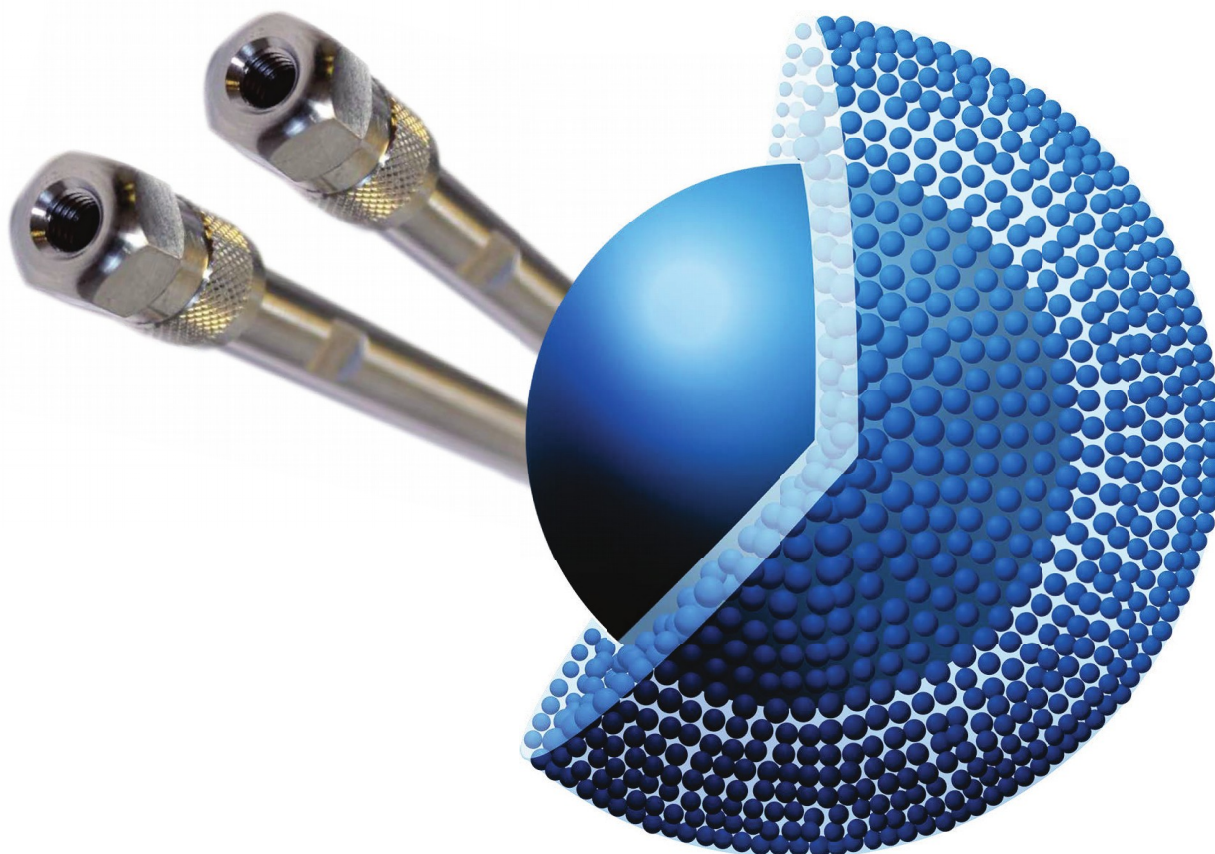


Chromatography Solutions



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Avantor® ACE® UltraCore



Avantor®ACE™

Avantor® ACE®

High throughput and high efficiency ultra-fast separations are achievable using Avantor® ACE® UltraCore – ultra-inert solid-core (core-shell) columns. Avantor® ACE® UltraCore columns utilise ultra-high purity solid-core silica with a monodisperse particle distribution to combine high efficiency with low backpressure. Achieve UHPLC-like performance using HPLC instrumentation with Avantor® ACE® UltraCore.

FASTER SEPARATION WITHOUT LOSS OF RESOLUTION

High efficiency separations comparable with sub-2 µm packings but lower back pressure. Ideal for a wide range of analytes differing in hydrophobicity.

Three product types within the range:

- UltraCore BIO for the separation of large, biologically derived molecules. Ideal for separating compounds with a molecular weight of 5 kDa and above.
- UltraCore for the separation of standard small organic molecules. Ideal for the fast separation of mixtures containing small organic molecules of either synthetic or natural origin.
- UltraCore Super featuring encapsulated bonding technology (EBT) for small organic molecules in extreme pH conditions. Ideal when high pH buffers are required.

ULTRACORE ADVANTAGE OVER FULLY POROUS MATERIAL (FPP)

Solid-core materials benefit from three key aspects;

- The size uniformity of the packing material means that it packs better, resulting in greater uniformity of the flow profile through the column.

- The dead volume in the column is reduced due to the solid-core, which significantly reduces the amount of longitudinal dispersion.
- Reduced mass transfer issues since the differential pathway between molecules entering the porous system and those that don't is substantially reduced.

This last phenomenon is exaggerated by higher flow rates and when using larger molecular weight molecules, due to their slower diffusion rates. Thus, using a silica particle with a solid-core is ideally suited to the analysis of biomolecules as it provides enhanced chromatographic performance coupled with the ability to run at elevated flow rates.

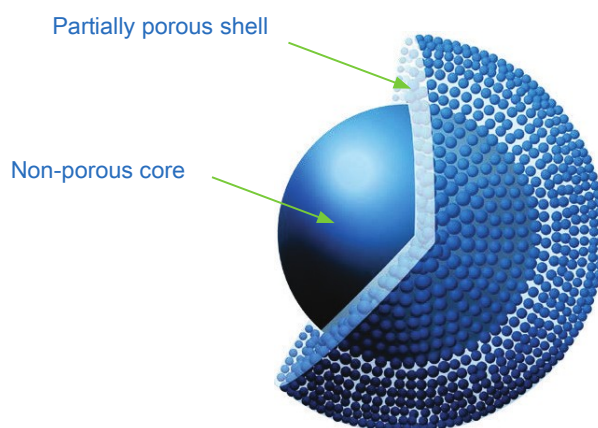


Figure 1: Schematic structure of a solid-core particle.

Biomolecule	Molecular weight kDa	Efficiency at 300 Å	Efficiency at 500 Å
Oxytocin Acetate	1.0072	234449	213719
Angiotensin I	1.2945	239231	203898
Angiotensin II	1.0642	349538	288695
Angiotensin III	1.1113	332637	269406
Bradykinin Acetate	1.060	106321	81543
Cytochrome C	12	245487	517929
Ribonuclease A	13.7	124969	154193
β-lactoglobulin	18.4	140722	280565
Ovalbumin	45	192084	324076
BSA	66.5	8551	33608

*NB comparison data collected using fully porous material to demonstrate effect of pore size change.

Table 1

retention and greater loading capacity.

THE IMPORTANCE OF PORE SIZE

When working with larger molecules, typically from around 5 kDa in molecular weight to 150 kDa, it is important to make sure that the molecules have full access to all of the available stationary phase. Typical analytical HPLC phases have a pore size around 95 Å, and for smaller molecules which have a molecular weight

less than 1 kDa, the molecules have full accessibility to the stationary phase. However, as the molecular weight increases the accessibility decreases, with individual molecules having different levels of access to a pore, dependant on the individual pore size and also the orientation of the molecule. This results in a loss of chromatographic efficiency evident by broad peaks with a limited Gaussian structure. Eventually the size of the molecule will become too big to enter the pore structure, resulting in a substantial reduction in the accessible available surface area causing a reduction in the retention time. Biomolecules above 5 kDa are too large to be used with a 95 Å material, as can be seen in the table 1, where a loss of efficiency occurs as molecules struggle to access all of the available surface. The correct selection of column according to pore size makes a significant difference in the chromatographic efficiency and consequently the resolution for larger molecules.

With both 300 Å and 500 Å pore sizes available in the UltraCore BIO range, the optimum pore size can be chosen. These particle formats are ideally suited for the efficient separation of larger molecules over 5 kDa in molecular weight.

ULTRACORE BENEFITS FOR BIOLOGICAL MACROMOLECULE SEPARATION

UltraCore particles are composed of a solid silica core, surrounded by a porous outer shell (Figure 1). Reduced mass transfer for large molecules using solid-core materials compared with a fully porous silica particles results in more efficient separations and increased analyte resolution.

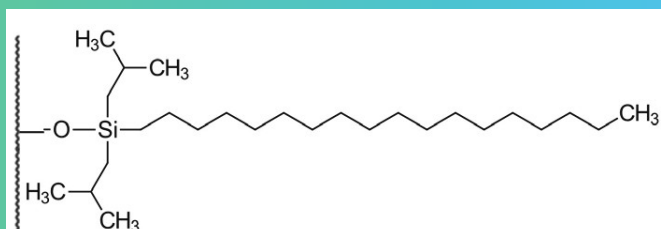
UltraCore columns give a reduced peak width and therefore improved resolution when compared to a fully porous alternative.

Avantor® ACE® UltraCore BIO for

PHASES AVAILABLE FOR AVANTOR ACE® ULTRACORE BIO

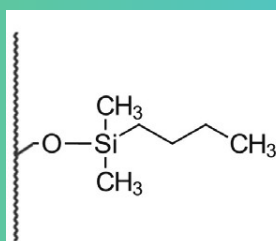
C18: Endcapped C18 phase, which can be used with 100% aqueous mobile phase. This is especially useful for the reversed-phase gradient separation of biologically derived compounds owing to the requirement for the start of the gradient often to be 0% organic. With excellent stability at low pH and high temperatures, these columns are ideal for the separation of a wide range of peptides and proteins.

ACE Ultra-Inert
Silica Surface



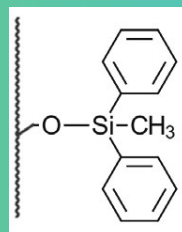
C4: Reversed-phase material for separations requiring a stationary phase with lower hydrophobicity. This is an important phase for large proteins given that the net interaction caused by the hydrophobic interaction is often strong in such molecules. The butyl ligand is well established in RP protein applications. This phase is well suited to the analysis of hydrophobic proteins over 5 kDa molecular weight.

ACE Ultra-Inert
Silica Surface



Phenyl2: Reversed-phase material in which π - π and hydrophobic interactions take place. This phase is similar in hydrophobicity to a C4 but given the different interaction of the phenyl groups, it displays a high degree of orthogonality to C18 and C4. It is a good choice if the required separation is not achieved on a C4 phase.

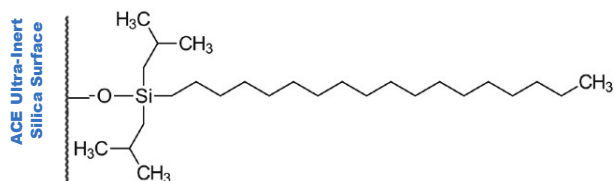
ACE Ultra-Inert
Silica Surface



Phase Comparison Table

Phase	USP Listing	Functional group	End-capping	Particle size (μm)	Pore size (\AA)	Surface area (m^2/g)	Carbon load (%)	pH range
UltraCore BIO C18	L1	Octadecyl	Yes	3.5	300	16	1.0	1-8
UltraCore BIO C18	L1	Octadecyl	Yes	2.5	500	23	1.4	1-8
UltraCore BIO C4	L26	Butyl	Yes	3.5	300	16	0.4	2-9
UltraCore BIO C4	L26	Butyl	Yes	2.5	500	23	0.6	2-9
UltraCore BIO Phenyl2	L11	Diphenyl	Yes	3.5	300	16	0.7	2-9
UltraCore BIO Phenyl2	L11	Diphenyl	Yes	2.5	500	23	1.0	2-9

Avantor® ACE® UltraCore BIO



SEPARATION OF SMALL PROTEINS USING AN AVANTORACE ULTRACORE BIO C18 300Å

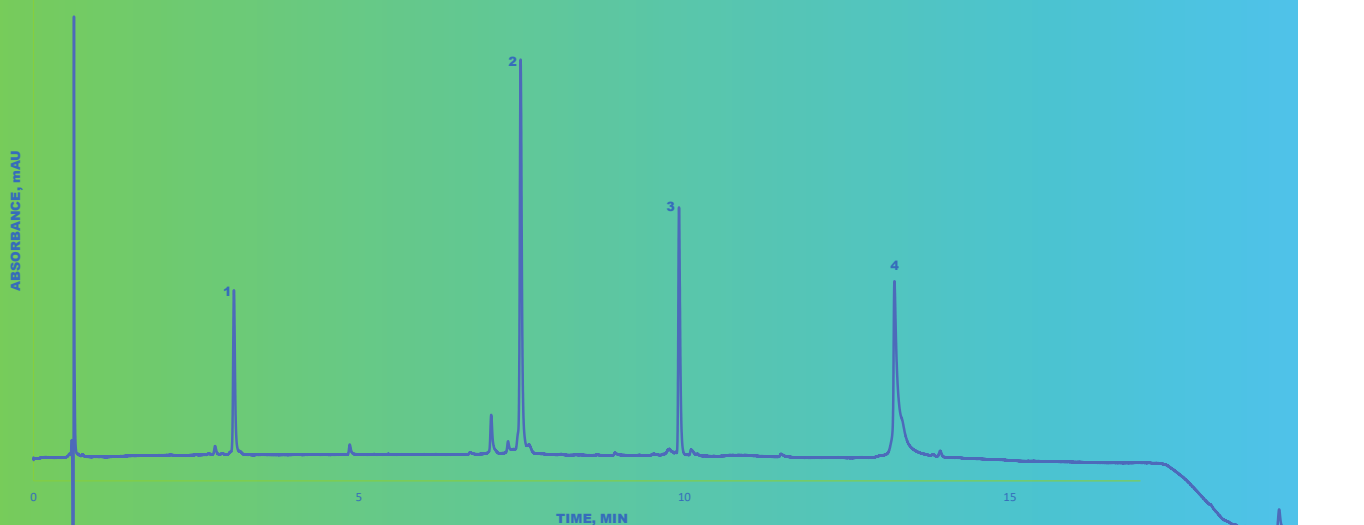
TESTING CONDITIONS:

Mobile Phase	A: Water + 0.1% TFA
	B: 80% ACN/20% Water + 0.085% TFA
Gradient:	Time %B
	0.0 28.5
	15.0 62.5
	16.0 62.5
	17.0 100
	22.0 100

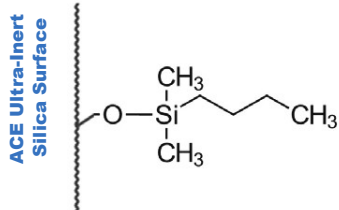
Wavelength:	215 nm
Injection:	5 µL Mix 1
Temperature:	60 °C
Flow Rate:	1.5 mL/min.
Column:	UltraCore BIO C18-300, 3.5 µm, 4.6 x 100 mm

Peak Number	Peak Identity	Retention Time	Molecular Weight (kDa)
1	Ribonuclease A	3.1	13.7
2	Lysozyme	7.5	14.3
3	Alpha-lactalbumin	9.9	14
4	Enolase	13.2	46

The C18 stationary phase with pore sizes of 300 Å is suitable for separation of proteins up to approximately 60 kDa. As seen here, it is ideal for the separation of proteins of approximately 14 kDa in size.



Avantor® ACE® UltraCore BIO

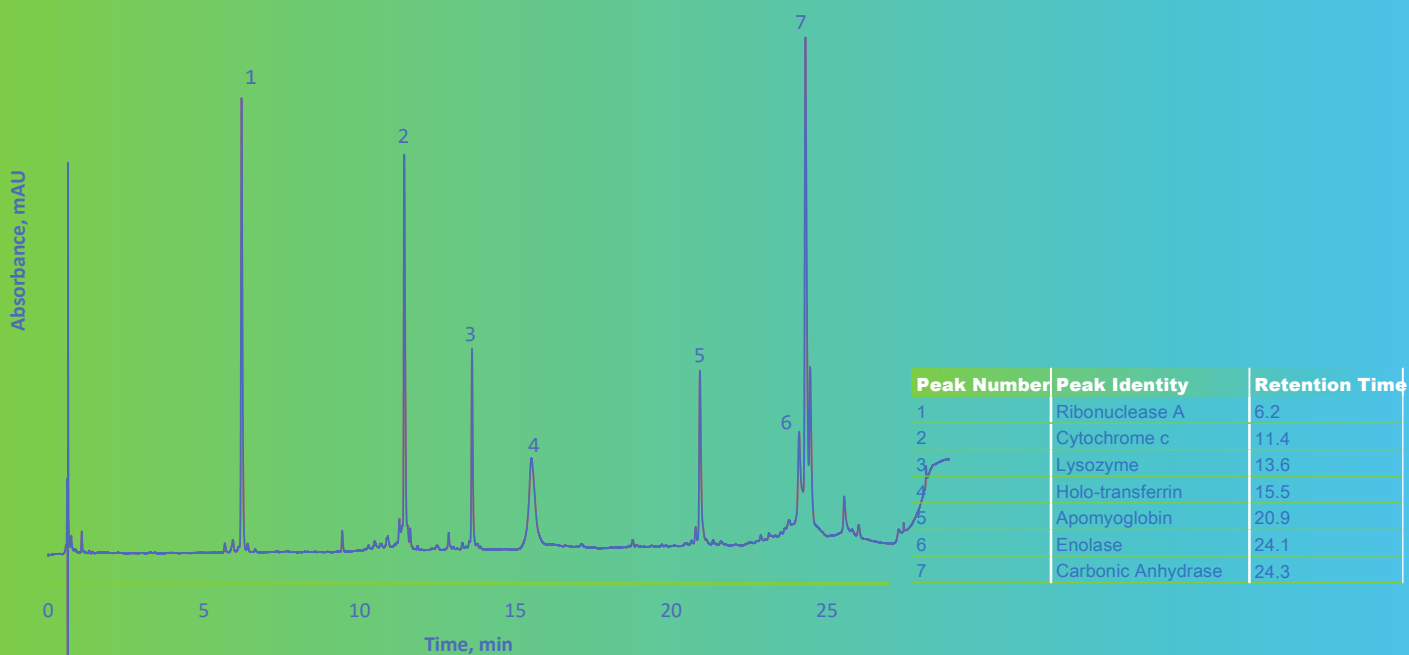


SEPARATION OF ENZYMES AND PROTEINS ON AN AVANTORACE ULTRACORE BIO C4

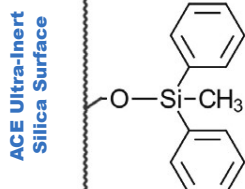
TESTING CONDITIONS:

Mobile Phase	A: Water + 0.1% TFA
	B: 80% ACN/20% Water + 0.085% TFA
Gradient:	Time %B
	0.0 25
	25.0 57.5
	26.0 57.5
	27.0 100
	29.0 100
Wavelength:	220 nm
Injection:	5 µL Mix 1
Temperature:	60 °C
Flow Rate:	1.5 mL/min.
Column:	UltraCore BIO C4-300, 3.5 µm, 4.6 x 100 mm

The 300 Å pore size is ideal for the separation of small proteins. The chromatogram shows narrow peak widths, giving well resolved peaks. The C4 phase, with lower hydrophobicity than the C18, is particularly well suited to separations of this type.



Avantor® ACE® UltraCore BIO



SEPARATION OF 4 PROTEINS TO SHOW SUPERIOR PERFORMANCE OF THE PHENYL2 PHASE

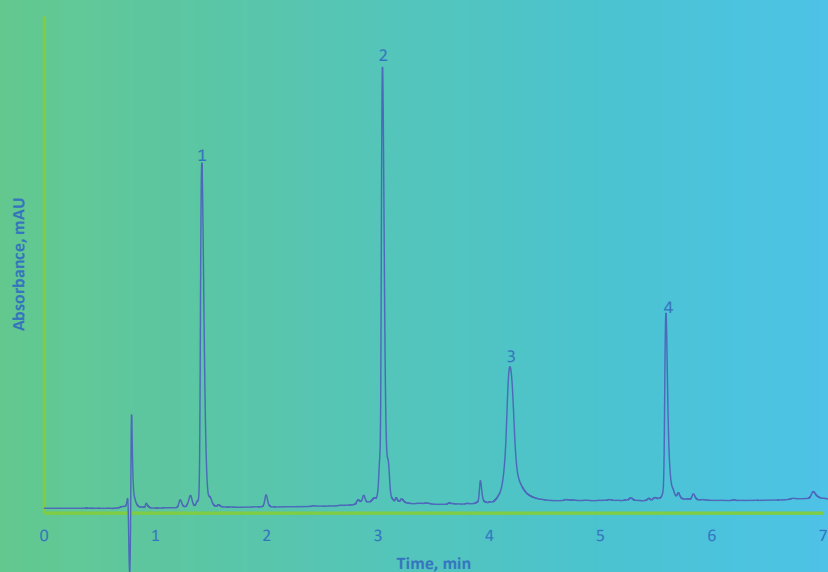
TESTING CONDITIONS:

Mobile Phase **A:** Water + 0.1% TFA
B: 80% ACN/20% Water + 0.085% TFA

Gradient:

Time	%B
0.0	31
7.0	60
8.0	60

Wavelength: 220 nm
Injection: 3 µL Mix 1
Temperature: 60 °C
Flow Rate: 0.4 mL/min.
Column: UltraCore BIO Phenyl2-300, 3.5 µm, 2.1 x 150 mm



Peak Number	Peak Identity	Retention Time
1	Ribonuclease A	1.4
2	Cytochrome c	3.0
3	Holo-transferrin	4.1
4	Apomyoglobin	5.6

The chromatogram shows the superior performance of the phenyl phase. It is clearly demonstrated how very fast, high efficiency separations can be achieved for the separation of biomolecules using a column of this type.

Avantor® ACE®

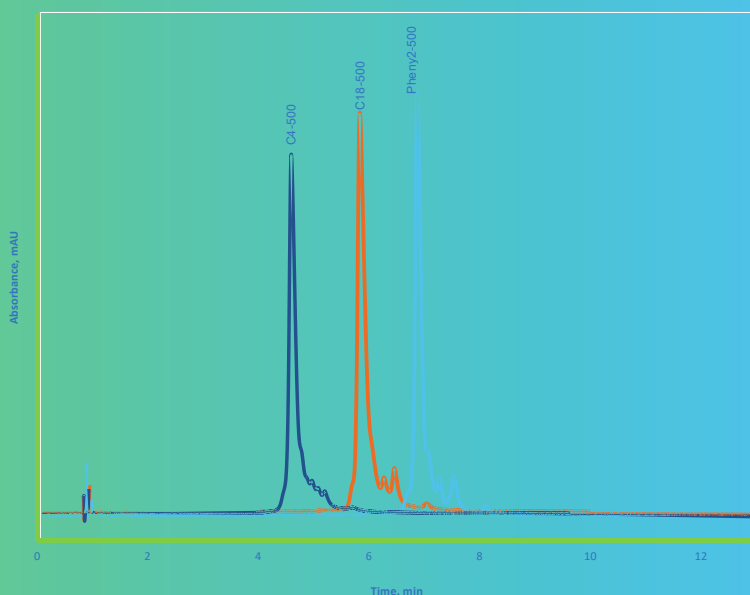
RETENTION OF TRASTUZUMAB SHOWING 3 OVERLAID CHROMATOGRAMS COMPARING RETENTION TIMES ACROSS THREE DIFFERENT PHASES ON 500 Å ULTRACORE SILICA.

TESTING CONDITIONS:

Mobile Phase	A: Water + 0.1% TFA	
	B: 80% ACN/20% Water + 0.085% TFA	
Gradient:	Time	%B
	0.0	40.0
	12.0	47.5
	12.5	47.5
	13.0	100
	13.5	100
	16.0	100
		Total Flow (mL/min)
		0.4
		0.4
		0.4
		0.7
		0.7
Wavelength:	280 nm	
Injection:	2 µL Trastuzumab	
Temperature:	80 °C	
Flow Rate:	0.4 mL/min.	

Retention Time (min)

4.7	UltraCore BIO C4-500, 2.5 µm, 2.1 x 150 mm
5.8	UltraCore BIO C18-500, 2.5 µm, 2.1 x 150 mm
6.8	UltraCore BIO Phenyl2-500, 2.5 µm, 2.1 x 150 mm



500 Å is a suitable pore size for the reversed-phase separation of antibodies of approximately 150 kDa in molecular weight. The 500 Å pore size retains the mAb well and a difference can clearly be observed when comparing the three different stationary phases. This is a useful tool to enable clear separation of the protein of interest from any similar compounds such as mAb fragments or impurities with similar retention.

All 300 Å phases have HPLC hardware with 600 bar (9000 psi) pressure limit. 500 Å columns have HPLC/UHPLC hardware with 600 bar (9000 psi) pressure limit for 4.6 mm ID or 1000 bar (14,500 psi) for 2.1 and 3.0 mm ID

ORDERING TABLE ULTRACORE BIO:

Dimensions	300 Å, 3.5 µm			500 Å, 2.5 µm		
	C18	C4	Phenyl2	C18	C4	Phenyl2
2.1 x 50 mm	BIO-350-0521	BIO-351-0521	BIO-352-0521	BIO-250-0521	BIO-251-0521	BIO-252-0521
2.1 x 100 mm	BIO-350-1021	BIO-351-1021	BIO-352-1021	BIO-250-1021	BIO-251-1021	BIO-252-1021
2.1 x 150 mm	BIO-350-1521	BIO-351-1521	BIO-352-1521	BIO-250-1521	BIO-251-1521	BIO-252-1521
3.0 x 50 mm	BIO-350-0530	BIO-351-0530	BIO-352-0530	BIO-250-0530	BIO-251-0530	BIO-252-0530
3.0 x 100 mm	BIO-350-1030	BIO-351-1030	BIO-352-1030	BIO-250-1030	BIO-251-1030	BIO-252-1030
3.0 x 150 mm	BIO-350-1530	BIO-351-1530	BIO-352-1530	BIO-250-1530	BIO-251-1530	BIO-252-1530
4.6 x 50 mm	BIO-350-0546	BIO-351-0546	BIO-352-0546	BIO-250-0546	BIO-251-0546	BIO-252-0546
4.6 x 100 mm	BIO-350-1046	BIO-351-1046	BIO-352-1046	BIO-250-1046	BIO-251-1046	BIO-252-1046
4.6 x 150 mm	BIO-350-1546	BIO-351-1546	BIO-352-1546	BIO-250-1546	BIO-251-1546	BIO-252-1546
Guard cartridges (3pk)	BIO-350-521G	BIO-351-521G	BIO-352-521G	BIO-250-521G	BIO-251-521G	BIO-252-521G
Guard cartridges (3pk)	BIO-350-530G	BIO-351-530G	BIO-352-530G	BIO-250-530G	BIO-251-530G	BIO-252-530G
Guard cartridges (3pk)	BIO-350-546G	BIO-351-546G	BIO-352-546G	BIO-250-546G	BIO-251-546G	BIO-252-546G

* Guard cartridges require guard holder BIO-GRD-0001

Avantor® ACE® UltraCore

The use of solid-core particles is well established, but has really gained prominence in recent years as they are able to generate higher efficiencies than comparably sized fully porous particles and show less of a performance drop-off at higher flow rates. This has been especially utilised in the small molecule application area.

The Avantor® ACE® UltraCore 95 Å range offers;

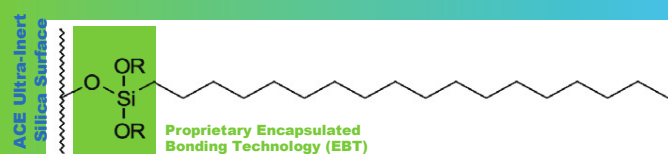
- High purity, base-deactivated silica with excellent reproducibility
- 2.5 µm solid-core particles, which give comparable efficiencies to sub 2 µm FPP with the advantage of lower back pressure
- 5 µm solid-core particles which can be used to replace 5 µm FPPs of the same chemistry to significantly increase separation speed and improve sample throughput
- 3.5 µm solid-core particles offering sub-3 µm FPP efficiencies and are available in alternative selectivities for method development

Avantor® ACE® UltraCore

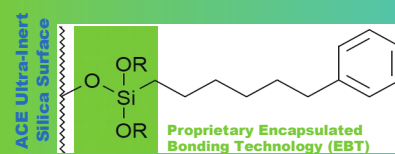
- Ultra-inert solid-core particles
- SuperC18 and SuperPhenylHexyl phases – with complementary selectivity for method development over a wide pH range
- Extended pH range 1.0–11.0
- Designed for LC-MS applications

Phase	USP Listing	Functional group	End-capping	Particle size (µm)	Pore size (Å)	Surface area (m ² /g)	Carbon load (%)	pH range
UltraCore SuperC18	L1	Octadecyl	Encapsulated	2.5	95	130	7.0	1.5-11
				5		100	5.4	
UltraCore SuperPhenylHexyl	L11	Phenyl-Hexyl	Encapsulated	2.5	95	130	4.6	1.5-11
				5		100	3.6	

SuperC18

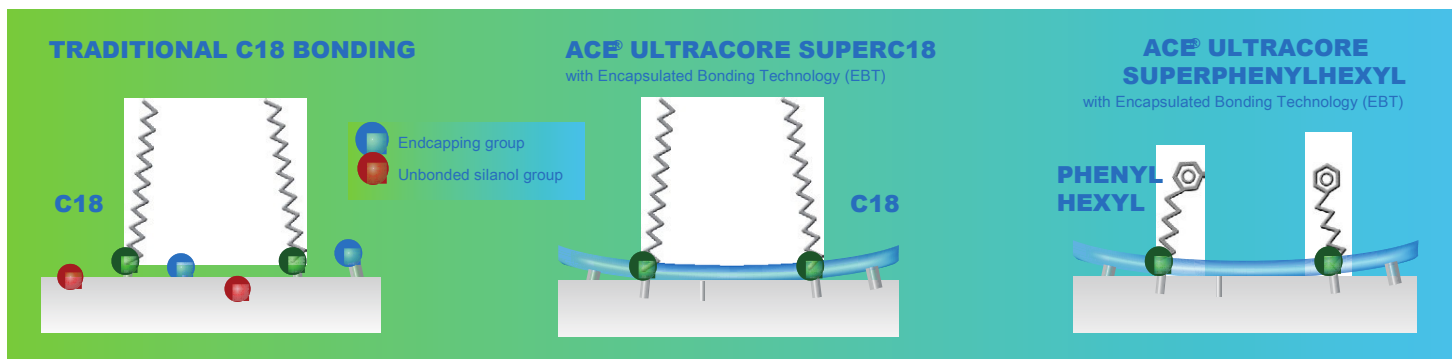


SuperPhenylHexyl



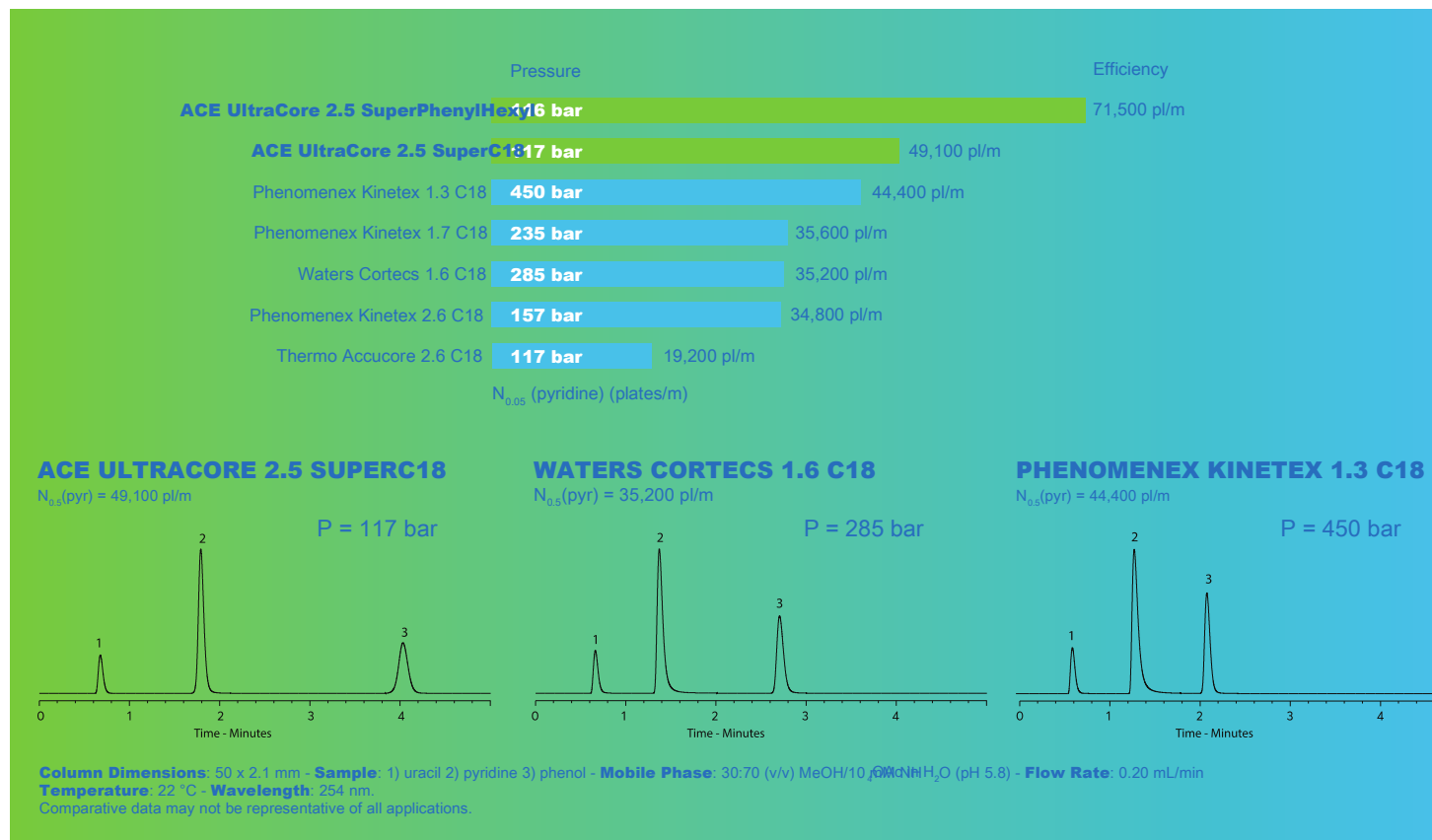
AVANTORACE® ULTRACORE

Avantor® ACE® UltraCore SuperC18 and SuperPhenylHexyl phases are manufactured using our unique Encapsulated Bonding Technology (EBT). This technology dramatically increases ligand coverage of the silica surface and effectively eliminates the negative effects of unbonded silanol groups. The higher ligand coverage results in improved inertness, chromatographic performance and stability.



Avantor® ACE® UltraCore columns are highly inert.

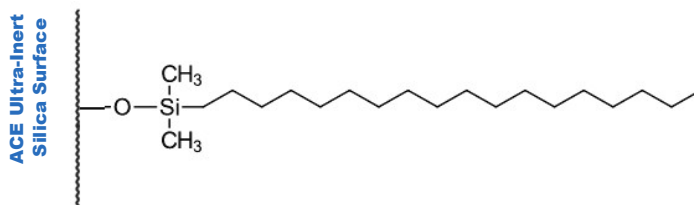
- Solid-core columns from leading manufacturers investigated
- Comparison of column efficiency for pyridine – a basic molecule



Avantor® ACE® UltraCore

This densely bonded octadecyl phase can be used to separate acidic, basic and neutral molecules, based on hydrophobic retention mechanisms. Often known as the workhorse stationary phase, it can be utilised for common application areas where high sensitivity is required.

- High efficiency separations comparable with sub-2 µm packings but lower back pressure
- Ideal for a wide range of analytes differing in hydrophobicity



Phase	USP Listing	Functional group	Endcapping	Particle size (µm)	Pore size (Å)	Surface area (m ² /g)	Carbon load (%)	Low pH/T limit	High pH/T limit	100% aqueous compatible
UltraCore C18	L1	Octadecyl	Yes	3.5	95	115	8.2	2/60°C	9/40°C	No

SEPARATION OF PHTHALATES

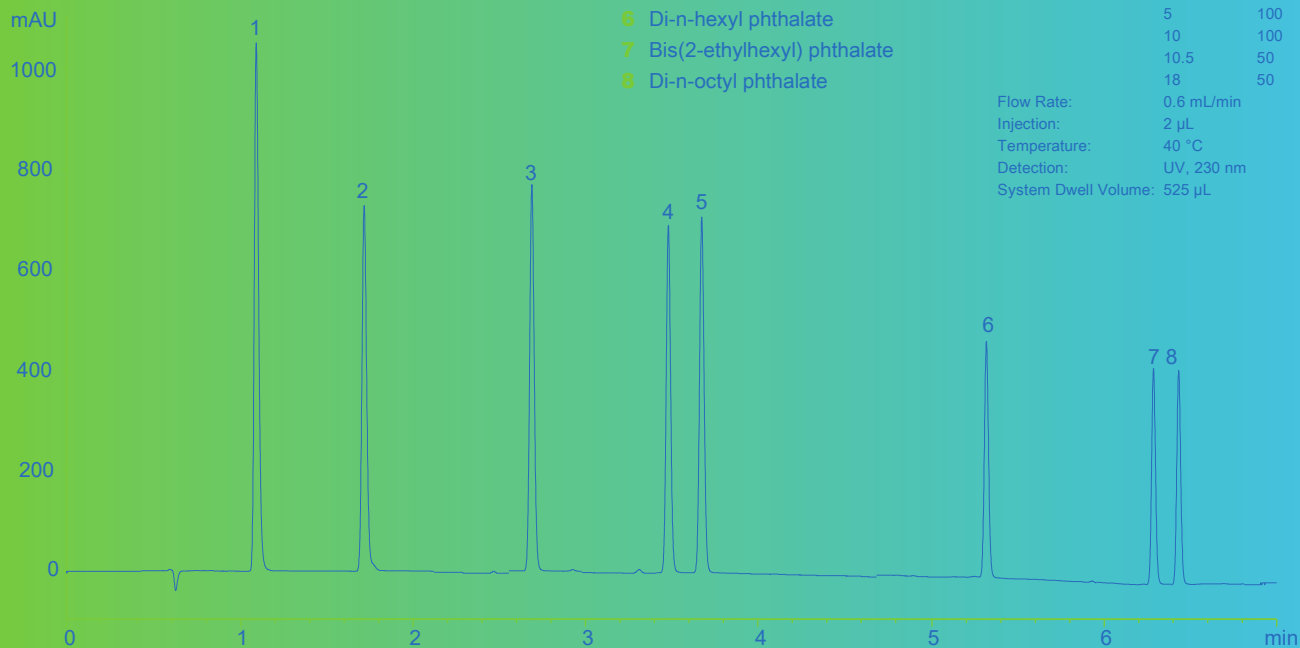
- 1 Dimethyl phthalate
- 2 Diethyl phthalate
- 3 Di-n-propyl phthalate
- 4 Benzyl butyl phthalate
- 5 Di-n-butyl phthalate
- 6 Di-n-hexyl phthalate
- 7 Bis(2-ethylhexyl) phthalate
- 8 Di-n-octyl phthalate

TESTING CONDITIONS

Column: UltraCore C18
 Dimensions: 100 x 3.0 mm
 Mobile Phases: **A:** 0.1% Formic acid in H₂O
B: 0.1% Formic acid in MeCN
 Gradient: **Time (mins)**

0	50
5	100
10	100
10.5	50
18	50

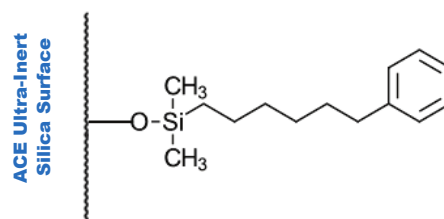
 Flow Rate: 0.6 mL/min
 Injection: 2 µL
 Temperature: 40 °C
 Detection: UV, 230 nm
 System Dwell Volume: 525 µL



Avantor® ACE® UltraCore

The Phenylhexyl phase is especially well suited for retention of aromatic compounds where π - π interactions can be exploited.

- Ideal for analytes with π -bonding
- Complementary selectivity to other UltraCore bonded phases

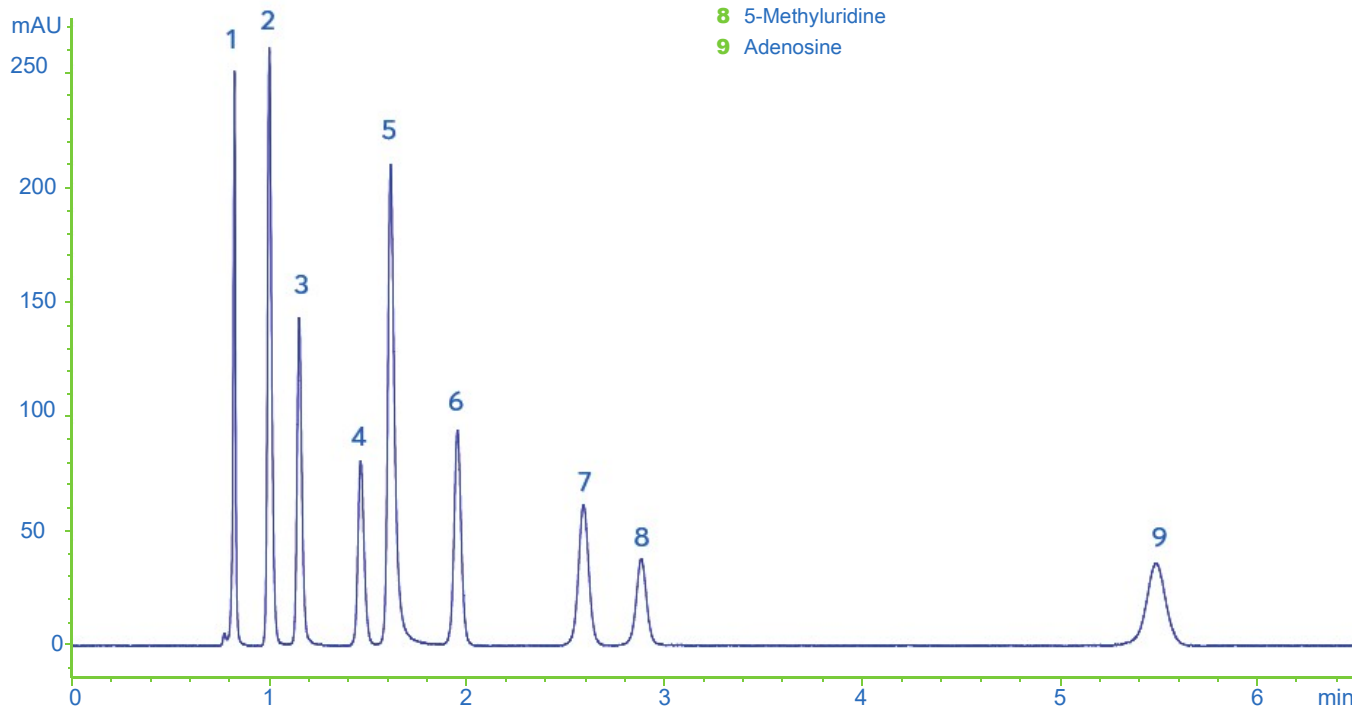


Phase	USP Listing	Functional group	End-capping	Particle size (μm)	Pore size (\AA)	Surface area (m^2/g)	Carbon load (%)	Low pH/T limit	High pH/T limit	100% aqueous compatible
UltraCore Phenylhexyl	L11	Phenylhexyl	Yes	3.5	95	115	6.2	2/60°C	9/40°C	Yes

SEPARATION OF NUCLEOSIDES & NUCLEOBASES

- 1 Cytosine
- 2 Uracil
- 3 Guanine
- 4 Uridine
- 5 Adenine
- 6 Thymine
- 7 Guanosine
- 8 5-Methyluridine
- 9 Adenosine

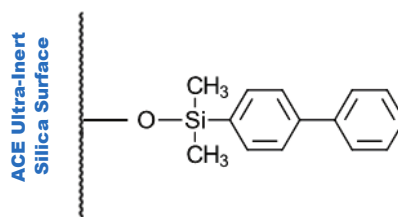
Column: UltraCore 3.5 Phenylhexyl
 Dimensions: 100 x 3.0 mm
 Mobile Phase: 10 mM ammonium formate pH 4.2 in H₂O
 Flow Rate: 0.6 mL/min
 Injection: 3 μL
 Temperature: 40 °C
 Detection: UV, 254 nm



Avantor® ACE® UltraCore

This biphenyl phase is especially well suited for retention of aromatic compounds that elute early on alkyl phases.

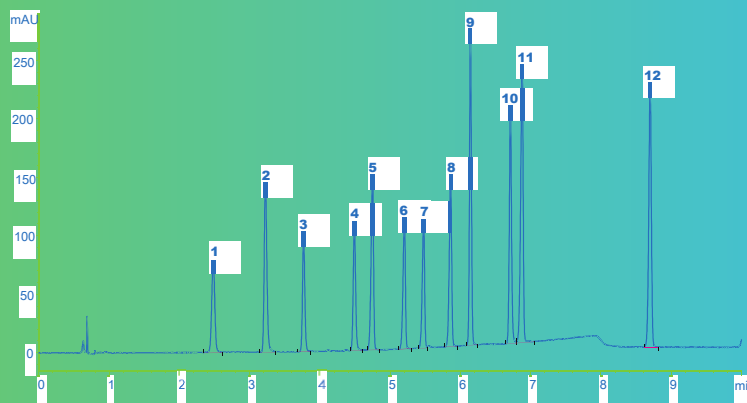
- Ideal for analytes with π -bonding, electron withdrawing or delocalised groups
- Alternative selectivity to alkyl phases and traditional phenyl phases



Phase	USP Listing	Functional group	End-capping	Particle size (µm)	Pore size (Å)	Surface area (m ² /g)	Carbon load (%)	Low pH/T limit	High pH/T limit	100% aqueous compatible
UltraCore Biphenyl	L11	Biphenyl	Yes	3.5	95	115	6.5	2/60°C	9/40°C	Yes

SEPARATION OF BENZIMIDAZOLES

Column: UltraCore 3.5 Biphenyl
 Dimensions: 100 x 3.0 mm
 Mobile Phases: **A:** 20 mM KH₂PO₄ pH 6.5 in H₂O
B: 20 mM KH₂PO₄ pH 6.5 in MeCN/H₂O 70:30 v/v
 Gradient: **Time (mins) %B**
 0 25
 10 100
 12 100
 13 25
 20 25



- 1 Carbendazim
- 2 Thiabendazole
- 3 Amino Mebendazole
- 4 Cambendazole
- 5 Oxibendazole
- 6 Mebendazole
- 7 Flubendazole
- 8 Albendazole
- 9 Triclabendazole Sulphone
- 10 Triclabendazole Sulphoxide
- 11 Fenbendazole
- 12 Triclabendazole

ORDERING INFORMATION

Avantor® ACE® Ultracore 3.5µm particle sizes

HPLC hardware format with 6000 bar / 9,000 psi pressure limit

Column dimensions	C18	Phenylhexyl	Biphenyl
2.1 x 50 mm	CORE-35F-521	CORE-35G-521	CORE-35D-521
3.0 x 50 mm	CORE-35F-530	CORE-35G-530	CORE-35D-530
4.6 x 50 mm	CORE-35F-546	CORE-35G-546	CORE-35D-546
2.1 x 100 mm	CORE-35F-1021	CORE-35G-1021	CORE-35D-1021
3.0 x 100 mm	CORE-35F-1030	CORE-35G-1030	CORE-35D-1030
4.6 x 100 mm	CORE-35F-1046	CORE-35G-1046	CORE-35D-1046
2.1 x 150 mm	CORE-35F-1521	CORE-35G-1521	CORE-35D-1521
3.0 x 150 mm	CORE-35F-1530	CORE-35G-1530	CORE-35D-1530
4.6 x 150 mm	CORE-35F-1546	CORE-35G-1546	CORE-35D-1546

HPLC hardware format with 6000 bar / 9,000 psi pressure limit

Column dimensions	C18	Phenylhexyl	Biphenyl
Guards (for 2.1 mm id columns) 3 pk*	CORE-35F-21GD	CORE-35G-21GD	CORE-35D-21GD
Guards (for 3.0 mm id columns) 3 pk*	CORE-35F-30GD	CORE-35G-30GD	CORE-35D-30GD
Guards (for 4.6 mm id columns) 3 pk*	CORE-35F-46GD	CORE-35G-46GD	CORE-35D-46GD

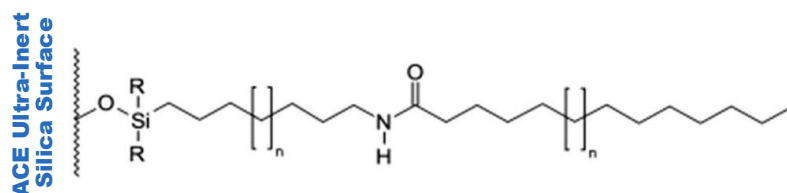
*Guards require holder BIO-GRD-0001

For any application support or additional questions please contact chromsupport@avantorsciences.com

Avantor® ACE® UltraCore

The C18-Amide phase incorporates additional mechanisms of interaction such as H-bonding to give an alternative selectivity for moderately polar analytes.

- Can retain more polar compounds in 100% aqueous mobile phase
- Alternative selectivity to alkyl phases



Phase	USP Listing	Functional group	End-capping	Particle size (µm)	Pore size (Å)	Surface area (m ² /g)	Carbon load (%)	Low pH/T limit	High pH/T limit	100% aqueous compatible
UltraCore C18-Amide	L60	Polar Embedded Amide	Yes	3.5	95	120	5.5	2/60°C	9/40°C	Yes

SEPARATION OF PHENOLIC COMPOUNDS

Column: Avantor® ACE® UltraCore C18-Amide

Dimensions: 100 x 3.0 mm

Mobile Phases: **A:** 0.1% Formic acid in H₂O

B: 0.1% Formic acid in ACN

Gradient:	Time (mins)	%B
	0	20
	20	40
	22	95
	24	95
	25	20
	35	20

Flow Rate: 0.43 mL/min

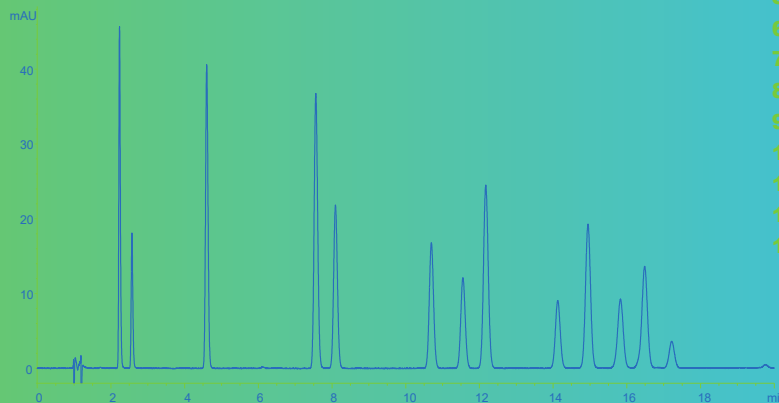
Injection: 5 µL

Temperature: 40 °C

Detection: UV, 274 nm

System Dwell

Volume: 525 µL



- 1 Resorcinol
- 2 Catechol
- 3 Phenol
- 4 m-Cresol
- 5 o-Cresol
- 6 3,4-Xylenol
- 7 3,5-Xylenol
- 8 2,4-Xylenol
- 9 3,4,5-Trimethylphenol
- 10 2-Naphthol
- 11 2,4,6-Trimethylphenol
- 12 2,3,5-Trimethylphenol
- 13 1-Naphthol

ORDERING INFORMATION

Avantor® ACE® UltraCore C18-Amide 3.5µm particle size

HPLC hardware format with 6000 bar / 9,000 psi pressure limit

Column dimensions	Catalogue number
2.1 x 50 mm	CORE-35E-521
3.0 x 50 mm	CORE-35E-530
4.6 x 50 mm	CORE-35E-546
2.1 x 100 mm	CORE-35E-1021
3.0 x 100 mm	CORE-35E-1030
4.6 x 100 mm	CORE-35E-1046
2.1 x 150 mm	CORE-35E-1521
3.0 x 150 mm	CORE-35E-1530

HPLC hardware format with 6000 bar / 9,000 psi pressure limit

Column dimensions	Catalogue number
4.6 x 150 mm	CORE-35E-1546
Guards (for 2.1 mm id columns) 3pk*	CORE-35E-21GD
Guards (for 3.0 mm id columns) 3pk*	CORE-35E-30GD
Guards (for 4.6 mm id columns) 3pk*	CORE-35E-46GD

*Guards require holder BIO-GRD-0001

For any application support or additional questions please contact chromsupport@avantorsciences.com