucore PFP

Alternative selectivity to C18, particularly for halogenated analytes

HILIC

Accucore HILIC

Enhanced retention of polar and hydrophilic analytes

Thermo Scientific Accucore RP-MS

Hydrophobicity

Low   High

pH Range

0   2 to 9   14

Pore Size

0   80Å   300

Carbon Load (%)

0   7   25

Particle Size 2.6μm

- 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
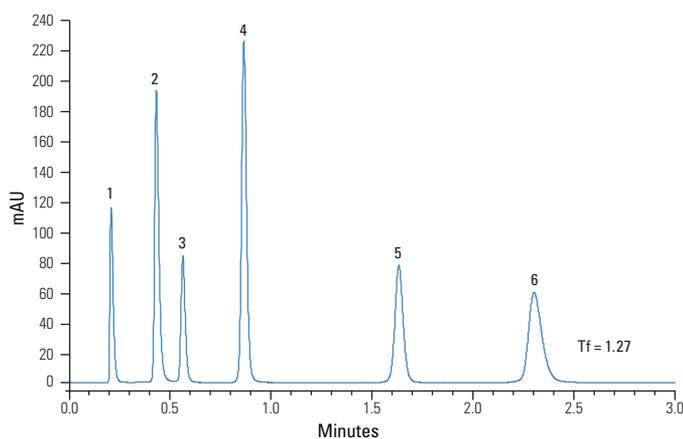
Accucore RP-MS 2.6μm, 50mm x 2.1mm	
Mobile phase:	65:35 (v/v) Methanol/25mM Potassium Phosphate pH7.0
Flow rate:	500μL/min
Temperature:	30°C
Detection:	UV at 215nm
Injection volume:	1μL
Back pressure:	232 bar
Analytes:	1.Uracil
	2. Propranolol
	3. Butylparaben
	4. Naphthalene
	5. Acenaphthene
	6. Amitriptyline

Tf = 1.27

Thermo Scientific Accucore C18

Hydrophobicity

Low — High

pH Range

0 — 1 to 11 — 14

Pore Size

0 — 80Å — 300

Carbon Load (%)

0 — 9 — 25

Particle Size 2.6µm   **USP**   L1

Radar plot axes: HR /10, HS, SS, HBC, IEX (7.6), BA, C, IEX (2.7), AI
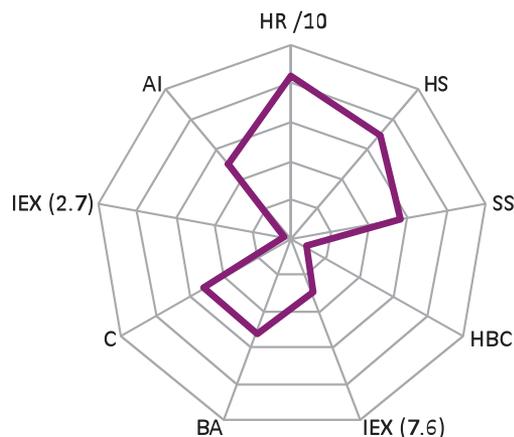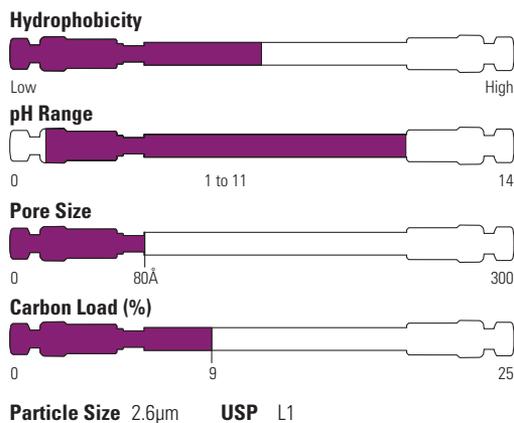
- Optimum retention of non-polar compounds
- Hydrophobic interaction mechanism
- Separates a broad range of analytes

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
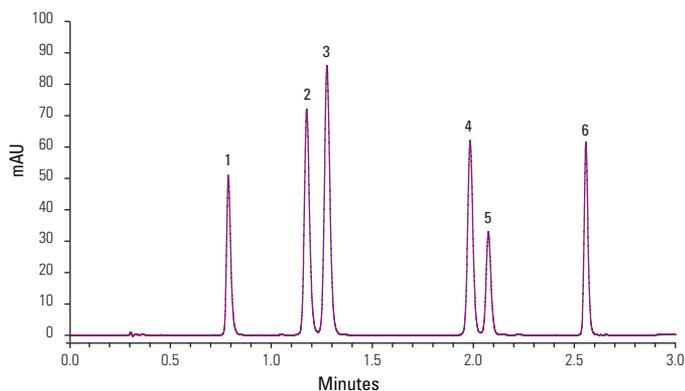
Accucore C18 2.6µm, 50mm x 2.1mm		
Mobile phase:	A – Water ; B – Acetonitrile	
Gradient:	Time (min)	% B
	1.0	35
	2.5	70
Flow rate:	600µL/min	
Temperature:	25°C	
Detection:	UV at 280nm	
Injection volume:	2µL	
Backpressure:	298 bar	
Analytes:	1. Simazine	
	2. Simetryn	
	3. Atrazine	
	4. Ametryn	
	5. Propazine	
	6. Prometryn	

Thermo Scientific Accucore aQ

Hydrophobicity

Low   High

pH Range

0   2 to 9   14

Pore Size

0   80Å   300

Carbon Load (%)

0   9   25

**Particle Size** 2.6µm   **USP**   L1
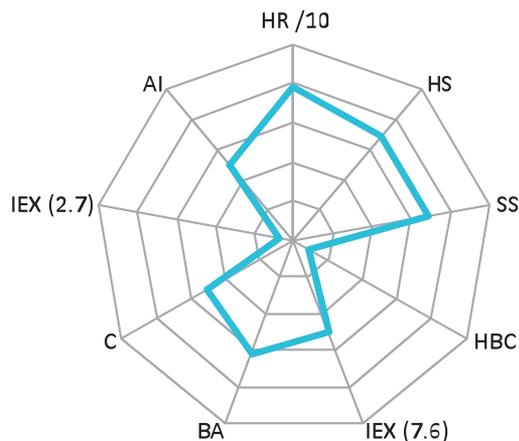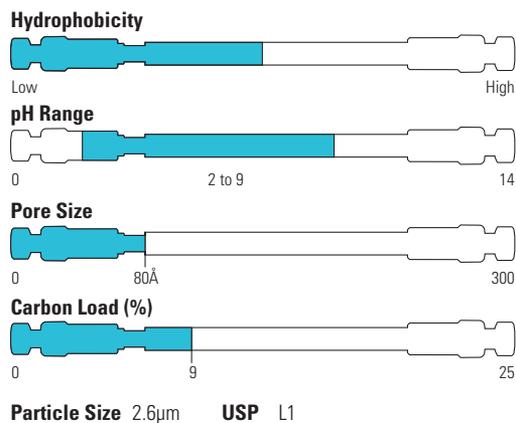
- Retention and Resolution of Polar Analytes
- Polar Endcapped C18 Stationary Phase for Alternative Selectivity
- Ideal for Highly Aqueous Mobile Phases

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)
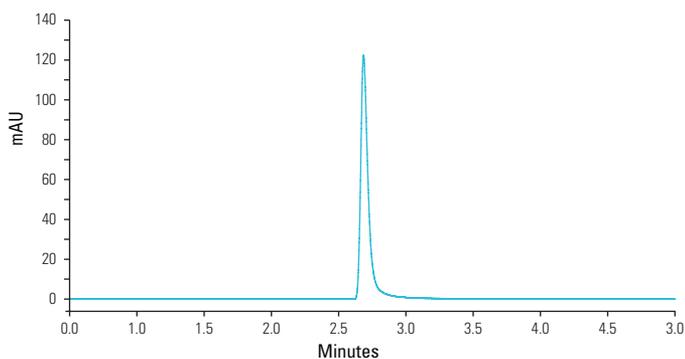
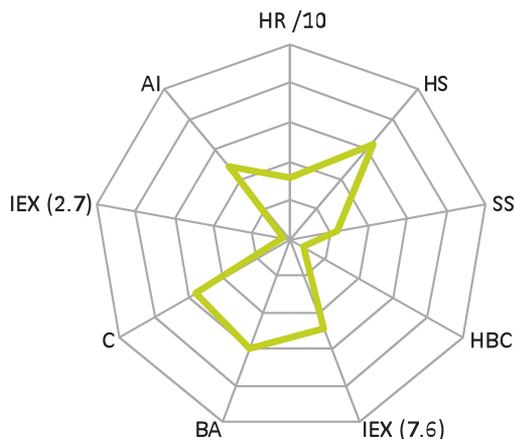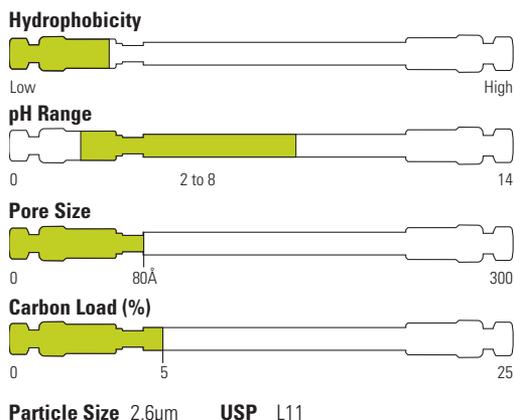| Accucore aQ 2.6µm, 50mm x 2.1mm	
Mobile phase:	95:5 (v/v) Ammonium Acetate, pH 3.80 / Methanol
Flow rate:	200µL/min
Temperature:	35°C
Detection:	UV at 277nm
Injection volume:	1µL
Analytes:	Lamivudine
%RSD t_r	0.00
%RSD Peak area	1.72
(%RSD calculated from 6 replicate injections)	

USP acceptance criteria: % RSD (t_r, Peak Area) <2.0

Thermo Scientific Accucore Phenyl-Hexyl

Hydrophobicity

Low — High

pH Range

0 — 2 to 8 — 14

Pore Size

0 — 80Å — 300

Carbon Load (%)

0 — 5 — 25

**Particle Size** 2.6µm   **USP** L11

- 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.

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
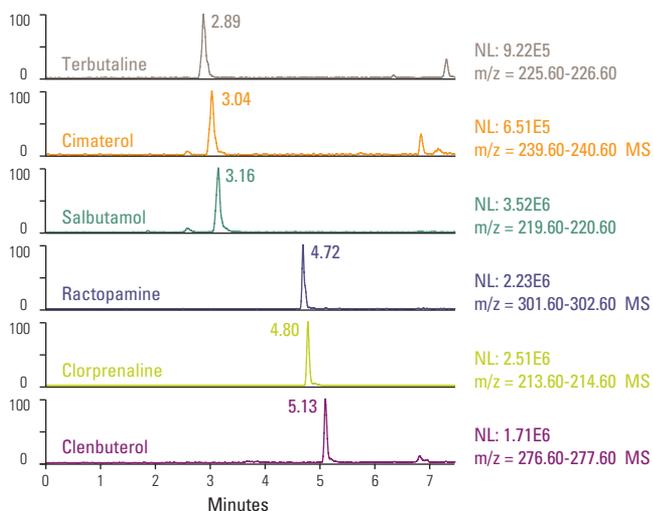
Accucore Phenyl-Hexyl 2.6µm, 100mm x 2.1mm		
Mobile phase:	A – Ammonium acetate 5mM, pH 4 B – Acetonitrile	
Gradient:	Time (min)	% B
	0	5
	1	5
	10	100
Flow rate:	0.25mL/min	
Temperature:	40°C	
Detection:	+ESI-MS (45ºC, 4.5kV, 60V, scan 150 – 350)	
Injection volume:	1µL	
Backpressure:	120 bar	

Thermo Scientific Accucore PFP

Hydrophobicity

Low / High

pH Range

0 / 2 to 8 / 14

Pore Size

0 / 80Å / 300

Carbon Load (%)

0 / 5 / 25

Particle Size 2.6μm **USP** L43

(Radar chart axes: HR /10, HS, SS, HBC, IEX (7.6), BA, C, IEX (2.7), AI)
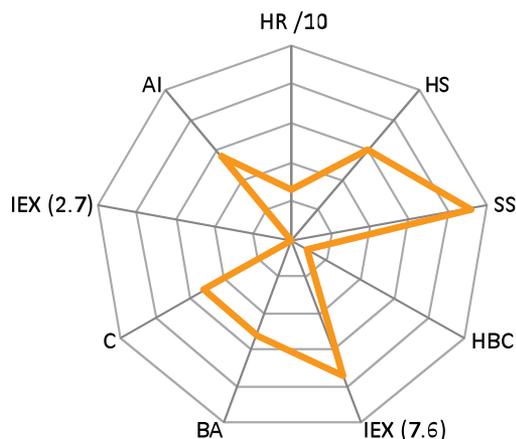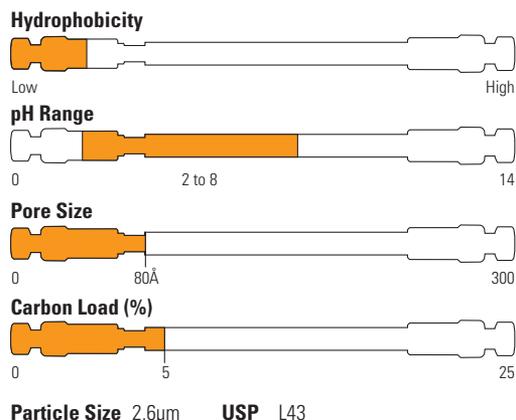
- Alternative Selectivity to C18
- Extra Retention for Halogenated Species
- Unique Selectivity for Non-halogenated Polar Compounds

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
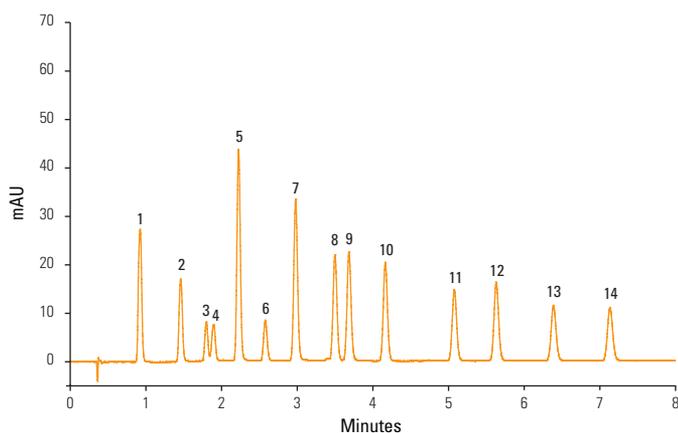
| Accucore PFP 2.6μm, 50mm x 2.1mm	
Mobile phase:	A – Water + 0.1% Formic Acid
	B – Acetonitrile + 0.1% Formic Acid
Gradient:	15 – 30% B in 7 minutes
Flow rate:	600μL/min
Temperature:	50°C
Detection:	UV at 270nm
Injection volume:	2μL

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

Thermo Scientific Accucore HILIC

Hydrophobicity

Low   Very Low (5%)   High

pH Range

0   2 to 8   14

Pore Size

0   80Å   300
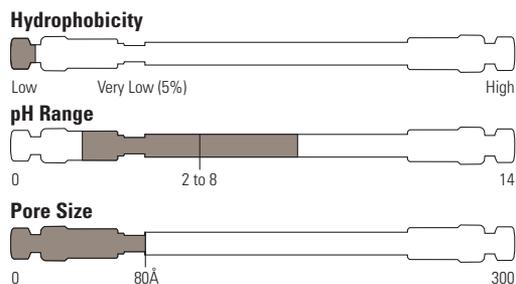
Particle Size 2.6µm   **USP** L3

HILIC

- Enhanced Retention of Polar and Hydrophilic Analytes

- Alternative Selectivity to C18 without Ion-pair or Derivatization

- Improved Sensitivity for MS detection

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
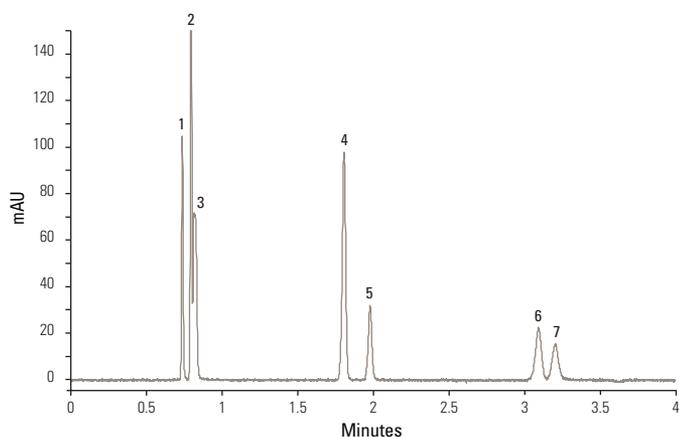
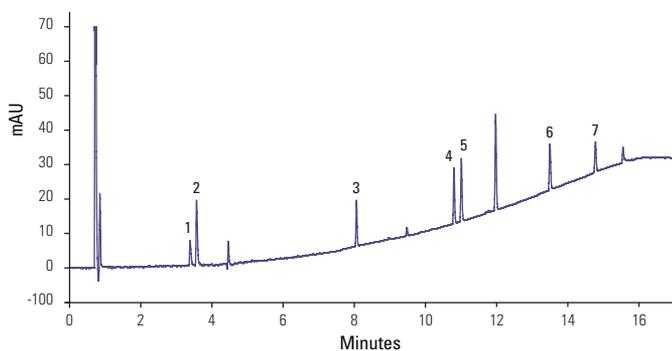| Accucore HILIC 2.6µm, 150 x 4.6mm	
Mobile phase:	85:15 (v/v) Acetonitrile/100mM Ammonium Formate, pH 3.2
Flow rate:	2mL/min
Temperature:	40°C
Detection:	UV at 280nm
Injection volume:	5µL
Backpressure:	157 bar
Analytes:	1. Catechol
	2. 5-HIAA
	3. DOPAC
	4. Serotonin
	5. L-tyrosine
	6. Dopamine
	7. L-DOPA

Applications

Food

Azo Dyes

| Accucore RP-MS 2.6µm, 150mm x 4.6mm		
Mobile phase:	A – Water + 0.1% Trifluoroacetic Acid B – Acetonitrile + 0.1% Trifluoroacetic Acid	
Gradient:	Time (min)	% B
	0	25
	3	30
	15	95
	17	95
Flow rate:	1.8mL/min	
Temperature:	50°C	
Detection:	UV at 225nm	
Injection volume:	2µL	
Analytes:	1. Fast Garnet 2. Orange II 3. Dimethyl Yellow 4. Sudan Red G 5. Sudan I 6. Sudan II 7. Sudan IV	

Melamine and Cyanuric Acid

m/z = 126.50- 128.20
167.50- 168.50
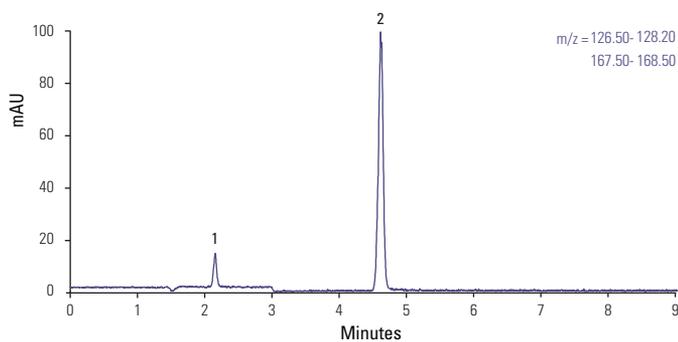
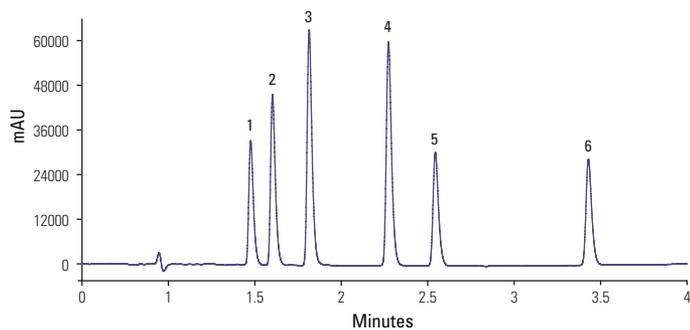| Accucore HILIC 2.6µm, 150mm x 4.6mm	
Mobile phase:	90:10 (v/v) Acetonitrile:50mM Ammonium Acetate, pH 5
Flow rate:	1mL/min
Temperature:	40°C
Detection:	MS at m/z 127, 128, 168 (negative mode 0-3 mins, positive mode 3-10 mins)
Injection volume:	5µL
Backpressure:	117 bar
Analytes:	Cyanuric Acid: m/z 128.1 (-1) Melamine: m/z 127.1 (+1), 168.1 (+1 with Acetonitrile adduct)

Environmental

Uron Herbicides

Accucore RP-MS 2.6μm, 100mm x 2.1mm	
Mobile phase:	A – Water; B – Acetonitrile
Gradient:	35 – 60% B in 3.20 min
Flow rate:	0.43mL/min
Temperature:	25°C
Detection:	UV at 240nm
Injection volume:	3μL
Analytes:	1. Monuron
	2. Metoxuron
	3. Linuron
	4. Diuron
	5. Terbuthiuron
	6. Chlortoluron

Banned Aromatic Amines
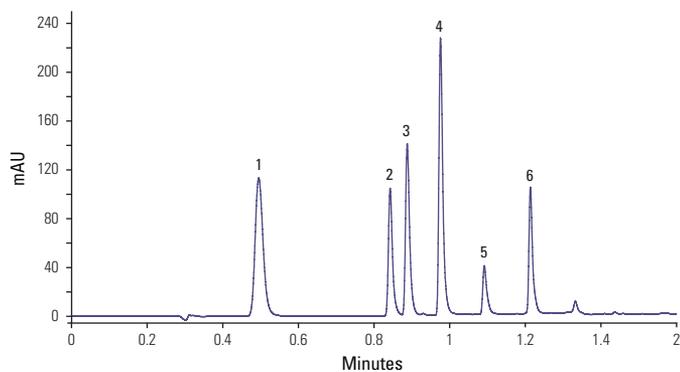
Accucore PFP 2.6μm, 100mm x 2.1mm	
Mobile phase:	A – 25mM Ammonium Acetate pH 5.0 B – Acetonitrile
Gradient:	20 – 100% B in 1.5 minutes
Flow rate:	0.6mL/min
Temperature:	40°C
Detection:	UV at 254nm
Injection volume:	1μL
Backpressure:	300 bar
Analytes:	1. 2,4 – Diaminotoluene
	2. 4,4 – Oxydianiline
	3. o-Toluidine
	4. 2-Methyoxy-5-methylaniline
	5. 2,4,5-Trimethylaniline
	6. 4,4-Methylene-bis (2-chloroaniline)

Basic polar compounds, volatile buffer in the mobile phase – good peak shape.

Clinical

Epinephrine and Norepinephrine
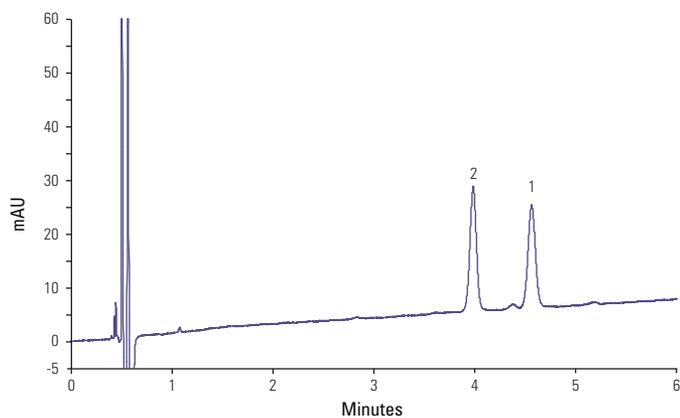
Accucore HILIC 2.6μm, 150mm x 4.6mm	
Mobile phase:	92:8 (v/v) Acetonitrile/100mM Ammonium Formate, pH 3.2
Flow rate:	3.5mL/min
Temperature:	40°C
Detection:	UV at 230nm
Injection volume:	10μL
Backpressure:	250 bar
Analytes:	1. Epinephrine 2. Norepinephrine
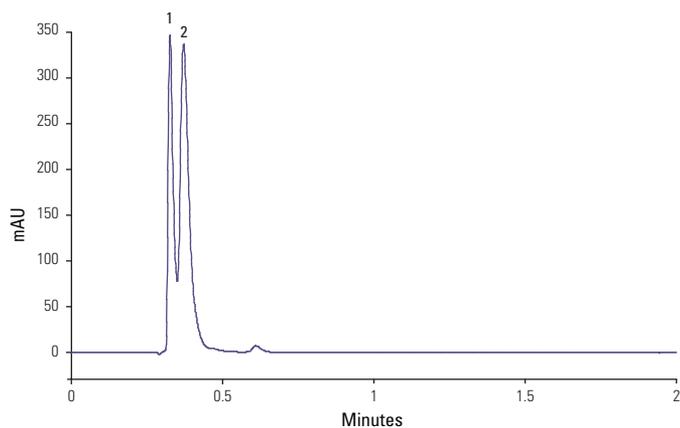
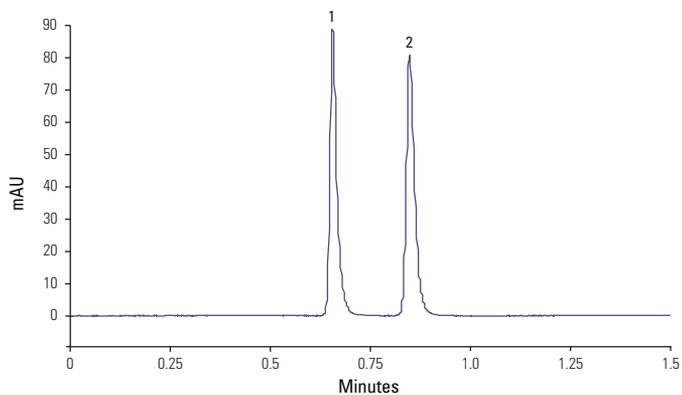| Accucore aQ 2.6μm, 100mm x 2.1mm	
Mobile phase:	5mM Ammonium Formate, pH3.2
Flow rate:	0.6mL/min
Temperature:	30°C
Detection:	UV at 230nm
Injection volume:	2μL
Analytes:	1. Norepinephrine (0.33 min) 2. Epinephrine (0.37 min)

Good retention and resolution of the two analytes in HILIC compared with the aQ column in reversed phase mode.

Steroids

Accucore RP-MS 2.6μm, 100mm x 2.1mm	
Mobile phase:	50:50 (v/v) Water / Acetonitrile
Flow rate:	0.6mL/min
Temperature:	40°C
Detection:	UV at 220nm
Injection volume:	1μL
Analytes:	1. Estradiol
	2. Estrone

Speed – Sub 1 minute separation.
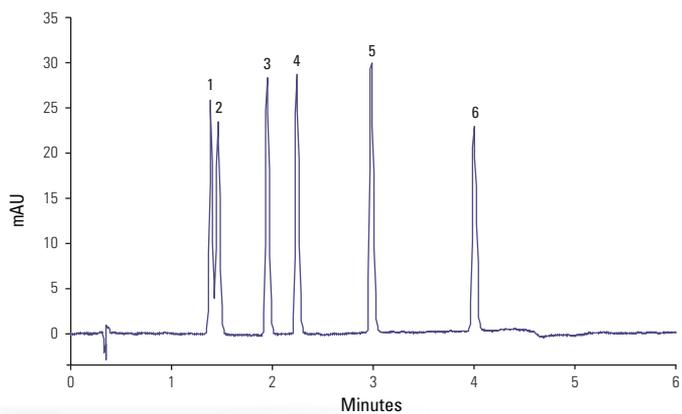
| Accucore RP-MS 2.6μm, 100mm x 2.1mm	
Mobile phase:	A – Water; B – Acetonitrile
Gradient:	25 – 70% B in 4 minutes
Flow rate:	0.6mL/min
Temperature:	25°C
Detection:	UV at 254nm
Injection volume:	1μL
Analytes:	1. Hydrocortisone
	2. Cortisone
	3. Corticosterone
	4. 11-alpha Hydroxyprogesterone
	5. 17-alpha Hydroxyprogesterone
	6. Progesterone

Forensic Toxicology

Testosterones
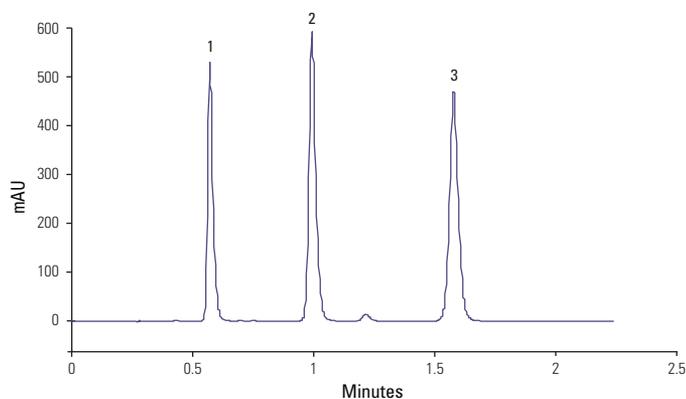
| Accucore RP-MS 2.6µm, 100mm x 2.1mm	
Mobile phase:	60:40 (v/v) Water/Acetonitrile
Flow rate:	0.6mL/min
Temperature:	40°C
Detection:	UV at 254nm
Injection volume:	1µL
Analytes:	1. 11-Ketotestosterone
	2. 19-Nortestosterone (Nandrolone)
	3. Epitestosterone

Forensics

Explosives
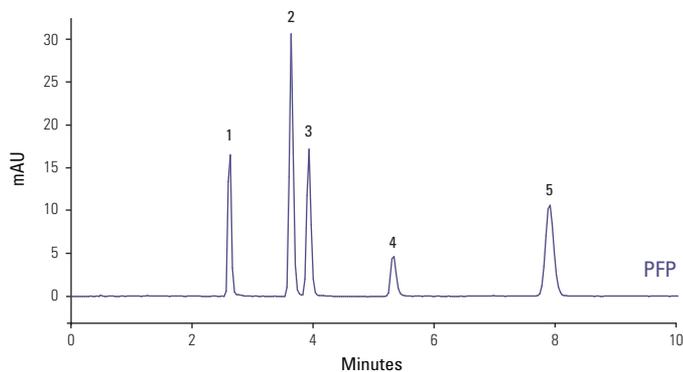
Accucore PFP 2.6µm, 100mm x 2.1mm	
Accucore C18 2.6µm, 100mm x 2.1mm	
Mobile phase:	60:40 (v/v) Water/Methanol
Flow rate:	0.4mL/min
Temperature:	40°C
Detection:	UV 254nm
Injection volume:	1µL
Backpressure:	260 bar
Analytes:	1. 1,3,5-trinitrobenzene
	2. Nitrobenzene
	3. 1,3-dinitrobenzene
	4. 2,4,6-trinitrotoluene
	5. 2,4-dinitrotoluene
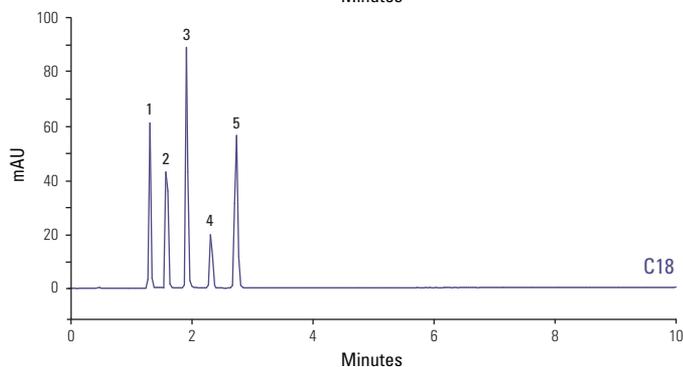
The carbon-fluorine bond on the PFP bonded phase is more polar than the carbon-hydrogen bond in the C18 phase, which results in extra retention and selectivity of these polar substituted aromatic compounds containing nitro functional groups.

Pharmaceuticals

Acidic and Neutral Drugs
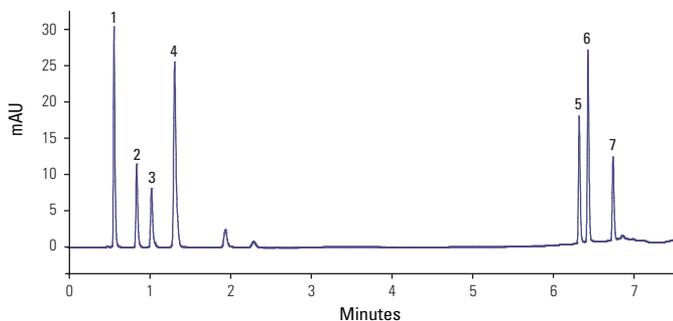
Accucore C18 2.6μm, 100mm x 2.1mm		
Mobile phase:	A – Water + 0.1% Phosphoric Acid	
	B – Acetonitrile + 0.1% Phosphoric Acid	
Gradient:	Min	% B
	0	30
	2	40
	4	95
Flow rate:	0.4mL/min	
Temperature:	25°C	
Detection:	UV at 215nm	
Injection volume:	1.5μL	
Analytes:	1. Hydrochlorothiazide	
	2. Prednisolone	
	3. Pravastatin	
	4. Carbamazepine	
	5. Diclofenac	
	6. Ibuprofen	
	7. Progesterone	

Excellent resolution.

Zidovudine (USP)
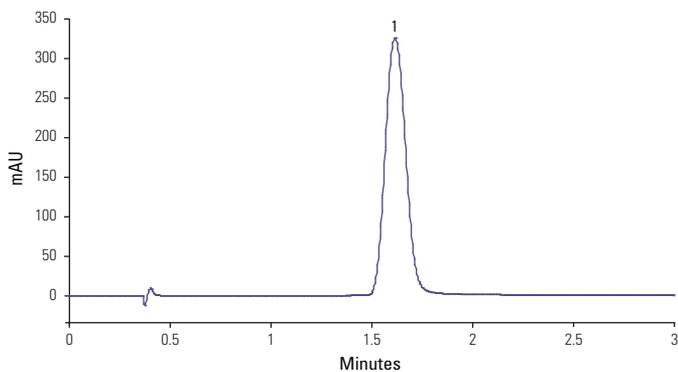
Accucore C18 2.6μm, 100mm x 2.1mm		
Mobile phase:	80:20 (v/v) Water/Methanol	
Flow rate:	0.5mL/min	
Temperature:	25°C	
Detection:	UV at 265nm	
Injection volume:	1μL	
Analytes:	1. Zidovudine	USP specification
Asymmetry	1.13	< 2.0
%RSD t_r	0.0	< 2.0
%RSD Peak area	0.34	< 2.0

The Accucore C18 column demonstrated excellent performance for the analysis of Zidovudine. The USP criteria was exceeded.

Ibuprofen
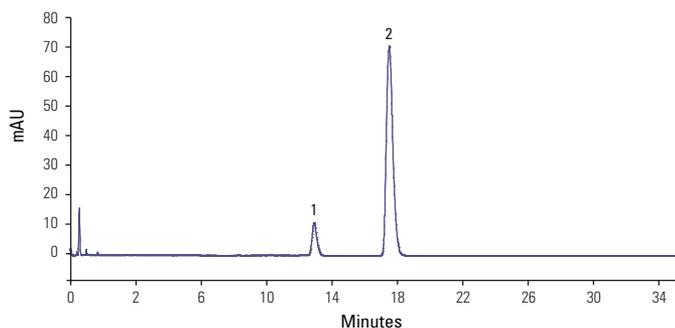
| Accucore C18 2.6μm, 100mm x 4.6mm	
Mobile phase:	66.3% Water adjusted to pH 2.5 with Phosphoric Acid / 33.7% Acetonitrile
Flow rate:	2.0mL/min
Temperature:	30°C
Detection:	UV at 214nm
Injection volume:	5μL
Back pressure:	276 bar
Analytes:	1. Valerophenone (USP grade) 2. Ibuprofen (USP grade)

The Accucore C18 column demonstrated excellent performance for the analysis of Ibuprofen, using a method based on the USP.

Fenoprofen and Gemfibrozil (USP)
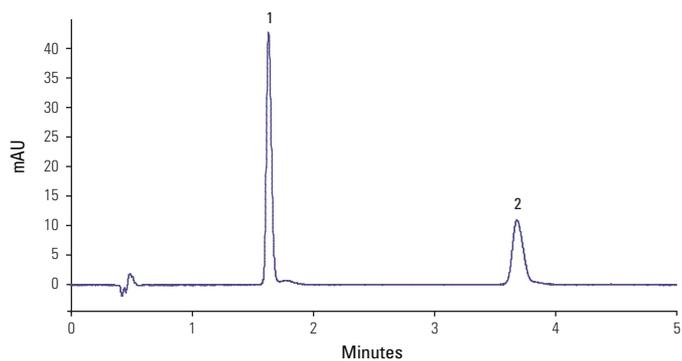
Accucore RP-MS 2.6μm, 100mm x 2.1mm

Mobile phase:	50:49.6:0.4 (v/v/v) Acetonitrile/Water/Phosphoric Acid		
Flow rate:	0.4mL/min		
Temperature:	30°C		
Detection:	UV at 272nm		
Injection volume:	3μL		
Analytes:	1. Fenoprofen	2. Gemfibrozil	USP specification
Asymmetry	1.23	1.22	< 2
%RSD t_r	0.39	0.39	< 2
%RSD Peak area	0.39	0.00	< 2
Resolution			> 8
Efficiency	7464	8259	> 3000

Lisinopril
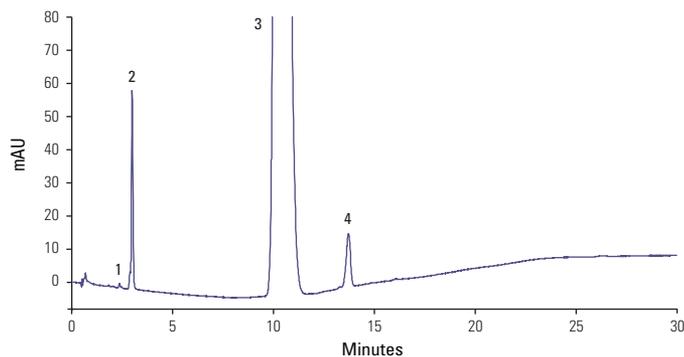
Accucore RP-MS 2.6µm, 100mm x 2.1mm	
Mobile Phase:	A – 0.026M Monobasic Sodium Phosphate pH 3.75 with Phosphoric Acid B – 80:20 (v/v) Mobile Phase A/Acetonitrile

Gradient:	Time (min)	% B
	0	3
	15	30
	30	30
	35	3

|	
Flow rate:	0.4mL/min
Temperature:	50°C
Detection:	UV at 210nm
Injection volume:	5µL
Backpressure:	159 bar
Analytes:	1. Impurity 1 2. 2-amino-4-Phenylbutyric Acid 3. Lisinopril 4. Lisinopril R,S.S isomer

Analytes:	Lisinopril	USP specification
Resolution 2,1	4.7	> 1.5
Resolution 2,3	12.7	> 1.5
Resolution 4,3	4.7	> 2.0
%RSD Peak Area Lisinopril	1.74	< 5%

The Accucore RP-MS column demonstrated excellent performance for the analysis of Lisinopril. The USP criteria was exceeded.

Bio-pharmaceuticals

Peptide Test Mix
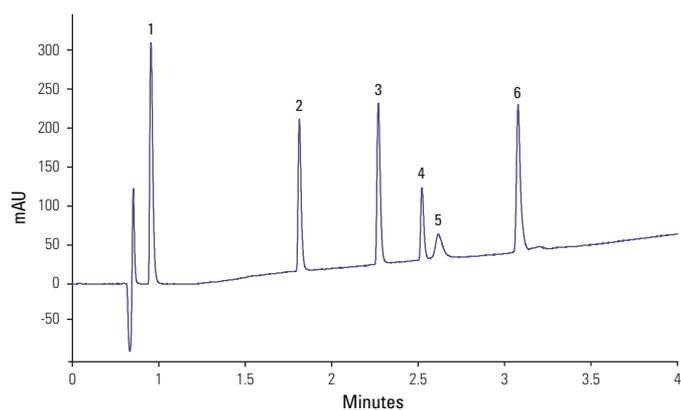
|	
Accucore C18 2.6µm, 100mm x 2.1mm	
Mobile phase:	A – Water + 0.1% Trifluoroacetic Acid B – Acetonitrile + 0.1% Trifluoroacetic Acid
Gradient:	10 – 70 % B in 6 min
Flow rate:	0.50mL/min
Temperature:	40°C
Detection:	UV at 220nm
Injection volume:	2µL
Analytes:	1. Glycyl-L-Tyrosine (MW=238.3) 2. Val-Tyr-Val (MW=379.5) 3. Methionine Enkephalin (MW=573.7) 4. Leucine Enkephalin (MW=555.6) 5. Ribonuclease A (MW=13700) 6. Insulin (MW=5733.5)

The Accucore C18 column can be used to analyze small peptides; demonstrated up to MW 13700.

General

High Throughput Ketones
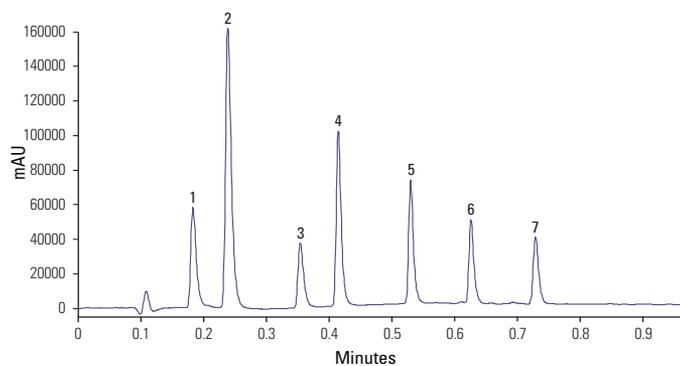
Accucore C18 2.6µm, 50mm x 2.1mm

Mobile phase:	A – Water; B – Acetonitrile	
Gradient:	Min	% B
	0	40
	0.4	95
	0.80	95
Flow rate:	1.0mL /min	
Temperature:	45°C	
Detection:	UV at λ = 258nm	
Injection volume:	1µL	
Backpressure:	113 bar	
Analytes:	1. 2-Pentanone 2. Acetophenone 3. 2-Heptanone 4. Butyrophenone 5. Hexanophenone 6. Octanophenne 7. Decanophenone	
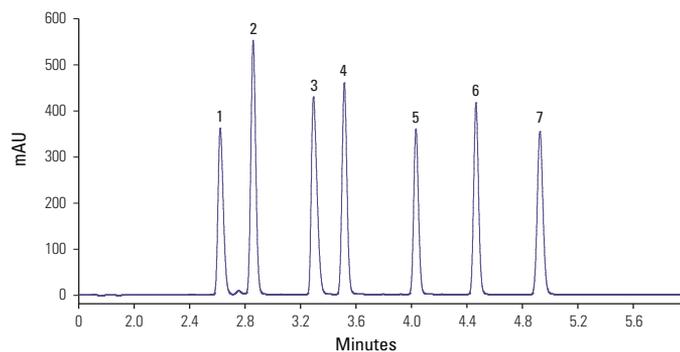
fully porous C18 5µm, 150mm x 4.6mm

Mobile phase:	A – Water; B – Acetonitrile	
Gradient:	Min	% B
	0.00	40
	2.30	95
	4.60	95
Flow rate:	1.0 mL/min	
Temperature:	45°C	
Detection:	UV at λ = 258nm	
Injection volume:	10µL	
Pressure:	47 bar	
Analytes:	1. 2-Pentanone 2. Acetophenone 3. 2-Heptanone 4. Butyrophenone 5. Hexanophenone 6. Octanophenne 7. Decanophenone	

Separation with the Accucore HPLC column is 6 times faster than the 150 x 4.6mm, 5µm fully porous column.

Thermo Scientific Options for Fast HPLC

Uniquely in the market Thermo Scientific HPLC columns offer chromatographers three different options for fast HPLC.

Hypersil GOLD 1.9µm and Syncronis 1.7µm – Small Particle Advantage

When used with UHPLC systems Hypersil GOLD 1.9µm and Syncronis 1.7µm HPLC columns offer optimum efficiency over a range of flow rates.

This high efficiency results in higher throughput, lower costs and better separations.

Accucore – Core Enhanced Technology

The core enhanced technology used in Accucore HPLC columns provides better speed, sensitivity and resolving power than 5µm and 3µm columns without the higher backpressures associated with sub-2µm materials.

Accucore HPLC columns do not require UHPLC systems.

Hypercarb – High Temperature HPLC

Hypercarb is a porous graphitized carbon material suitable for use at temperatures up to 200°C. As a result it is uniquely suited for use with high temperature HPLC.

High temperatures in HPLC increase efficiency, reduce backpressure and reduce analysis time.

Ordering Information

Accucore HPLC Columns

Description	Particle Size	Length (mm)	2.1mm ID	3.0mm ID	4.6mm ID
Accucore RP-MS	2.6µm	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
Accucore C18	2.6µm	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
Accucore aQ	2.6µm	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
Accucore Phenyl-Hexyl	2.6µm	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
Accucore PFP	2.6µm	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
Accucore HILIC	2.6µm	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

Accucore Defender Guard Columns (4/pk)

Description	Particle Size	Length (mm)	2.1mm ID	3.0mm ID	4.6mm ID
Accucore RP-MS	2.6µm	10	17626-012105	17626-013005	17626-014005
Accucore C18	2.6µm	10	17126-012105	17126-013005	17126-014005
Accucore aQ	2.6µm	10	17326-012105	17326-013005	17326-014005
Accucore Phenyl-Hexyl	2.6µm	10	17926-012105	17926-013005	17926-014005
Accucore PFP	2.6µm	10	17426-012105	17426-013005	17426-014005
Accucore HILIC	2.6µm	10	17526-012105	17526-013001	17526-014005

UNIGUARD Direct-Connection Guard Cartridge Holders

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

Accucore Kits

Accucore kits allow validation of the reproducibility of Accucore HPLC columns, or verification of which Accucore HPLC column offers the best separation.

Accucore Validation Kit

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

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

Accucore Narrow Selectivity Kit

Verify which Accucore HPLC column offers the best separation over a narrow selectivity range. Contains 1 each of Accucore C18, RP-MS and aQ columns.

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

Accucore Wide Selectivity Kit

Verify which Accucore HPLC column offers the best separation over a wide selectivity range. Contains 1 each of Accucore C18, Phenyl-Hexyl and PFP columns.

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

Accucore Polar Selectivity Kit

Verify which Accucore HPLC column offers the best separation for polar analytes. Contains 1 each of Accucore aQ, PFP and HILIC columns.

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