HPLC and UHPLC Column Selection Guide

The comprehensive Supelco® portfolio of analytical solutions to meet your U/HPLC and LC-MS needs

Supelco®
Analytical Products

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The Supelco® portfolio of analytical solutions is developed by analytical chemists for analytical chemists to ensure your results are accurate, precise and reproducible. Every product is meticulously quality controlled to maintain the integrity of your testing protocols and, with our dedicated scientists, the expertise you need is always on hand.

We provide a premier selection of proven analytical tools and consumables that meet the requirements of scientists who primarily use HPLC and LC-MS for separation and analysis of drugs and biomolecules, or for other analytical assays. Our selection of columns, solvents, standards and sample preparation products are exclusively designed for HPLC and LC-MS, meeting the critical need for purity.

HPLC and LC-MS Workflow

Sample Collection

- Full Labware Portfolio
- Pipettes, Pipettors and Tips
- Multiwell Plates
- Microcentrifuge Tubes
- Vials

Sample Preparation

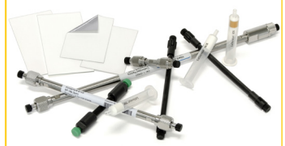
- SPE Cartridges and 96-well Plates
- BioSPME 96-pin device
- QuEChERS (dispersive SPE)
- Dispersive Pipette Extraction (DPX) Tips
- SLE Cartridges
- Millex® Filters
- Samplicity® Filtration System

Standardization & Calibration

- Certified Reference Materials
- Reference Materials

Chromatographic Separation & Analysis

- U/HPLC-MS Columns
- U/HPLC-MS Solvents
- LC-MS Reagents
- TLC-MS
- Accessories



HPLC Columns

Supelco® HPLC and UHPLC Columns

Our HPLC & UHPLC column portfolio meets today's challenging needs of fast HPLC including UHPLC, LC-MS, biopolymer separation, high pH conditions, as well as traditional pharmacopeia and agency methods within pharmaceutical, environmental, clinical, and food industries etc. – from nano LC to preparative applications

Fused-Core® technology, monolithic silica, ultra-pure silica, and polymeric particles are some of the particle platforms that make up the Supelco® HPLC product line so you can find the right column for your specific application.

We have a tradition of providing innovative HPLC Columns; our trusted SUPELCOSIL™, LiChrospher®, Discovery®, Ascentis®, SeQuant® and Purospher® columns have a proven track record. Our Purospher® STAR columns deliver leading edge UHPLC performance at high quality standards, and Ascentis® Express and BIOshell™ columns (based on Fused-Core® technology) have the capability to turn any HPLC system into a fast HPLC workhorse. Chromolith® HPLC and UHPLC columns, based on monolithic silica, enable rapid separations at extremely low column backpressure and provide a very high matrix-tolerance making this material suitable for the separation of matrix-rich samples.

Our broad range of chiral HPLC & LC-MS columns, derivatization reagents and mobile phase additives make us one of the leaders in chiral chromatography technology. Our comprehensive range of well-respected brands includes Astec® CHIROBIOTIC®, CYCLOBOND™, ChiraDex®, and CLC columns.

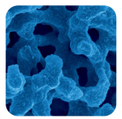
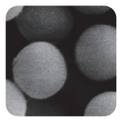
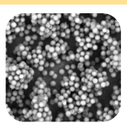
We can help you find a solution for your chiral separation application, including clinical, food, environmental, or drug discovery. Depending on your chromatography needs, our chiral columns can be used with several of the most common mobile phase modes, and many are suitable for LC-MS.

NEW

Supel™ Carbon HPLC columns enable the use of extreme pH and temperature without a compromise in efficiency. This column is an excellent choice for the retention and separation of polar compounds using reversed phase conditions

Supel™ Carbon HPLC Columns

Stationary Phase

		Stationary phase base material	Methods / Use			Separation mode	Solution for small molecule separation		Page	Solution for Biomolecule separation	Page
Silica based stationary phases											
Type B $Si(OC_2H_5)_4$ $[-Si(OC_2H_5)_2 - O - Si(OC_2H_5)_2 - O -]_n$ $[-Si(O)_2 - Si(O)_2 - O -]_n$ SiO_2 • NO Metal content • Provides optimal peak shape for basic and chelating compounds		Superficially porous silica particles (SPP) / Fused-Core® SPP - Superficially porous silica HPLC and UHPLC columns provide very high efficiencies which are typically 40% higher in comparison to fully porous particles of the same particle size.	First choice for new methods under development or during method transfer in Pharma. Excellent for UHPLC applications (2 µm particles)	Silica SPP		Reversed Phase HILIC	**Ascentis® Express** HPLC columns UHPLC columns Capillary columns	MS ☆	28	**BIOshell™** HPLC columns UHPLC columns Capillary columns MS ☆	41
		Monolithic silica Monolithic silica HPLC, UHPLC and semi-preparative columns enable rapid separations at very low column back-pressure with high matrix-tolerance and extended column lifetime.	Best choice for matrix-rich samples and applications where lifetime and robustness are a concern. Great choice for rapid separations at low column backpressure.	Monolithic silica		Reversed Phase HILIC Affinity	**Chromolith®** HPLC columns Capillary columns Semi-preparative columns	MS ☆	51	**Chromolith® WP 300** HPLC columns MS ☆	58
		**Fully porous silica particles (FPP)** **Type B (high purity silica)** Fully porous silica particles provide the full loadability of the stationary phase due to its fully porous physical characteristics. This aspect ensures high sensitivities because the peak broadening effect of overloading the stationary phase is minimized. Type B silica particles are produced from tetraalkoxysilane in a sol-gel process. This metal free stationary phase base material can be used for the analysis of acidic, basic, and chelating compounds, providing excellent peak symmetries with less need for strong buffer concentrations.	For established HPLC methods, for special selectivities such as HILIC and Chiral as well as columns for biomolecule separation. These columns are in use in thousands of methods and ensure reliable results over the complete range of use, particle sizes and column dimensions in Nano-LC (Capillary columns) UHPLC Analytical HPLC Semi-preparative LC Preparative LC	Silica FPP		Reversed Phase HILIC Normal phase	**Purospher® STAR** HPLC and UHPLC columns **Discovery®** HPLC columns **Ascentis®** HPLC columns **SeQuant®** HILIC HPLC columns and capillary columns	MS ☆ MS MS ☆ MS ☆	66 74 80 84	**Discovery® BIO** HPLC columns	78
				Silica FPP		Chiral	**Astec®** Chiral HPLC and U/HPLC columns		96		
				Silica FPP		Size Exclusion Ion Exchange HIC				**Sepax** U/HPLC columns **TSKgel®** U/HPLC columns	104 109
Type A $M_2O - nSiO_2$ M=Na, K, Fe $[-Si(OH)_2 - O - Si(OH)_2 - O -]_n$ $[-Si(O)_2 - Si(O)_2 - O -]_n$ SiO_2 (Na, K, Fe) • Contains metals • Peak-Tailing for basic and chelating compounds		**Fully porous silica particles (FPP)** **Type A (conventional silica)** Spherical Traditional silica made from sodium waterglass established in many applications and methods.		Silica FPP		Reversed Phase Normal Phase HILIC Ion Exchange	**LiChrospher®** HPLC columns Semi-preparative columns **Superspher®** HPLC columns **SUPELCOSIL™** HPLC columns Semi-preparative columns		88 90 92		
		**Irregular** Reliable stationary phase providing same properties for HPLC and large scale preparative LC (bulk sorbent) as well as TLC		Silica FPP irregular		Reversed Phase Normal Phase	**LiChrosorb®** HPLC columns Semi-preparative columns Bulk sorbent		91		
Other stationary phase materials											
		**Carbon particles** Fully porous graphitic carbon (PGC) particles manufactured using a patented synthetic process, enable extreme pH and temperature stability without a compromise in efficiency.	Excellent choice for the retention and separation of polar compounds using reversed phase conditions.	Porous Graphitic Carbon		Reversed Phase	**Supel™ Carbon LC**	MS ☆	116	Supel™ Carbon LC MS ☆	116
		Fully porous polymeric particles Enable the use of the full pH-range for the mobile phase (0-14)		Polymeric FPP		HILIC Reversed Phase Size Exclusion Ion Exchange HIC	**apHera™** HPLC columns **SUPELCOGEL™** HPLC columns **Hamilton®** HPLC columns **SeQuant®** ZIC®-pHILIC	MS ☆	121 84	**TSKgel®** HPLC and UHPLC columns	109

Note: As chromatographic separation depends on many physical and chemical parameters, we cannot guarantee the success of a separation based on the recommended column modification.

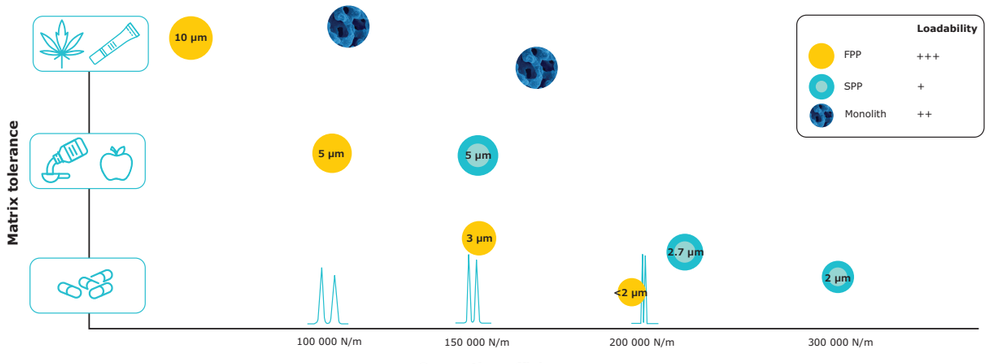
☆ 1st choice for method development MS Recommended for LC-MS use

Selection of Stationary Phase Carrier by Their Benefits

The performance of HPLC columns has improved dramatically in recent years, particularly in terms of separation power as measured by the number of theoretical plates per meter. The improvement in performance has been achieved primarily by a reduction in particle size.

Superficially Porous Particles (SPP) show higher separation efficiencies than Fully Porous Particles (FPP) with the same particle size. To achieve this high efficiency, it is essential to avoid any column overloading that could cause peak broadening effects.

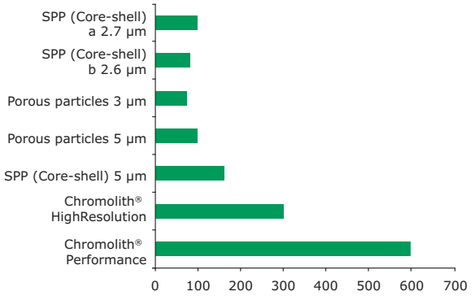
HPLC columns packed with small and very small particles can be plugged or blocked more easily. Matrix-rich samples require extensive sample preparation when analyzed with HPLC and UHPLC columns using small particles. This added sample preparation step is a substantial time and cost factor. When analyzing challenging, matrix-rich samples, the benefit of monolithic silica columns is significant.



Need for Speed

Plates per pressure (N/bar)

The chromatographic support's capability to generate a high number of plates (efficient separation) at relatively low column back pressure might be demonstrated by this artificial term "plates per pressure". It is obtained by dividing column efficiency by the column back pressure at the moment of peak elution.



All columns are C_{18} modified, 100 x 4.6 mm I.D. Sample: anthracene, eluted isocratically using acetonitrile/water (60/40) at 2 mL/min flow rate. Injection volume: 5 µL, detection at 254 nm UV. All analyses performed at room temperature.

The development of faster separation processes is one of the most important issues in HPLC. Particularly in industry, chromatographers wish to speed up separations, and analyze more samples with the limited financial and human resources available. One of the main issues preventing speed is backpressure. With conventional particle-packed HPLC columns, higher efficiency always comes at the expense of higher back pressure. Even core-shell particle (equal to SPP) columns, which are designed for lower resistance, still exhibit rather high back pressure. Hence, the task is to minimize back pressure in order to maximize speed.

HPLC Columns

Selection of Stationary Phase Bonding by Compound Class

Selecting the most suitable column is highly dependent upon the sample undergoing analysis. Compound structure, solubility, and log P values of analytes all need to be taken into consideration when selecting column phase chemistry and mode of separation. While compounds can often be separated using various column chemistries, some column selectivities are better suited than others for certain compound classes. The table below shows a selection of classes of compounds typically analyzed by HPLC methods.

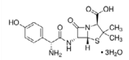
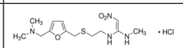
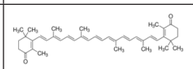
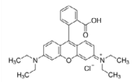
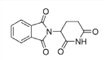
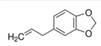
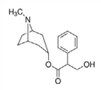
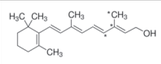
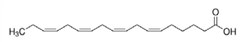
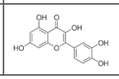
Compound Class	ZIC®-HILIC	ZIC®-cHILIC	ZIC®-pHILIC	NH2	Si	OH5	DIOL	CN	F5	RP-Amide	Propyl Phenyl	Phenyl Hexyl	Biphenyl	RP-4	RP-8	RP-8e	RP-18	RP-18e	PAH	C30
Aflatoxins					2										2	2	1	1		
Alcohols	1	1		2		1	2	2		2							2	2		
Aldehydes											2	2			2	2	1	1		
Alkaloids	2	2							1		2	2			2	2	1	1		
Aliphatic amines	1	2							1	1							2	2		
Amino Acids	1	1				1	1								2	1	2	2		
Antibiotics	2	2							1		2	2	1	2	2	2	1	1		
Aromatic amines	1	2							1		1	1	1				2	2		
Carboxylic acids	2	1	2	1		2				1										1
Carotenoids					1		1								2	2	2	2		
Catecholamines										2							1	1		
Explosives										2							1	2		
Oils					1		2	2									2	2		
Oligonucleotides																	1	1		
Esters	2	2				2									2	1	1	1		
Fat soluble vitamins				1	1						2	2	2				1	1		1
Lipids	2	2			1												1	1		
Fatty acids					1										2	2	1	1		
Flavonoids			2		2			1			1	1	1				1	1		
Glycans	1	1	1		2	2											1	1		
Glycols	1	1			2	1	2	2												
Inorganic ions	1	1	1	2																
Ketones					2						2	2			2	2	1	1		
Nitrosamines	1	1				1									2	2	1	1		
Nucleosides															2	2	1	1		
Nucleotides	1	1	1	1	2						2	2					2	2		
PAH											1	1					1	1	1	
PCB											2	2			2	2	1	1		
Peptides	1	1	2			1					1	1		2	2	2	1	1		
Pesticides	2	2											2				1	1		
Phenols			2					1	1	1					2	2	1	1		
Phospholipids	1	1			2		2			2							2	2		
Phthalates								1									1	1		
Preservatives											1	1	2				1	1		
Proteins													2	1	1	1	2	2		
Organic phosphates	1	1															2	2		
Steroids				1	2			1			2	2	2		2	2	1	1		
Metabolized steroids	2	2									2	2	2		2	2	1	1		
Sugars	2	2	1	1		2	1			1										
Sugar Alcohols	1	1	2	2		1														
Sulfonamides	2					2	2	2									1	1		
Sweeteners	1	1		1		1					2	2	2				1	1		
Water soluble vitamins	1	1		1		1	1										1	1		

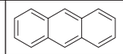
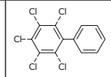
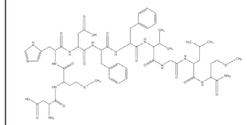
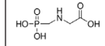
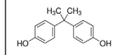
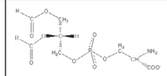
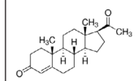
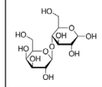
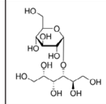
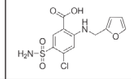
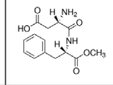
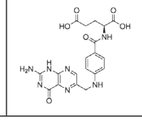
1 Most suitable column chemistry 2 Possible alternative column chemistry

HPLC Columns

Selection by Chemical Structure of the Analyte Using the Analyte log P Value

The selection of the most appropriate stationary phase depends on the chemical structure of the compound to be separated. One important parameter that describes the chemical structure of a compound is the log P value (water octanol logarithmic partition coefficient). This table shows the log P value of representative compounds of important analyte groups.

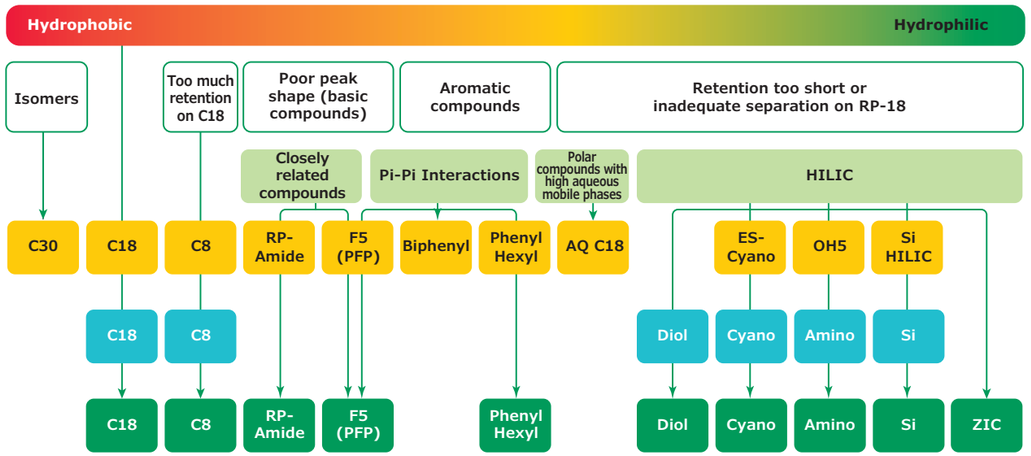
	Analyte group	Example	Structure	log P value
A	Aflatoxins	Aflatoxin G1		1.8
	Alcohols	Ethyl alcohol	CH_3CH_2OH	-0.1
	Aldehydes	Benzaldehyde		1.5
	Alkaloids	Quinine		2.9
	Amino Acids	Aspartic acid		2
	Antibiotics	Amoxicillin		-2
		Ranitidine		0.3
	Aromatic amines	Aniline		0.9
C	Carboxylic acids	Glucuronic acid		-2.3
	Carotenoids	Canthaxanthin		11.4
D	Dyes	Rhodamine		4.4
E	Enantiomers	Thalidomide		0.3
	Essential oils	Safrole		3
	Esters	Atropine		1.8
F	Fat soluble vitamins	Retinol		5.7
	Fatty acids	Stearidonic acid		5.9
	Flavonoids	Quercetin		1.5

	Analyte group	Example	Structure	log P value
G	Glycols	Ethylene glycol	$HOCH_2CH_2OH$	-1.4
I	Inorganic ions	Chloride	Cl^-	0.8
K	Ketones	Cyclohexanone		0.8
N	Nitrosamines	N-Nitrosodimethylamine		-0.6
P	PAH	Anthracene		4.4
	PCB	Polychlorinated biphenyls		7.3
	Peptides	Neurokinin B		-1.6
	Pesticides	Glyphosate		-4.6
	Phenols	Bisphenol A		2.2
	Phospholipids	Phosphatidylserine		-3.5
S	Steroids	Progesterone		3.9
	Sugars	Lactose		-4.7
	Sugar Alcohols	Maltitol		-5.2
	Sulfonamides	Furosemide		2
	Sweeteners	Aspartame		-2.7
W	Water soluble vitamins	Folic Acid		-1.1

Selection of Chromatographic Mode and Stationary Phase by log P Value

C18 is usually the first choice for starting a new method. However, when a C18 does not give the desired separation, or your sample contains compounds that are known to be difficult to retain or resolve on a C18, then you should consider changing the stationary phase base material, modification or both.

If a compound is predominantly hydrophobic with a positive log P value (water octanol logarithmic partition coefficient), then the use of a reversed phase column is recommended. For low/medium polarity analytes, normal phase HPLC or HILIC are viable techniques.



(SPP) Ascentis® Express (monolithic) Chromolith® (FPP) Purospher® STAR, Discovery®, Ascentis®, SeQuant®

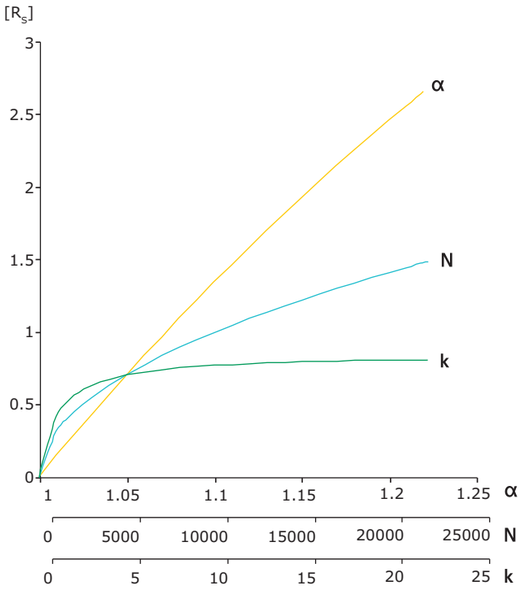
Choose the Right HPLC Column

Chromatographic resolution is most influenced by the selectivity (α) (when k>2 and N> 3000). Changing the mobile phase composition or the stationary phase is the most powerful way of optimizing selectivity whereas the particle size, pore size, length of the column, temperature, or mobile phase strength have much less effect. Therefore, if satisfactory results are not met, or no retention is achieved, it is better to change to another selectivity using a different column type and/or a different mobile phase.

Resolution is Mainly Controlled by Selectivity

Resolution (R_s or R) is impacted by three parameters (k, α, and N) which are directly related to experimental conditions. k is the average retention factor for the two bands, N is the number of theoretical plates and α is the separation factor (or selectivity factor).

The parameters k and α are determined by the experimental conditions (composition of the mobile phase; stationary phase chemistry and temperature), and N is affected by column length, particle size and pore size.



HPLC Columns

Selection by Specific Chromatographic Need (C18)

Prioritizing specific needs helps to select the best suitable column material for different chromatographic demands. Depending on the sample, analyte or matrix, the lab environment (e.g. instrumentation) and the separation goal, the best column choice can be very different from task to task. While the selection of the column chemistry is the first step in this process, the next step is to determine the most suitable column material. Many different selectivities are available based on different column materials. Nevertheless, C18 is still the most widely used column modification. The table below lists a selection of specific needs in HPLC on column materials with C18 modifications. The ranking is from 1 (lowest ranking) to 5 (highest ranking).

	Fused® Core Silica (SPP)						Monolithic Silica		Fully porous silica particles (FPP) Type B								FPP Type A							
Need	Ascentis® Express			Ascentis® Express			Chromolith®		Purospher® STAR			Discovery®			Ascentis®		Supersphер®	LiChrospher®		SUPELCOSIL™				
	C18			AQ C18			RP-18e	HR RP-18e	RP-18e			C18	HS-C18		C18		RP-18 (e)	RP-18 (e)		LC-18		LC-18-DB		
Particle size (µm)	2	2.7	5	2	2.7	5			2	3	5	5	3	5	3	5	10	4	5	10	3	5	12	3	5
Separation efficiency	5	5	4	5	5	4	3	4	5	4	3	2	3	2	1	4	3	2	3	2	1	3	2	1	3	2
Peak symmetry	5	5	5	5	5	5	3	4	5	5	5	3	3	3	3	4	4	4	3	3	3	3	3	3	3	3
Need for sample preparation	1	2	3	1	2	3	5	4	1	2	3	3	2	3	4	2	3	4	2	3	4	1	2	3	1	2
Lifetime (for Matrix-rich samples)	1	2	3	1	2	3	5	4	1	2	3	3	2	3	4	2	3	4	2	3	4	1	2	3	1	2
Lifetime (based on particle mechanical stability)	3	3	3	3	3	3	5	4	4	4	2	5	5	5	5	4	4	4	5	5	5	5	5	5	5	5
100% Aqueous mobile phase compatibility	1	1	1	5	5	5	1	1	5	5	5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
pH stability (range)	3	3	3	3	3	3	1	2	4	4	4	2	2	2	2	2	2	2	1	1	1	2	2	2	2	2
Bleeding (for MS)	5	5	5	5	5	5	5	5	4	4	4	3	3	3	3	5	5	5	2	2	2	3	3	3	3	3
Reproducibility (Column-to-Column)	4	4	4	4	4	4	3	4	4	4	5	4	4	4	4	4	4	4	3	5	3	3	3	3	3	3
Column Back Pressure	1	2	3	1	2	3	5	4	1	2	3	3	2	3	4	2	3	4	2	3	4	2	3	4	2	3
Useable flow rate ranges	5	4	2	5	4	2	5	5	5	3	2	2	3	2	1	3	2	1	2	2	1	3	2	1	3	2
Loadability	3	3	3	3	3	3	4	4	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Quantitation - linear response range	4	4	4	4	4	4	4	5	5	5	5	3	4	3	3	4	3	3	3	3	3	3	2	2	3	2
Sample throughput	5	5	4	5	5	4	5	5	5	4	3	3	4	3	2	4	3	2	3	2	4	4	3	2	4	3
UHPLC use (Stability at high pressure)	5	4	4	5	4	4	1	1	5	5	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Temperature stability	4	4	4	4	4	4	3	3	5	5	5	5	5	5	5	5	5	5	4	4	4	5	5	5	5	5
Up-Scalability (above 4.6 mm id)	1	1	1	1	1	1	5	1	1	1	4	1	1	3	4	1	4	5	1	5	5	1	4	5	1	4
Down-Scalability (to below 1 mm id)	1	5	1	1	5	1	5	3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Criteria - Ranking:

Separation efficiency (N/m)	Temperature (Max °C)
1 = < 50,000	1 = 30
2 = 50,000 - 75,000	2 = 40
3 = 75,001 - 100,000	3 = 50
4 = 100,001 - 150,000	4 = 60
5 = > 150,000	5 = 70

pH stability (range)	UHPLC (Stability at high pressure)
1 = 2-7.5	1 = 200 bar
2 = 2-8	2 = 400 bar
3 = 2-9	3 = 500 bar
4 = 1.5-10.5	4 = 600 bar
5 = 1.5-12	5 = 1000 bar

HPLC Columns

Validation kits*

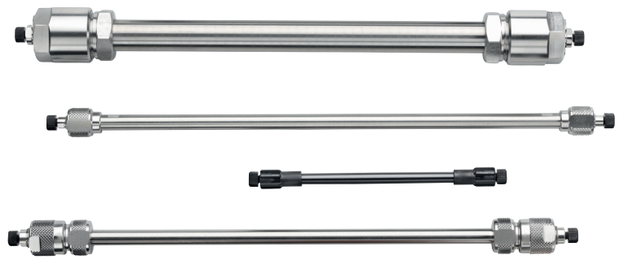
The success of an HPLC method depends strongly on the consistent quality of the stationary phase. Long-term reproducibility is a key factor in achieving reliable results. Supelco® validation kits consist of three HPLC columns, packed with three different sorbent lots to confirm the reliability of HPLC methods and their robustness.

* In case the needed column dimension or stationary phase is not availabe from stock, please send a request to "Custom HPLC Column Request."

Selection by USP Classification

HPLC Packings for USP Compendial Methods

The following list describes the main USP classes and the corresponding Supelco® stationary phases. The official pharmaceutical analysis monographs in the United States Pharmacopeia (USP) detail the methods used by pharmaceutical manufacturers for quality control of bulk drug substances and dosage form preparations. Each method specifies a particular high pressure liquid chromatography (HPLC) or gas chromatography (GC) column or column type and the conditions under which the analysis is performed. This table lists the USP Codes for the HPLC phases used in these methods, descriptions of the columns, and information about our products that comply to these descriptions.



USP Code [1]	Description [1]	Available Columns [2] [3]				
		Particles				Monolithic
		Fused-Core® Silica Particles	Fully Porous Silica Particles		Non-Silica Particles	Silica Based
		Type B Silica [4]	Type B Silica [4]	Type A Silica [5]		Type B Silica [4]
L1	Octadecyl silane chemically bonded to porous or nonporous silica particles, superficially porous particles, or ceramic microparticles, 1.5–10 µm in diameter, or a monolithic silica rod.	Ascentis® Express C18 ★ BIOshell™ Peptide C18 ★ Ascentis® Express PAH ★ Ascentis® Express PFAS ★ BIOshell™ IgG C18 ★ Ascentis® Express AQ C18 ★ Ascentis® Express PCS C18 BIOshell™ PCS C18 Ascentis® Express ES C18 ★ BIOshell™ Protein C18 ★	Ascentis® C18 Discovery® C18 Discovery® HS C18 Discovery® BIO Wide Pore C18 Purospher® RP-18e Purospher® RP-18 Purospher® STAR RP-18e ★	LiChrosorb® RP-18 LiChrospher® RP-18e LiChrospher® RP-18 LiChrospher® PAH SUPELCOSIL™ LC-18 SUPELCOSIL™ LC-18-DB SUPELCOSIL™ LC-18-S SUPELCOSIL™ LC-18-T Supersper® RP-18e Supersper® RP-18		Chromolith® CapRod® HighResolution RP-18e Chromolith® CapRod® RP-18e Chromolith® HighResolution RP-18e ★ Chromolith® Performance RP-18e Chromolith® Prep RP-18e Chromolith® SemiPrep RP-18e Chromolith® WP 300 RP-18 ★
L3	Porous silica particles or superficially porous particles, 1.5–10 µm in diameter, or a monolithic silica rod.	Ascentis® Express HILIC ★	Ascentis® Si Purospher® STAR Si ★	LiChrosorb® Si 60 LiChrospher® Si 60 SUPELCOSIL™ LC-Si SUPELCOSIL™ LC-3Si Supersper® Si 60		Chromolith® Performance Si Chromolith® Prep Si Chromolith® SemiPrep Si

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HPLC Packings for USP Compendial Methods

USP Code [1]	Description [1]	Available Columns [2] [3]				
		Particles				Monolithic
		Fused-Core® Silica Particles	Fully Porous Silica Particles		Non-Silica Particles	Silica Based
		Type B Silica [4]	Type B Silica [4]	Type A Silica [5]		Type B Silica [4]
L7	Octylsilane chemically bonded to totally or superficially porous silica particles, 1.5 to 10 µm in diameter, or a monolithic silica rod.	Ascentis® Express C8 ★	Ascentis® C8 Discovery® C8 Discovery® BIO Wide Pore C8 Purospher® STAR RP-8e ★	LiChrosorb® RP-8 LiChrospher® RP-8e 100 LiChrospher® RP-8 100 LiChrospher® RP-Select B 60 SUPELCOSIL™ LC-8 SUPELCOSIL™ LC-8-DB Supersper® RP-8e 60 Supersper® RP-8 60 Supersper® RP-Select B 60		Chromolith® CapRod® RP-8e Chromolith® HighResolution RP-8e ★ Chromolith® Performance RP-8e Chromolith® WP 300 RP-8 ★
L8	An essentially monomolecular layer of aminopropylsilane chemically bonded to totally porous silica gel support, 1.5 to 10 µm in diameter, or a monolithic silica rod.		Purospher® STAR NH_2 ★	LiChrospher® NH_2 100 SUPELCOSIL™ LC-NH_2		Chromolith® NH_2
L9	Irregular or spherical, totally porous silica gel having a chemically bonded, strongly acidic cation-exchange coating, 3 to 10 µm in diameter.		*TSKgel® SP-2SW ★	SUPELCOSIL™ LC-SCX		
L10	Nitrile groups chemically bonded to porous silica particles or superficially porous particles, 1.5–10 µm in diameter, or a monolithic silica rod.	Ascentis® Express ES-Cyano ★ BIOshell™ Peptide CN ★	Ascentis® ES Cyano Discovery® Cyano	LiChrospher® CN SUPELCOSIL™ LC-CN		Chromolith® CN
L11	Phenyl groups chemically bonded to porous or superficially porous silica particles, 1.5–10 µm in diameter, or a monolithic silica rod.	Ascentis® Express Phenyl-Hexyl ★ Ascentis® Express PCS Phenyl-Hexyl BIOshell™ IgG Diphenyl ★	Ascentis® Phenyl ★ Purospher® STAR Phenyl ★	SUPELCOSIL™ LC-DP		
L13	Trimethylsilane chemically bonded to porous silica particles, 3 to 10 µm in diameter.		*TSKgel® TMS-250 ★	SUPELCOSIL™ LC-1		
L14	Silica gel having a chemicallly bonded strongly basic quaternary ammonium anion-exchange coating, 5 to 10 µm in diameter.		*TSKgel® QAE-2SW ★	SUPELCOSIL™ SAX1		

*Tosoh Bioscience columns are available in select countries. For a list, please go to the page 109 of this brochure

HPLC Packings for USP Compendial Methods

USP Code [1]	Description [1]	Available Columns [2] [3]				
		Particles				Monolithic
		Fused-Core® Silica Particles	Fully Porous Silica Particles		Non-Silica Particles	Silica Based
		Type B Silica [4]	Type B Silica [4]	Type A Silica [5]		Type B Silica [4]
L17	Strong cation-exchange resin consisting of sulfonated cross-linked styrene-divinylbenzene copolymer in the hydrogen form, 6 to 12 µm in diameter.				Proteomix® WCX-NP10 SUPELCOGEL™ C-610H SUPELCOGEL™ H	
L19	Strong cation-exchange resin consisting of sulfonated cross-linked styrene-divinylbenzene copolymer in the calcium form, 5 to 15 µm in diameter.				Proteomix® SCX-NP5 Proteomix® SCX-NP10 SUPELCOGEL™ Ca	
L20	Dihydroxypropane groups chemically bonded to porous silica or hybrid particles, 1.5 to 10 µm in diameter, or a monolithic silica rod.		TSKgel®* QC-PAK GFC TSKgel®* SuperSW TSKgel®* SW TSKgel®* SW_{XL}	LiChrosorb® Diol LiChrospher® Diol SUPELCOSIL™ LC-Diol		
L21	A rigid, spherical styrene-divinylbenzene copolymer, 3 to 30 µm in diameter.				Hamilton® PRP-1 Hamilton® PRP-3 TSKgel®* SuperH TSKgel®* SuperHZ TSKgel®* H_{HR} TSKgel®* H_{XL}	
L22	A cation-exchange resin made of porous polystyrene gel with sulfonic acid groups, 5 to 15 µm in diameter.				Hamilton® PRP-X200 Hamilton® PRP-X300 SUPELCOGEL™ C-610H SUPELCOGEL™ H	
L25	Packing having the capacity to separate compounds with a molecular weight range from 100 – 5000 (as determined by polyethylene oxide), applied to neutral, anionic, and cationic water-soluble polymers.				TSKgel®* $G2500PW_{XL}$ TSKgel®* G2500PW TSKgel®* G2000PW TSKgel®* G1000PW	
L26	Butyl silane chemically bonded to totally porous or superficially porous silica particles, 1.5 to 10 µm in diameter.	BIOshell™ Protein C4 BIOshell™ IgG C4				Chromolith® WP 300 RP-4
L27	Porous silica particles, 30 to 50 µm in diameter.			Pelliguard LC-Si Supelclean™ LC-Si		

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*Tosoh Bioscience columns are available in select countries.
For a list, please go to the page 109 of this brochure

HPLC Columns

HPLC Packings for USP Compendial Methods (continued)

USP Code [1]	Description [1]	Available Columns [2] [3]				
		Particles				Monolithic
		Fused-Core® Silica Particles	Fully Porous Silica Particles		Non-Silica Particles	Silica Based
		Type B Silica [4]	Type B Silica [4]	Type A Silica [5]		Type B Silica [4]
L32	A chiral ligand-exchange resin packing-L-proline copper complex covalently bonded to irregularly shaped silica particles, 5 to 10 µm in diameter.		Astec® CLC-D Astec® CLC-L			
L33	Packing having the capacity to separate dextrans by molecular size over a range of 4,000 to 500,000 Da. It is spherical, silica-based, and processed to provide pH stability.		TSKgel®* $G2000SW_{XL}$ TSKgel®* $G4000SW_{XL}$			
L34	Strong cation-exchange resin consisting of sulfonated cross-linked styrene-divinylbenzene copolymer in the lead form, 7 to 9 µm in diameter.				SUPELCOGEL™ Pb	
L37	Packing having the capacity to separate proteins by molecular size over a range of 2,000 to 40,000 Da. It is a polymethacrylate gel.				TSKgel®* $G3000PW_{XL}$ TSKgel®* G3000PW	
L38	A methacrylate-based size-exclusion packing for water-soluble samples.				TSKgel®* SuperAW TSKgel®* PW TSKgel®* PW_{XL} TSKgel®* PW_{XL}-CL	
L39	A hydrophilic polyhydroxymethacrylate gel of totally porous spherical resin.				TSKgel®* SuperAW TSKgel®* PW TSKgel®* PW_{XL} TSKgel®* PW_{XL}-CL	
L40	Cellulose tris-3,5-dimethylphenylcarbamate coated porous silica particles, 3 to 20 µm in diameter.		Astec® Cellulose DMP			
L41	Immobilized α1-acid glycoprotein on spherical silica particles, 5 µm in diameter.		CHIRALPAK® AGP			

*Tosoh Bioscience columns are available in select countries.
For a list, please go to the page 109 of this brochure

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HPLC Columns

HPLC Packings for USP Compendial Methods

USP Code [1]	Description [1]	Available Columns [2] [3] — Particles: Fused-Core® Silica Particles — Type B Silica [4]	Particles: Fully Porous Silica Particles — Type B Silica [4]	Particles: Fully Porous Silica Particles — Type A Silica [5]	Particles: Non-Silica Particles	Monolithic: Silica Based — Type B Silica [4]
L43	Pentafluorophenyl groups chemically bonded to porous or superficially porous silica particles by a propyl spacer, 1.5–10 µm in diameter.	Ascentis® Express F5 ★	Discovery® HS F5			
L45	Beta cyclodextrin, R,S-hydroxypropyl ether derivative, bonded to porous silica particles, 3 to 10 µm in diameter.		Astec® CYCLOBOND™ I 2000 Series ★		ChiraDex® ChiraDex® HR	
L52	A strong cation exchange resin made of porous silica with sulfopropyl or sulfoethyl groups, 1 to 10 µm in diameter.			SUPELCOSIL™ LC-SCX ★		
L59	Packing for the size-exclusion separations of proteins (separation by molecular weight) over the range of 5 to 7,000 kDa. The packing is spherical 1.5 to 10 µm, silica or hybrid packing with a hydrophilic coating.		SRT SEC Series TSKgel®* SuperSW ★ TSKgel®* SW TSKgel®* SW_{XL} Unix series TSKgel® UP series Zenix® series			
L60	Spherical, porous silica gel, 10 µm or less in diameter, the surface of which has been covalently modified with alkyl amide groups and endcapped.	Ascentis® Express RP-Amide ★	Ascentis® RP-Amide Discovery® RP-AmideC16	SUPELCOSIL™ ABZ+Plus SUPELCOSIL™ LC-ABZ		
L62	C30 silane bonded phase on a fully porous spherical silica 3 to 15 µm in diameter.	Ascentis® Express C30 ★				
L63	Glycopeptide teicoplanin linked through multiple covalent bonds to a 100 Å units spherical silica.		Astec® CHIROBIOTIC® T Astec® CHIROBIOTIC® T2 ★ Astec® CHIROBIOTIC® TAG			
L67	Porous vinyl alcohol copolymer with a C18 alkyl group attached to the hydroxyl group of the polymer, 2 to 10 µm in diameter.				apHera™ C18 ★	

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*Tosoh Bioscience columns are available in select countries.
For a list, please go to the page 109 of this brochure

HPLC Columns

HPLC Packings for USP Compendial Methods (continued)

USP Code [1]	Description [1]	Available Columns [2] [3] — Particles: Fused-Core® Silica Particles — Type B Silica [4]	Particles: Fully Porous Silica Particles — Type B Silica [4]	Particles: Fully Porous Silica Particles — Type A Silica [5]	Particles: Non-Silica Particles	Monolithic: Silica Based — Type B Silica [4]
L68	Spherical, porous silica, 10 µm or less in diameter, the surface of which has been covalently modified with alkyl amide groups and not endcapped.			SUPLEX pKb-100 ★		
L82	Polyamine chemically bonded to cross-linked polyvinyl alcohol polymer, 4 to 5 µm in diameter.				apHera™ NH_2 ★	
L86	A 5 µm fused core particle with a highly polar ligand possessing 5 hydroxyl groups tethered to the silica gel outer layer.	Ascentis® Express OH5 ★ BIOshell™ Glycan				
L88	Glycopeptide vancomycin linked through multiple covalent bonds to 100 Å spherical silica.		Astec® CHIROBIOTIC® V Astec® CHIROBIOTIC® V2 ★			
L95	Highly polar alkyl ligand comprising five hydroxyl groups that are chemically bonded to totally porous or superficially porous silica or a monolithic silica rod.	Ascentis® Express OH5 ★ BIOshell™ Glycan				
L109	Spherical particles of porous graphitic carbon, 1.5 to 30 µm in diameter.				Supel™ Carbon LC ★	
L114	Sulfobetaine graft-polymerized to totally or superficially porous silica, 1.5 to 10 µm in diameter, or a monolithic rod. Packing having densely bonded zwitterionic groups with 1:1 charge balance.		SeQuant® ZIC®-HILIC ★			
L122	Sulfobetaine graft-polymerized to totally or superficially porous hydrophilic polymer particles, 1.0 to 10 µm in diameter, or a monolithic rod. Packing having densely bonded zwitterionic groups with 1:1 charge balance.				SeQuant® ZIC®-pHILIC ★	

Footnotes:

[1] United States Pharmacopeia. Request from United States Pharmacopeial Convention, Inc., 12601 Twinbrook Parkway, Rockville, MD USA 20852 (tel. 800-227-8772).
[2] Indicates availability of material(s) matching the description. We are not necessarily the manufacturer of the material.
[3] Purple text indicates our recommendation(s).
[4] Type B silica is obtained from a synthetic source and is virtually free of metal contant.
[5] Type A silica is obtained from a natural source so may contain varying degrees of metal content.

*Tosoh Bioscience columns are available in select countries.
For a list, please go to the page 109 of this brochure

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HPLC Columns

Selection by Column Dimension

Depending on the scale and/or efficiency of the separation required, the table below can help you to choose a column by the most appropriate inner diameter (I.D.) and column length for your needs.

Which column length is best for my needs?

- If you want to maximize the speed of your application . . . . .20 mm to 75 mm length
- If you want a balance of resolution and speed . . . . . .100 mm to 150 mm length
- If you want the best resolution possible . . . . . .150 mm to 250 mm length
- Also available in both **analytical** and **semi-prep** dimensions

Column dimension [length × I.D. in mm]	Application	Reason
4 × 4 5 × 2 / 3 / 4.6 10 × 4.6 / 10 / 25	Guard-column	Protection from mechanical contamination and non-specific adsorption Sample contaminated to low extent
25 × 4	Pre-column	Long pre-column for higher protection needs suitable for rapid separations
30 × 2 / 2.1 / 3 / 4 55 × 2 / 2.1 / 3 / 4 75 × 4	Method development Rapid HPLC and UHPLC (if pressure stable)	Short retention time Rapid equilibration Low solvent consumption (small I.D.) and more sustainable Low pressure drop
100 × 2.1 125 × 2 / 3 150 × 2.1 / 3	High detection sensitivity (mass selectivity)	Semi-micro column for low injection volumes and low peak dispersion Low solvent consumption and more sustainable
100 × 4.6 125 × 4 / 4.6 150 × 4.6	Standard column	Adequate performance for most applications (average performance 8000 – 10000 N/column)
250 × 2 / 2.1 / 3	High detection sensitivity High performance separation	Semi-micro column for low injection volumes and low peak dispersion Low solvent consumption and more sustainable For complex samples
250 × 4.6	High robustness/higher tolerance to injection volume	For very complex samples
250 × 10	Semi-preparative	For mg quantities of pure substance on lab scale
250 × 25	Preparative	For g quantities of pure substance

Guidelines for typical flow rates and orientation values for the loading capacities of analytical and semi-preparative particulate columns as well as capillary particulate packed columns

Column dimension Length x I.D. (mm)	Typical Flow Rates*	Max Sample Amount**	Sample Volume***
100 x 0.075	0.15 - 0.5 µL/min	9 ng	0.5 – 9 nL
100 x 0.1	0.25 - 1 µL/min	16 ng	1 – 16 nL
100 x 0.2	1 - 4 µL/min	60 ng	0.01 – 0.06 µL
100 x 0.3	2 - 9 µL/min	140 ng	0.01 – 0.14 µL
100 x 0.5	5 - 25 µL/min	0.4 µg	0.05 – 0.4 µL
100 x 1	20 - 100 µL/min	2 µg	0.1 – 2 µL
100 x 1.5	40 - 200 µL/min	4 µg	0.2 – 4 µL
100 x 2	0.08 - 0.4 mL/min	6 µg	0.4 – 6 µL
250 x 3	0.15 - 0.8 mL/min	40 µg	3 – 40 µL
250 x 4	0.3 - 1.5 mL/min	60 µg	4 – 60 µL
250 x 4.6	0.4 - 2 mL/min	80 µg	5 – 80 µL
250 x 10	2 - 10 mL/min	400 µg	30 – 400 µL
250 x 25	10 - 60 mL/min	2.5 mg	200 – 2500 µL

* Typical flow rate values calculated for fully porous 5 µm particulate material column, for smaller particle packed columns flow rates are higher by a factor of 1.6 shifting from 5 to 3 µm particles, and by a factor of 2.5 when shifting from 5 to 2 µm particles. For superficially porous materials, upper limit of flow rate is also higher comparing to fully porous particles. For monolithic silica columns, upper flow rate limit might be even up to 3 times higher. **Note:** In any case, it is important to monitor column backpressure ensuring it does not exceed maximum recommended limits.

** If a high amount of sample needs to be loaded onto the column, it is better to increase the sample concentration rather than the sample volume. The standard sample concentration in HPLC/UHPLC is 1 mg/mL.

*** The maximum injection volumes are calculated based on the maximum recommended volume limit, which is 2% of the empty column volume. For superficially porous particles and monolithic silica columns, maximum injection volume should be reduced by 50%.

HPLC Columns

Increase Sensitivity and Save Solvents with Small I.D. HPLC Columns

The use of smaller inner diameter (I.D.) columns results in decreased solvent usage. When using small I.D. columns, less mobile phase is required to achieve the same linear velocity; therefore, analysis time can be reduced by increasing flow rate. This attribute allows for significant cost and time savings.

In addition, the peak response is increased with small I.D. columns – the peak height increases as the column diameter decreases. The peak response for 2 or 2.1 mm I.D. columns is three times higher in comparison to 4.6 mm I.D. columns. This quality is beneficial when analyzing samples with limited mass, typically used in LC-MS applications.

Loadability / Sensitivity / Dead Volume / Solvent Saving

Column I.D.	Typical Particle Size	Technique
>20 mm	10 – 25 µm	Preparative
10 mm	5 - 10 µm	Semi-preparative
3 / 4 / 4.6 mm	3 - 5 µm	Conventional HPLC
2 / 2.1 mm	≤ 2 - 3 µm	UHPLC
1.5 mm	2-5 µm	UHPLC
1.0 mm	≤ 2 - 5 µm	Micro LC
300 µm	≤ 2 - 5 µm	Capillary LC
<100 µm	≤ 2 - 5 µm	Nano LC

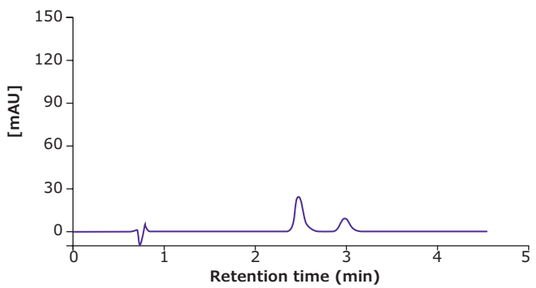
Column Length	250 mm	100 - 150 mm	30 - 50 mm	4 – 25 mm
Use	Very complex samples	Adequate performance for most applications	Rapid separations	Guard columns

Resolution / Speed

Increase Sensitivity and Save Solvents with 2 mm I.D. Chromolith® RP-18 Endcapped Columns

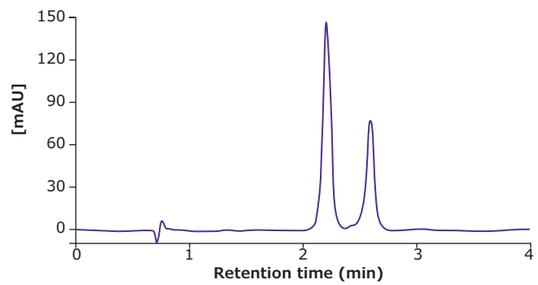
Chromolith® Performance RP-18e 100 x 4.6 mm I.D.

Column:	Chromolith® Performance RP-18 endcapped 100 x 4.6 mm I.D.
Mobile phase:	A: 100 % Acetonitrile B: 100 % Water + 0.1 % TFA (v/v) C: 100 % Methanol
Isocratic:	A/B/C 30/60/10 (v/v/v)
Flow rate:	2 mL/min
Pressure:	45 bar (4.5 MPa, 65.3 psi)
Detection:	Dionex Ultimate 3000 VWD-3400, 2.5 Hz, Response time 0.1 s, UV = 210 nm
Vol. detector cell:	11 µL
Temperature:	ambient
Injection volume:	1 µL
Sample:	Bimatoprost Bimatoprost free acid



Chromolith® Performance RP-18e 100 x 2.0 mm I.D.

Column:	Chromolith® Performance RP-18 endcapped 100 x 2.0 mm I.D.
Mobile phase:	A: 100 % Acetonitrile B: 100 % Water + 0.05 % TFA (v/v) C: 100 % Methanol
Isocratic:	A/B/C 30/60/10 (v/v/v)
Flow rate:	0.38 mL/min
Pressure:	48 bar (4.8 MPa, 70 psi)
Detection:	Dionex Ultimate 3000 VWD-3400, 2.5 Hz, Response time 0.1 s, UV = 210 nm
Vol. detector cell:	1.4 µL
Temperature:	ambient
Injection volume:	1 µL
Sample:	Bimatoprost Bimatoprost free acid



The same separation on a Chromolith® 2 mm I.D. column demonstrates improved sensitivity and solvent savings of 81%.

HPLC Columns

HPLC Column Hardware

Supelco® HPLC Columns perfectly fit to every HPLC and U/HPLC instrument. All Supelco® columns have a Parker type endfitting. A 1/16 inch outer diameter capillary connection of stainless steel or PEEK (Polyether ether ketone) is typically used to connect the HPLC column to the HPLC system. 0.5 mm outer dimension flexible stainless steel capillary connections are suitable as well as using a 1/16 inch connection part.

Trademark Hardware	Trademark Sorbent	Column	Use	Pre-column	Material	Pressure stability
LiChroCART®	Purospher® STAR	HPLC Cartridge	Requires manu-CART® to use	Direct integration of pre-column—no separate holder needed	Stainless Steel	250 bar
	LiChrospher®					
	Superspher®					
	LiChrosorb®					
Hibar® RT	Purospher® STAR	HPLC Column	Ready to use column	Separate pre-column holder required	Stainless Steel	400 bar
	LiChrospher®					
	Superspher®					
	LiChrosorb®					
Hibar® HR	Purospher® STAR	UHPLC Column	Ready to use column	No pre-columns available	Stainless Steel	1000 bar
	SeQuant®	U/HPLC Column	Ready to use column	Separate pre-column holder required	PEEK lined Stainless Steel	550 bar*
	Chromolith®	U/HPLC Column	Ready to use column	Separate pre-column holder required	PEEK	200 bar
	Discovery®	HPLC Column	Ready to use column	Separate pre-column holder required	Stainless Steel	400 bar
	Ascentis®					
	SUPELCOSIL™					
	Ascentis® Express	U/HPLC Column	Ready to use column	Separate pre-column holder required	Stainless Steel	600 bar
	BIOshell™					
	Ascentis® Express (2 µm)	UHPLC Column	Ready to use column	Separate pre-column holder required	Stainless Steel	1000 bar
	BIOshell™ (2 µm)					

*SeQuant® ZIC®-pHILIC: 200 bar

UHPLC columns are pressure stable up to 1000 bar, U/HPLC columns are suitable to be used in UHPLC instruments (preferred with 2 or 2.1 mm I.D.) with limited pressure stability

Supelco® HPLC, UHPLC and Capillary Columns are Available in Different Column Hardware Designs:

Ascentis® Express and BIOshell™ as well as SUPELCOSIL™, Discovery®, Ascentis®, and Astec® columns are designed for direct connection to any HPLC system. Supelguard pre-columns protect the analytical HPLC column.

SeQuant® PEEK-lined Stainless Steel Ready to Use U/HPLC and Capillary Columns

SeQuant® columns are PEEK-lined stainless steel columns making them best suitable for HILIC separations including phosphorylated compounds and meeting the need for bioinertness.

Chromolith® PEEK Columns

Chromolith® monolithic silica HPLC columns are cladded in inert PEEK (polyetheretherketone) polymeric material and can be connected directly to HPLC or UHPLC systems as a "ready to use" column.

Hibar® HR Ready to Use UHPLC Columns

Purospher® STAR Hibar® HR UHPLC columns are fully compatible with any UHPLC instrument and provide a pressure stability of 1000 bar.

Hibar® RT Ready to Use HPLC Columns

Hibar® RT HPLC columns are made of stainless steel providing a pressure stability of 400 bar. For the use of a pre-column, a separate pre-column holder is required.

LiChroCART® HPLC Cartridge

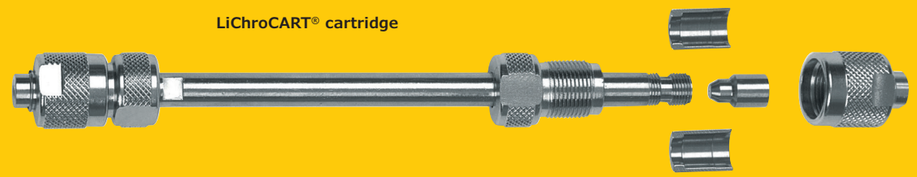
With LiChroCART® cartridges, the user works with reusable manu-CART® endittings which fit different cartridge lengths and inner dimensions. Since these cartridge holders may remain in the system, and the capillary connections do not need to be detached, the cartridges may be changed within the shortest possible time.

manu-CART® Holder for LiChroCART® HPLC Cartridges

The "one-turn" cartridge system for simple, rapid, hand tight fitting of cartridges and pre-columns. manu-CART® cartridge holders **1.51486.0001** for the LiChroCART® cartridge system are reusable and it allows for every cartridge length with different internal diameter. A simple turn permits an easy and problem-free integration of a guard cartridge. For coupling of two LiChroCART® cartridges, the coupling kit **1.50083.0001** can be used. For connecting a LiChroCART® 25-4 pre-cartridge to a LiChroCART® HPLC cartridge the coupling kit **1.50082.0001** is suitable. The manu-CART® cartridge holder is for 2, 3, 4 and 4.6 mm I.D. LiChroCART® cartridges and 75, 100, 125, 150 and 250 mm length.

manu-CART® NT cartridge holder [**1.51486.0001**] for LiChroCART® cartridge of 75, 100, 125, 150 and 250 mm length and 2, 3, 4 and 4.6 mm I.D.

LiChroCART® cartridge



Use of LiChroCART® 4-4 or 10-2 guard cartridges with manu-CART® NT

Column Accessories and Pre-column Holder

Supelco® HPLC Columns perfectly fit to every HPLC and U/HPLC instrument. All Supelco® columns have a Parker style endfitting. A 1/16 inch outer diameter capillary connection of stainless steel or PEEK is typically used to connect the HPLC column to the HPLC system. 0.5 mm outer dimension flexible stainless steel capillary connections are suitable as well using a 1/16 inch connection part. For protection of the analytical HPLC column from contamination, it is recommended to use a guard or pre-column. These short columns are placed typically into a guard column holder. For the different column hardwares described on the previous pages, corresponding pre-column holders are available.

Supelguard™

Cat. No.	Product	Content of pack	Description
59660-U	Stand-Alone, for use with Supelguard™ cartridges (2 cm L x 2.1 to 4.6 mm I.D.)	pkg of 1 ea fittings included	For 2.1, 3.0, 4.0 and 4.6 mm I.D. Supelco® columns
21150AST	Stand-Alone (Swivel-type), for use with Supelguard™ cartridges (2 cm L x 2 to 4.6 mm I.D.)	pkg of 1 ea	Holder fits 2 cm x 2, 3, and 4 mm I.D. Astec® CYCLOBOND™, CHIROBIOTIC®, Cellulose DMP, guard cartridges. Cartridges, tubing, nuts and ferrules are not included
55205	Direct-Connect (Swivel-type), for use with Supelguard™ cartridges (2 cm L x 3 to 4.6 mm I.D.)	pkg of 1 ea	Connects guard column directly to a 3.0, 4.0 and 4.6 mm I.D. Supelco® analytical column
504262	Direct-Connect (Swivel-type), for use with Supelguard™ cartridges (2 cm L x 2.1 mm I.D.)	pkg of 1 ea	For direct connection to 2.1 mm I.D. Supelco® columns

21150AST

For coupling the precolumn holder remove endfitting

[1.51487]

Hibar® RT column
2, 3, 4, and 4.6 mm I.D.

Ordering information

Guard column holder for Hibar® RT columns

Product	Cat. No.	Contents of one package
Pre-column holder for 4-4 LiChroCART® cartridges for capillary connection to Hibar® RT column	**1.16217.0001**	1 piece
Pre-column holder for 4-4 LiChroCART® cartridges for direct coupling to Hibar® RT column	**1.51487.0001**	1 piece

HPLC Columns

Ordering information

manu-CART® cartridge holder, manu-CART® endfittings for stainless steel cartridges LiChroCART®

Product	Cat. No.	Contents of one package
manu-CART® NT cartridge holder for 2, 3, 4 and 4.6 mm I.D. LiChroCART® cartridges	**1.51486.0001**	2 complete stainless steel units for mounting one LiChroCART® cartridge
manu-CART® "10" II cartridge holder for 10 mm I.D. LiChroCART® cartridges	**1.51419.0001**	2 complete stainless steel units for mounting one LiChroCART® cartridge
manu-CART® coupling kit for coupling with LiChroCART® 25-4 pre-cartridge	**1.50082.0001**	1 coupling unit 1 endfitting for LiChroCART® 25-4
manu-CART® coupling unit to connect two LiChroCART® cartridges	**1.50083.0001**	1 piece
manu-CART® holder 25-4 and 25-2	**1.50017.0001**	1 piece
manu-CART® holder 30 mm for 30-2, 30-3 and 30-4 LiChroCART® cartridges	**1.50227.0001**	1 piece
manu-CART® holder 55 mm for 55-2, 55-3 and 55-4 LiChroCART® cartridges	**1.50226.0001**	1 piece
Pressure cone for manu-CART® endfitting	**1.51258.0001**	2 pieces
Split collets for manu-CART® endfitting	**1.51257.0001**	4 pieces

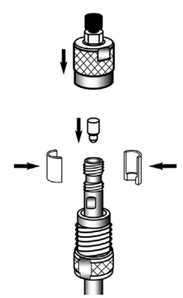
manu-CART® holder 30 mm [1.50227]
for LiChroCART® 30-4, 30-3 and 30-2

manu-CART® holder 55 mm [1.50226]
for LiChroCART® 55-4, 55-3 and 55-2

manu-CART® cartridge holder [1.51486]

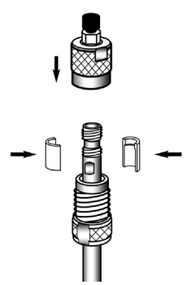
Mounting with guard cartridge 4-4 or 10-10

1. Slide sleeve over cartridge.
2. Fix split-collets in the groove in direction of the guard cartridge. Apply guard cartridge with its cone in direction of the main cartridge, slide sleeve on top and fasten with cap nut.



Mounting without guard cartridge

1. Slide sleeve with external thread over cartridge.
2. Using your finger, hold one split-collet at the grove. Apply the second split-collet, slide sleeve over it and fasten with cap nut.



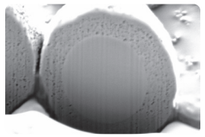
HPLC Columns

Fused-Core® (Superficially Porous Silica Particles, SPP) HPLC and UHPLC Columns

Maximum Resolution for Small and Large Molecule Separation

Ascentis® Express and BIOshell™ HPLC and UHPLC columns are based on Fused-Core® particle technology enabling fast results with highest resolution.

Fused-Core® columns feature narrower particle size distribution as well as a shorter diffusion path compared to Fully Porous Particles. The result is increased resolution, added sensitivity and higher throughput.



Fused-Core® particles consist of a solid silica core and a porous silica shell allowing a shorter diffusion path compared to conventional Fully Porous Particles.

Features of Fused-Core® particles over Fully Porous Particles:

- Narrower particle size distribution
- More consistently packed bed
- Shorter diffusion path

Superior for Small Molecule Separation:

Ascentis® Express HPLC and UHPLC Columns

Ascentis® Express HPLC and UHPLC columns provide about 40% more efficiency in comparison to columns with Fully Porous Particles of the same size. This performance enhancement is applicable to all HPLC instruments (in addition to UHPLC systems).

The very broad range of column chemistries makes it easy to select the best, suitable column for any HPLC and UHPLC application – from capillary column dimensions to analytical 4.6 mm I.D. dimensions.

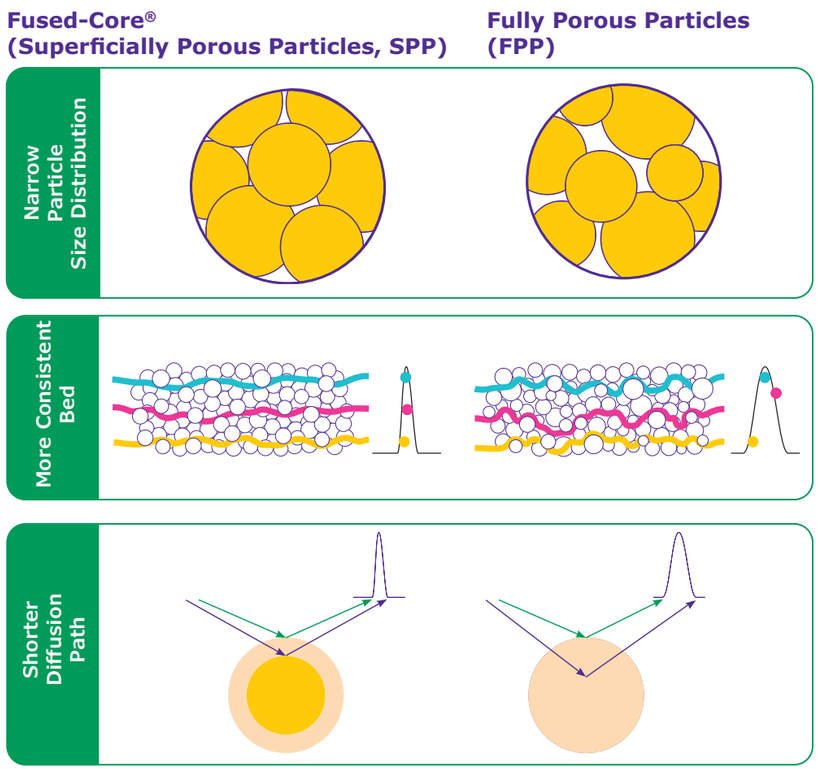
As well as for Biomolecule Separation:

BIOshell™ UHPLC and HPLC Columns

BIOshell™ UHPLC and HPLC columns deliver maximum speed and efficiency for the separation of biomolecules on both UHPLC and HPLC systems. The Fused-Core® superficially porous silica particles (SPP) with pore sizes from 90 Å up to 1000 Å allow superior separation of glycans as well as very large proteins. In particular, a pore size of 1000 Å shows very clear advantages over common 300 Å pores for the separation of very large proteins in biotherapeutic drug development such as monoclonal antibodies (mAbs) or proteins with molecular weights greater than 100 kDa.

Fused-Core® Particles (SPP)

HPLC Columns

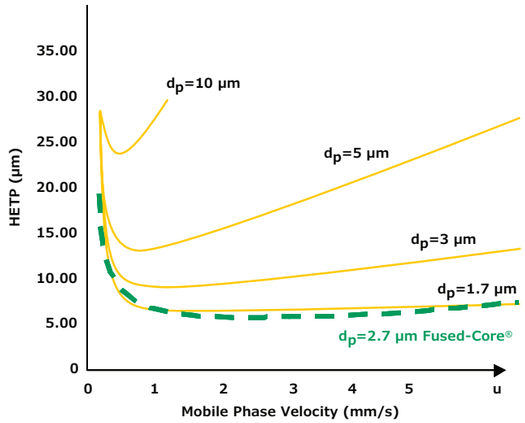


The innovative manufacturing process for Fused-Core® particles produces a very narrow particle size distribution. This attribute allows for the use of larger porosity frits that clog less, resulting in a more rugged column. Traditional, fully porous particles provide a larger particle size distribution, requiring smaller pore frits that clog more easily.

The "A" term in the van Deemter equation accounts for the effects of heterogeneities in the packed bed of an HPLC column. Narrow particle size distributions form a more consistently packed bed and more consistent path lengths, minimizing analyte dispersion (peak broadening) through the column. This eddy diffusion is effectively independent of mobile phase velocity.

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

The factors that affect chromatographic efficiency are Eddy diffusion, longitudinal diffusion, and resistance to mass transfer, the A, B and C 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

HPLC Columns

Ascentis® Express HPLC and UHPLC columns

Maximum Resolution on any System

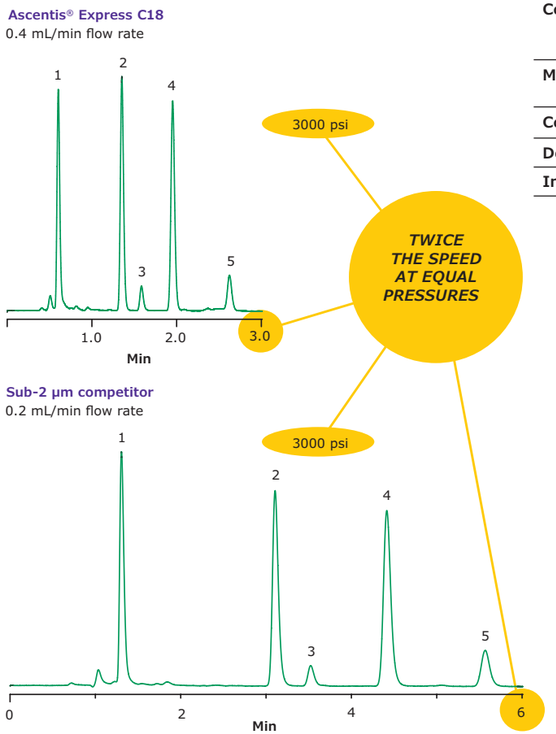
Based on Fused-Core® particle technology, Ascentis® Express columns provide an exceptional advancement in HPLC column performance and the benefits of high sample throughput at maximum resolution.

Feature and benefits:

- Fused-Core® technology (Superficially Porous Particles; SPP)
- Maximum speed and efficiency on both UHPLC and HPLC systems (particle sizes: 2 µm, 2.7 µm and 5 µm)
- 40% more efficiency in comparison to Fully Porous Particles (FPP) of same particle size
- UHPLC columns with 2 µm particles (pressure stable 1000 bar)
- Column dimensions from (capillary columns to 4.6 mm I.D. (analytical HPLC columns)
- Broadest range of phases/selectivities for optimal method development

Twice the Speed at Equivalent Pressure vs. sub-2 µm Fully Porous Particles

Compared to fully porous sub-2 µm particles typically used in UHPLC, Ascentis® Express Fused-Core® 2.7 µm particles generate approximately half the backpressure while providing the same high resolution. This trait permits both longer columns, for more resolving power, and faster flow rates, for higher throughput. Demonstrating this point, the separation below shows a steroid mixture on Ascentis® Express (top) and a sub-2 µm UHPLC column (bottom) of the same dimensions. Due to the lower backpressure of the Ascentis® Express 2.7 µm column an increased flow rate (double in this case) can be applied, providing the same back pressure, separation efficiency and resolution as on a sub-2 µm UHPLC column, just with a 50% shorter runtime, increasing sample throughput.



Chromatographic Conditions:

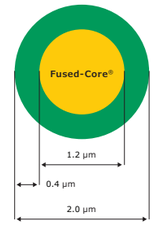
Columns:	Ascentis® Express C18, 10 cm x 2.1 mm I.D., 2.7 µm particles (53823-U) and sub-2 µm particle column (same dimensions)
Mobile phase:	water/acetonitrile 49:51 (for Ascentis® Express); water/acetonitrile 55:45 (for sub-2 µm)
Column temp:	ambient
Detector:	UV, 200 nm
Injection:	1 µL

1. Estradiol
2. β-Estradiol
3. Impurity
4. Estrone
5. Estrone degradant

HPLC Columns

Best fit for HPLC and UHPLC

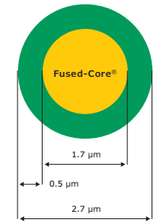
Best Fused-Core® UHPLC Column



An optimized solution for high throughput small molecule analysis

Pressure stability: 2 µm: 1000 bar

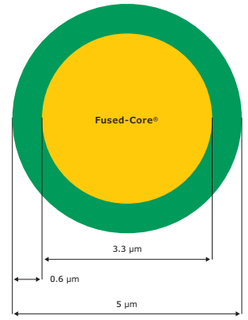
Fast HPLC on any System



A practical solution that delivers UHPLC performance from any HPLC

2.7 µm: 600 bar

The Lab Work-horse Column

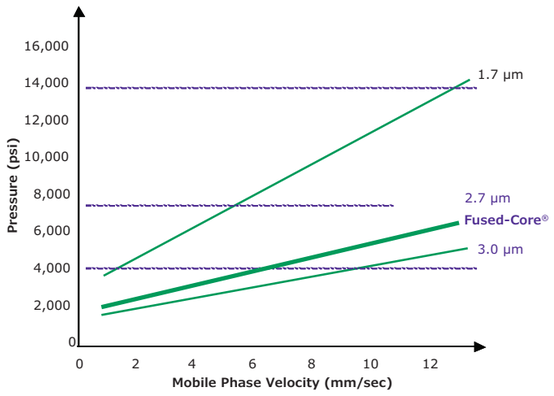


True plug and play solution for improving separations on existing 3 µm or 5 µm fully porous particle HPLC columns

5 µm: 600 bar

More Separation Power per Unit Pressure

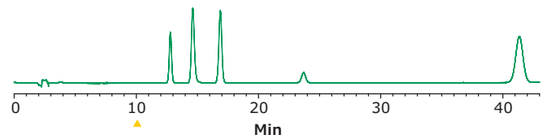
Designed to deliver speed and resolution on all UHPLC and HPLC systems, Ascentis® Express columns with Fused-Core® technology exceed the benefits of sub-2, 3 and 5 µm particles. Ascentis® Express 2.7 µm particles deliver more resolving power per unit pressure than even sub-2 µm particles on any HPLC system (including UHPLC). Ascentis® Express 5 µm columns are able to achieve greater speed and efficiency than any other 5 µm particle-based column. This fact means that Ascentis® Express 5 µm columns can be the standard column for all fully porous 5 µm-based methods.
With the addition of 2.0 µm Ascentis® Express UHPLC columns, we now offer three U/HPLC Fused-Core® particle sizes, making the Ascentis® Express column line truly scalable from HPLC to UHPLC.



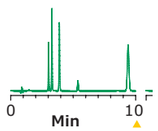
Higher Sample Throughput Without Compromises

The outstanding separating power of Ascentis® Express HPLC columns allows the use of shorter column length while maintaining good resolution. This trait results in higher sample throughput and reduction in solvent consumption and waste generation.

Fully porous particle C18
25 cm × 4.6 mm I.D., 5 µm particles



Ascentis® Express C18 column
10 cm × 4.6 mm I.D., 2.7 µm particles

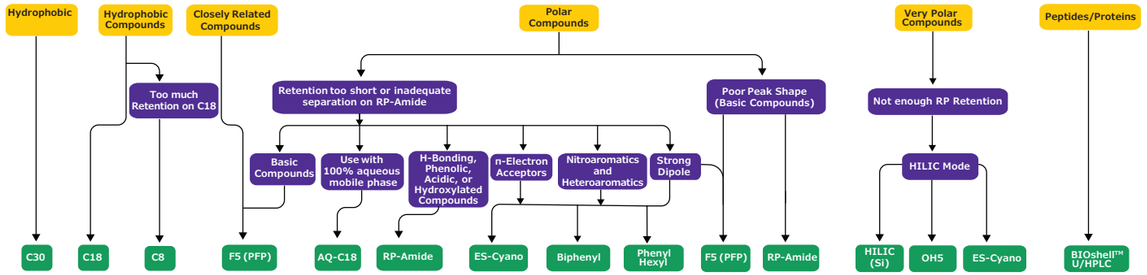


HPLC Columns

Ascentis® Express HPLC and UHPLC columns

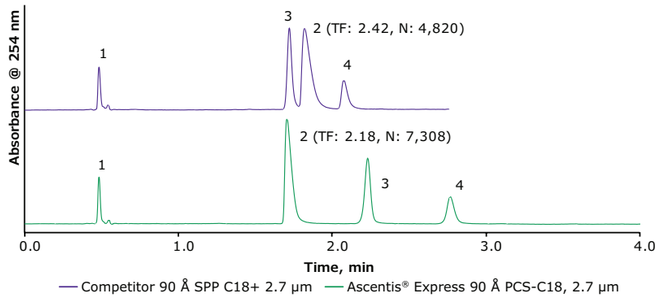
A Broad Range of Column Selectivities for all Compound Classes

Column selectivity has the highest influence on resolution in chromatography. Selection of the best column chemistry for your target analytes is therefore an important selection parameter. C18 column chemistries are typically the first choice. Nevertheless, when a C18 does not give the desired separation or the sample contains compounds that are known to be difficult to retain or resolve on a C18, consider changing stationary phase chemistry early in method development for more optimal applications. The range of selectivity provided by Ascentis® Express columns makes this easy.



Introducing the New Ascentis® Express PCS-C18 Columns

Ascentis® Express PCS-C18 columns are designed for effective separation of a broad range of compounds using low ionic strength (formic acid) mobile phase conditions. These columns utilize advanced Fused-Core® particles with 90 Å pores, making them ideal for small molecule analysis in pharmaceutical method development. With its unique Positive Charged C18 Chemistry, this column offers exceptional peak shape and improved loading capacity for basic compounds compared to traditional C18 chemistries.



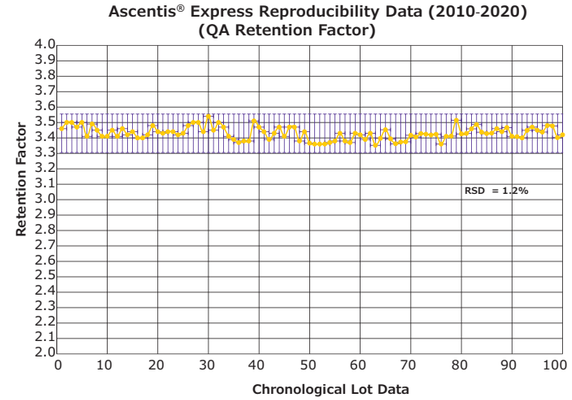
In a direct comparison of SPP columns, the Ascentis® Express PCS-C18 column outperforms the SPP column of a leading competitor by offering superior resolution, improved tailing factors, and increased plate count.

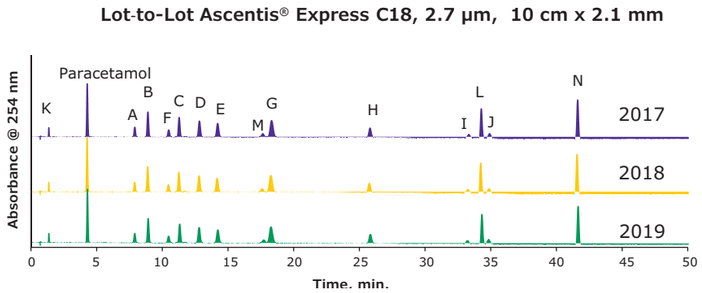
Chromatographic Conditions:	
Column:	Ascentis® Express 90 Å PCS-C18, 2.7 µm, 100 mm x 2.1 mm I.D. Competitor SPP C18+, 2.7 µm 100 mm x 2.1 mm I.D.
Mobile Phase:	A: Water, 0.1% Formic Acid
	B: Acetonitrile, 0.1% Formic Acid
Isocratic:	Ascentis® Express 90 Å PCS-C18: 24% B Competitor C18+: 26% B
Flow Rate:	0.4 mL/min
Temperature:	35 °C
Injection:	0.5 µL
Sample Solvent:	70/30 Water/Acetonitrile
Wavelength:	PDA, 254 nm
Sample:	1. Uracil 2. Imipramine 3. 4-Methoxybenzoic Acid 4. 2-Chlorobenzoic Acid

Excellent Lot-to-Lot Reproducibility

The consistency of chromatographic results depends on many factors. One major contributor to consistent and reliable results is the HPLC column. Therefore, the lot-to-lot and column–to-column reproducibility is a major concern. Ascentis® Express HPLC and UHPLC columns show excellent reproducibility. Over the last 10 years, the relative standard deviation (RSD) of the QA retention factor was 1.2%.

SigmaAldrich.com/express

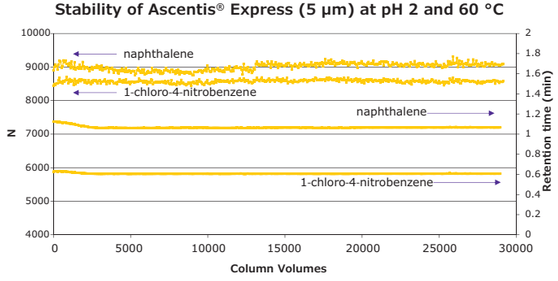




Chromatographic Conditions:	
Gradient Elution	
Mobile Phase:	[A] pH 7 phosphate buffer [B] methanol
Wavelength:	254 nm
Injection:	1.0 µL
Temperature:	30 °C
Flow Rate:	0.3 mL/min.

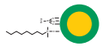
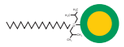
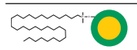
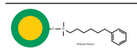
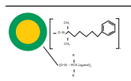
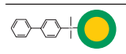
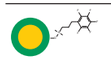
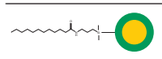
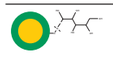
Temperature Stability

In addition to high reproducibility, the stability of HPLC column materials is important. The test-set below demonstrates the stability of an Ascentis® Express, C18 column, 5 µm, at 60 °C, pH 2.



Chromatographic Conditions:	
Column:	Ascentis® Express C18, 5 cm x 4.6 mm I.D., 5 µm (50530-U)
Temperature:	60 °C;
Mobile phase:	50% acetonitrile/50% aqueous 0.1% trifluoroacetic acid;
Flow rate:	1.8 mL/min
Injection:	1.0 µL
Solutes:	1-chloro-4-nitrobenzene, naphthalene, k = 1.7 and 3.8, respectively.

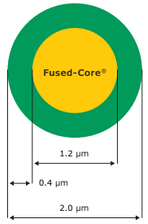
Ascentis® Express HPLC and UHPLC Columns

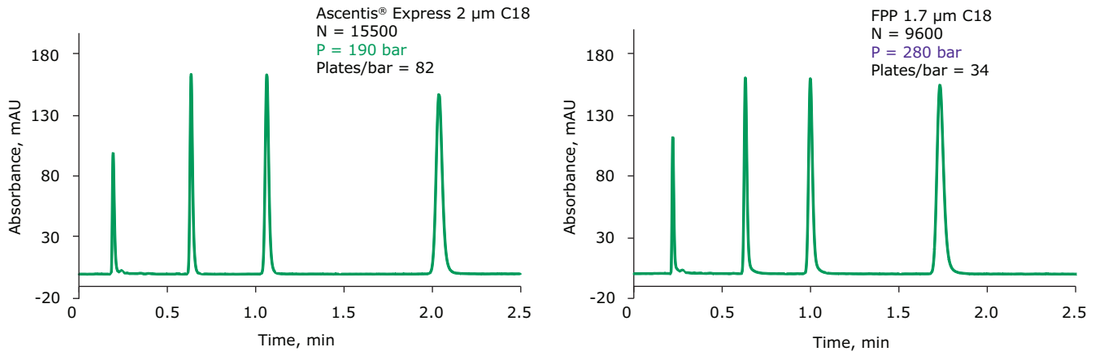
Ascentis® Express	Phase Bonding	USP Designation	Bonding Chemistry	Chromatographic Properties / Use		Particle Size (µm)	Pore Size (Å)	Surface Area (m^2/g)	Carbon Load (%)	Surface Coverage ($\mu mol/m^2$)	Low pH Limit/ Max T	High pH Limit/ Max T	Endcapped
	C18	L1	Dimethyloctadecyl	Outstanding performance for a broad range of analytes. Excellent peak shape for acids, bases and neutral compounds.		2	90	120	7.2	3.6	2/60 °C	9/40 °C	Yes
						2.7		135	7.7	3.4			
						5		90	6.4	4			
	AQ-C18	L1	Polar modified Octadecyl	Resistant to dewetting; compatible with 100% aqueous mobile phase. Suitable for the separation of polar compounds in RP-mode.		2	90	120	6.5	3.1	2/60 °C	9/40 °C	Yes
						2.7		135	6.7	3.2			
						5		90	5.6	3.6			
	ES-C18	L1	Diisobutyloctadecyl	Separation of parabens on a Ascentis® Express ES-C18 column at pH 1 and 60 °C compared to a standard C18 SPP column. Due to the special design of the stationary phase modification, the Ascentis® Express ES-C18 column can withstand these harsh conditions significantly longer than the standard C18 SPP column.		2	90	120	6.5	3.1	2/60 °C	9/40 °C	Yes
						2.7		135	6.5	2.7	1/90 °C	8/40 °C	No
	Peptide ES-C18	L1	Diisobutyloctadecyl	Fast separation of peptides and polypeptides with high peak capacity. Ideal for pharmaceutical/therapeutic peptide separation, peptide mapping, natural and synthetic peptide analysis and oligonucleotide analysis.		2.7	160	90	4.6	2.1	1/90 °C	8/40 °C	No
	PCS-C18	L1	Dimethyloctadecylsilane and positively charged ligand	Designed for performance with formic acid avoiding LC-MS signal suppression from TFA. Provides significantly improved peak widths and symmetry for basic compounds compared to traditional peptide C18 stationary phases.		2.7	90	125	7.4		2/60 °C	7/40 °C	Yes
	C30	L62	Triacontyldimethyl	Excellent selectivity for hydrophobic, long chain and structurally related isomers.		2.7	160	90	4.5	1.4	2/60 °C	9/40 °C	Yes
	C8	L7	Dimethyloctyl	Enhanced retention for less hydrophobic compounds or faster separation if retention on C18 is too high.		2	90	120	4.8	3.6	2/60 °C	9/40 °C	Yes
						2.7		135	5.4	3.6			
						5		90	3.7	3.6			
	Phenyl-Hexyl	L11	Dimethylphenyl-hexyl	Enhanced selectivity for aromatic compounds; strong pi-pi donor. Ideal for the separation of ketones, nitriles and alkenes.		2	90	120	6.3	3.4	2/60 °C	9/40 °C	Yes
						2.7		135	7.1	3.5			
						5		90	5.2	3.7			
	PCS-Phenyl Hexyl	L11	Dimethylphenylhexyl and positively charged ligand	Provides enhenced pi-pi interactions between the analytes and the phenyl stationary phase, resulting in increased retention for basic compounds and decreased retention for neutral compounds.		2.7	90	125	6.1		2/60 °C	7/40 °C	Yes
						5		90	5.2	3.7			
	Biphenyl	L11	Dimethylbiphenyl	High selectivity for aromatic compounds with enhanced pi-pi and mild steric interactions due to the two sequential phenyl groups. Ideal for rapid, efficient drug and metabolite analysis using conditions that are compatible with MS detection.		2.7		135	7.0	3.4			
						5		90	5.5	3.9			
	F5 (PFP)	L43	Pentafluorophenylpropyl	Outstanding selectivity for stereoisomers, strong pi-pi acceptor. Enhanced selectivity for aromatic and electron-rich compounds. Can be used in Reversed-phase and HILIC mode. In comparison with the C18 phases, the F5 phase shows longer retention time of basic analytes and less retention of hydrophobic analytes.		2	90	120	5.3	3.8	2/60 °C	8/40 °C	Yes
						2.7		135	5.5	3.5			
						5		90	3.9	3.6			
	ES-Cyano	L10	Diisopropylcyanopropyl	Enhanced retention for polar compounds and much less retention for hydrophobic compounds. Ideal for the separation of non-polar bases in HILIC mode (Ion-exchange mechanism). Compatible to 100% aqueous mobile phase and stable at low pH and high temperature.		2	90	120	3.4	2.5	1/80 °C	8/40 °C	Yes
						2.7		135	3.5	2.3			
						5		90	2.5	2.4			
	RP-Amide	L60	C16-Amide	Complementary selectivity to alkyl phases with improved peak shape for basic compounds compared to older EPG amide phases. Ideal for the separation of organic acids, phenols, catechins, alcohols, and bases. Compatible in 100% aqueous mobile phase.		2	90	120	7.3	3	2/60 °C	9/40 °C	Yes
						2.7		135	8.2	3			
						5		90	5.1	3			
	HILIC	L3	Bare silica	Enhanced separation of polar compounds. Can be used in HILIC and normal-phase mode. Offers both ion-exchange and partition mechanisms of separation in HILIC mode.		2	90	120	Unbonded	Unbonded	1/60 °C	8/40 °C	N.A.
						2.7		135					
						5		90					
	OH5	L95	Penta-hydroxy	Ideal for the HILIC separation of very polar compounds with a LogP value close to 0 or less than 0. Exhibits predominantly HILIC partitioning retention, limited silanol anionic character, and is relatively insensitive to ionic strength.		2	90	120	2.8	3	2/60 °C	9/40 °C	No
						2.7		135	3.2	3			
						5		90	2.1	3			

Ascentis® Express UHPLC Columns

Ascentis® Express columns with 2 µm particles are dedicated UHPLC columns which deliver reliable high speed and high resolution separations at pressures lower than fully porous sub-2 µm columns.

- Highest UHPLC performance possible without the ultra-high pressure of sub-2 µm fully porous columns
- Highest efficiency and best performance obtained with UHPLC instruments
- Excellent for fast method development due to full range of column chemistries
- 1 µm inlet frit optimized for ruggedness
- Pressure stability: 1000 bar/14,500 psi





Chromatographic Conditions:

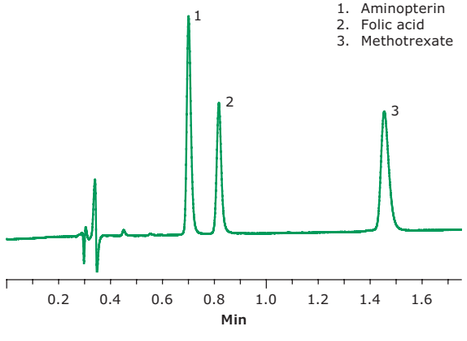
Dimensions of both Columns:	2.1 x 50 mm
Mobile Phase:	[A] Water; [B] Acetonitrile
Isocratic:	A/B: 15/85
Flow rate:	0.5 mL/min
Injection Volume:	0.2 µL
Detection:	254 nm
Temperature:	25 °C

Peak Identities (in elution order):

1. Uracil
2. Pyrene
3. Decanophenone
4. Dodecanophenone

Ascentis® Express 2 µm UHPLC Columns enable full suitability in UHPLC applications providing very high efficiencies at lower column backpressure in comparison to 1.7 µm fully Porous Particles (FPP)

UHPLC Analysis of Methotrexate and Related Compounds on Ascentis® Express C18, 2.0 µm

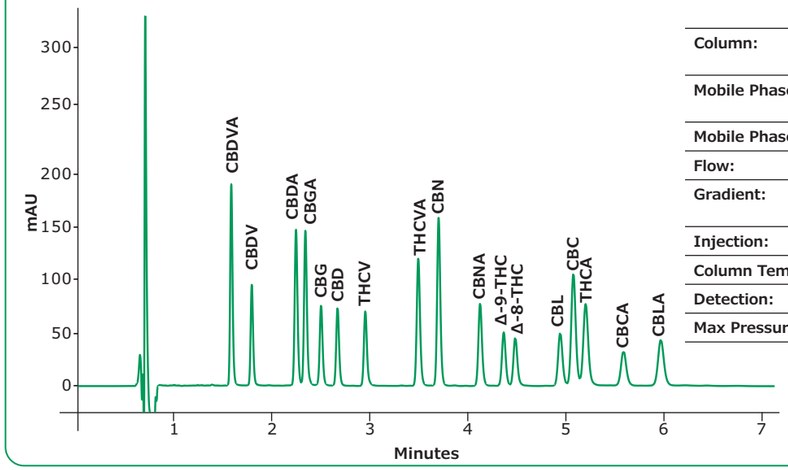


Column:	Ascentis® Express C18, 10 cm x 2.1 mm I.D., 2.0 µm particles (50813-U)
Column temp.:	35 °C
Mobile phase:	[A] 10 mM ammonium formate, pH 3.0 with formic acid; [B] Acetonitrile; (90:10, A:B)
Flow rate:	0.7 mL/min
Pressure:	1153 psi (795 bar)
Sample:	50 µg/mL in 85:15, water:methanol
Injection:	1 µL
Detector:	UV, 254 nm

HPLC Columns

High resolution UHPLC Analysis of Cannabinoids on Ascentis® Express C18, 2.0 µm

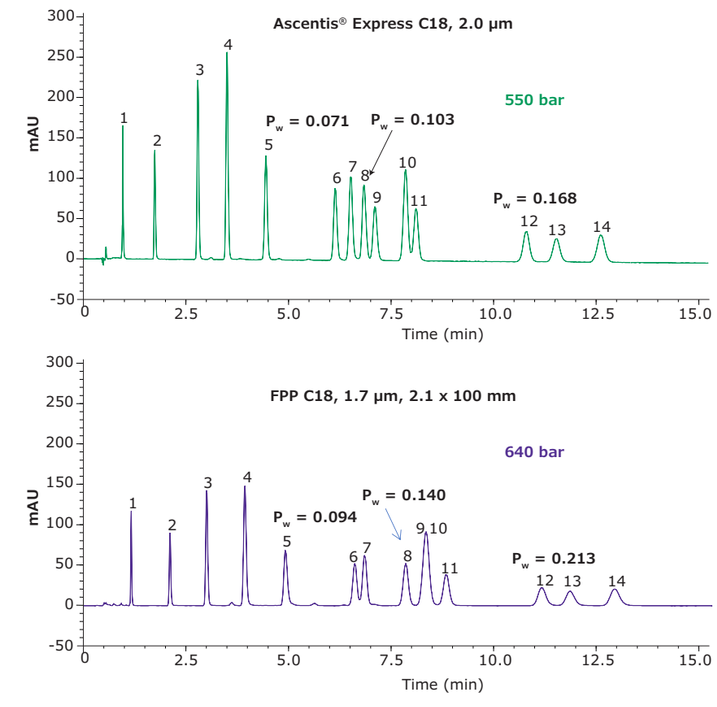
With increasing cannabis and hemp legislation, there has been increased demand for development and validation of accurate and precise testing methods for potency quantitation. Ascentis® Express 2 µm UHPLC columns enable the high resolution separation of 17 Cannabinoids in 6 minutes.



Column:	Ascentis® Express C18, 2.7 µm, 150 x 3 mm (53816-U)
Mobile Phase A:	5 mM Ammonium Formate + 0.1% Formic acid in water
Mobile Phase B:	0.1% Formic acid in acetonitrile
Flow:	0.4 mL/min
Gradient:	75% B to 90% B in 2 min, hold at 90% B 5 min
Injection:	3 µL, 25 µg/mL
Column Temp.:	25 °C
Detection:	UV, 228 nm
Max Pressure:	530 bar (7690 psi)

Learn more

UHPLC Separation of Explosives on Ascentis® Express C18 2 µm UHPLC Column compared to FPP C18 UHPLC Column



Chromatographic Conditions:

Dimensions of both Columns:	100 mm x 2.1 mm ID
Mobile Phase:	[A] Water; [B] Methanol
Isocratic:	A/B: 72/28
Flow rate:	0.4 mL/min
Detection:	PDA @ 254 nm
Temperature:	42 °C

Peak Identities:

1. Peak Identities:
2. HMX
3. RDX
4. 1,3,5-Trinitrobenzene
5. 1,3-Dinitrobenzene
6. Nitrobenzene
7. Tetryl
8. 2,4,6-Trinitrotoluene
9. 2-Amino-4,6-Dinitrotoluene
10. 4-amino-2,6-dinitrotoluene
11. 2,4-Dinitrotoluene
12. 2,6-Dinitrotoluene
13. 2-Nitrotoluene
14. 4-Nitrotoluene
15. 3-Nitrotoluene

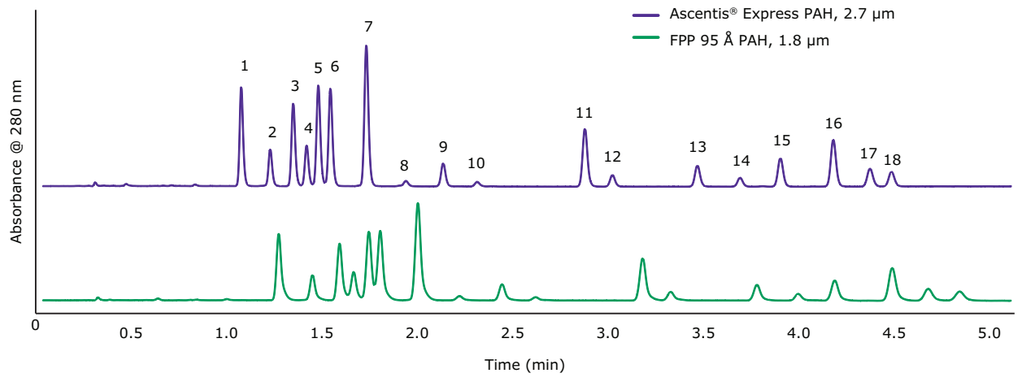
HPLC Columns

Ascentis® Express UHPLC columns

Ascentis® Express Columns for Environmental Testing

Ascentis® Express PAH HPLC columns deliver a fast and high efficiency separation of 16 standard PAH compounds with a resolution value of at least 1.5 in under five minutes for EPA 8310 and EPA 610.

- Application-assured through method qualified lot analysis
- 2.7 µm Fused-Core® particle for UHPLC-like separation with maximum resolution at lower back-pressure in comparison to sub-2 µm particles
- Well suited for UV, fluorescence and MS detection



Column:	Ascentis® Express PAH, 2.7µm, 5cm x 4.6mm, 90A
Cat. No.:	53539-U
Competitor Column:	FPP 95 Å PAH, 1.8 µm, 4.6 x 50mm
Mobile Phase:	[A] Water; [B] Acetonitrile
Gradient:	Time %B
	0.0 50
	4.0 100
	5.0 100
	5.01 50
Flow Rate:	1.8 mL/min

Ascentis® Express Back Pressure:	256 bar
FPP Back Pressure:	344 bar
Temperature:	30 °C
Detection:	280 nm
Injection Volume:	2 µL
Sample Solvent:	Methanol
Data Rate:	100 Hz
Response Time:	0.025 sec.
Flow Cell:	1 µL

Peak Identities

1. Naphthalene
2. Acenaphthylene
3. 1-Methylnaphthalene
4. 2-Methylnaphthalene
5. Acenaphthene
6. Fluorene
7. Phenanthrene
8. Anthracene
9. Fluoranthene
10. Pyrene
11. Benzo[a]anthracene
12. Chrysene
13. Benzo[b]fluoranthene
14. Benzo[k]fluoranthene
15. Benzo[a]pyrene
16. Dibenzo[a,h] anthracene
17. Benzo[g,h,i]perylene
18. Indeno[1,2,3-c,d] pyrene

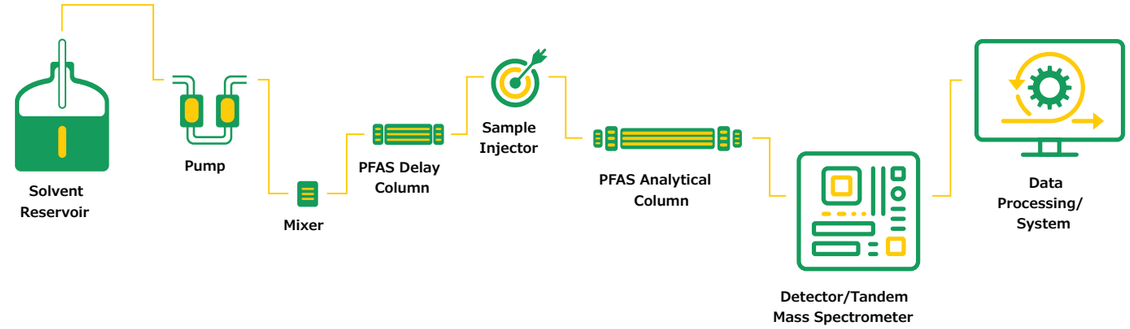
Ascentis® Express PFAS HPLC Columns and Delay Columns

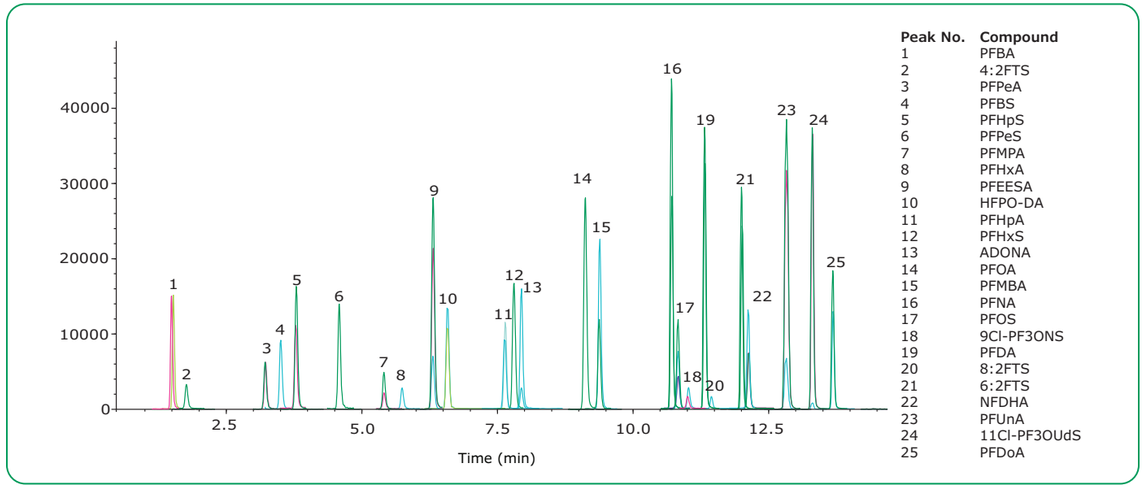
The **Ascentis® Express PFAS** HPLC column is designed for the separation of novel and legacy short chain and long chain PFAS compounds containing branched and linear isomers, whilst adhering to EPA methodology requirements. Furthermore, a specific PFAS delay column prevents background PFAS contamination from interfering with the sample results in quantitative LC-MS methods. The Ascentis® Express PFAS HPLC column, with its Fused-Core® technology and a particle size of 2.7 µm, delivers fast and high-resolution separations with excellent selectivity, peak shape, and necessary retention to perform in EPA methods 537.1, 533 and 8327. These advantages are demonstrated, in one particular example, by the separation of all PFAS analytes from EPA methods 537.1, 533, and 8327 in under five minutes.

- Application-assured through method qualified lot analysis
- 2.7 µm Fused-Core® particle for UHPLC-like separation with maximum resolution at lower back-pressure in comparison to sub-2 µm particles
- Endcapped alkyl phases for high sensitivity (no bleed) LC-MS analysis

PFAS Testing | Ascentis® Express PFAS HPLC Columns and Delay Columns | HPLC Columns

Ascentis® Express PFAS column is a high-performance liquid chromatography column based on Fused-Core® particle design. The highly retentive silane of the endcapped Ascentis® Express PFAS Delay column provides high retention of PFAS compounds across various mobile phase conditions and is used to delay background instrument PFAS contamination from interference with analyzed samples. For this reason, the Ascentis® Express PFAS Delay column is placed upstream of the sample injector and after the mixer. See diagram below.





Peak No.	Compound
1	PFBA
2	4:2FTS
3	PFPeA
4	PFBS
5	PFHpS
6	PFPeS
7	PFMPA
8	PFHxA
9	PFEESA
10	HFPO-DA
11	PFHpA
12	PFHxS
13	ADONA
14	PFOA
15	PFMBA
16	PFNA
17	PFOS
18	9Cl-PF3ONS
19	PFDA
20	8:2FTS
21	6:2FTS
22	NFDHA
23	PFUnA
24	11Cl-PF3OUdS
25	PFDoA

Access full application data:
LC-MS Analysis of PFAS Compounds in EPA Methods 537.1, 533 and 8327 | LC-MS Analysis of 33 PFAS Compounds in 5 minutes

Ordering Information Ascentis® Express PAH and PFAS HPLC Columns

Column dimension

Length (mm)		I.D. (mm)	PAH	PFAS
50	x	2.1	53513-U	53557-U
100	x	2.1	53532-U	53559-U
150	x	2.1	53533-U	53560-U
250	x	2.1		53562-U
50	x	3	53534-U	53563-U
100	x	3	53535-U	53564-U
150	x	3	53538-U	53565-U
250	x	3		53570-U
50	x	4.6	53539-U	

Length (mm)		I.D. (mm)	PAH	PFAS
100	x	4.6	53540-U	
150	x	4.6	53541-U	
250	x	4.6	53550-U	
			Guard Columns	**Delay Columns**
50	x	2.1	53551-U	
50	x	3	53555-U	53572-U
50	x	4.6	53556-U	53573-U

HPLC Columns

Ordering information

Ascentis® Express (2.7 µm)

Length (mm)		I.D. (mm)	C30	C18	ES-C18	NEW PSC-C18	AQ-C18	C18-PCP*	Peptide ES-C18	C8	RP-Amide	Phenyl-Hexyl		Peptide Phenyl-Hexyl	PCS-Phenyl-Hexyl NEW	Biphenyl	F5 (PFP)	ES-Cyano	OH5	HILIC (Si)	PAH	PFAS	PFAS Delay
50	x	1.5		50629-U	50582-U	50637-U									50686-U								
100	x	1.5		50630-U	50584-U	50638-U									50688-U								
150	x	1.5		50636-U	50586-U	50639-U									50689-U								
20	x	2.1	on request	53799-U	on request	on request	577320-U		on request	53795-U	on request	on request		on request	on request	64043-U	53592-U	53494-U	53779-U	on request			
30	x	2.1	on request	53802-U	on request	on request	577321-U		53299-U	53839-U	53910-U	53332-U		on request	on request	64054-U	53566-U	53468-U	53748-U	53933-U			
50	x	2.1	577100-U	53822-U	50587-U	50640-U	577322-U		53301-U	53831-U	53911-U	53334-U		584609-U	50690-U	64057-U	53567-U	53470-U	53749-U	53934-U	53513-U	53557-U	
75	x	2.1	on request	53804-U	on request	on request	577323-U		53304-U	53843-U	53912-U	53335-U		on request	on request	on request	53568-U	53472-U	53755-U	53938-U			
100	x	2.1	577101-U	53823-U	50589-U	50638-U	577324-U		53306-U	53832-U	53913-U	53336-U		584610-U	50691-U	64065-U	53569-U	53473-U	53757-U	53939-U	53532-U	53559-U	
150	x	2.1	577102-U	53825-U	50590-U	50639-U	577325-U		53307-U	53834-U	53914-U	53338-U		584611-U	50692-U	64068-U	53571-U	53475-U	53764-U	53946-U	53533-U	53560-U	
250	x	2.1	577103-U	on request	on request	on request	on request		on request	on request	on request	on request		584612-U	on request	on request	on request	on request	on request	on request		53562-U	
20	x	3.0	on request	on request	on request	on request	577326-U		on request	on request	on request	on request		on request	on request	64047-U	on request	on request	on request	on request			
30	x	3.0	577104-U	53805-U	on request	on request	577327-U		53308-U	53844-U	53915-U	53341-U		584613-U	on request	64055-U	53574-U	53476-U	53766-U	53964-U			53572-U
50	x	3.0	577105-U	53811-U	50594-U	50643-U	577328-U		53311-U	53848-U	53916-U	53342-U		584614-U	50693-U	64058-U	53576-U	53478-U	53767-U	53967-U	53534-U	53563-U	
75	x	3.0	on request	53812-U	on request	on request	577329-U		on request	53849-U	53917-U	53343-U		on request	on request	on request	53577-U	53479-U	53768-U	53969-U			
100	x	3.0	577106-U	53814-U	50595-U	50644-U	577330-U		53313-U	53852-U	53918-U	53345-U		584615-U	50694-U	64066-U	53578-U	53481-U	53769-U	53970-U	53535-U	53564-U	
150	x	3.0	577107-U	53816-U	50596-U	50645-U	577331-U		53314-U	53853-U	53919-U	53346-U		584616-U	50696-U	64069-U	53579-U	53483-U	53771-U	53972-U	53538-U	53565-U	
250	x	3.0	on request	on request	on request	on request	on request		on request	on request	on request	on request		on request	on request	on request	on request	on request	on request	on request		53570-U	
20	x	4.6	on request	on request	on request	on request	577332-U		on request	on request	on request	on request		on request	on request	64051-U	on request	on request	on request	on request			
30	x	4.6	577108-U	53818-U	on request	on request	577333-U		53316-U	53857-U	53921-U	53347-U		584617-U	on request	64056-U	53581-U	53484-U	53772-U	53974-U			
50	x	4.6	577134-U	53826-U	50598-U	50646-U	577334-U		53318-U	53836-U	53922-U	53348-U		584618-U	50697-U	64059-U	53583-U	53486-U	53774-U	53975-U	53539-U		53573-U
75	x	4.6	on request	53819-U	on request	on request	577335-U		53323-U	53858-U	53923-U	53351-U		on request	on request	64064-U	53584-U	on request	on request	on request			
100	x	4.6	577135-U	53827-U	50599-U	50647-U	577336-U	50461-U	53324-U	53837-U	53929-U	53352-U		584619-U	50700-U	64067-U	53590-U	53491-U	53776-U	53979-U	53540-U		
150	x	4.6	577136-U	53829-U	50600-U	50648-U	577337-U	50462-U	53328-U	53838-U	53931-U	53353-U		584620-U	50702-U	64071-U	53591-U	53492-U	53778-U	53981-U	53541-U		
250	x	4.6	577140-U	on request	on request	on request	577341-U		on request	on request	on request	on request		584626-U	on request	on request	on request	on request	on request	on request	53550-U		
Guard Columns, Three Pack																							
5	x	1.5				50649-U									50706-U								
5	x	2.1	577137-U	53501-U	50601-U	50650-U	577338-U		53536-U	53509-U	53514-U	53524-U		584621-U	50703-U	64074-U	53594-U	53495-U	53780-U	53520-U	53551-U		
5	x	3.0	577138-U	53504-U	50602-U	50651-U	577339-U		53537-U	53511-U	53516-U	53526-U		584622-U	50704-U	64076-U	53597-U	53496-U	53781-U	53521-U	53555-U		
5	x	4.6	577139-U	53508-U	50606-U	50652-U	577340-U		53542-U	53512-U	53519-U	53531-U		584623-U	50705-U	64078-U	53599-U	53497-U	on request	53523-U	53556-U		

*Ascentis® Express C18 PCP: pre-conditioned with phosphoric acid.
HPLC Columns "on request" are available as Custom Product. Please see page 126/127

Ascentis® Express (5 µm)

Length (mm)		I.D. (mm)	C18	AQ-C18	C8	RP-Amide	Phenyl-Hexyl	Biphenyl	F5 (PFP)	ES-Cyano	OH5	HILIC (Si)
20	x	2.1	50507-U	on request	50362-U	50732-U	50442-U	on request	50603-U	50557-U	50313-U	50255-U
30	x	2.1	50508-U	on request	50363-U	50733-U	50443-U	on request	50604-U	50558-U	50314-U	50256-U
50	x	2.1	50509-U	581363-U	50364-U	50734-U	50446-U	584585-U	50605-U	50559-U	50317-U	50257-U
75	x	2.1	50511-U	on request	50367-U	on request	on request	on request	on request	on request	50321-U	on request
100	x	2.1	50517-U	582702-U	50368-U	50737-U	50454-U	584586-U	50612-U	50563-U	50322-U	50260-U
150	x	2.1	50518-U	584572-U	50372-U	50739-U	50455-U	584587-U	50613-U	50564-U	50327-U	50261-U
250	x	2.1	50521-U	584573-U	50373-U	50747-U	50456-U	584588-U	50614-U	50566-U	50328-U	50262-U
30	x	3.0	50522-U	584574-U	50376-U	50749-U	50459-U	584589-U	50615-U	50567-U	50329-U	50264-U
50	x	3.0	50523-U	584575-U	50377-U	50751-U	50464-U	584590-U	50616-U	50568-U	50335-U	50265-U
75	x	3.0	50525-U	on request	50378-U	on request	on request	on request	50619-U	on request	on request	50268-U
100	x	3.0	50526-U	584576-U	50381-U	50753-U	50469-U	584591-U	50622-U	50570-U	50338-U	50269-U
150	x	3.0	50527-U	584577-U	50382-U	50758-U	50470-U	584592-U	50623-U	50574-U	50339-U	50270-U
250	x	3.0	50528-U	on request	50385-U	50759-U	50472-U	on request	50624-U	50575-U	50341-U	50276-U
30	x	4.6	50529-U	584578-U	50386-U	50767-U	50474-U	584593-U	50625-U	50577-U	50343-U	50278-U
50	x	4.6	50530-U	584579-U	50389-U	50768-U	50477-U	584594-U	50626-U	50581-U	50344-U	50284-U
75	x	4.6	50533-U	on request	50390-U	on request	on request	on request	on request	on request	on request	on request
100	x	4.6	50536-U	584580-U	50391-U	50773-U	50482-U	584595-U	50628-U	50585-U	50346-U	50288-U
150	x	4.6	50537-U	584581-U	50392-U	50774-U	50483-U	584596-U	50631-U	50588-U	50347-U	50289-U
250	x	4.6	50538-U	584526-U	50394-U	50775-U	50487-U	584624-U	50632-U	50591-U	50348-U	50294-U
Guard Columns, Three Pack												
5	x	2.1	50539-U	584582-U	50395-U	50776-U	50496-U	584597-U	50633-U	50592-U	50349-U	50295-U
5	x	3.0	50541-U	584583-U	50396-U	50777-U	50497-U	584598-U	50634-U	50593-U	50350-U	50297-U
5	x	4.6	50542-U	584584-U	50399-U	50779-U	50498-U	584599-U	50635-U	50597-U	50355-U	50298-U

HPLC Columns

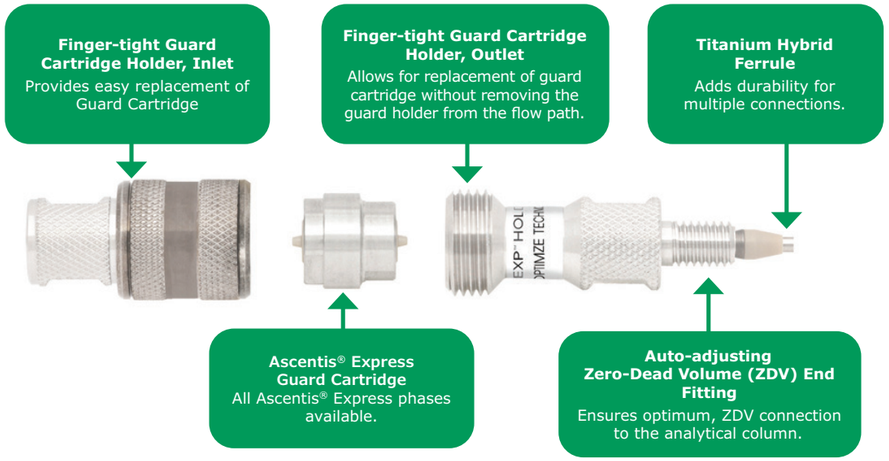
Ascentis® Express UHPLC Columns

Ascentis® Express (2 µm)

Length (mm)		I.D. (mm)	C18	ES-C18	AQ-C18	C8	RP-Amide	Phenyl-Hexyl	Biphenyl	F5 (PFP)	ES-Cyano	OH5	HILIC (Si)
20	x	2.1	50805-U	on request	on request	51652-U	51567-U	51600-U	on request	50857-U	51709-U	50951-U	51403-U
30	x	2.1	50809-U	on request	on request	51654-U	51568-U	51601-U	on request	50858-U	51712-U	50952-U	51404-U
50	x	2.1	50811-U	50608-U	565349-U	51656-U	51569-U	51603-U	584600-U	50859-U	51717-U	50957-U	51406-U
75	x	2.1	50812-U	on request	on request	51657-U	51571-U	51605-U	on request	50861-U	on request	on request	51408-U
100	x	2.1	50813-U	50609-U	565351-U	51658-U	51576-U	51608-U	584601-U	50863-U	51724-U	50959-U	51409-U
150	x	2.1	50814-U	50610-U	565362-U	51661-U	51577-U	51609-U	584602-U	50867-U	51725-U	50962-U	51418-U
250	x	2.1	on request	on request	565363-U	on request	on request	on request	584603-U	on request	on request	on request	on request
30	x	3.0	50815-U	on request	565364-U	51663-U	51582-U	51611-U	584604-U	50869-U	51727-U	50963-U	51419-U
50	x	3.0	50816-U	50611-U	565365-U	51664-U	51583-U	51614-U	584605-U	50871-U	51728-U	50964-U	51421-U
75	x	3.0		on request	on request	51672-U	on request	on request	on request	50876-U	on request	on request	on request
100	x	3.0	50819-U	50617-U	577168-U	51673-U	51588-U	51617-U	584625-U	50879-U	51732-U	50967-U	51428-U
150	x	3.0	50821-U	50618-U	577160-U	51674-U	51589-U	51618-U	584606-U	50881-U	51734-U	50968-U	51429-U
Guard Columns, Three Pack													
5	x	2.1	50822-U	50620-U	577161-U	51676-U	51594-U	51619-U	584607-U	50884-U	51736-U	50969-U	51430-U
5	x	3.0	on request	50621-U	577162-U	51679-U	51595-U	51623-U	584608-U	50886-U	51739-U	50973-U	51433-U

HPLC Columns

Ascentis® Express Guard Cartridge Holder 53500-U



Ascentis® Express Capillary Columns

Increase Resolution and Speed

Ascentis® Express columns break new ground with a 1.5 mm internal diameter column in addition to typical capillary column dimensions of 1 mm I.D. or below, pushing the boundaries of UHPLC systems. Based on all the benefits of Fused-Core® particles, these 1.5 mm I.D. columns deliver increased sensitivity and reduced solvent consumption allowing scientists to experience the benefits of capillary columns without the pains of specialized, microflow systems.

Ascentis® Express Capillary Columns

Ascentis® Express (2.7 µm)					NEW			NEW	
Length (mm)		I.D. (mm)	C18	ES-C18	PSC-C18	Peptide ES-C18	C8	PCS-Phenyl-Hexyl	C30
50	x	0.3	53992-U			53546-U	53997-U		
150	x	0.3	54271-U			53554-U	54272-U		
50	x	0.5	53998-U			53547-U			
150	x	0.5	54273-U						577141-U
50	x	1.0				53548-U			
150	x	1.0				53561-U			
50	x	1.5	50629-U	50582-U	50637-U			50686-U	
100	x	1.5	50630-U	50584-U	50638-U			50688-U	
150	x	1.5	50636-U	50586-U	50639-U			50689-U	
Guard columns (3 pack)									
5	x	1.5			50649-U			50706-U	

Ascentis® Express Nano/Capillary columns
HPLC Columns

BIOshell™ HPLC and UHPLC Columns

Maximum Resolution for Glycan, Peptide, Protein, and IgG Separation

As the pharmaceutical and biotechnology industries continuously evolve into the development of “large molecule” biotherapeutics to treat a myriad of diseases, both fast and high-resolution separations are required in order to resolve the numerous structural variants that exist in these complex samples. The BIOshell™ line of superficially porous particle (SPP) packed columns has been developed to aid research into understanding the subtleties of the molecule that is being developed.

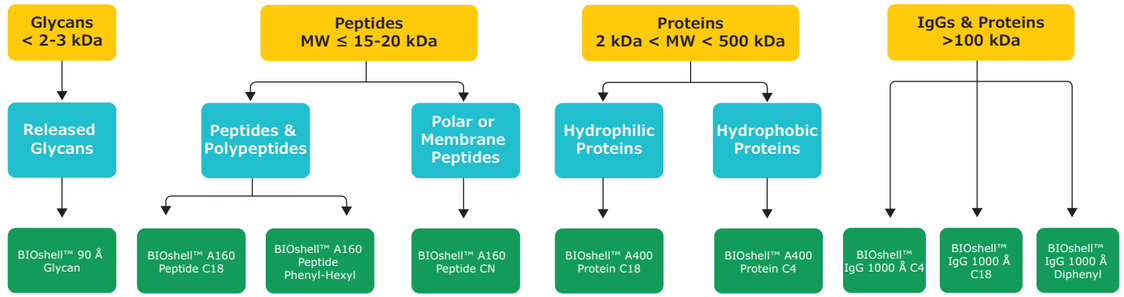
Highlighted Applications for these Columns include:

- Top-down analysis of intact proteins, monoclonal antibodies (mAbs), antibody-drug conjugates (ADCs), and other large biomolecules.
- Bottom-up analysis (peptide mapping) of proteins for primary structure confirmation.
- Middle-up analysis of mAb fragments (light and heavy chains).
- High resolution separation of released N- and O-linked glycans.

Features and Benefits

- Application specific columns for bioseparations that outperform fully porous particulate silica columns.
- Significantly higher separation efficiency.
- Offer better peak shape and peak capacity.
- Breakthrough 1000 Å pore diameter particles for large molecule enablement.

Column Selection Guide for Biomolecule Separations

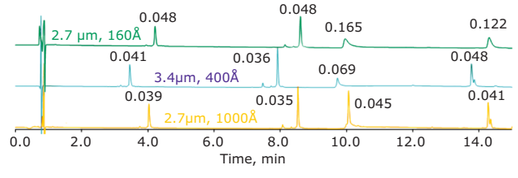


Pore Size Mismatch can Lead to Significant Losses in Efficiency

Column:	BIOshell™ A160 Peptide C18, 15 cm x 2.1 mm I.D., 2.7 µm; BIOshell™ A400 Protein C18, 15 cm x 2.1 mm I.D., 3.4 µm; BIOshell™ IgG 1000 Å C18, 15 cm x 2.1 mm I.D., 2.7 µm;
Mobile phase:	[A] Water (0.1% v/v trifluoroacetic acid); [B] 20:80 Water:Acetonitrile (0.085% v/v trifluoroacetic acid)
Gradient:	27% B to 60% B in 15 min
Flow rate:	0.4 mL/min
Column temp.:	60 °C
Detector:	UV, 280 nm
Injection:	4 µL
Sample:	Proteins, varied concentration, water (0.1% v/v trifluoroacetic acid)

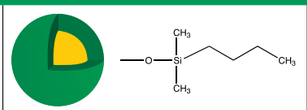
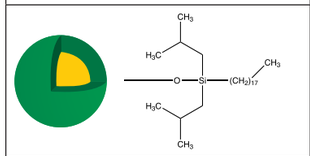
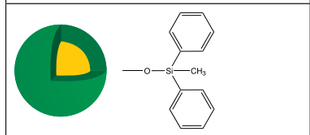
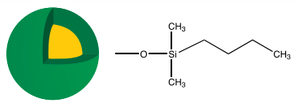
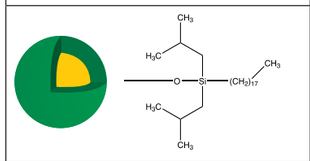
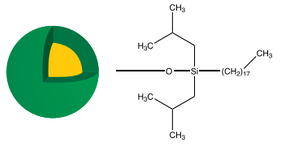
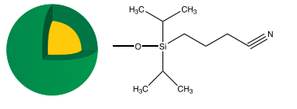
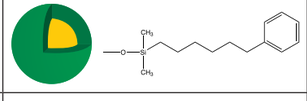
Higher efficiencies and better sensitivity can be realized with proper pore diameter selection. Here, the 1000 Å pore diameter is the only one capable of providing good peak shape of the mAb (peak 3) analyte.

1. Ribonuclease A (13.8 kDa)
2. Lysozyme (14.4 kDa)
3. SILu™ Lite SigmaMAb Antibody (~150 kDa)
4. Enolase (46.7 kDa)



BIOshell™ HPLC and UHPLC Columns
HPLC Columns

BIOshell™ HPLC and UHPLC Columns

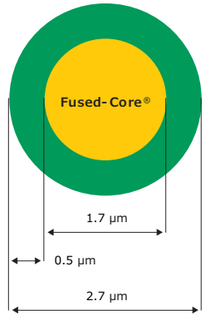
	Molecule size	Properties	Applications		Bonded Phase	USP Designation	Bonding Chemistry	Particle Size (s) (µm)	Pore Size (Å)	Carbon Load (%)	Surface Area (m^2/g)	Low pH /T Limit	High pH /T Limit	Endcapped
IgG	Large (> 50 kDa)	Largest pore diameter in the portfolio allowing for unrestricted access of proteins and other large molecules. Compatible for UHPLC, HPLC, and mass spectrometry (MS). C4, C18, and Diphenyl phase chemistries provide different selectivities. Resolution of very large proteins with superior peak shape and efficiency as compared to separations on FPP-packed columns.	mAbs; ADCs; Biosimilars; H/D Exchange; mAb Fragments		C4	L26	Dimethylbutylsilane	2.7	1000	0.6	22	2/90 °C	9/40 °C	Yes
					C18	L1	Diisobutyloctadecylsilane	2.7	1000	1.4	22	1/90 °C	8/40 °C	Yes
					Diphenyl	L11	Diphenylmethylsilane	2.7	1000	1.0	22	2/90 °C	9/40 °C	Yes
Protein	Large (2 kDa < MW < 500 kDa)	Fast separation of biomolecules due to a more shallow shell. Temperature stable up to 90 °C enabling high efficiency separations perfect for UHPLC, HPLC, and LC-MS assays. C4 and C18 chemistries provide different selectivities for hydrophobic and hydrophilic proteins. Resolution of large proteins with superior peak shape and efficiency as compared to separations on FPP-packed columns.	mAbs; ADCs; Biosimilars; Proteins; mAb Fragments		C4	L26	Dimethylbutylsilane	3.4	400	0.4	15	2/90 °C	9/40 °C	Yes
					C18	L1	Diisobutyloctadecylsilane	3.4	400	1.0	15	1/90 °C	8/40 °C	Yes
Peptide	Medium (0.1 kDa < MW < 15 kDa)	Wide range of particle sizes for both high efficiency separations and high throughput. Peak capacities of columns capable of resolving complex peptide mixtures.	Tryptic Digests; Post-Translational Modifications (PTMs); Polypeptides		C18	L1	Diisobutyloctadecylsilane	2	160	4.0	65	1/90 °C	8/40 °C	No
								2.7	160	4.6	90	1/90 °C	8/40 °C	No
								5	160	4.0	60	1/90 °C	8/40 °C	No
					Cyano	L10	Diisopropylcyanopropylsilane	2.7	160	2.2	90	1/90 °C	8/40 °C	Yes
								5	160	1.5	60	1/90 °C	8/40 °C	Yes
					Phenyl-Hexyl	L11	Dimethylphenyl-hexylsilane	2.7	160	4.7	90	2/90 °C	9/40 °C	Yes
Glycan	Small (< 20 kDa*) *for glycans, glycopeptides, and glycoproteins	Improved retention of polar compounds and zwitterions as compared to bare silica. Resolution of peaks unaffected by slight changes in buffer concentration. Capable of resolving complex mixtures of glycans (isobaric glycans with different linkages).	Resolution of oligosaccharides (released and labeled glycans) via hydrophilic interaction liquid chromatography (HILIC).		Penta-hydroxy	L95	Proprietary Ligand	2.7	90	3.2	135	2/65 °C	9/40 °C	No

HPLC Columns

HPLC Columns

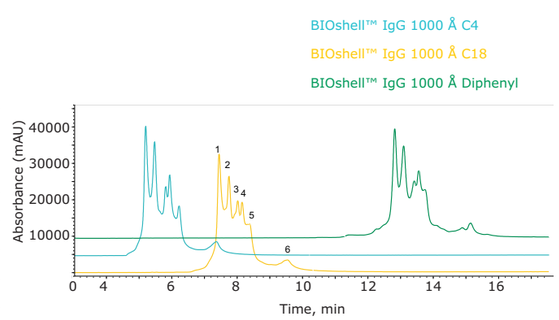
BIOshell™ IgG 1000 Å U/HPLC Columns: Maximizing Pore Diameter to Minimize Size Exclusion Effects

- A 1000 Å pore diameter allows unrestricted access of large biomolecules into the particles.
- Superficially Porous Particles (SPPs) provide narrower peak widths and improved resolution for characterization of biomolecules in comparison to Fully Porous Particles (FPPs).
- Post-translational modifications (PTMs) of expressed proteins can lead to subtle differences in molecular structure and function of the protein. These minor variants can be resolved with BIOshell™ IgG 1000 Å columns.
- Three different phase chemistries (C4, C18 and Diphenyl) for optimal selectivity.



Effect of Phase Chemistry on Protein Selectivity

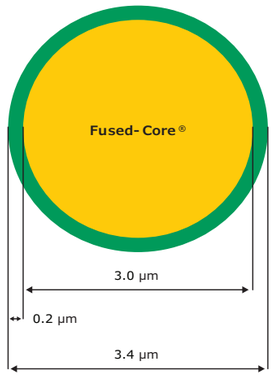
Column:	BIOshell™ IgG 1000 Å C4, 15 cm x 2.1 mm I.D., 2.7 µm; BIOshell™ IgG 1000 Å C18, 15 cm x 2.1 mm I.D., 2.7 µm; BIOshell™ IgG 1000 Å Diphenyl, 15 cm x 2.1 mm I.D., 2.7 µm
Mobile phase:	[A] 2:10:88 n-propanol/acetonitrile/water (0.1% v/v difluoroacetic acid); [B] 70:20:10 n-propanol/acetonitrile/water (0.1% v/v difluoroacetic acid)
Gradient:	16% B to 26% B in 20 min
Flow rate:	0.2 mL/min
Column temp.:	80 °C
Detector:	UV, 280 nm
Injection:	2 µL
Sample:	Denosumab, 2 mg/mL, water (0.1% v/v trifluoroacetic acid)



Monoclonal antibodies are unique molecules and therefore can interact differently with different phase chemistries.
The numbered peaks correspond to IgG2 disulfide bond variants.

BIOshell™ A400 Protein U/HPLC Columns: Minimizing Mass Transfer for Maximum Throughput

- A 0.2 µm-thin, porous shell with 400 Å pores leads to rapid and efficient separations of proteins.
- Superficially Porous Particles (SPPs) provide narrower peak widths and improved resolution for characterization of biomolecules in comparison to Fully Porous Particles (FPPs).
- Ability to tolerate high temperature applications (up to 90 °C) in acidic mobile phases.
- Two different phase chemistries (C4 and C18) for rapid protein separation.



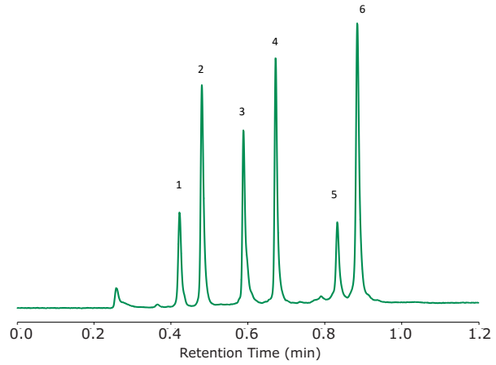
HPLC Columns

Rapid Protein Separations on BIOshell™ A400 Column

Column:	BIOshell™ A400 Protein C4, 5 cm x 2.1 mm I.D., 3.4 µm
Mobile phase:	[A] 75:25 (0.1% TFA in water):(0.1% TFA in acetonitrile); [B] 25:75 (0.1% TFA in water):(0.1% TFA in acetonitrile)
Gradient:	12 to 100% B in 1 min; held at 100% B for 1 min
Flow rate:	0.4 mL/min
Column temp.:	90 °C
Detector:	UV, 215 nm
Injection:	1 µL
Sample:	Protein mix, varied concentration, water (0.1% TFA)

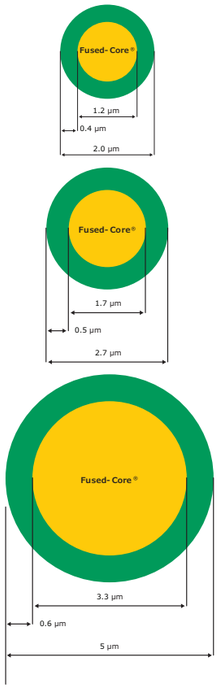
Due to the shallow, porous shell, protein separations can be performed in less than one minute with the BIOshell™ A400 column.

Peak Name	kDa (native)
1. lysozyme; chicken	14.3
2. haptoglobulin, phenotype 1-1; human	96
3. glutamate dehydrogenase; bovine	332
4. ß-galactosidase; *E. coli*	465
5. lactate dehydrogenase contaminant	
6. lactate dehydrogenase; rabbit	140



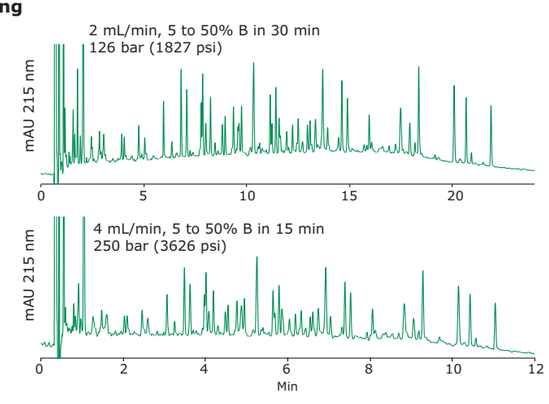
BIOshell™ A160 Peptide U/HPLC Columns: High Resolution Peptide Separations

- BIOshell™ A160 Peptide U/HPLC columns offer a broad portfolio of particle sizes and phase chemistries to create a superior solution for fast and efficient separations of peptides up to 20 kDa.
- Higher resolutions and higher peak capacities of peptides at ~ 50% backpressure of sub-2 µm Fully Porous Particle (FPP)-packed columns.
- Lower backpressure allows for columns to be used in series to maximize resolution and peak capacity of complex proteomic samples or tryptic digests.
- Compatible with all UHPLC and LC-MS instrumentation.
- Three different particle sizes and phase chemistries (Phenyl-Hexyl, C18 and Cyano) for fast peptide separations.



Rapid Tryptic Digest Analysis Using BIOshell™ A160 Peptide C18

Column:	BIOshell™ A160 Peptide C18, 15 cm x 4.6 mm I.D., 5.0 µm
Mobile phase:	[A] Water (0.1% TFA); [B] Acetonitrile (0.1% TFA)
Gradient:	As indicated
Flow rate:	As indicated
Column temp.:	60 °C
Detector:	UV, 215 nm
Injection:	15 µL
Sample:	Tryptic digest, 1 mg/mL, water (0.1% TFA)

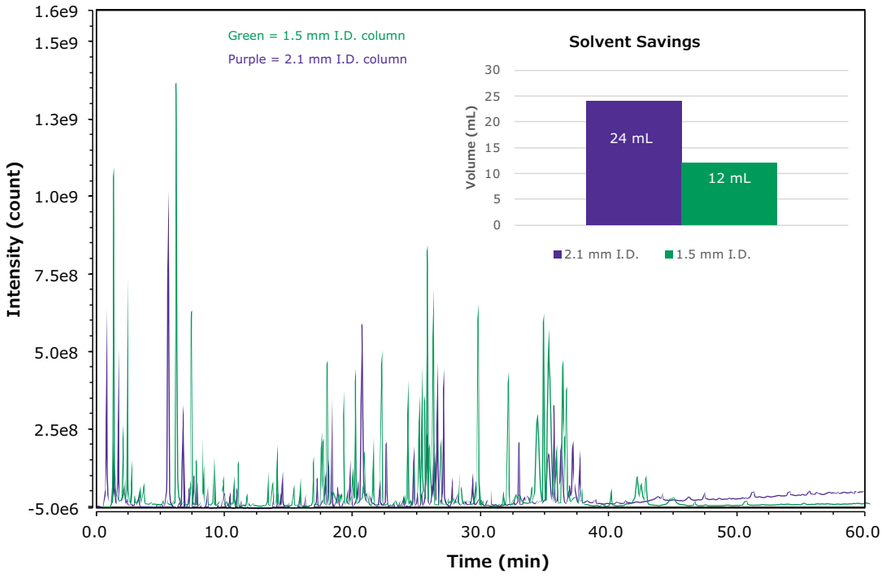


BIOshell™ A160 Peptide columns can more than double sample throughput by allowing the analyst to operate at higher flow rates without a concomitant drop in efficiency.

HPLC Columns

BIOshell™ 1.5 mm I.D. Columns for Improved Sensitivity and Solvent Savings

As available sample volume might be small, and the need for higher sensitivity increases, a solution is required for this type of analysis. Outside of purchasing a high-end mass spectrometer, another solution could be to use more narrow I.D. columns. One significant advantage of using 1.5 mm I.D. HPLC columns is their enhanced efficiency and improved separation capabilities. The smaller column diameter allows for increased column efficiency, resulting in higher resolution and better peak shapes for the analyzed compounds. Additionally, 1.5 mm inner diameter columns require lower mobile phase flow rates and consume less solvent, making them more cost-effective and environmentally friendly (more sustainable). These columns are particularly advantageous when dealing with complex samples or trace-level analysis, as they offer higher sensitivity and improved detection limits. The below figure shows the sensitivity gains and solvent savings when using a 1.5 mm I.D. column over a 2.1 mm I.D. column when analyzing a digested sample of trastuzumab.



Chromatographic Conditions

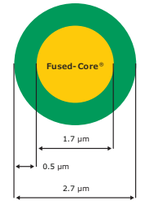
Column:	BIOshell™ A160 Peptide C18, 2.7 µm 15 cm x 1.5 mm I.D., 2.7 µm (66922-U) 15 cm x 2.1 mm I.D., 2.7 µm (66905-U)
Mobile phase:	[A] Water (0.1% (v/v) DFA); [B] Acetonitrile (0.1% (v/v) DFA)
Gradient:	2 - 50% B in 60 min
Flow rate:	0.2 mL/min (1.5 mm I.D.) or 0.4 mL/min (2.1 mm I.D.)
Column temp.:	60 °C
Detector:	MSD, ESI-(+)
Injection:	2.0 µL
Sample:	Trastuzumab tryptic digest, 1.25 mg/mL, 1.5 M guanidine hydrochloride, 0.5% (v/v) formic acid

Spray voltage:	3.8 kV
Capilary temp:	320 °C
Sheath gas:	35
Aux gas:	10
RF lens	50

HPLC Columns

BIOshell™ Glycan U/HPLC Columns: High Resolution Glycan Separations

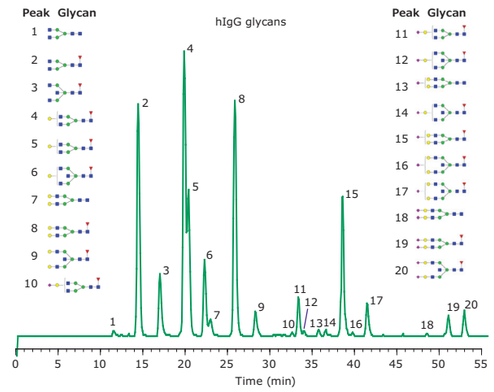
- BIOshell™ Glycan U/HPLC columns consist of a proprietary, pentahydroxy chemistry tethered to a 2.7 µm, 90 Å superficially porous particle (SPP).
- Appropriate for USP L95 methods.
- Main application is for the resolution of oligosaccharides (released and labeled glycans) via hydrophilic interaction liquid chromatography (HILIC).
- Increased retention of acidic and zwitterionic analytes than bare silica columns.
- Each lot of BIOshell™ Glycan particles is tested for quality control by separation of a series of labeled glycans having 2 – 25 glucose units (GU).



HPLC Analysis of Procainamide-Labeled Human IgG Glycans on BIOshell™ Glycan using HILIC-FLR

Column:	BIOshell™ Glycan, 15 cm x 2.1 mm I.D., 2.7 µm
Mobile phase:	[A] 50 mM ammonium formate, pH 4.4 (50 mM ammonium hydroxide, adjusted to pH 4.4 with formic acid); [B] acetonitrile
Gradient:	75% B to 59% B in 75 min
Flow rate:	0.3 mL/min
Temp.:	60 °C
Detector:	FLR, ex 308 nm, em 359 nm
Injection vol:	10 µL
Sample:	Released human IgG glycans

Excellent resolution and peak shape of released glycans.



BIOshell™ IgG 1000 Å U/HPLC Columns

Dimensions Length (cm)		I.D. (mm)	C_4	C_{18}	Diphenyl
5	x	0.075	**	**	**
100	x	0.075	**	**	**
150	x	0.075	**	**	**
5	x	0.1	**	**	**
100	x	0.1	**	**	**
150	x	0.1	**	**	**
5	x	0.2	**	**	**
10	x	0.2	**	**	**
150	x	0.2	**	**	**
5	x	0.3	63336-U	581367-U	577436-U
10	x	0.3	**	**	**
15	x	0.3	63335-U	581366-U	577435-U
5	x	0.5	63338-U	581381-U	577438-U
10	x	0.5	**	**	**
15	x	0.5	63337-U	581380-U	577437-U
3	x	1.0	**	**	**
5	x	1.0	63340-U	581383-U	577440-U
7.5	x	1.0	**	**	**
10	x	1.0	**	**	**
15	x	1.0	63339-U	581382-U	577439-U
5	x	1.5	581385-U	**	577451-U
15	x	1.5	581384-U	**	577450-U
2	x	2.1	63281-U	**	**
3	x	2.1	63282-U	**	**
5	x	2.1	63283-U	581362-U	577419-U
7.5	x	2.1	63284-U	**	**
10	x	2.1	63288-U	582701-U	577420-U
15	x	2.1	63289-U	582703-U	577421-U
25	x	2.1	**	582704-U	577422-U
2	x	3.0	63306-U	**	**
3	x	3.0	63307-U	582705-U	577423-U
5	x	3.0	63308-U	582706-U	577424-U
7.5	x	3.0	63311-U	**	**
10	x	3.0	63313-U	582707-U	577425-U
15	x	3.0	63314-U	582708-U	577426-U
25	x	3.0	**	**	**
2	x	4.6	63322-U	**	**
3	x	4.6	63324-U	582709-U	577427-U
5	x	4.6	63325-U	582710-U	577428-U
7.5	x	4.6	63327-U	**	**
10	x	4.6	63328-U	582713-U	577429-U
15	x	4.6	63329-U	581348-U	577430-U
25	x	4.6	**	**	**
Guard Columns, Three Pack					
0.5	x	2.1	63291-U	581349-U	577431-U
0.5	x	3.0	63315-U	581360-U	577432-U
0.5	x	4.6	63334-U	581361-U	577433-U
Guard Column Holder:			66841-U		

For geometries marked **, please contact technical service for custom quote options.

HPLC Columns

BIOshell™ Glycan U/HPLC Columns

Dimensions Length (cm)		I.D. (mm)	BIOshell™ Glycan
5	x	2.1	50991-U
10	x	2.1	50993-U
15	x	2.1	50994-U
5	x	4.6	50997-U
10	x	4.6	50998-U
15	x	4.6	50999-U
Guard Columns, Three Pack			
0.5	x	2.1	**
0.5	x	4.6	**
Guard Column Holder:			66841-U

BIOshell™ A400 Protein U/HPLC Columns

Dimensions Length (cm)		I.D. (mm)	C_4	C_{18}
5	x	0.075		
100	x	0.075	**	**
150	x	0.075		
5	x	0.1		
100	x	0.1	**	**
150	x	0.1		
5	x	0.2		
10	x	0.2	**	**
150	x	0.2	67037-U	67495-U
5	x	0.3	67038-U	67496-U
10	x	0.3	**	**
15	x	0.3	67039-U	
5	x	0.5		
10	x	0.5	**	**
15	x	0.5	67041-U	
3	x	1.0	**	**
5	x	1.0		
7.5	x	1.0	**	**
10	x	1.0	**	**
15	x	1.0	67045-U	
2	x	2.1	**	67457-U
3	x	2.1	**	67458-U
5	x	2.1	66824-U	67459-U
7.5	x	2.1	**	67462-U
10	x	2.1	66825-U	67463-U
15	x	2.1	66826-U	67469-U
25	x	2.1	**	**
2	x	3.0	**	67471-U
3	x	3.0	**	67472-U
5	x	3.0	**	67473-U
7.5	x	3.0	**	67474-U
10	x	3.0	**	67475-U
15	x	3.0	**	67477-U
25	x	3.0	**	**
2	x	4.6	**	67478-U
3	x	4.6	**	67482-U
5	x	4.6	66827-U	67483-U
7.5	x	4.6	**	67485-U
10	x	4.6	66828-U	67487-U
15	x	4.6	66829-U	67488-U
25	x	4.6	**	**
Guard Columns, Three Pack				
0.5	x	2.1	66830-U	67505-U
0.5	x	3.0	**	67506-U
0.5	x	4.6	66831-U	67508-U
Guard Column Holder:			66841-U	

For geometries marked **, please contact technical service for custom quote options.

BIOshell™ A160 Peptide U/HPLC Columns

Dimensions Length (cm)		I.D. (mm)	160 Å, 2.0 µm C18	160 Å, 2.7 µm C18	160 Å, 2.7 µm CN	160 Å, 2.7 µm Phenyl-Hexyl	160 Å, 5.0 µm C18	160 Å, 5.0 µm CN
5	x	0.075	**			**		
100	x	0.075	**	**	**	**	**	**
150	x	0.075	**			**		
5	x	0.1	**			**		
100	x	0.1	**	**	**	**	**	**
150	x	0.1	**			**		
5	x	0.2	**			**		
10	x	0.2	**	**	**	**	**	**
150	x	0.2	**			**		
5	x	0.3	**		67159-U	577546-U		
10	x	0.3	**	**	**	**	**	**
15	x	0.3	**	67093-U	67160-U	577545-U		
5	x	0.5	**		67161-U	577548-U		
10	x	0.5	**	67096-U	**	**	**	**
15	x	0.5	**	67097-U	67163-U	577547-U		
3	x	1.0	**	**	**	**	**	**
5	x	1.0	**		67164-U	577550-U		
7.5	x	1.0	**	**	**	**	**	**
10	x	1.0	**	**	**	**	**	**
15	x	1.0	**	67099-U	67165-U	577549-U		
5	x	1.5	67284-U	66923-U	**	**	**	**
15	x	1.5	67283-U	66922-U	**	**	**	**
2	x	2.1	67234-U	**	**	577523-U	**	**
3	x	2.1	67238-U	66901-U	66965-U	577524-U	67001-U	67061-U
5	x	2.1	67239-U	66902-U	66966-U	577525-U	67002-U	67062-U
7.5	x	2.1	67241-U	66903-U	66967-U	577526-U	67003-U	67063-U
10	x	2.1	67242-U	66904-U	66968-U	577527-U	67004-U	67064-U
15	x	2.1	67243-U	66905-U	66969-U	577528-U	67006-U	67065-U
25	x	2.1	**	**	**	**	**	**
2	x	3.0	67257-U	**	**	577529-U	**	**
3	x	3.0	67263-U	66906-U	66970-U	577530-U	67007-U	67066-U
5	x	3.0	67267-U	66907-U	66971-U	577531-U	67008-U	67067-U
7.5	x	3.0	67274-U	**	**	577532-U	**	**
10	x	3.0	67275-U	66908_U	66972-U	577533-U	67011-U	67068-U
15	x	3.0	67277-U	66909-U	66973-U	577534-U	67012-U	67069-U
25	x	3.0	**	**	**	**	**	**
2	x	4.6	**	**	**	577535-U	**	**
3	x	4.6	**	**	**	577536-U	**	**
5	x	4.6	**	66913-U	66974-U	577537-U	67013-U	67071-U
7.5	x	4.6	**	**	**	577538-U	**	**
10	x	4.6	**	66915-U	66975-U	577539-U	67014-U	67080-U
15	x	4.6	**	**	66976-U	577540-U	67015-U	67081-U
25	x	4.6	**	**	**	**	**	**
Guard Columns, Three Pack								
0.5	x	2.1	67281-U	66918-U	66977-U	577541-U	67016-U	67082-U
0.5	x	3.0	67282-U	66919-U	66978-U	577542-U	67017-U	67083-U
0.5	x	4.6	**	66921-U	66979-U	577543-U	67018-U	67084-U
Guard Column Holder:			66841-U					

For geometries marked **, please contact technical service for custom quote options.

Monolithic Silica HPLC Columns

Revolutionary Monolithic Silica for Rapid and Robust Separations

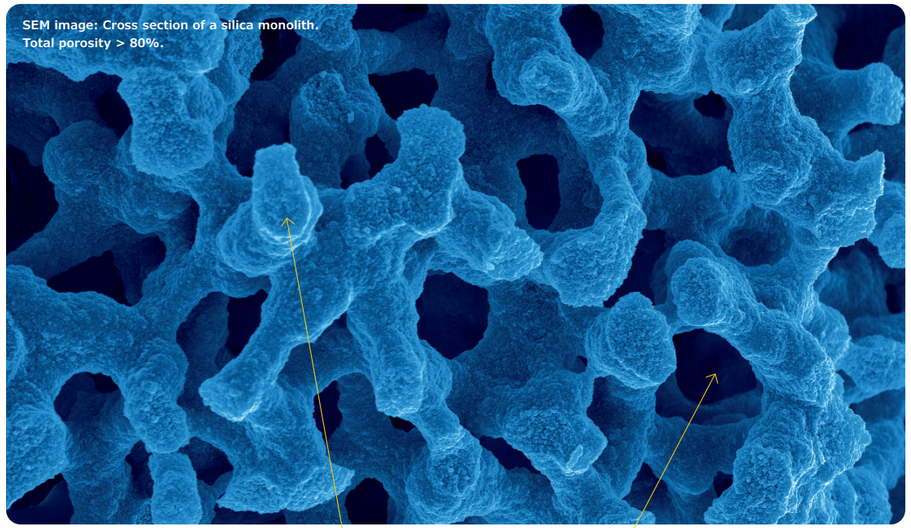
Thanks to their patented, monolithic silica technology, Chromolith® HPLC columns allow you to race through separations with maximum robustness and selectivity- at minimal back pressure.

Bi-modal pore structure: Macropores and Mesopores

To truly accelerate chromatographic separations, there is no better choice than Chromolith® HPLC columns. Due to their revolutionary, monolithic technology, Chromolith® columns provide excellent and rapid separations with extremely high robustness and matrix tolerance compared to particulate columns.

The secret to the speed of Chromolith® columns is their exceptionally low back pressure. Produced from a continuous piece of porous silica using a sol-gel process, Chromolith® columns possess a defined bimodal pore structure with macro and mesopores in the micro and nanometer range. The high permeability and porosity of the silica skeleton, and the resulting low back pressure, allow for more flexible flow rates than particle-packed columns. As a result, Chromolith® HPLC columns enable high-throughput analysis without loss of separation efficiency or peak capacity.

The revolutionary, bimodal pore structure of Chromolith® columns provides a unique combination of macropores and mesopores.



Mesopores: Average pore size is 13 nm for Chromolith®, 15 nm for Chromolith® HR, and 30 nm for Chromolith® WP 300.
Forms a fine porous structure with a large, uniform surface area on which adsorption takes place, thus enabling high-performance chromatographic separation.

Macropores: Average pore size is 1.5 µm for Chromolith® 2 mm I.D. , 1.15 µm for Chromolith® HR, and 2 µm for all others.
Allows rapid flow of the mobile phase at low back pressure.

Chromolith® Pore Sizes

	Mesopores	Macropores
Chromolith® Performance	13 nm (130 Å)	2 µm
Chromolith® 2 mm ID	13 nm (130 Å)	1.5 µm
Chromolith® HighResolution	15 nm (150 Å)	2 µm
Chromolith® WP 300	30 nm (300 Å)	2 µm

HPLC Columns

Monolithic Silica Technology

Chromolith® HPLC Columns for Small and Large Molecule Separation

Several key benefits result directly from the bimodal pore structure of the silica gel:

- Rapid separations at very low column back pressure.
- Standard HPLC instruments are fully compatible with all Chromolith® columns and UHPLC instruments are fully compatible with Chromolith® 2 mm I.D. columns.
- Matrix-rich samples (such as food or biological samples) can be analyzed without the need for sophisticated and time-consuming sample preparation. Guard column cartridges are also available.
- Cost-savings are achieved as the column lifetimes are much longer than for particulate HPLC columns, in particular when analyzing matrix-rich samples.
- Complex, multi-component samples can be separated either by using Chromolith® HighResolution (HR) columns or by using long, high-efficiency columns formed by connecting two or more Chromolith® columns together. The low column backpressure makes this possible.
- Easy transfer of methods from a particulate column to a Chromolith® column.

The columns are available with several surface modifications such as octadecyl (RP-18e) or octyl (RP-8e) endcapped, CN (cyano), Diol and NH_2 (amino) as well as an unmodified pure silica. The available column dimensions range from capillary (nano) columns to preparative HPLC columns with 25 mm I.D.

Column Dimensions	
HighResolution RP-18e	2 and 4.6 mm I.D. and Capillary columns
HighResolution RP-8e	4.6 mm I.D. and Capillary columns
RP-18e	2, 3, 4.6, 10, 25 mm I.D. and Capillary columns
RP-8e	4.6 mm I.D.
CN	4.6 mm I.D.
Diol	4.6 mm I.D.
NH_2	4.6 mm I.D.
Si	4.6, 10 and 25 mm I.D.

Chromolith® WP 300 Columns for Biomolecule Separations

Chromolith® columns have shown great potential for the analysis of proteins, antibodies and large peptides where columns with good permeability, along with better mass transfer and selectivity are required. Chromolith® columns remove back pressure as the primary consideration in method development and allow flow rate flexibility for much higher throughput, a choice of column lengths for superior resolution, and more solvent options for optimum selectivity. With no individual particles to shift or break, column performance is consistent over a much longer lifetime, making them ideal for matrix-rich sample analysis.

Column Dimensions	
WP 300 RP-18	2 and 4.6 mm I.D.
WP 300 RP-8	4.6 mm I.D.
WP 300 RP-4	4.6 mm I.D.
WP 300 Protein A	2 and 4.6 mm I.D.
WP 300 Epoxy	2 and 4.6 mm I.D.

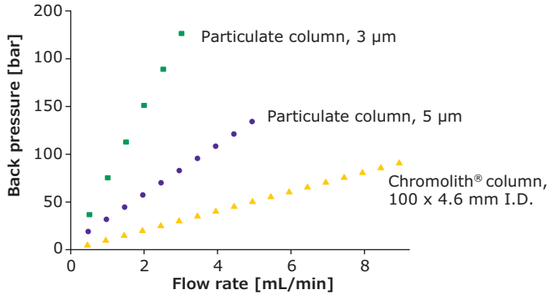
Chromolith® HPLC and U/HPLC Columns

Chromolith® WP 300 HPLC Columns

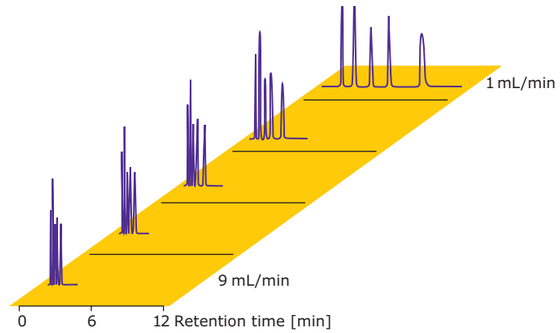
HPLC Columns

Analysis Speed

Chromolith® columns owe their rapid separation speed to their unique, bimodal pore structure of macro and mesopores. The macropores reduce column back pressure and allow the use of faster flow rates, thereby considerably reducing analysis time. The mesopores form a fine porous structure, which creates a very large, active surface area for high-efficiency separations.



With Chromolith® columns, flow rates can now easily be varied from 1 mL up to 9 mL per minute with the same high quality resolution. A mixture of five beta-blocker drugs was analyzed to demonstrate the extreme time savings and high separation efficiency made possible with Chromolith® columns. Due to excellent mass transfer properties of the monolithic skeleton, high-speed separation was possible, even at high flow rates. The beta-blockers were well separated with excellent peak symmetry. At 9 mL/min, analysis time was less than 1 minute, and the column back pressure was only 153 bar.

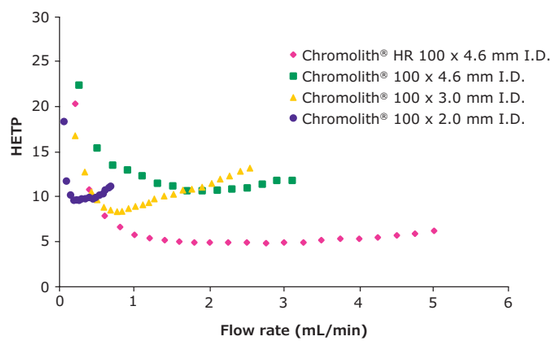


Chromolith® Performance RP-18e 100 x 4.6 mm I.D.

Column:	Chromolith® Performance RP-18 endcapped 100 x 4.6 mm I.D.	
Mobile phase:	Isocratic, Acetonitrile / 0.1 % trifluoroacetic acid in water, 20/80 (v/v)	
Pressure:	Total pressure (including HPLC system) 25 °C	
Detection:	UV, 220 nm	
Injection volume:	5 µL	
Sample:	Atenolol	63 µg/mL
	Pindolol	29 µg/mL
	Metoprolol	108 µg/mL
	Celiprolol	104 µg/mL
	Bisoprolol	208 µg/mL

High Separation Efficiency

The traditional plate-count method of measuring quality shows that the separation efficiency of Chromolith® columns is better than standard 5 µm particulate columns, and just as good as 3.5 µm columns, but with the ability to continue up to 9 mL/min without reaching HPLC system pressure limits. The van Deemter plot of the Chromolith® column clearly demonstrates that separation efficiency does not decrease significantly when flow rate is increased, as is the case with particulate columns. It is, therefore, possible to operate Chromolith® columns at high flow rates with minimal loss of peak resolution. For complex separations, it is still necessary to use long columns in order to provide the separation efficiency required for resolution of all compounds of interest. Chromolith® HPLC columns can be connected in series to produce a column with high plate count at low back pressure. (Please see: Chromolith® column coupler). With particulate columns, further column length is prevented by excessive back pressure.

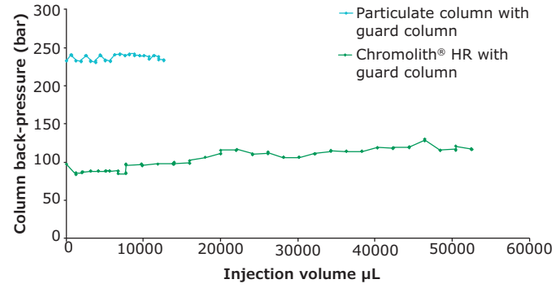


HPLC Columns

Long-term Stability

Besides lower back pressure and greater flow rate flexibility, Chromolith® columns also achieve faster equilibration after gradient elution than particle-packed columns of similar dimensions. These features allow high-throughput analysis without loss of separation efficiency or peak capacity.

The significantly longer column lifetime of Chromolith® monolithic HPLC columns, in comparison to particulate HPLC columns, is over-compensating the higher cost of this column material in comparison to particulate columns. In particular, if matrix-rich samples are used with simplified sample preparation; therefore, the cost per sample can be significantly lower in direct comparison under equal conditions and the same sample.

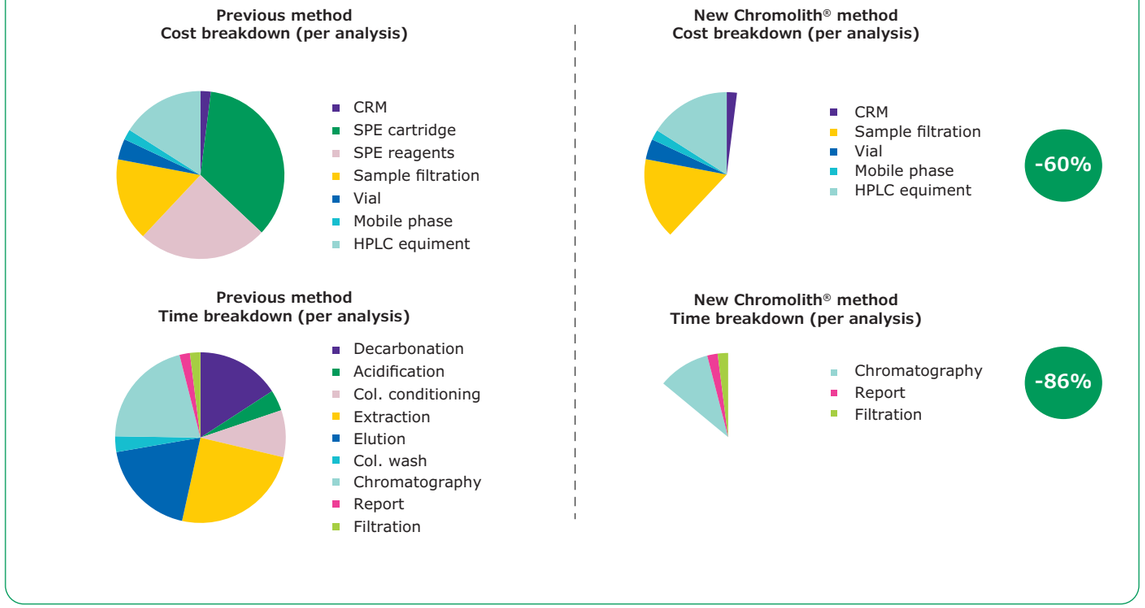


Note: Comparison of long-term stability by injection volume under equal conditions and simplified sample preparation using a matrix-rich sample for a Chromolith® monolithic HPLC column (green line) and a fully porous particulate HPLC column with 3 µm particles (blue line)

Column Robustness

Chromolith® columns offer excellent robustness and unsurpassed column lifetime. This trait not only ensures maximum reliability and versatility, but also minimizes maintenance on the HPLC system. As a result, Chromolith® columns reduce cost per analysis while enhancing data integrity.

When analyzing challenging, matrix-rich samples with traditional HPLC columns, the benefit of monolithic silica columns is significant. Sample preparation is time-consuming and costly. The transfer from particulate columns to monolithic columns can, therefore, save a substantial amount of cost and time.



Time and cost per analysis for the determination of Iso-alpha-acids in Beer using a particulate and a monolithic column.

HPLC Columns

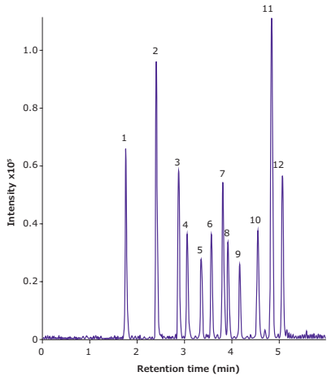
Chromolith® and Chromolith® HighResolution (HR) HPLC Columns

Robust and rapid separation of small molecules

Chromolith® HPLC columns "Performance" with 100 mm length; "SpeedRod" with 50 mm length and "Flash" with 25 mm length provide the highest matrix-tolerance due to their large marcopores (2 µm) and are available with several column chemistries for a broad selectivity range.

Chromolith® columns with 2 mm internal diameter "Performance" with 100 mm length, "FastGradient" with 50 mm length and "Flash" with 25 mm length are ideal for use with UHPLC or UPLC instruments, thanks to their very small internal volumes. A particular benefit is the very fast analysis, reduced sample-preparation and the low column backpressure. Ultra-high performance and extremely low operating pressure make Chromolith® 2 mm columns truly unique.

Excellent, ultra-fast results are obtained, not only in UHPLC and UPLC, but equally well in all standard HPLC systems with low dead volume. Chromolith® 2 mm columns have macropores of 1.5 µm in diameter, resulting in a column efficiency that exceeds 100,000 plates/meter.

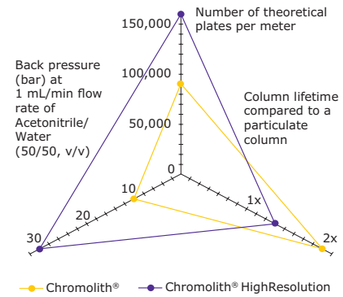


Column:	Chromolith® FastGradient RP-18 endcapped 50-2 mm		
Mobile phase:	A: ACN + 0.1 % HCOOH B: Water + 0.1 % HCOOH		
Gradient:	**Time**	**% A**	**% B**
	0	15	85
	4.5	70	30
	6	70	30
Flow rate:	0.5 mL/min		
Pressure:	55 – 85 bar		
Detection:	MS; Ion Source: ESI; Ion Trap		
Sample:	1. Metabolite of Fluoxymesterone		353 m/z
	2. Metabolite of Stanozolol		345 m/z
	3. Metabolite of Danazol		343 m/z
	4. Testosterone		289 m/z
	5. Epitestosterone		289 m/z
	6. Metabolite of Methyltestosterone		271 m/z
	7. Metabolite of Calusterone		285 m/z
	8. Metabolite of Clostebol		305 m/z
	9. Boldenone-acetate		329 m/z
	10. Testosterone-acetate		331 m/z
	11. Nandrolone-17-Propionate		331 m/z
	12. Testosterone-Propionate		345 m/z

Phase Bonding	USP Designation	Bonding Chemistry	Silica Type	Macropore Size (µm)	Mesopore Size (Å)	Pore Volume (mL/g)	Surface Area (m^2/g)	Carbon Load (%)	pH Stability	Max Temperature	Endcapped
RP-18 endcapped	L1	Octadecylsi-lane	Monolithic Type B silica	2 [2 mm I.D. Columns: 1.5 µm]	130	1	300	18	2 - 7.5	50	Yes
High Resolution RP-18 endcapped	L1	Octadecylsi-lane	Monolithic Type B silica	1.15	150	1	250	18	1.5 - 8	50	Yes
RP-8 endcapped	L7	Octylsilane	Monolithic Type B silica	2	130	1	300	11	2 - 7.5	50	Yes
High Resolution RP-8 endcapped	L7	Octylsilane	Monolithic Type B silica	1.15	150	1	300	11	1.5 - 8	50	Yes
CN	L10	Cyanosilane	Monolithic Type B silica	2	130	1	300	6	2 - 7.5	50	No
Diol	L20	Diolsilane	Monolithic Type B silica	2	130	1	300	9	2 - 7.5	50	No
NH2	L8	Aminopropyl-silane	Monolithic Type B silica	2	130	1	300	5	2 - 7.5	50	No
Si	L3	unbonded	Monolithic Type B silica	2	130	1	300	n.a.	2 - 7.5	50	No

Chromolith HPLC Columns
HPLC Columns

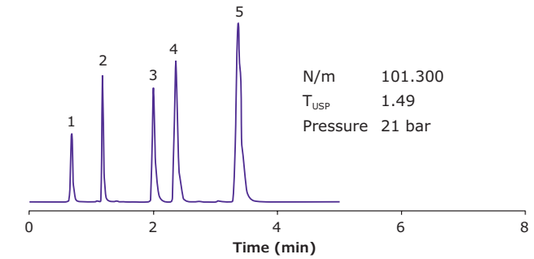
Chromolith® HighResolution (HR) columns have around 50% higher efficiency, which is approximately equivalent to 2.6 µm particulate columns, whereas the backpressure is approximately equivalent to 5 µm columns, excellent peak symmetry and still more than 30 % longer lifetime compared with particulate columns. Two Chromolith® HighResolution columns could be easily coupled in order to achieve even higher resolution. The completely endcapped stationary phase enables peak-tailing free elution of basic compounds.



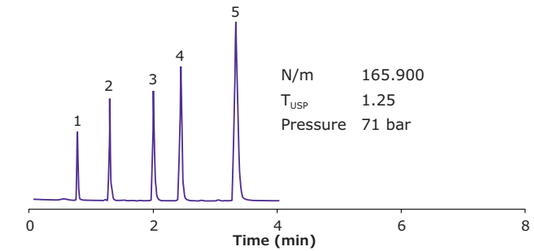
Excellent batch-to-batch reproducibility

The batch-to-batch reproducibility of Chromolith® HPLC columns is strictly controlled and fulfills the requirements of QA and QC laboratories.

Chromolith® Performance RP-18e 100-4.6 mm

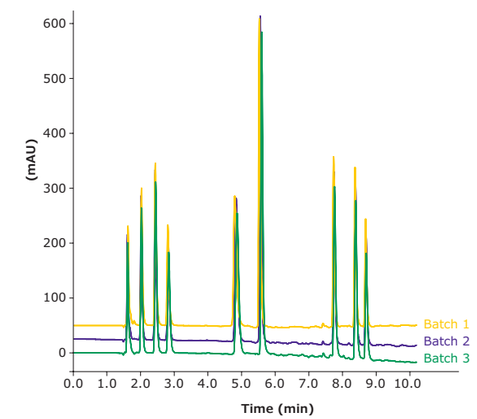


Chromolith® HighResolution RP-18e 100-4.6 mm



Mobile phase:	Acetonitrile / water 60/40
Flow rate:	2 mL/min
Detection:	UV 254 nm
Temperature:	ambient
Injection volume:	5 µL
Sample:	1. Urea 2. Biphenyl-2-ol 3. Progesterone 4. Hexanophenon 5. Anthracene

Chromolith® HighResolution RP-18e - Batch consistency



Column:	Chromolith® HighResolution RP-18 endcapped 100-4.6 mm
Mobile phase:	A: Acetonitrile + 0.1 % TFA B: Water + 0.1 % TFA
Gradient:	2 min 0% A 10 min 30% A
Flow rate:	1 mL/min
Detection:	UV 210 nm
Temperature:	25 °C
Injection volume:	2 µL
Sample:	1. Norepinephrine 2. Octopamine 3. Epinephrine tartrate 4. Dopamine 5. DOPA 6. Norephedrine 7. Ephedrine 8. N-Methylephedrine

HPLC Columns

Chromolith® Semi-preparative and Preparative HPLC Columns

Chromolith® SemiPrep 10 mm I.D. columns

Combine high separation speed with excellent performance. These traits make them the perfect alternative to particulate columns of 10 mm I.D. (and even 21.2 mm I.D.). These columns have the same bimodal porous silica rod structure as Chromolith® analytical columns with an internal diameter of 4.6 mm. Their macropores are 2 µm in diameter and the mesopores are 13 nm. This combination dramatically reduces separation time while increasing efficiency.

Chromolith® Prep columns

Preparative HPLC involves much higher sample volumes than analytical chromatography. Consequently, greater sample throughput and separation speed are essential for optimal productivity. These criteria are best fulfilled by Chromolith® Prep columns. The combination of macro and mesopores maximizes separation efficiency and flow rate, while minimizing resistance.

Ready-to-use Chromolith® Prep column

Ordering Information

Column dimension										
Length (mm)		I.D. (mm)	RP-18e	HR RP-18e	RP-8e	HR RP-8e	CN	Diol	NH2	Si
25	x	4.6	1.51463.0001	1.52020.0001			1.52046.0001	1.53170.0001	1.52026.0001	
25	x	3	1.52003.0001							
25	x	2	1.52014.0001	1.52320.0001						
50	x	4.6	1.51450.0001	1.52021.0001			1.52047.0001	1.53171.0001	1.52027.0001	
50	x	3	1.52002.0001							
50	x	2	1.52007.0001	1.52321.0001						
100	x	4.6	1.02129.0001	1.52022.0001	1.51468.0001	1.52064.0001	1.52048.0001	1.53172.0001	1.52028.0001	1.51465.0001
100	x	3	1.52001.0001							
100	x	2	1.52006.0001	1.52322.0001						
150	x	4.6		1.52023.0001						
100	x	10	1.52016.0001							1.52015.0001
100	x	25	1.25252.0001							1.25251.0001
Validation Kits [3 Chromolith® HPLC cartridges from 3 different sorbent batches]										
50	x	2	1.52062.0001							
100	x	4.6	1.51466.0001	1.52019.0001						
100	x	3	1.52063.0001							
10	x	10	1.52036.0001							1.52035.0001
Chromolith® Guard cartridges [3 units]										
5	x	4.6	1.51451.0001	1.52025.0001	1.52013.0001		1.52050.0001	1.53175.0001	1.52030.0001	1.52011.0001
10	x	4.6	1.51452.0001							
5	x	3	1.52005.0001							
5	x	2	1.52009.0001	1.52325.0001						
Chromolith® Guard cartridges set [1 starter kit with holder and 3 guard cartridges]										
5	x	4.6	1.52008.0001							
5	x	3	1.52004.0001							

Validation kits are available

HPLC Columns

Chromolith® Guard Column Holder

Chromolith® HPLC guard cartridges are extremely easy to use. The guard cartridges are simply added directly in front of the main column to protect it from chemical or mechanical contamination. Due to the benefits of monolithic technology, and the convenience of Chromolith® guard columns, they are also popular for use with classical particulate columns. Moreover, guard columns can be used as trap columns when large sample volumes are to be injected. Guard columns should be changed frequently in order to avoid excessive accumulation of impurities.

Guard cartridge holders

Depending on your needs, we offer several different guard cartridge holders: made out of PEEK for 2 and 3 mm I.D. cartridges; bioinert PEEK lined stainless steel holder and standard stainless steel holder for 4.6 mm I.D. cartridges and holders for 10 and 25 mm I.D. cartridges.

a) b) c) d)

Guard cartridge holder type	Material holder is made of	Max. back pressure	How to tighten holder	Guard cartridge I.D.	Guard cartridge length
a)	PEEK	200 bar (2,940 psi)	Finger-tight	2, 3 mm	5 mm
b)	PEEK lined SS	400 bar (5,880 psi)	Finger-tight + tool (not included)	4.6 mm	5 mm
c)	SS	400 bar (5,880 psi)	Finger-tight + tool (not included)	4.6 mm	5 mm, 10 mm
d)	PEEK / SS	150 bar (2,205 psi)	Finger-tight + tool (not included)	10 mm	10 mm

Ordering Information

Chromolith® Guard cartridge holder				
for dimension			Type	Material	Item No.
5	x	2 and 3	a	PEEK	1.52004.0001
5	x	4.6	b	Bioinert	1.52255.0001
5	x	4.6	c	SST	1.52032.0001
10	x	4.6	c	SST	1.52033.0001
10	x	10	d	PEEK/SST	1.52037.0001

Chromolith® Column Coupler
Make your column longer for extra high resolution

The Chromolith® HPLC column coupler is designed for linking several monolithic columns together in order to further increase separation efficiency and column performance. The combination results in a theoretical plate count that is significantly higher than any particulate column available. At the same time, pressure is kept well below the HPLC system limit.

Ordering Information

Chromolith® Column coupler	
for analytical columns	1.51467.0001

HPLC Columns

Chromolith® WP 300 Columns

Monolithic HPLC Columns for Biomolecule Separation

Biotherapeutics, for example bio-engineered drugs and peptide therapeutics, represent the promise of new medical treatments for the future. Production costs have been falling, leading to an extremely high demand for suitable, analytical methods for process monitoring and quality control of these biomolecules. HPLC is the preferred method of analysis, and it is important to use the right column for these larger molecules.

Accurate analysis of proteins, antibodies and large peptides requires columns with good permeability, along with better mass transfer and selectivity. In order for size-exclusion not to influence the separation, the pore size should be approximately ten-times larger than the molecule being analyzed.

In contrast to conventional, packed-particle columns, wide pore (300 Å) monolithic silica columns are made of a single, continuous rod of high purity, porous silica that is then bonded with C18, C8, C4, epoxy and Protein A depending on the use of the column.

Monolithic columns remove backpressure as the primary consideration in method development and allow:

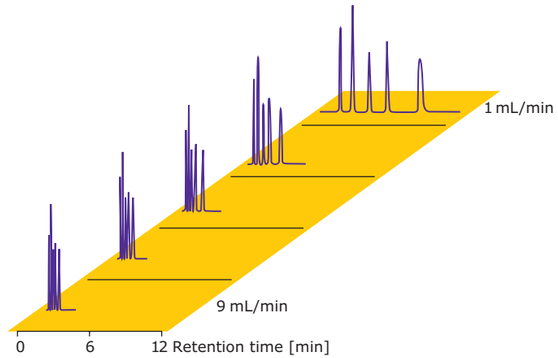
- Flow rate flexibility for much higher throughput
- Choice of column lengths for superior resolution
- More solvent options for optimum selectivity

With no individual particles to shift or break, column performance is consistent over a much longer lifetime, making them ideal for relatively ”dirty or matrix-rich” sample analysis. High permeability also makes them forgiving of less rigorously prepared samples, in addition to making it easier to aggressively flush out for re-equilibration.

Features and Benefits

- Completely bioinert column hardware
- High biorecovery
- Selectivity for a range of biomolecules
- Very low column backpressure
- High-speed separation possible
- Longer column lifetime
- High resistance to column blockage
- Cost savings from higher sample throughput and column durability
- Possibility to use flow gradients

Fast chromatography with low column backpressure



Owing to the very high porosity of the Chromolith® WP 300 column, very high flow rates can be applied with very low pressures as seen above.

A mixture of five compounds demonstrates the extreme time savings and high separation efficiency made possible with Chromolith® WP 300 columns. Due to excellent mass transfer properties of the monolithic skeleton, high-speed separation is possible even at high flow rate.

HPLC Columns

Chromolith® WP 300

Chromolith® WP 300 Protein A Fast Monoclonal Antibody Quantitation

Affinity chromatography is a selective technique which takes advantage of specific molecular interactions, for example antigen and antibody. The Chromolith® WP 300 Protein A HPLC column is designed to monitor monoclonal antibody titer and yield determination from cell-culture supernatants. The analytical scale procedure helps to monitor the titer of monoclonal antibody for the optimal time for harvest of the monoclonal antibody products. Chromolith® WP 300 Protein A column could be used for separation of all IgGs (except class 3). These columns provide extremely fast separations and could be used longer minimizing overall analysis costs.

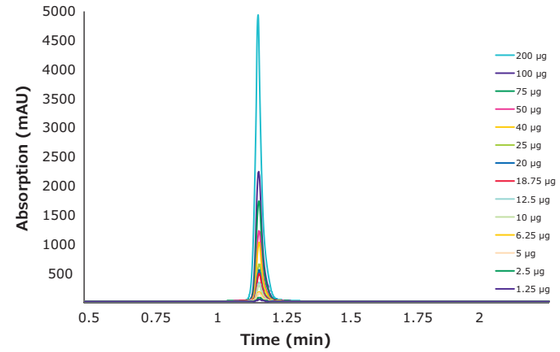
Monoclonal Antibody Analysis

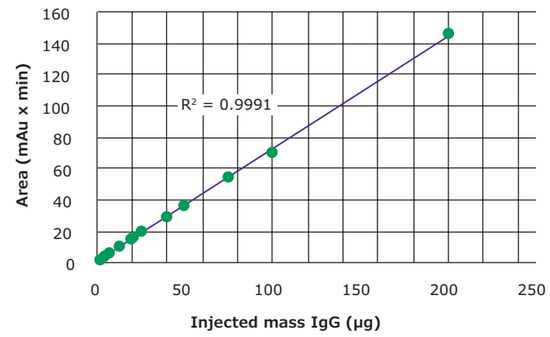
Mobile Phase A	100 mM sodium phosphate pH 7.4		
Mobile Phase B	100 mM sodium phosphate pH 2.5		
Flow rate	2.0 mL/min		
Detection	280 nm		
Temperature	25 °C		
Injection volume	10 µL		
Gradient	Time	%A	%B
	0.00	100	0
	0.25	100	0
	0.26	0	100
	1.25	0	100
	1.26	100	0
	2.50	100	0

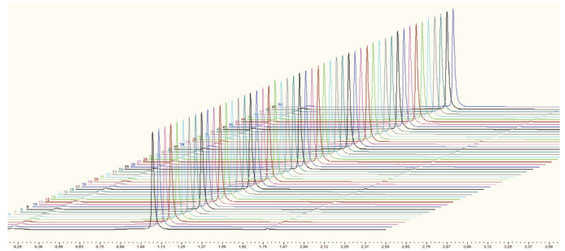
Constant binding efficiency at any flow rate

- High-speed separation at high flow rate due to excellent mass transfer properties of the monolithic skeleton.
- Separation of IgG demonstrates the extreme time savings and high separation efficiency made possible with Chromolith® Protein A columns.
- IgG was well separated with excellent peak symmetry.
- At 5 mL/min, the total analysis time is less than 1 minute and the net column backpressure is only 21 bar.
- Antibody binding is not affected by flow rate.
- As depicted at right, antibody capture and release is reproducible across 50 injections.

Linearity







Parameter	RSD
Retention time	< 0.1%
Peak Height	< 0.9%
Peak area	< 0.5%

HPLC Columns

Chromolith® WP 300 Epoxy Column
Create Your Own Column Selectivity On Demand

Chromolith® WP 300 Epoxy columns are specially designed for the user-specific immobilization of ligands and their later application in HPLC. The unique, bimodal pore structure of silica monoliths allows efficient coupling independent of molecule size. The wider mesopores also enable the use of both proteins and antibodies as ligands immobilized on the column, and as analytes, separated by an immobilized column.

Potential applications: Attach trypsin to obtain HPLC column-protein digestion reactor, attach a protein for protein-protein interaction analysis, attach any chiral selector to obtain a chiral column, or attach any affinity ligand for a custom made affinity column, among other options.

The Chromolith® WP 300 Epoxy column is shipped in 100% 2-Propanol. The column has to be washed with 20 column volumes (CV) deionized water before immobilization.

Immobilization via Epoxide Functions

Step I - Equilibration

Column equilibration with 50 mL 50 mM sodium phosphate + 1.9 M ammonium sulfate, pH 8.0

2.0 mL/min flow rate at room temperature

Step II - Immobilization

Dissolving of ligand in 25 mL 50 mM sodium phosphate + 1.9 M ammonium sulfate, pH 8.0

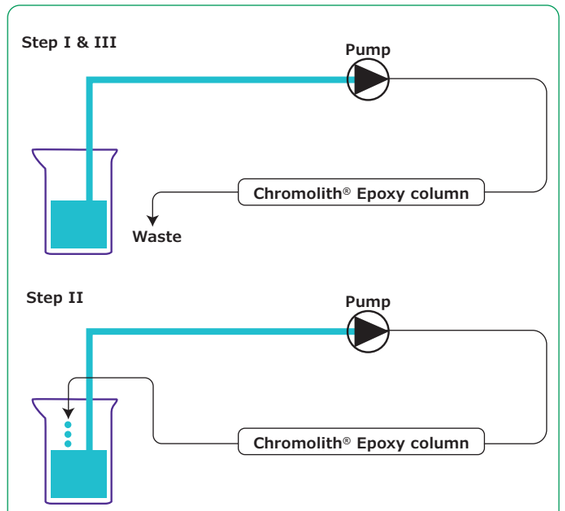
Connection of ligand solution to pump

Immobilization in cycles with 0.2 mL/min flow rate at room temperature for 4 – 24 h

Step III - Quenching & Washing

Quenching of remaining epoxide functions with 150 mM phosphoric acid or 1 M glycine (optional)

Washing of the immobilized column with 100 mM sodium phosphate, pH 7.4

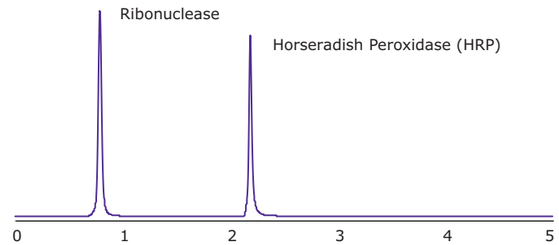


After immobilization, the column is ready to use for the desired purpose of the immobilized ligand. The type of required solvent or buffers depends on the type of ligand used. Chromolith® WP Epoxy columns can be used with all commonly used HPLC grade organic solvents, with the following restrictions. The mobile phase should NOT contain more than 50% Tetrahydrofuran (THF), 5% Chlorinated solvent (e.g. Dichloromethane) or 5% Dimethylsulfoxide (DMSO). However pure DMSO can be used as solvent for samples. Buffers, organic modifiers and ion pair reagents present no problems as long as the appropriate pH range is not exceeded. Nevertheless, be careful not to expose the column to conditions which could cause denaturation of the ligand.

Affinity Column Created by Immobilization of Concanavalin A onto Chromolith® WP300 Epoxy Column

Preparing the Column:

Immobilization according to Epoxy method using a Chromolith® WP 300 Epoxy 100 x 4.6 mm I.D. column with 50 mg concanavalin A from Jack bean dissolved in 25 mL, 50 mM disodium hydrogen phosphate, 1 mM calcium chloride + 1.9 M ammonium sulfate, pH 8.0. Immobilization for 4 hours at 0.2 mL/min and quenching of remaining epoxide functions with glycine.



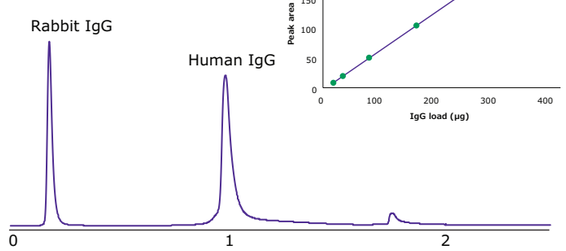
Chromatographic Conditions

Mobile Phase A:	50 mM sodium acetate, 200 mM sodium chloride, 1 mM calcium chloride, pH 5.3
Mobile Phase B:	Eluent A + 100 mM Methyl-α-D-mannopyranoside
Flow rate:	2.0 mL/min
Detection:	214 nm
Temperature:	25 °C
Injection volume:	5 µL

Gradient:

Time	%A	%B
0	100	0
1	100	0
1.25	0	100
3.5	0	100
3.6	100	0
5	100	0

Protein G Chromatography



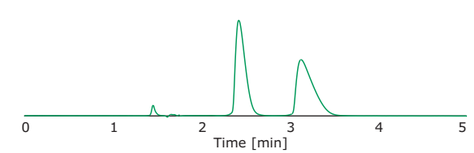
Chromatographic Conditions

Mobile Phase A:	100 mM sodium phosphate, pH 7.4
Mobile Phase B:	100 mM sodium phosphate, pH 2.5
Flow rate:	2.0 mL/min
Detection:	280 nm
Temperature:	25 °C
Injection volume:	10 µL

Gradient:

Time	%A	%B
0	100	0
0.5	0	100
0.6	0	100
1.2	100	0

Separation of Racemic Thalidomide



Chromatographic Conditions

Column:	Chromolith® Epoxy immobilized with vancomycin
Mobile Phase:	10 mM disodium hydrogen phosphate, pH 7.0
Flow:	1.0 mL/min
Temperature:	25 °C
Detection:	214 nm
Injection:	1.0 µL 1 mM thalidomide in acetonitrile

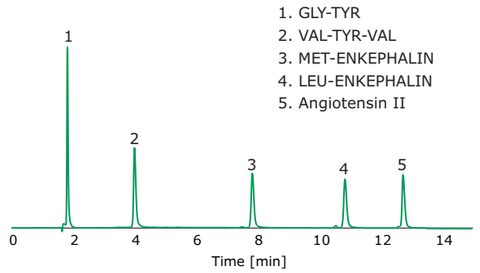
Chromolith® WP 300 HPLC Columns

Chromolith® WP 300 RP-18, RP-8 and RP-4: Reversed-phase HPLC Columns for Bioapplications

Reversed-phase chromatography is often used for protein and peptide separations. The longer octadecyl (C18) chains can efficiently separate complex peptide mixtures; shorter C8 modified columns are used for small, less hydrophobic proteins; C4 is mainly applied for separation of hydrophobic proteins.

Separation of Five Peptides Using Chromolith® WP 300 RP-18

Column:	Chromolith® WP 300 RP18 100 x 4.6 mm I.D.
Mobile phase:	A: Water 0.1% TFA B: Acetonitrile 0.1% TFA
Flow rate:	1.0 mL/min
Detection:	220 nm
Temperature:	60 °C
Injection volume:	1.0 µL
Sample:	HPLC peptide standard mixture (H2016)



Chromolith® WP 300 Specifications

Phase Bonding	USP Designation	Bonding Chemistry	Silica Type	Macropore Size (µm)	Mesopore Size (Å)	Pore volume (mL/g)	Surface Area (m^2/g)	Carbon Load (%)	pH Stability	Max Temp.	Endcapped
RP-18	L1	Octadecylsilane	Monolithic Type B silica	2	300	1	300	8-9	2 - 7.5	60	No
RP-8	L7	Octylsilane	Monolithic Type B silica	2	300	1	300	4-5	2 - 7.5	60	No
RP-4	L26	Butylsilane	Monolithic Type B silica	2	300	1	300	3-4	2 - 7.5	60	No
Protein A		Protein A	Monolithic Type B silica	2	300	1	300	n.a.	2 - 7.5	45	No
Epoxy		Glycidoxipropylsilane	Monolithic Type B silica	2	300	1	300	5-6	2 - 7.5	60	No

Ordering Information

Column dimension							
Length (mm)		I.D. (mm)	RP-18	RP-8	RP-4	Protein A	Epoxy
Chromolith® WP 300 HPLC column [1 unit]							
25	x	4.6				1.52258.0001	1.52252.0001
25	x	2				1.52358.0001	1.52352.0001
50	x	4.6	1.52271.0001	1.52266.0001	1.52261.0001		1.52251.0001
50	x	2	1.52371.0001				1.52351.0001
100	x	4.6	1.52270.0001	1.52265.0001	1.52260.0001		1.52250.0001
100	x	2	1.52370.0001				1.52350.0001
Chromolith® Guard cartridges [3 units]							
5	x	4.6	1.52273.0001	1.52268.0001	1.52263.0001		1.52254.0001
5	x	2	1.52372.0001				1.52353.0001
10	x	4.6	1.52272.0001	1.52267.0001	1.52262.0001		1.52253.0001
Chromolith® Guard cartridges Holder							
5	x	4.6	1.52032.0001				
10	x	4.6	1.52033.0001				
Chromolith® Column coupler							
			1.51467.0001				

HPLC Columns

Chromolith® CapRod® Capillary Columns

A Chromolith® CapRod® capillary column combines the speed of monolithic silica technology with the sensitivity of nano-LC. These traits enable superior productivity for high throughput, highly sensitive proteomics-LC applications. Compared to particulate, capillary columns, Chromolith® CapRod® capillaries demonstrate better performance with optimal resolution (narrow peak widths), increased productivity (higher sample throughput), and extended column lifetime. Furthermore, column length is less limited than with any other type of column. The capillaries can even be slightly bent to fit any LC configuration or instrument. Chromolith® CapRod® columns are designed to work with various nano or capillary-LC systems. This trait provides the highest efficiency and performance when coupled to mass spectrometers, both on-line (ESI, nanospray) and off-line (MALDI). Compared to classical, micro-particulate sorbents, Chromolith® CapRod® columns can be operated at higher flow rates—without loss of performance, resolution, or limitations due to column back pressure. Separations can be achieved at 1–3 µL/min, compared to 200–400 nL/min for conventional media on a standard 100 µm LC capillary column. For more complex, biological samples, a trapping capillary can be used to protect the separation column and optimize separation efficiency.

Recommended use and flow rate ranges

Recommended use	RP-18e 150 x 0.05 mm	RP-8e 150 x 0.1 mm	RP-18e 50 x 0.1 mm Trap	RP-18e 150 x 0.1 mm	RP-18e 300 x 0.1 mm	RP-18e 150 x 0.1 mm HR	RP-18e 50 x 0.2 mm Trap	RP-18e 150 x 0.2 mm	RP-18e 150 x 0.2 mm HR
Separation of small molecules	●		●	●	●	●	●	●	●
– of peptides	●	●	●	●	●	●	●	●	●
– of proteins		●							
Micro ESI		●		●	●	●		●	●
Nano ESI	●	●		●	●	●			●
High Resolution						●			●
Flow rates (µL/min)	0.2 – 0.8	0.4 – 3	1 – 10	0.4 – 3	0.2 – 1.5	0.1 – 0.4	10 – 50	5 – 20	0.5 – 2
Max back pressure (bar)	200	200	200	200	200	218	218	218	218

Ordering Information

Column dimension						
Length (mm)		I.D. (mm)		HR RP-18e	RP-18e	RP-8e
Chromolith® CapRod® HPLC capillary columns [1 unit]						
50	x	0.1	Trap		1.50426.0001	1.52031
50	x	0.2	Trap		1.50409.0001	
150	x	0.05			1.50403.0001	
150	x	0.1		1.50404.0001	1.50402.0001	1.50400.0001
150	x	0.2		1.50407.0001	1.50405.0001	
300	x	0.1			1.50424.0001	

HPLC Columns

Fully Porous Particulate Silica (FPP) Columns

High Loadability and Scalable from Nano LC to Preparative LC

Fully porous silica particles (FPP) are well-established in the chromatographic community over the past several decades. These columns are in use in thousands of methods and ensure reliable results over the complete range of use, particle sizes and column dimensions in:

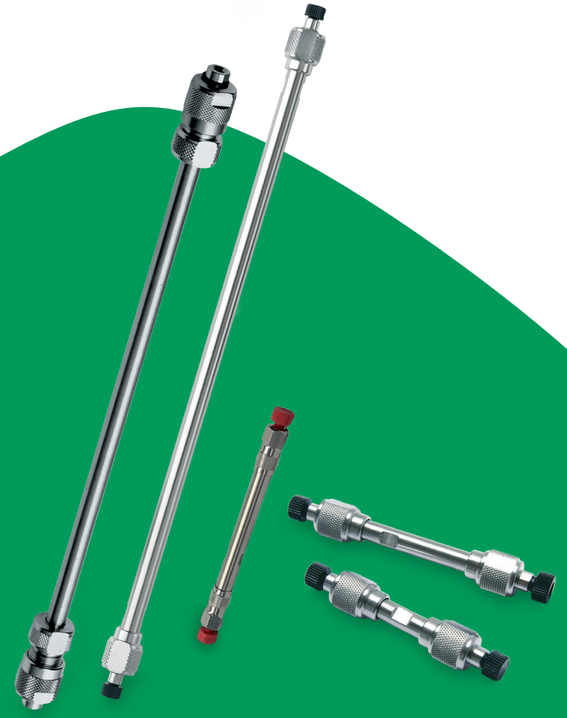
- Micro and Nano-LC (Capillary columns)
- UHPLC
- Analytical HPLC
- Semi-preparative LC
- Preparative LC

Fully porous silica particles provide the highest loadability of the stationary phase due to their fully porous physical characteristics. This trait ensures high sensitivities because the peak broadening effect of overloading the stationary phase is minimized.

Traditional fully porous silica particles such as LiChrosorb®, LiChrospher®, Superspher® and SUPELCOSIL™ are based on Type A silica which is produced from sodium waterglass.

The more modern, high purity Type B silica was introduced in the early 1990´s. Type B silica particles are produced from tetraalkoxysilane in a sol-gel process. This metal-free stationary phase base material can be used for the analysis of acidic, basic, and chelating compounds providing excellent peak symmetries with less need for strong buffer concentrations.

Therefore, this type of stationary phase base material is the preferred FPP option for method development, method improvement and LC-MS use.



FPP Type B Stationary Phases (RP and HILIC):

UHPLC (pressure stability of 1000 bar):

- Purospher® STAR
  - 2 µm particle size
  - superior peak symmetry
  - extended pH stability
  - available modifications: RP-18e (C18), RP-8e (C8), Phenyl, Si

Analytical HPLC:

- Purospher® STAR
  - 3 µm and 5 µm particle size
  - superior peak symmetry
  - extended pH stability up to pH 10.5
  - available modifications: RP-18e (C18), RP-8e (C8), Phenyl, NH_2, Si
- Discovery® and Ascentis®
  - 3 µm and 5 µm particle size
  - broad range of selectivities
  - available modifications: C18, C8, Phenyl, RP-Amide; F5 (PFP), CN, Si
- Discovery® BIO
  - 5 µm particle size
  - 300 A pores
  - available modifications: C18, C8, C5
- SeQuant® ZIC®
  - 3/3.5 µm and 5 µm particle size
  - superior separation of polar compounds
  - available modifications: Sulfobetaine (ZIC®-HILIC), Phosphorylcholine (ZIC®-cHILIC)
  - additional: polymeric particle (5 µm) with Sulfobetaine (ZIC®-pHILIC) for extended pH stability

Semi-Preparative/Preparative LC:

- Ascentis® Discovery® and Discovery® BIO
  - 10 µm particle size
  - column dimensions up to 21.2 mm I.D.
- SeQuant® ZIC®
  - 5 µm particle size
  - column dimensions up to 21.2 mm I.D.

Nano LC:

- SeQuant® ZIC®

Validation kits*

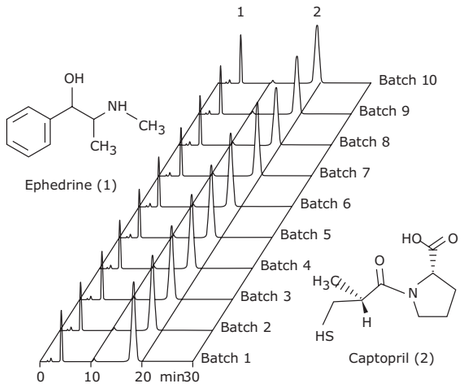
The success of an HPLC method depends strongly on the consistent quality of the stationary phase. Long-term reproducibility is a key factor in achieving reliable results. Supelco® validation kits consist of three HPLC columns, packed with three different sorbent lots to confirm the reliability of HPLC methods and their robustness.

* In case the needed column dimension or stationary phase is not availabe from stock, please send a request to Custom HPLC Column Request.

Purospher® STAR HPLC and UHPLC Columns

Accuracy and precision made simple

Purospher® STAR HPLC and UHPLC columns are based on 99.999% ultra-pure fully porous silica (Type B). These columns are designed for universal use and allow the separation of basic, neutral, and metal chelating compounds with simple mobile phases and excellent peak symmetry. These columns offer an outstanding stability from pH 1.5 to 10.5 over a wide temperature range, and suitability in up to 100% aqueous mobile phases. Plus, the columns demonstrate best all around retention characteristics, as proven by the Tanaka test. Thanks to these features, Purospher® STAR HPLC columns allow high-throughput applications and allow maximum flexibility for use with the best chromatographic conditions for any separation including reversed phase.



Features and Benefits

- Ultra-pure silica (99.999%) for excellent peak symmetry
- High separation efficiency
- Reproducible results from run-to-run and batch-to-batch
- Best overall performance according to Tanaka test
- Outstanding pH stability from pH 1.5 to 10.5
- No column dewetting when using highly aqueous mobile phases
- LC-MS compatible

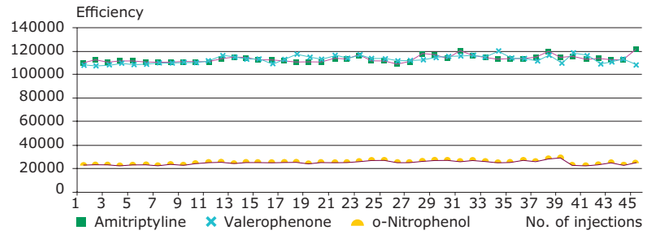
Consistent Results

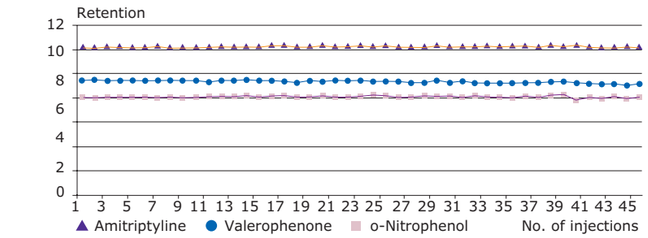
The success of any method depends on the quality of the stationary phase. Precise, long-term reproducibility is a key factor in achieving reliable results. The base silica of Purospher® STAR columns is 99.999% pure. Furthermore, meticulous care is given to quality control all aspects of silica structure and chemistry. These factors ensure that the columns will always perform consistently, resulting in method reproducibility you can trust.

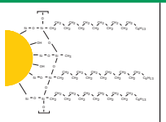
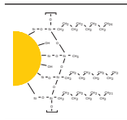
Outstanding Stability

The combination of extremely high purity silica, best overall retention characteristics, outstanding pH stability up to pH 10.5 and suitability for use with 100% aqueous mobile phases makes Purospher® STAR RP-18 endcapped an all-round top performance column, almost universal in its range of applications. The present stability test shows the suitability with 100% aqueous mobile phase at high temperature at 60 °C.

Column:	Purospher® STAR RP-18 endcapped, 3 µm LiChroCART® 55-4
Mobile phase:	0.1 v/v% H_3PO_4 in Water
Flow rate:	1.5 mL/min
Temperature:	60 °C
Sample:	Amitriptyline
	Valerophenone
	o-Nitrophenol





Purospher® STAR	Phase Bonding	USP Designation	Bonding Chemistry	Chromatographic Properties / Use		Particle Size (µm)	Pore Size (Å)	Pore Volume mL/g	Surface Area (m^2/g)	Carbon Load (%)	Surface Coverage µmol/m^2	pH Stability	Max Temperature	Endcapped	Shipping Solvent
	RP-18 endcapped	L1	Octadecylsilane with polymeric endcapping	Best in class C18 column for excellent peak symmetry, performance and pH stability. Accurate results with excellent peak shape for all types of analytes • Outstanding resolution due to high separation efficiency • Proven reliability and reproducibility from run to run and batch to batch • Universal compatibility with best all around performance according to Tanaka test • Maximum flexibility in method development and choice of mobile phase • pH stability from pH 1.5 – 10.5 • Suitable in up to 100% aqueous mobile phases • Highest sensitivity and suitability for LC-MS applications		2, 3 and 5	120	1.1	330	17	3	1.5 - 10.5	65 °C	Yes	Acetonitrile/ Water
	RP-8 endcapped	L7	Octylsilane	Less hydrophobic compounds, faster retention of very hydrophobic compounds. Excellent peak symmetry for acidic, basic and chelating compounds; Excellent resolution due to high separation efficiency; Excellent stability from pH 1.5 to 10.5; Enhanced selectivity for positional isomers		2, 3 and 5	120	1.1	330	11.2	3	1.5 - 10.5	65 °C	Yes	Acetonitrile/ Water
	Phenyl	L11	Phenylsilane	Enhanced selectivitiy for separation of aromatic compounds due to π-π interactions. • Low silanol activity • Excellent pH stability from 1.5 to 10.5 • Suitable in up to 100% aqueous mobile phases		2, 3 and 5	120	1.1	330	12.5	3	1.5 - 10.5	65 °C	Yes	Acetonitrile/ Water
	NH_2	L8	Amino	Separation of carbohydrates and polar compounds with normal-phase or HILIC chromatography. Very high separation efficiency as measured by the plate count • Absence of metal impurities, thus giving consistently symmetrical peaks • Extended column lifetime		5	120	1.1	330	3.5	3	2 - 7.5	60 °C	No	Acetonitrile/ Water
	Si	L3	unbonded	Separation of polar compounds with normal-phase or HILIC chromatography Very high separation efficiency as measured by the plate count • Absence of metal impurities, thus giving consistently symmetrical peaks • Extended column lifetime		5	120	1.1	330	n.a.	n.a.	2 - 7.5	65 °C	N.A.	Acetonitrile/ Water

HPLC Columns

Purospher® STAR HPLC and UHPLC Columns

HPLC Columns

Ordering Information

Purospher® STAR (5 µm)

Column dimension Length (mm)		I.D. (mm)	RP-18 endcapped	RP-8 endcapped	Phenyl	NH2	Si
LiChroCART® HPLC Cartridge [1 unit]							
100	x	2	1.50623.0001	on request	on request	on request	on request
100	x	3	1.50625.0001	on request	on request	on request	on request
100	x	4.6	1.50627.0001	on request	on request	on request	on request
125	x	2	1.50255.0001	1.50274.0001	on request	on request	on request
125	x	3	1.50253.0001	on request	on request	on request	on request
125	x	4	1.50251.0001	1.50271.0001	on request	1.50244.0001	1.50268.0001
150	x	2	1.50624.0001	on request	on request	on request	on request
150	x	3	1.50626.0001	on request	on request	on request	on request
150	x	4.6	1.50358.0001	1.50031.0001	1.51922.0001	1.50247.0001	1.50356.0001
250	x	2	1.50256.0001	on request	on request	on request	on request
250	x	3	1.50254.0001	on request	on request	on request	on request
250	x	4	1.50252.0001	1.50272.0001	on request	1.50245.0001	1.50269.0001
250	x	4.6	1.50359.0001	1.50032.0001	1.51921.0001	1.50248.0001	1.50357.0001
250	x	10	on request	on request	on request	on request	on request
Validation Kits [3 LiChroCART® HPLC cartridges from 3 different sorbent batches]							
125	x	4	1.50251.1003	1.50271.1003	on request	on request	on request
150	x	4.6	1.50358.1003	1.50031.1003	1.51922.1003	on request	on request
250	x	4	1.50252.1003	1.50272.1003	on request	on request	on request
250	x	4.6	1.50359.1003	1.50032.1003	1.51921.1003	on request	on request
Guard cartridges LiChroCART® [10 units]							
4	x	4	1.50250.0001	1.50270.0001	on request	1.50267.0001	1.50249.0001

The LiChroCART® columns (100, 125, 150 and 250 mm length) in the list above (2, 3, 4 and 4.6 mm I.D.) require part number **1.51486.0001** manu-CART® cartridge column holder, which can be used to hold one cartridge column with or without a 4-4 mm guard column. LiChroCART® columns 250-10 mm require part number **1.51419.0001** manu-CART® 10. Additional dimensions and validation kit available as customized packings see page 126.

Column dimension Length (mm)		I.D. (mm)	RP-18 endcapped	RP-8 endcapped	Phenyl	NH2	Si
Hibar® RT HPLC Column [1 unit]							
50	x	2	1.50593.0001	on request	on request	on request	on request
50	x	3	1.50607.0001	on request	on request	on request	on request
50	x	4	1.50621.0001	on request	on request	on request	on request
100	x	2	1.50595.0001	on request	on request	on request	on request
100	x	3	1.50612.0001	on request	on request	on request	on request
100	x	4.6	1.50622.0001	1.51917.0001	on request	on request	on request
125	x	2	1.50596.0001	on request	on request	on request	on request
125	x	3	1.50615.0001	on request	on request	on request	on request
125	x	4	1.50036.0001	1.50033.0001	on request	on request	on request
125	x	4.6	1.51914.0001	1.51916.0001	on request	on request	on request
150	x	2	on request	on request	on request	on request	on request
150	x	3	1.50617.0001	1.50644.0001	1.51920.0001	on request	on request
150	x	4.6	1.51455.0001	1.51453.0001	on request	on request	on request
250	x	2	1.50598.0001	on request	on request	on request	on request
250	x	3	1.50620.0001	on request	on request	on request	on request
250	x	4	1.50037.0001	1.50035.0001	on request	on request	on request
250	x	4.6	1.51456.0001	1.51454.0001	1.51918.0001	1.51913.0001	1.51911.0001
250	x	10	on request	on request	on request		1.51912.0001
Validation Kits [3 Hibar® RT HPLC Columns from 3 different sorbent batches]							
125	x	4	1.50036.1003	1.50033.1003	on request		
150	x	3	1.50617.1003	1.50644.1003	1.51920.1003		
150	x	4.6	1.51455.1003	1.51453.1003	on request		
250	x	4	1.50037.1003	1.50035.1003	on request		
240	x	4.6	1.51456.1003	1.51454.1003	1.51918.1003		

The Hibar® columns are complete with endfittings. When using a guard column with a Hibar® column, we recommend part number **1.51487.0001** guard column cartridge holder for 4-4 mm guard column cartridges LiChroCART®. Additional dimensions available as customized packings see page 126.

HPLC Columns

HPLC Columns "on request" are available as Custom Product. Please see page 126/127

Validation kits are available

Purospher® STAR (3 µm)

Column dimension Length (mm)		I.D. (mm)	RP-18 endcapped	RP-8 endcapped	Phenyl
LiChroCART® HPLC Cartridge [1 unit; * 3 units] [One set contains: 1 LiChroCART® cartridge and one holder]**					
30	x	2	on request	on request	on request
30	x	4	1.50225.0001*	on request	on request
55	x	2	1.50241.0001*	on request	on request
55	x	2; Set**	1.50240.0001	on request	on request
55	x	4	1.50231.0001*	on request	on request
55	x	4; Set**	1.50242.0001	on request	on request
75	x	4	1.51460.0001	on request	on request

LiChroCART® cartridges with 30 mm length require a manu-CART® 30 mm cartridge holder for LiChroCART® 30-2 and 30-4 mm HPLC cartridges (**1.50227**). LiChroCART® cartridges with 55 mm length require a manuCART® 55 mm cartridge holder for LiChroCART® 55-2 and 55-4 mm HPLC cartridges (**1.50226**). LiChroCART® cartridges with 75 mm length require a manuCART® NT holder (**1.51486**)

Hibar® RT HPLC Column [1 unit]					
50	x	3	1.50393.0001	on request	on request
50	x	4	1.50428.0001	on request	on request
100	x	3	1.50398.0001	on request	on request
100	x	4.6	1.50469.0001	on request	on request
125	x	3	1.50413.0001	on request	on request
125	x	4	1.50431.0001	on request	on request
150	x	3	1.50414.0001	1.50750.0001	on request
150	x	4.6	1.50470.0001	on request	on request
250	x	3	1.50427.0001	on request	on request
250	x	4	1.50468.0001	on request	on request
250	x	4.6	1.50471.0001	on request	on request
Validation Kits [3 Hibar® RT HPLC Columns from 3 different sorbent batches]					
125	x	4	1.50431.1003	on request	
150	x	3	1.50414.1003	1.50750.1003	
150	x	4.6	1.50470.1003	on request	
250	x	3	1.50427.1003	on request	
250	x	4	1.50468.1003	on request	
250	x	4.6	1.50471.1003	on request	

The Hibar® columns are complete with endfittings. When using a guard column with a Hibar® column, we recommend part number **1.51487.0001** guard column cartridge holder for 4-4 mm guard column cartridges LiChroCART®. Additional dimensions available as customized packings see page 126.

Other Purospher® Columns (5 µm)

Purospher® HPLC columns can easily be replaced by the advanced Purospher® STAR HPLC columns for many applications. Purospher® STAR HPLC columns provide an extended pH stability and outstanding peak symmetry for basic and chelating compounds.

Column dimension Length (mm)		I.D. (mm)	RP-18	RP-18 endcapped
LiChroCART® HPLC Cartridge [1 unit]				
125	x	3	on request	1.50798.0001
125	x	4	1.50142.0001	1.50168.0001
250	x	3	on request	1.51384.0001
250	x	4	1.50144.0001	1.50169.0001
Guard cartridges LiChroCART® [10 units]				
4	x	4	1.50141.0001	1.50167.0001

The LiChroCART® columns (125 and 250 mm length) in the list above 3, 4 and I.D. require part number 1.51486.0001 manu-CART® cartridge column holder, which can be used to hold one cartridge column with or without a 4-4 mm guard column. Additional dimensions and validation kit available as customized packings see page 126.

HPLC Columns

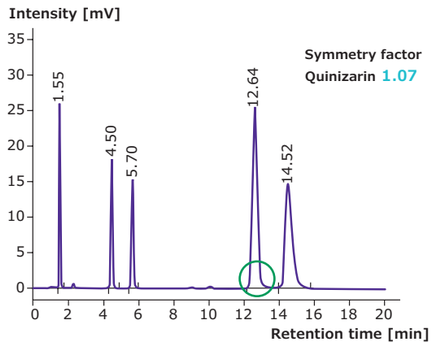
HPLC Columns "on request" are available as Custom Product. Please see page 126/127

Validation kits are available

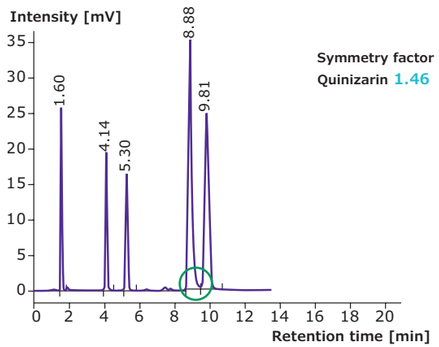
Perfect peak shape

Accurate results rely on two important chromatographic properties of the stationary phase: resolution and peak shape. With Purospher® STAR columns, high efficiency and bonded phase surface coverage produce sharp, symmetrical peaks for acidic, basic and chelating compounds. This makes Purospher® STAR RP-18 endcapped and RP-8 endcapped columns the optimal choice for pharmaceutical applications, USP methods as well as for general method development.

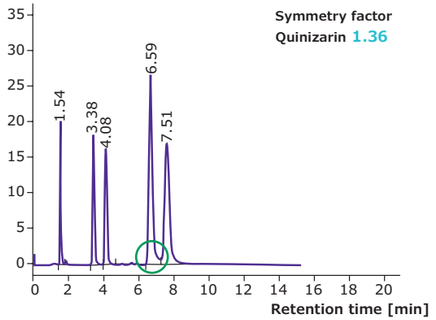
Purospher® STAR RP-18 endcapped



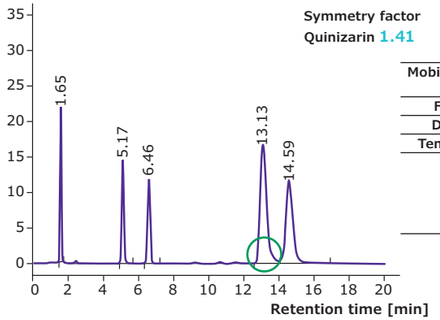
Column L



Column X



Column I

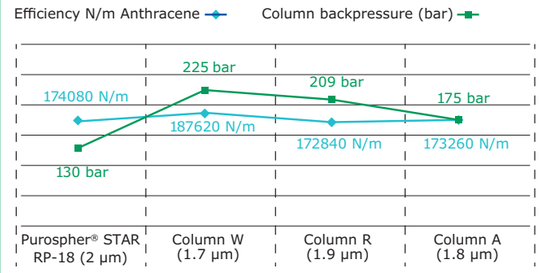


Mobile phase:	Methanol/Buffer pH 7.0 80/20
Flow rate:	1.0 mL/min
Detection:	UV 254 nm
Temprature:	22 °C
Sample:	1. Uracil; 2. Toluene; 3. Ethylbenzene; 4. Quinizarin; 5. Amitryptyline

Purospher® STAR UHPLC Columns

High resolution at lower column backpressure

Although UHPLC is typically performed with a particle size smaller than 2 µm, we employ 2 µm particles due to two important factors. Firstly, column efficiency and backpressure depends on the particle size of the column material. Secondly, column efficiency is also highly influenced by instrument effects. When UHPLC columns with 1.7 µm, 1.8 µm, 1.9 µm and 2 µm particles are compared on the same instrument and under the same conditions, results show no significant difference in efficiency. However, column pressure varies substantially among the different particle size materials. For example, a 1.7 µm particulate material significantly higher column backpressure, compared to a 2 µm material.



Column dimension:	50-2.1 mm
Mobile phase:	Acetonitrile/Water 60/40
Flow rate:	0.350 mL/min
Injection:	0.2 µL
Sample:	Thiourea; Biphenyl-2-ol; Progesterone; Hexanophenone; Anthracene

Chromatographic conditions

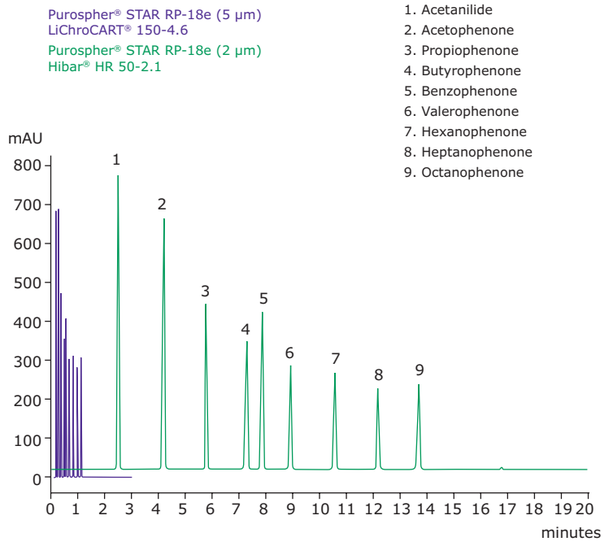
Column temperature:	40 °C
Eluents:	A. Water, B. Acetonitrile
UV:	247 nm
Injection volume:	10 µL

Green: Purospher® STAR RP-18e (5 µm) LiChroCART® 150-4.6

Gradient:	0 min 45 % B, from 45 to 95 % B in 15 min, from 15.1 to 20 min reequilibration with 45 % B
Flow rate:	1.0 mL/min
Pressure:	105 bar
Total run time:	20 min

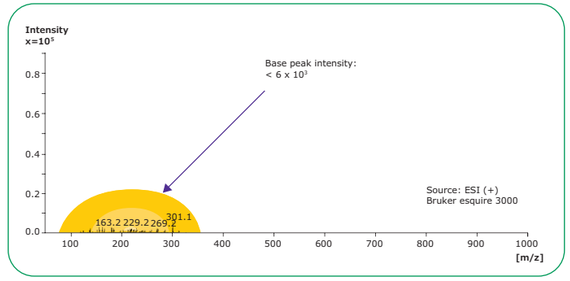
Purple: Purospher® STAR RP-18e (2 µm) Hibar® HR 50-2.1

Gradient:	0 min 45 % B, from 55 to 100 % B in 0.8 min from 0.9 to 2 min reequilibration with 55 % B
Flow rate:	1.1 mL/min
Pressure:	505 bar
Total run time:	2 min

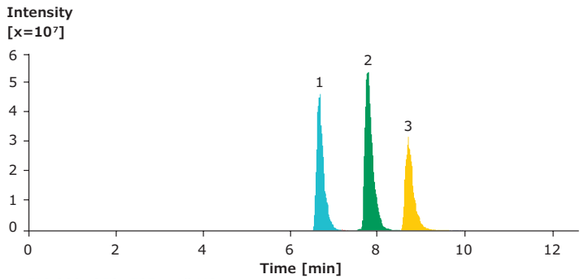


Excellent for LC-MS

In order to obtain sensitive results with LC-MS, it is essential to avoid trace impurities in the column and solvents. Purospher® STAR HPLC and UHPLC columns are highly suitably for LC-MS. To ensure low and stable background signals, it is recommended to wash columns with an eluent of isopropanol and 0.1% formic acid. Displayed here is an extracted ion chromatogram of NSAIDs in negative ion mode separated on Purospher® STAR RP-18 endcapped



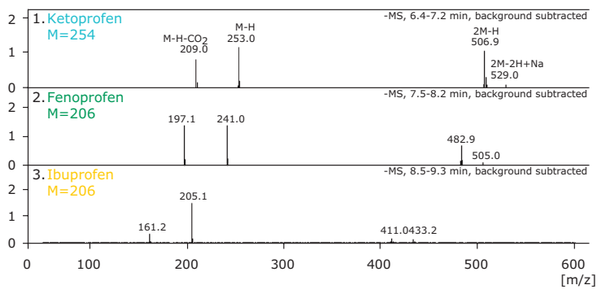
Extracted ion chromatograms of NSAIDs in negative ion mode separated on Purospher® STAR RP-18 endcapped



Chromatographic conditions

Column:	Purospher® STAR RP-18 endcapped, 3 µm, LiChroCART® 55-2	
Mobile phase A:	0.1 % Acetic acid in Acetonitrile	
Mobile phase B:	0.1 % Acetic acid in Water	
Gradient:	From 25 % A to 50 % A in 3 min, then isocratic	
Flow Rate:	300 µL, without split	
Detection:	UV 220 nm, Ion Trap MS	
Temperature:	ambient	
Injection volume:	1 µL	
Sample:	1. Ketoprofen	0.1 µg/µL
	2. Fenoprofen	0.1 µg/µL
	3. Ibuprofen	0.1 µg/µL

Intensity [x=10⁷]



Ketoprofen, Fenoprofen and Ibuprofen (100 ng) MS spectra without interfering signals using LiChrosolv® Acetonitrile hypergrade and Purospher® STAR RP-18 endcapped columns.

MS conditions

Ionization:	ESI(-)
Nebulizer:	36 psi
Dry gas:	8.5 L/min
Dry temperature:	330 °C
Smart mode optimization:	Target mass 205
Ion charge control:	Target 50,000, max 50 ms
Scan mode:	Standard/Normal
Scan range:	50 – 600 m/z

HPLC Columns

Purospher® STAR Hibar® HR UHPLC Columns [2 µm and 3 µm particle size]

Column dimension Length (mm)		I.D. (mm)	RP-18 e (2 µm)	RP-18 e (3 µm)	RP-8 e (2 µm)	RP-8 e (3 µm)	Phenyl (2 µm)	Phenyl (3 µm)
Hibar® HR UHPLC Column [1 unit]								
30	x	2.1	1.50645.0001	1.50650.0001	on request	on request	on request	on request
50	x	2.1	1.50646.0001	1.50651.0001	1.50630.0001	1.50674.0001	1.51013.0001	on request
100	x	2.1	1.50648.0001	1.50653.0001	1.50629.0001	1.50675.0001	1.51014.0001	1.50673.0001
150	x	2.1	1.50649.0001	1.50654.0001	on request	on request	on request	on request
Validation Kits [3 Hibar® HR UHPLC Columns from 3 different sorbent batches]								
100	x	2.1	1.50648.1003	1.50653.1003	1.50629.1003	1.50675.1003	on request	on request

Hibar® HR UHPLC columns are designed for use in UHPLC instruments. The pressure stability is set at 1000 bar.

HPLC Columns "on request" are available as Custom Product. Please see page 126/127

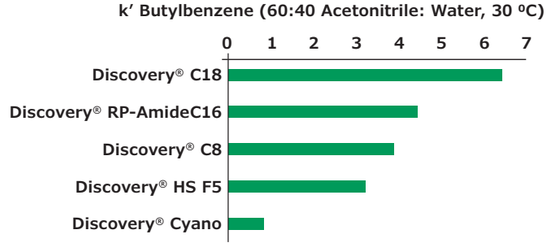
HPLC Columns

Discovery® HPLC Columns

Alternative selectivities for straightforward method development

The Discovery® HPLC columns are based on Type B silica, available with a broad range of modifications providing the best suitable selectivity for many applications. Although designed to meet the exacting requirements of pharmaceutical analysis and purification, Discovery® columns are also ideal for all application segments requiring reversed-phase HPLC.

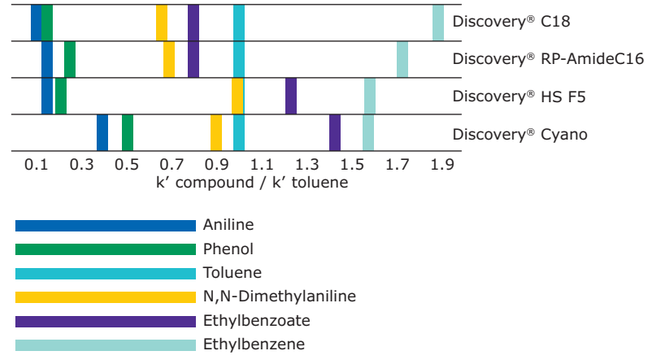
Method development scientists often choose a single stationary phase for development. If the chosen phase is not the best chemistry to yield a given separation, many hours may be spent studying mobile phase compositions that may or may not yield a suitable separation. Screening several stationary phase chemistries up front during method development and choosing the best phase for further optimization can save many precious hours. In addition, the use of a more effective stationary phase chemistry often eliminates the need for mobile phase additives that can greatly complicate separation conditions.



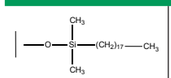
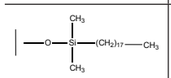
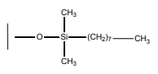
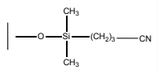
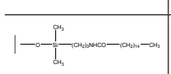
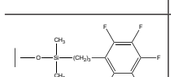
Hydrophobic Retention Ranking of Discovery® Reversed-Phases

As a visual representation of how the different phase chemistries provide different selectivities, the chart at right shows the k' of various analytes relative to toluene on Discovery® columns.

The polar functional group-containing solutes - aniline, phenol, N,N-dimethylaniline (N,N-DMA) and ethylbenzoate - clearly illustrate the very different selectivities of the functionalized reversed-phases vs. C18. The colors representing solutes containing polar groups dramatically change positions from phase to phase. Also, observe the changing hydrophobic selectivity by looking at the ethylbenzene bar.



Mobile phase:	45:55, 25mM Potassium Phosphate (pH 7.0): MeOH
Flow Rate:	1.0 mL/min

	Phase Bonding	USP Designation	Bonding Chemistry	Chromatographic Properties / Use		Particle Size (µm)	Pore Size (Å)	Surface Area (m^2/g)	Carbon Load (%)	Surface Coverage ($\mu mol/m^2$)	pH Stability	Max Temperature	Endcapped	Shipping Eluent
	C18	L1	Octadecyl	Classic reversed-phase selectivity and retention with excellent peak shape for all compounds. Very high stability and no-bleed properties for LC-MS applications		5	180	200	12	3	2 - 8	70 °C	No	34% Water: 66% Methanol
	HS C18	L1	Octadecyl	Non-polar, reversed-phase, higher surface (HS) column with excellent, no bleed LC-MS performance. Higher hydrophobicity for better resolution of difficult analytes		3, 5, 10	120	300	20	3.8	2 - 8	70 °C	Yes	30% Water: 70% Methanol
	C8	L7	Octyl	Less hydrophobic reversed-phase selectivity and retention with excellent peak shape for all compounds. Very high stability and no-bleed properties for LC-MS applications		5	180	200	7.5	3.4	2 - 8	70 °C	Yes	100% Methanol
	Cyano	L10	Cyanopropyl	Cyanopropyl reversed-phase column with lower hydrophobicity than C18 or C8 and unique selectivity. Excellent peak shape, significantly less retention than C18 (typically requires lower % organic mobile phase) and high stability with mobile phase). Low-bleed for LC-MS separations		5	180	200	4.5	3.5	2 - 8	70 °C	Yes	Acetonitrile
	RP-Amide C16	L60	Palmitamidopropyl	Polar-embedded, palmitamidopropyl reversed-phase column with unique retention and selectivity. Excellent peak shape and efficiency. Due to the nature of the bonded phase, we do not recommend the RP-AmideC16 be used for LC-MS applications		5	180	200	12	2.6	2 - 8	70 °C	Yes	Acetonitrile
	HS F5 (PFP)	L43	Pentafluorophenylpropyl	Pentafluorophenyl terminated reversed-phase, higher surface (HS) column with unique retention and selectivity (e.g. basic & halogenated compounds). The Discovery® HS F5 bonded phase provides reversed-phase separations that are distinctly different from C18 columns. However, compounds will generally elute within the same retention time window, making most C18 methods easily transferable. Excellent peak shape and stable, low-bleed LC-MS separations		3, 5, 10	120	300	12	4	2 - 8	70 °C	Yes	Acetonitrile

HPLC Columns

HPLC Columns

Discovery® (5 µm)

Column dimension								
Length (mm)		I.D. (mm)	HS C18	C18	C8	RP-Amide C16	HS F5 (PFP)	Cyano
20	x	2.1	on request	on request	on request	on request	on request	on request
30	x	2.1	on request	on request	on request	on request	on request	on request
50	x	2.1	568500-U	50494721	on request	on request	567508-U	on request
100	x	2.1	568501-U	569220-U	on request	on request	567510-U	on request
125	x	2.1	on request	569229-U	on request	on request	on request	on request
150	x	2.1	568502-U	50495521	59353-U21	50501321	567511-U	on request
250	x	2.1	on request	on request	on request	on request	567512-U	on request
20	x	3	on request	on request	on request	on request	on request	on request
30	x	3	on request	on request	on request	on request	on request	on request
50	x	3	on request	on request	on request	on request	on request	on request
100	x	3	on request	on request	on request	on request	on request	569522-U
125	x	3	on request	on request	on request	on request	on request	on request
150	x	3	on request	on request	on request	on request	on request	on request
250	x	3	on request	on request	on request	on request	on request	on request
50	x	4	on request	on request	on request	on request	on request	on request
100	x	4	on request	569222-U	on request	on request	on request	on request
125	x	4	on request	569231-U	569426-U	569331-U	on request	on request
150	x	4	568512-U	on request	on request	on request	567535-U	on request
250	x	4	568513-U	on request	on request	on request	567536-U	on request
20	x	4.6	on request	on request	on request	on request	on request	on request
30	x	4.6	on request	on request	on request	on request	on request	on request
50	x	4.6	568520-U	504947	59352-U	505005	567513-U	on request
100	x	4.6	568521-U	569223-U	on request	on request	567515-U	on request
125	x	4.6	on request	569232-U	569427-U	on request	on request	on request
150	x	4.6	568522-U	504955	59353-U	505013	567516-U	59356-U
250	x	4.6	568523-U	504971	59354-U	505064	567517-U	59357-U
50	x	10	on request	on request	on request	on request	on request	on request
100	x	10	on request	on request	on request	on request	on request	on request
50	x	10	on request	on request	on request	on request	on request	on request
100	x	10	on request	on request	on request	on request	on request	on request
150	x	10	on request	on request	on request	on request	on request	on request
250	x	10	568533-U	569224-U	on request	on request	567520-U	on request
50	x	21.2	on request	on request	on request	on request	on request	on request
100	x	21.2	on request	on request	on request	on request	on request	on request
150	x	21.2	on request	on request	on request	on request	on request	on request
250	x	21.2	568543-U	569226-U	on request	on request	567523-U	on request
Discovery® Validation Packs (3 columns, each from a different lof of bonded phase)								
50	x	2.1	on request	on request	on request	on request	on request	on request
100	x	2.1	on request	on request	on request	on request	on request	on request
150	x	2.1	on request	on request	on request	on request	on request	on request
50	x	4.6	on request	on request	on request	on request	on request	on request
100	x	4.6	on request	on request	on request	on request	on request	on request
150	x	4.6	on request	on request	on request	on request	on request	on request
250	x	4.6	on request	on request	on request	on request	on request	on request
Discovery® Supelguard Guard Cartridge (2 pack)								
20	x	2.1	on request	505188	59588-U	on request	567574-U	on request
20	x	3	on request	59576-U	on request	59578-U	on request	on request
20	x	4	568572-U	505137	on request	505099	567576-U	on request
Discovery® Supelguard Guard Cartridge (2 pack)								
20	x	2.1	on request	505161	on request	on request	567575-U	on request
20	x	3	on request	59575-U	on request	on request	on request	on request
20	x	4	568573-U	505129	59589-U	505080	567577-U	on request

HPLC Columns "on request" are available as Custom Product. Please see page 126/127

Discovery® (3 µm)

Column dimension				
Length (mm)		I.D. (mm)	HS C18	HS F5 (PFP)
30	x	2.1	on request	567501-U
50	x	2.1	569253-U	567500-U
75	x	2.1	569254-U	on request
100	x	2.1	on request	567502-U
150	x	2.1	569255-U	567503-U
30	x	3	on request	on request
150	x	3	on request	567542-U
50	x	4	on request	567530-U
75	x	4	on request	on request
100	x	4	on request	567531-U
150	x	4	on request	567532-U
50	x	4.6	569250-U	567504-U
75	x	4.6	569251-U	
100	x	4.6	on request	567506-U
150	x	4.6	569252-U	567507-U
Discovery® Supelguard Guard Cartridge (2 pack)				
20	x	2.1	569276-U	567570-U
20	x	4	569274-U	567572-U
Discovery® Supelguard Guard Kit (Guard cartridge, stand-alone holder, tubing, 2 nuts and ferrules)				
20	x	2.1	on request	567571-U
20	x	4	569275-U	567573-U

HPLC Columns "on request" are available as Custom Product. Please see page 126/127

Discovery® (10 µm)

Column dimension			
Length (mm)		I.D. (mm)	HS C18
50	x	10	on request
100	x	10	on request
150	x	10	on request
250	x	10	on request
50	x	21.2	on request
100	x	21.2	on request
150	x	21.2	on request
250	x	21.2	568643-U

HPLC Columns

Validation kits are available

Discovery® BIO Columns

Reversed-Phase Solutions to Protein and Peptide Separation Challenges

Discovery® BIO Wide Pore reversed-phase columns satisfy the need for efficiency, selectivity, LC-MS sensitivity, stability, scalability, and reproducibility for reversed-phase HPLC analyses of proteins, peptides, and small biomolecules. Three phase chemistries, C18, C8, and C5, give unmatched selectivity and performance. Separations are completely scalable from analytical to preparative column dimensions. The low-bleed, inert surface chemistry makes them ideal for proteomics and LC-MS applications.

Features and Benefits

- Better protein and peptide resolution compared to leading RP-HPLC FPP columns
- High efficiency for peptide mapping
- Complementary selectivity choices with C5, C8, and C18 phase chemistries
- C5 has enhanced stability and lifetime compared to conventional C4 phases
- Excellent LC-MS properties
- Reliable reproducibility, column-to column, batch-to-batch

Choosing a Discovery® BIO Wide Pore Reversed Phase for Sample and Separation Modes

Sample or Usage	Separation Mode	Discovery® BIO Product
Peptide Mapping / Proteolytic Digests	Reversed-phase	Discovery® BIO Wide Pore C18 Discovery® BIO Wide Pore C8
Hydrophobic Peptides	Reversed-phase	Discovery® BIO Wide Pore C5
Proteins	Reversed-phase	Discovery® BIO Wide Pore C5

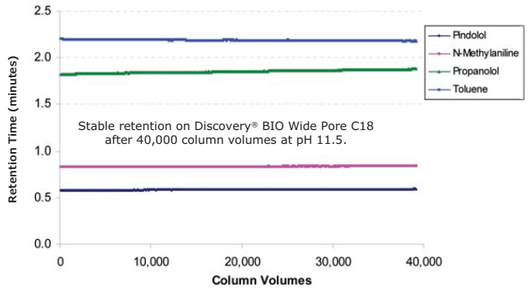
Discovery® BIO Wide Pore HPLC columns are packed with C5, C8, or C18 ligands bonded to 3, 5, or 10 µm, spherical, high purity silica particles containing 300 Å pores. All Discovery® BIO Wide Pore products provide stable, efficient, and reproducible separations of proteins and peptides. The low-bleed character and excellent peak shape without TFA in the mobile phase makes these columns ideal for proteomics and other LC-MS applications and preparative purifications.

Phase Bonding	Particle Size (µm)	Pore Size (Å)	Surface Area (m^2/g)	Carbon Load (%)	Surface Coverage ($\mu mol/m^2$)	pH Stability	Max Temperature	Endcapped	Shipping Eluent
C18	3, 5, and 10	300	100	9	3.6	2 to 8	70 °C	Yes	Acetonitrile/Water
C8	5 and 10	300	100	5	4	2 to 8	70 °C	Yes	Acetonitrile/Water
C5	3, 5, and 10	300	100	3.5	4.5	2 to 8	70 °C	Yes	Acetonitrile/Water

HPLC Columns

Discovery® BIO Wide Pore C18 Stability at pH 11.5

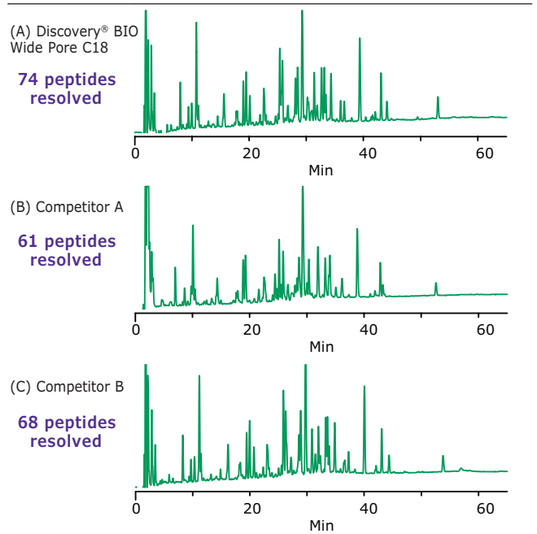
Column:	Discovery® BIO Wide Pore C18, 5 cm x 2.1 mm I.D., 3 µm (567200-U)
Mobile phase:	65:35, 50 mM pyrrolidine HCl, pH 11.5: acetonitrile
Flow rate:	0.7 mL/min
Column temp.:	35 °C



Note: Stability was measured using small molecule probes because they are generally more sensitive to changes in the silica and bonded phase chemistry than peptides and proteins. If the retention and selectivity for the small molecule probes does not change, it is very likely that the retention and selectivity for proteins and peptides will be stable as well.

Tryptic Digest of Carboxymethylated Apohemoglobin on a Discovery® Wide Pore C18 versus Competitive Columns

Columns:	(A) Discovery® BIO Wide Pore C18, 15 cm x 4.6 mm, 5 µm (568222-U), (B) and (C) Competitive protein and peptide C18, 15 cm x 4.6 mm, 300 Å, 5 µm;
Mobile phase:	[A] 95:5, (0.1% (v/v) TFA in water):(0.1% (v/v) TFA in acetonitrile); (B) 50:50, (0.1% (v/v) TFA in water): (0.1% (v/v) TFA in acetonitrile)
Gradient:	0-100% B in 65 min
Flow rate:	1.0 mL/min
Column temp.:	30 °C
Detector:	UV, 215 nm
Injection:	50 µL
Sample:	Carboxymethylated apohemoglobin tryptic digest, varied concentration, 50 mM aqueous ammonium bicarbonate



Note: The absolute number of peptides detected depends on the detector settings. In this comparison, the relative number of detected peptides is important, not the absolute number. The Discovery® BIO Wide Pore C18 column detected more peptides relative to the competitive columns under the same conditions.

Ordering Information

Discovery® BIO (3.0 µm)

Length (mm)		I.D. (mm)	C18	C5
50	x	1.0	on request	65511-U
50	x	2.1	567200-U	567226-U
100	x	2.1	567201-U	567227-U
150	x	2.1	567202-U	567228-U
50	x	4.6	on request	567229-U
100	x	4.6	567204-U	
150	x	4.6	567205-U	567231-U
Guard 2 pk	x	2.1	567270-U	567278-U
Guard 2 pk	x	4.0	on request	567280-U
Guard Kit	x	2.1	567271-U	on request
Guard Kit	x	4.0	567273-U	567281-U

Discovery® BIO (10.0 µm)

Length (mm)		I.D. (mm)	C18	C8	C5
250	x	4.6	on request	on request	567232-U
50	x	10.0		on request	on request
150	x	10.0	567208-U	on request	567234-U
250	x	10.0	567209-U	on request	on request
150	x	21.2		on request	on request
250	x	21.2	567212-U	567225-U	on request

HPLC Columns "on request" are available as Custom Product.
Please see page 120/121

HPLC Columns

Discovery® BIO (5.0 µm)

Length (mm)		I.D. (mm)	C18	C8	C5
50	x	2.1	on request	on request	on request
100	x	2.1	on request	on request	on request
150	x	2.1	on request	on request	568402-U
250	x	2.1	568203-U	on request	on request
250	x	4.0	568213-U	on request	on request
50	x	4.6	on request	on request	
100	x	4.6	on request	on request	
150	x	4.6	568222-U	on request	568422-U
250	x	4.6	on request	568323-U	568423-U
250	x	10.0	568230-U	on request	568430-U
250	x	21.2	on request	on request	on request
Guard 2 pk	x	2.1	on request	on request	on request
Guard 2 pk	x	4.0	568272-U	on request	568472-U
Guard Kit	x	2.1	568271-U	on request	on request
Guard Kit	x	4.0	568273-U	on request	on request

Ascentis® HPLC Columns

Ascentis® HPLC Columns are optimized to the three terms of the resolution equation: efficiency, retention and selectivity. Ascentis® bonded phases have a wide range of selectivities. It is likely that one or more Ascentis® phase will accomplish any small molecule HPLC separation. Packed in micro- to preparative hardware dimensions, Ascentis® products cover all HPLC application areas, including the most sensitive trace-level analyses.

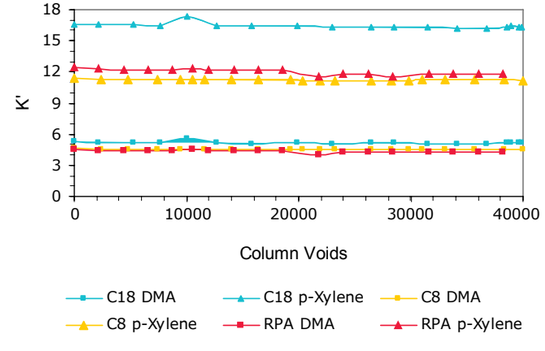
The general features of the Ascentis® family include:

- High purity, type B silica for inertness, reproducibility and stability
- Modern bonding processes that optimize bonded phase coverage and maximize stability, while minimizing bleed and unwanted secondary interactions
- Wide selection of bonded phase chemistries and bare silica
- Phases with enhanced polar compound retention
- Compatible with LC-MS and all of today's sensitive instruments and methods
- Scalable selectivity from analytical to preparative
- High surface area silica for high preparative loading capacity

Phosphate buffers are often preferred in HPLC applications, but they are aggressive at high pH and can cause dissolution of silica, stripping of phase and voiding in columns and usually should be avoided at high pH conditions.

Ascentis® columns have a pH stability of pH 2-8. Under special conditions, especially at lower temperature, Ascentis® C18, C8 and RP-Amide can be used with mobile phase conditions at pH 1.5 and pH 10, even with aggressive mobile phase conditions with phosphate and methanol.

Column:	Ascentis® C18, Ascentis® C8 or Ascentis® RP-Amide, 5 cm x 3.0 mm I.D., 5 µm particles
Mobile phase:	40:60, 10 mM ammonium phosphate (pH 10):methanol
Flow rate:	0.9 mL/min.
Temp.:	25 °C
Et.:	UV at 220 nm
Stability test mix:	Uracil was used as a void marker. Dimethylaniline (DMA) was used as a polar basic analyte that is sensitive to phase loss and silanol activity. As end-capping is lost, peak shape and efficiency should suffer. Toluene provided neutral k', efficiency and asymmetry marker. p-Xylene is a highly retained neutral efficiency marker that is very sensitive to loss of main phase due to its high k'



Phase Bonding	USP Designation	Bonding Chemistry	Chromatographic Properties / Use		Particle Size (µm)	Pore Size (Å)	Surface Area (m^2/g)	Carbon Load (%)	Surface Coverage ($\mu mol/m^2$)	pH Stability	Max Temperature	Endcapped	Shipping Eluent
C18	L1	Octadecyl	The classic reversed-phase column suitable for any method that specifies a C18-type column. Its high surface area gives Ascentis® C18 strong hydrophobic retention and high loading capacity for preparative applications. Ascentis® C18 is low-bleed for clean ESI and APCI traces. The high retentivity means that the mobile phase can contain high levels of organic modifier.		3, 5	100	450	25	3.7	2 - 8*	70 °C	Yes	40% Water: 60% Acetonitrile
C8	L7	Octyl	Ascentis® C8 is suitable for any method that specifies a C8-type column. Although C8 columns often show similar selectivity to C18 columns, shorter alkyl chains sometimes show different selectivity toward polar compounds because they can solvate differently with the mobile phase and interact differently due to the size and shape of certain molecules. Also, C8 reagents are smaller than C18 reagents and have improved primary phase coverage, thereby requiring less endcapping. Ascentis® C8 has excellent peak shape and very high phase stability.		3, 5	100	450	15	4.0	2 - 8*	70 °C	Yes	30% Water: 70% Methanol
Cyano	L10	Cyanopropyl	Useful for selectivity in the reversed-phase mode, including π-π and dipole-dipole interacting compounds. Can also be used in HILIC mode and normal phase chromatography.		5	100	450	10	2.5	1 - 8	70 °C	Yes	Acetonitrile
RP-Amide	L60	Palmitamidopropyl	Ascentis® RP-Amide can be used for many of the same separations as a C18 while avoiding some of the disadvantages of C18 such as poor wettability in high aqueous mobile phases. In addition, it is much more retentive for those molecules that can interact by hydrophobic interactions and also by H-bonding with the amide group. Compared to alkyl only phases, Ascentis® RP-Amide has enhanced retention and selectivity for phenols, organic acids and other polar solutes due to strong H-bonding between the amide carbonyl (H-bond acceptor) and H-bond donors, like phenols and acids. Compared to other embedded polar group (EPG) phases, like carbamates, ureas, sulfonamides and ethers, Ascentis® RP-Amide gives retention comparable to C18 and C8 for easy column comparison without the need to change mobile phase conditions.		3, 5	100	450	19.5	2.7	2 - 8*	70 °C	Yes	Acetonitrile
Phenyl	L11	Phenyl ring with short butyl spacer	Phenyl phases are π-basic (electron donating) and are similar in overall retention to alkyl and EPG phases for easy column screening. The alternate selectivity of phenyl phases is often explained by the π-π interactions available through the phenyl ring. They provide low-bleed for MS or UV gradient applications due to the use of trifunctional bonding reagent, outstanding phenyl selectivity due to high phase loading and short butyl spacer and 100% aqueous-compatible for highly-polar compounds.		3, 5	100	450	19	5.2	2 - 8*	70 °C	Yes	35% Water: 65% Methanol
Silica	L3	unbonded	The classic use of silica columns is for normal phase HPLC. The rigid structure of the silica surface, as opposed to the flexible nature of bonded phases, allows it to distinguish between molecules with different molecular shape that may have the same hydrophobicity. Ascentis® Si columns provide a high-loading capacity and operates in both normal-phase and HILIC modes.		3, 5	100	450	n.a.	n.a.	2 - 6	70 °C	n.a.	Ethanol

*Under certain conditions such as low temperature, the column can be operated in the extended pH range from pH 1.5 - 10.

HPLC Columns

HPLC Columns

Ordering Information

Ascentis® (5 µm)

Column dimension Length (mm)		I.D. (mm)	C18	C8	Phenyl	RP-Amide	ES Cyano	Si
50	x	2.1	on request	on request	on request	565303-U	on request	on request
100	x	2.1	581326-U	on request	on request	565304-U	577301-U	581500-U
150	x	2.1	581304-U	on request	on request	565305-U	on request	581509-U
250	x	2.1	on request	on request	on request	565306-U	on request	581510-U
50	x	3	on request	on request	on request	on request	on request	on request
100	x	3	on request	on request	on request	on request	on request	on request
150	x	3	on request	on request	on request	on request	on request	on request
250	x	3	on request	on request	on request	on request	on request	on request
250	x	4	on request	on request	on request	on request	on request	on request
50	x	4.6	581323-U	on request	581615-U	on request	on request	on request
100	x	4.6	on request	on request	on request	565328-U	on request	on request
150	x	4.6	581324-U	581424-U	581616-U	565324-U	577306-U	581512-U
250	x	4.6	581325-U	581425-U	581617-U	565325-U	577307-U	581513-U
50	x	10	on request	on request	on request	on request	on request	on request
100	x	10	on request	on request	on request	on request	on request	on request
150	x	10	581342-U	on request	on request	on request	on request	on request
250	x	10	581343-U	on request	on request	565344-U	on request	581514-U
50	x	21.2	on request	on request	on request	on request	on request	on request
150	x	21.2	581346-U	on request	on request	565347-U	on request	on request
250	x	21.2	581347-U	581442-U	on request	565348-U	on request	581515-U
Ascentis® Validation Packs (3 columns, each from a different lof of bonded phase)								
150	x	4.6	on request	on request	on request	on request	on request	on request
250	x	4.6	on request	on request	on request	on request	on request	on request
Ascentis® Supelguard Guard Cartridge (2 pack)								
20	x	2.1	581370-U	on request	on request	565372-U	on request	on request
20	x	3	581374-U	on request	on request	on request	on request	on request
20	x	4	581372-U	581426-U	581620-U	565370-U	on request	581518-U
Ascentis® Supelguard Guard Kit (Guard cartridge, stand-alone holder, tubing, 2 nuts and ferrules)								
20	x	2.1	on request	on request	on request	565373-U	on request	on request
20	x	4	581373-U	581427-U	581621-U	565371-U	on request	581519-U

Ascentis® (3 µm)

Column dimension Length (mm)		I.D. (mm)	C18	C8	RP-Amide	Si
20	x	2.1	on request	on request	on request	on request
30	x	2.1	on request	on request	on request	581522-U
50	x	2.1	581300-U	581400-U	565300-U	581500-U
75	x	2.1	on request	on request	on request	on request
100	x	2.1	581301-U	581401-U	565301-U	on request
150	x	2.1	581302-U	581402-U	565302-U	581502-U
20	x	3	on request	on request	on request	on request
30	x	3	on request	on request	on request	on request
100	x	3	581308-U	on request	565312-U	581503-U
20	x	4.6	on request	on request	on request	on request
50	x	4.6	581320-U	on request	565320-U	on request
100	x	4.6	581321-U	581407-U	565321-U	on request
150	x	4.6	581322-U	581408-U	565322-U	on request
Ascentis® Supelguard Guard Cartridge (2 pack)						
20	x	2.1	581377-U	on request	on request	on request
20	x	4	on request	on request	on request	on request
Ascentis® Supelguard Guard Kit (Guard cartridge, stand-alone holder, tubing, 2 nuts and ferrules)						
20	x	2.1	581376-U	on request	on request	on request
20	x	4	581379-U	on request	on request	on request

Validation kits are available

HPLC Columns "on request" are available as Custom Product. Please see page 126/127

HPLC Columns

All Supelco® HPLC columns, including the complete range of Fully Porous Particles (FPP), Superficially Porous Particles (SPP) and Monolithic Columns (Chromolith®), perfectly fit to every HPLC, UHPLC and UPLC® instrument independent of the instrument supplier.

SeQuant® HILIC HPLC and Capillary Columns

ZIC®-HILIC, ZIC®-cHILIC and ZIC®-pHILIC are the ideal columns for all classes of polar and hydrophilic compounds

SeQuant® HILIC (**hydrophilic interaction liquid chromatography**) HPLC columns constitute a range of high-performance tools for separating polar, hydrophilic compounds. All columns carry densely bonded, truly zwitterionic functional groups with a charge balance of 1:1. Separation is achieved by hydrophilic partitioning combined with weak ionic interactions for maximum selectivity, high loadability and easy optimization of methods.

Features and Benefits

- High-performance HPLC and LC-MS separations of polar hydrophilic compounds
- Zwitterionic stationary phase ensures reproducible retention
- Two complementary phase chemistries
- Maximum LC-MS compatibility with minimized column bleed
- Excellent reproducibility and robustness
- Available in a variety of lengths, particle sizes, and pore dimensions

Choose your SeQuant® HILIC selectivity

SeQuant® Column	Functional Group	Features	Base Particle	Particle Size	Pore Size	Column Types
SeQuant® ZIC®-HILIC	Sulfobetaine	High-performance selectivity and robustness	silica	3.5 µm, 5 µm	100 Å, 200 Å	analytical, capillary, semi-prep, guards
SeQuant® ZIC®-cHILIC	Phosphoryl-choline	Complementary selectivity with favorable LC-MS performance	silica	3 µm	100 Å	analytical, capillary, guards
SeQuant® ZIC®-pHILIC	Sulfobetaine	High pH-stability and low noise with ion detectors	polymer	5 µm		analytical, guards

Your ideal choice for separation of all types of polar and hydrophilic compounds are the SeQuant® HILIC HPLC columns. Reproducible retention for compounds that have proved difficult to separate on reversed-phase HPLC columns is ensured by the high-performance zwitterionic sorbents in these columns.

Straightforward separation of compounds such as acids and bases, anions and cations, carbohydrates, metabolites, metal complexes, amino acids, peptides, protein digests and oligonucleotides can therefore be achieved with a selectivity complementary to reversed-phase columns. Enhanced LC-MS sensitivity is an additional benefit of using these columns.

Columns are available in a wide range of formats from capillary to semi-preparative dimensions, and with several different particle sizes and pore sizes.

SeQuant® HILIC Analytical and Capillary HPLC Columns

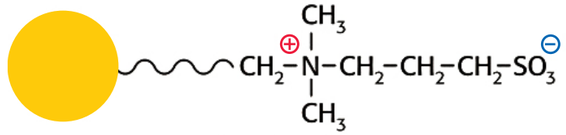
HPLC Columns

SeQuant® ZIC®-HILIC and ZIC®-cHILIC

The ZIC®-HILIC column is designed to retain and separate all types of polar and hydrophilic compounds and for robust chromatography with high selectivity and reproducibility. The silica-based ZIC®-HILIC sorbent has a bonded stationary phase consisting of a highly polar, permanent zwitterion. Separation selectivity is favored by the 1:1 zwitterion charge balance, which makes the ZIC®-HILIC column overall neutral, with weak, but important, ionic interactions. Tuning of the selectivity on the ZIC®-HILIC Column during method development is facilitated by the pH-independent, permanent zwitterion, ensuring that only the analytes (and not the column) is affected during eluent optimization.

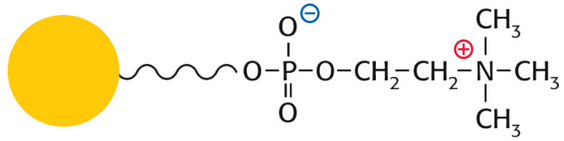
SeQuant® ZIC®-cHILIC is designed for excellent HPLC and LC-MS of polar, hydrophilic compounds. This zwitterionic stationary phase with phosphorylcholine functional group provides you with complementary selectivity for easier method development for analytes that have been difficult to separate by previous types of HPLC columns operated in reversed-phase or HILIC mode.

SeQuant® ZIC®-HILIC



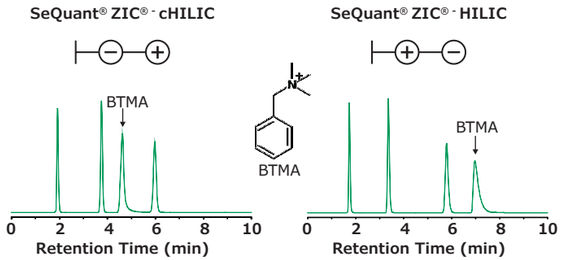
Sulfobetaine (SB)

SeQuant® ZIC®-cHILIC



Phosphorylcholine (PC)

Isocratic separations of the positively charged benzyltrimethylamine (BTMA, peaks indicated with arrows) and the neutral toluene (void marker), uracil and cytosine on ZIC®-cHILIC (left) and ZIC®-HILIC (right) illustrating differences and similarities in selectivity caused by the different charge orientation of the zwitterionic functional groups (see illustrations).



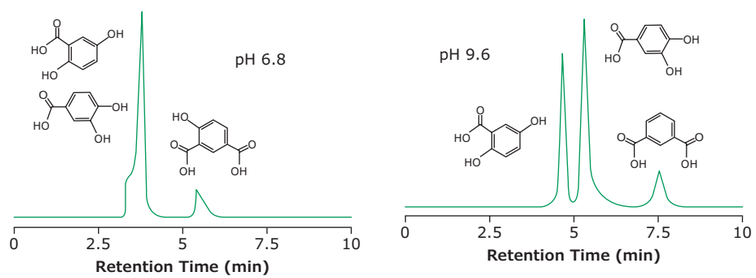
Chromatographic conditions

Column dimensions were 100 x 4.6 mm, particles size 3 or 3.5 µm, and pore size 100 Å. Eluent was 80 : 20 acetonitrile/ 25 mM aqueous ammonium acetate pH 6.8 pumped at 0.5 mL/min at 23 °C. Detection by UV absorption at 254 nm.

SeQuant® ZIC®-pHILIC

The ZIC®-pHILIC stationary phase has the same highly polar, bonded, permanent zwitterion, functional group as the silica-based ZIC®-HILIC column. The user can therefore expect the same selectivity, however, with a trade-off in flow-rate range, and separation efficiency, common with polymeric materials. The more durable support allows for use in an extended pH range, which can be beneficial for certain applications.

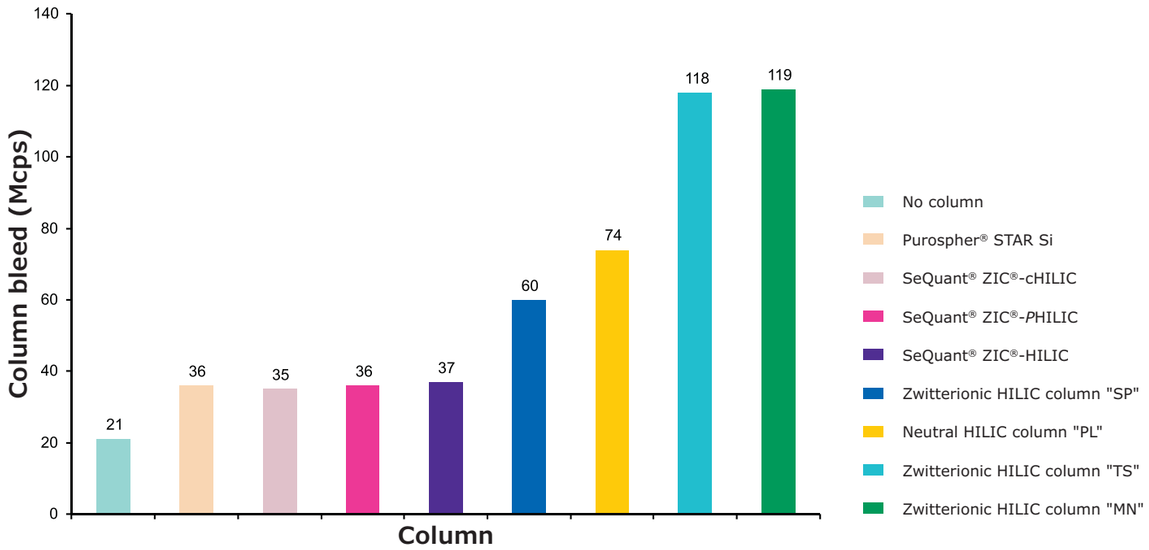
The application example below illustrates how the selectivity of the ZIC®-pHILIC material can be enhanced by performing the separation at elevated pH. The chromatograms show isocratic separations of gentisic acid, protocatechuic acid and isophthalic acid on a ZIC®-pHILIC column. The increase in pH also results in higher retention and improved peak shape for these analytes.



HPLC Columns

Outstanding Suitability for LC-MS

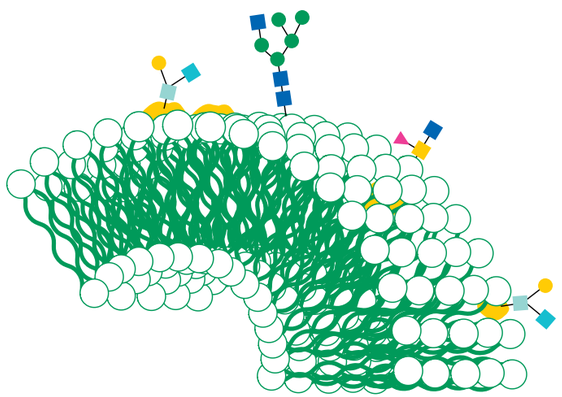
Thanks to their favorable retention and robust performance, SeQuant® HILIC columns have become extremely popular in numerous laboratories around the world. For example, the US FDA (United States Food and Drug Administration) recommended SeQuant® ZIC®-HILIC for analysis of melamine and related substances. SeQuant® HILIC columns excel in stability and low bleed. These features make them particularly suitable for LC-MS applications where high background can lead to signal suppression and interference with quantitative measurements while also increasing instrument wear. The column's high stability makes these high-performance HILIC columns also attractive for traditional HPLC applications. SeQuant® HILIC columns have been used in numerous methods for analysis of a wide variety of polar, hydrophilic compounds such as acids and bases, anions and cations, carbohydrates, metabolites, metal complexes, amino acids, peptides, and protein digests.



Note: SeQuant® ZIC®-HILIC columns show an exceptional low bleeding in LC-MS compared to other sulfobetaine modified HILIC columns.

Glycomics and Glyco-proteomics

SeQuant® ZIC® is an ideal sorbent for the separation and extraction of glycans and glycopeptides. A particle pore size of 200 Å is recommended to avoid size-exclusion effects from the relatively large hydrodynamic volume of the hydrated glycan structures. Small I.D. HPLC columns are suitable for LC-MS methods, whereas larger, conventional column dimensions are more appropriate for separations of labeled glycan structures detected with other techniques.



HPLC Columns

Peptide Mapping with High-Sequence Coverage

SeQuant® ZIC® HPLC columns are suitable for peptide mapping, especially so when exploring information from hydrophilic peptide fragments. Thanks to their advantageous combination of reversed phase chromatography and HILIC technology, the columns provide complementary information and may be coupled to an online 2-D peptide mapping approach.

Ordering Information

Column dimension			ZIC®-HILIC 200A, 5 µm	ZIC®-HILIC 100 A, 3.5 µm	ZIC®-HILIC 200A, 3.5 µm	ZIC®-cHILIC 100A, 3 µm	ZIC®-pHILIC, 5 µm
Length (mm)		I.D. (mm)					
50	x	2.1	1.50450.0001	1.50440.0001	1.50445.0001	1.50656.0001	1.50459.0001
100	x	2.1	1.50452.0001	1.50441.0001	1.50447.0001	1.50657.0001	1.50462.0001
150	x	2.1	1.50454.0001	1.50442.0001	1.50448.0001	1.50658.0001	1.50460.0001
250	x	2.1	1.50457.0001	1.50443.0001			
50	x	4.6	1.50451.0001		1.50446.0001	1.50659.0001	1.50463.0001
100	x	4.6	1.50453.0001			1.50660.0001	1.50464.0001
150	x	4.6	1.50455.0001	1.50444.0001	1.50449.0001	1.50661.0001	1.50461.0001
250	x	4.6	1.50458.0001			1.50662.0001	
150	x	10	1.50493.0001				
250	x	10	1.50494.0001				
50	x	21.2	1.50496.0001				
150	x	21.2	1.50497.0001				
SeQuant® Capillary columns							
30	x	0.3			1.50489.0001		
30	x	1			1.50478.0001		
150	x	0.075	1.50465.0001				
150	x	0.3	1.50481.0001		1.50479.0001	1.50669.0001	
150	x	1	1.50482.0001	1.50487.0001	1.50480.0001	1.50670.0001	
SeQuant® Guard (1 piece; * 3 Pieces; **5 pieces)							
5	x	0.3	1.50492.0001**			1.50765.0001*	
5	x	1	1.50490.0001**			1.50766.0001*	
20	x	2.1	1.50435.0001				1.50437.0001
SeQuant® Guard Kit (3 guard columns including column coupler)							
20	x	2.1				1.50764.0001	1.50438.0001
20	x	1	1.50436.0001				

HPLC Columns

LiChrospher® HPLC Columns

Classical silica carrier for consistent results

LiChrospher® is the name given to reliable and versatile, traditionally produced spherical silica carriers (Type A). LiChrospher® silica carriers are available in a number of different modifications. The polar modified phases LiChrospher® CN, LiChrospher® NH2 and LiChrospher® DIOL as well as LiChrospher® Si with no modification are best for normal-phase HPLC. The non-polar modified phases LiChrospher® RP-8, RP-8 endcapped, RP-select B, RP-18, RP-18 endcapped are made for reversed-phase separations. Furthermore, LiChrospher® PAH is highly selective for the separation of PAHs.

LiChrospher® packing materials are available as Hibar® RT columns and as LiChroCART® cartridges of various lengths and internal diameters (10 mm, 4.6 mm, 4 mm, 3 mm and 2 mm). LiChroCART® 3 mm I.D. and 2 mm I.D. narrow bore cartridges for HPLC save costs by reducing solvent consumption and allow the handling of very small quantities with excellent sensitivity and resolution. LiChroCART® cartridges 4.6 mm, 4 mm I.D., 3 mm I.D. and 2 mm I.D. are compatible with manu-CART® "4". This trait facilitates faster and more flexible method adaptation to smaller bore columns. LiChroCART® cartridges 10 mm I.D. have to be used with manu-CART® "10".

For improvements in resolution, column efficiency and peak symmetry as well as for fast and UHPLC applications, we recommend alternatives based on more advanced column technology such as Type B silica, Superficially Porous Particles or monolithic columns.

Phase Bonding	USP Designation	Bonding Chemistry	Particle Size (µm)	Pore Size (Å)	Pore Volume (mL/g)	Surface Area (m^2/g)	Carbon Load (%)	Surface Coverage (µmol/m^2)	pH Stability	Max Temperature	Endcapped	Shipping Eluent
RP-18	L1	Octadecylsilane	5, 10	100	1.25	350	21	3.61	2 - 7.5	65°C	No	Acetonitrile/ Water (80:20)
RP-18 endcapped	L1	Octadecylsilane with endcapping	5, 10	100	1.25	350	21.6	4.09	2 - 7.5	65°C	Yes	Acetonitrile / Water (80:20)
PAH	L1	Octadecylsilane	5	150		200			2 - 7.5	65°C	No	Acetonitrile / Water (80:20)
RP-8	L7	Octylsilane	5, 10	100	1.25	350	12.5	4.04	2 - 7.5	65°C	No	Acetonitrile / Water (80:20)
RP-8 endcapped	L7	Octylsilane with endcapping	5, 10	100	1.25	350	13.0	4.44	2 - 7.5	65°C	Yes	Acetonitrile / Water (80:20)
RP-selectB	L7	Octylsilane deactivated for the separation of basic compounds	5, 10	60	0.9	360	11.5	3.55	2 - 7.5	65°C	No	Acetonitrile / Water (80:20)
Diol	L20	Diol	5, 10	100	1.25	350	8.0	3.87	2 - 7.5	65°C	No	n-Heptane
CN	L10	Cyanosilane	5, 10	100	1.25	350	6.6	3.52	2 - 7.5	65°C	No	n-Heptane
NH2	L8	Aminosilane	5, 10	100	1.25	350	4.6	4.10	2 - 7.5	65°C	No	n-Heptane
Si	L3	unbonded	5, 10	60 100	0.85 1.25	700 400	n.a.	n.a.	2 - 7.5	65°C	N.A.	n-Heptane

HPLC Columns

Ordering Information

LiChrospher® (5µm)

Length (mm)		I.D. (mm)	RP-18	RP-18 endcapped	PAH	RP-8 (5 µm)	RP-8 endcapped	RP-select	Diol	CN	NH2	Si 60	Si 100
LiChroCART® HPLC Cartridge [1 unit; * 3 units]													
25	x	4	1.50931.0001*	1.50936.0001*	on request	1.50930.0001*	on request	1.50937.0001*	on request	on request	on request	on request	on request
75	x	4	1.50987.0001*	on request	on request	on request	on request	1.50993.0001*	on request	on request	on request	on request	on request
100	x	4.6	1.50600.0001	1.50603.0001	on request	1.50634.0001	on request	1.50640.0001	on request	on request	on request	on request	on request
125	x	3	1.50159.0001	on request	on request	on request	on request	1.50158.0001	on request	on request	on request	on request	on request
125	x	4	1.50823.0001	1.50828.0001	on request	1.50822.0001	1.50827.0001	1.50829.0001	1.50826.0001	1.50825.0001	1.50824.0001	1.50820.0001	on request
125	x	4	1.50943.0001*	1.50734.0001*	on request	1.50942.0001*	on request	1.50981.0001*	on request	on request	on request	on request	on request
125	x	4.6	on request	1.51908.0001	on request	on request	on request	on request	on request	on request	on request	on request	on request
150	x	4.6	1.50601.0001	1.50604.0001	on request	1.50635.0001	1.50638.0001	1.50641.0001	on request	on request	on request	on request	on request
250	x	3	1.50154.0001	on request	1.50156.0001	on request	on request	1.50155.0001	on request	on request	on request	on request	on request
250	x	4	1.50833.0001	1.50838.0001	1.50149.0001	1.50832.0001	1.50837.0001	1.50839.0001	1.50836.0001	1.50892.0001	1.50834.0001	1.50830.0001	on request
250	x	4	1.50983.0001*	1.50995.0001*	on request	on request	on request	1.50984.0001*	on request	on request	on request	on request	on request
250	x	4.6	1.50602.0001	on request	on request	1.50636.0001	1.50639.0001	on request	on request	on request	on request	on request	on request
Validation Kits [3 LiChroCART® HPLC Cartridges from 3 different sorbent batches]													
100	x	4.6	1.50600.1003	on request	on request	1.50634.1003	on request	1.50640.1003	on request	on request	on request	on request	on request
125	x	3	1.50159.1003	on request	on request	on request	on request	on request	on request	on request	on request	on request	on request
125	x	4	1.50823.1003	on request	on request	1.50822.1003	on request	1.50981.1003	on request	on request	on request	on request	on request
150	x	4.6	on request	1.50604.1003	on request	on request	on request	1.50641.1003	on request	on request	on request	on request	on request
250	x	3	1.50154.1003	on request	on request	on request	on request	1.50155.1003	on request	on request	on request	on request	on request
250	x	4	1.50833.1003	1.50838.1003	on request	1.50832.1003	on request	1.50839.1003	on request	on request	on request	on request	on request
250	x	4.6	1.50602.1003	1.50605.1003	on request	1.50636.1003	on request	1.50642.1003	on request	on request	on request	on request	on request
Guard Cartridges LiChroCART® [10 units]													
4	x	4	1.50957.0001	1.50962.0001	1.50148.0001	1.50956.0001	1.50961.0001	1.50963.0001	1.50960.0001	1.50959.0001	1.50958.0001	1.50955.0001	

The LiChroCART® columns (75, 100, 125, 150 and 250 mm length) in the list above (3, 4 and 4.6 mm I.D.) require part number 1.51486.0001 manu-CART® cartridge column holder, which can be used to hold one cartridge column with or without a 4-4 mm guard column. LiChroCART® columns 250-10 mm require part number 1.51419.0001 manu-CART® 10. Additional dimensions and validation kit available as customized packings see page 126.

Length (mm)		I.D. (mm)	RP-18	RP-18 endcapped	PAH	RP-8 (5 µm)	RP-8 endcapped	RP-select	Diol	CN	NH2	Si 60	Si 100
Hibar® RT HPLC Column [1 unit]													
100	x	4.6	1.50545.0001	on request	on request	1.50578.0001	1.50581.0001	1.50573.0001	on request	on request	on request	on request	on request
125	x	2	on request	1.51907.0001	on request	on request	on request	on request	on request	on request	on request	on request	on request
125	x	4	1.50477.0001	on request	on request	on request	on request	on request	on request	on request	on request	on request	on request
125	x	4.6	on request	on request	on request	on request	on request	on request	on request	on request	on request	on request	on request
150	x	4.6	1.50546.0001	1.50549.0001	on request	1.50579.0001	1.50582.0001	1.50574.0001	on request	on request	1.51905.0001	on request	on request
250	x	4	1.50377.0001	on request	on request	1.50329.0001	on request	on request	on request	on request	on request	on request	1.50316.0001
250	x	4.6	1.50547.0001	1.50550.0001	on request	1.50580.0001	1.50583.0001	1.50575.0001	on request	on request	1.51904.0001	on request	on request
Validation Kits [3 Hibar® RT HPLC Columns from 3 different sorbent batches]													
100	x	4.6	1.50545.1003	on request	on request	1.50578.1003	on request	1.50573.1003	on request	on request	on request	on request	on request
125	x	4	1.50477.1003	on request	on request	on request	on request	on request	on request	on request	on request	on request	on request
150	x	4.6	on request	1.50549.1003	on request	on request	on request	1.50574.1003	on request	on request	on request	on request	on request
250	x	4	1.50377.1003	on request	on request	1.50329.1003	on request	on request	on request	on request	on request	on request	on request
240	x	4.6	on request	1.50550.1003	on request	on request	on request	on request	on request	on request	on request	on request	on request

The Hibar® columns are complete with endittings. When using a guard column with a Hibar® column, we recommend part number 1.51487.0001 guard column cartridge holder for 4-4 mm guard column cartridges LiChroCART®. Additional dimensions available as customized packings see page 126.

Length (mm)		I.D. (mm)	RP-18	RP-18 endcapped	PAH	RP-8 (5 µm)	RP-8 endcapped	RP-select	Diol	CN	NH2	Si 60	Si 100
Stainless steel ready to use HPLC Column													
150	x	3.2	54775										
250	x	3.2	54777										
125	x	4				50141-U		50146-U					
250	x	4	50137-U										
125	x	4.6				50140-U							
150	x	4.6	54774			54778						54790-U	
250	x	4.6	54776			54780				54788		54792	
SupelGuard Column													
10	x	4.6	54794									54797-U	
LiChrospher® - Bulk Sorbents													
10 g Sorbent in glass bottle			1.16177.0010	1.19637.0010		1.16129.0010	1.19636.0010	1.19641.0010	1.16152.0010	1.19638.0010		1.19640.0010	

HPLC Columns "on request" are available as Custom Product. Please see page 126/127

HPLC Columns

Superspher® HPLC Columns

Classical silica carrier for highly efficient separations

Superspher®, a spherical silica carrier with a mean particle size of 4 µm, providing higher separation performance in HPLC applications. The number of theoretical plates for Superspher® is approx. 100,000 N/m. Thus, Superspher® HPLC columns are a good choice when complex mixtures demand high peak capacity. The following modifications are available on Superspher®: non-polar derivatives (RP-8, RP-8 endcapped, RP-18, RP-18 endcapped and RP-select B) and polar derivatives (Si 60).

Phase Bonding	USP Designation	Bonding Chemistry	Particle Size (µm)	Pore Size (Å)	Pore Volume (mL/g)	Surface Area (m^2/g)	Carbon Load (%)	Surface Coverage (µmol/m^2)	pH Stability	Max Temperature	Endcapped
RP-18	L1	Octadecylsilane	4	100	1.25	350	21.0	3.61	2 - 7.5	65 °C	No
RP-18 endcapped	L1	Octadecylsilane	4	100	1.25	350	21.6	4.09	2 - 7.5	65 °C	Yes
RP-8	L7	Octylsilane	4	60	1.25	350		4.04	2 - 7.5	65 °C	No
RP-8 endcapped	L7	Octylsilane	4	60	1.25	350	13.0	4.44	2 - 7.5	65 °C	Yes
RP-select B	L7	Octylsilane deactivated for the separation of basic compounds	4	60	0.9	360	11.5	3.55	2 - 7.5	65 °C	No
Si	L3	unbonded	4	60	0.85	700	n.a.	n.a.	2 - 7.5	65°C	n.a.

Superspher® packing materials are available as LiChroCART® cartridges in various lengths and internal diameters (4.6 mm, 4 mm, 3 mm and 2 mm). LiChroCART® 3 mm I.D. and 2 mm I.D. narrow bore cartridges for HPLC save costs by reducing solvent consumption and allow the handling of very small quantities with excellent sensitivity and resolution. LiChroCART® cartridges 4.6 mm, 4 mm I.D., 3.9 mm I.D., 3 mm I.D. and 2 mm I.D. are compatible with manu-CART® "4". This facilitates faster and more flexible method adaptation to smaller bore columns.

Ordering Information

Superspher® (4 µm)

Column dimension								
Length (mm)		I.D. (mm)	RP-18	RP-18 endcapped	RP-8	RP-8 endcapped	RP-select B	Si
LiChroCART® HPLC Cartridge [1 unit; * 3 units]								
25	x	4	1.16039.0001*	1.16869.0001*	on request	on request	on request	on request
30	x	3	on request	on request	on request	on request	on request	on request
75	x	4	1.50980.0001*	on request	on request	on request	on request	on request
125	x	2	on request	1.50198.0001	on request	on request	1.50197.0001	on request
125	x	3	1.50792.0001	1.51909.0001	on request	on request	1.50791.0001	on request
125	x	4	1.16051.0001	1.16855.0001	1.16052.0001	1.16854.0001	1.50975.0001	1.16054.0001
150	x	4.6	on request	on request	on request	on request	on request	on request
250	x	2	on request	1.50193.0001	on request	on request	1.51308.0001	on request
250	x	3	1.51299.0001	1.51910.0001	on request	on request	1.51288.0001	on request
250	x	4	1.16056.0001	1.16858.0001	1.16010.0001	1.16857.0001	1.50973.0001	1.16009.0001
250	x	4.6	on request	on request	on request	on request	on request	on request
Validation Kits [3 LiChroCART® HPLC cartridges from 3 different sorbent batches]								
125	x	2	on request	on request	on request	on request	on request	on request
125	x	3	1.50792.1003	on request	on request	on request	on request	on request
125	x	4	on request	on request	on request	on request	on request	on request
250	x	3	on request	on request	on request	on request	on request	on request
250	x	4	1.16056.1003	on request	on request	on request	on request	on request
Guard cartridges LiChroCART® [3 units]								
10	x	2	on request	on request	on request	on request	on request	on request
Superspher® - Bulk Sorbents								
10 g Sorbent in glass bottle			1.19613.0010		1.19612.0010		1.19643.0010	

The LiChroCART® columns (75, 125, 150 and 250 mm length) in the list on the left (2, 3, 4 and 4.6 mm I.D.) require part number **1.51486.0001** manu-CART® cartridge column holder, which can be used to hold one cartridge column with or without a 4-4 mm guard column. Additional dimensions available as customized packings see page 120. As guard column we recommend LiChroCART® 4-4 LiChrospher® guard cartridges.

HPLC Columns

LiChrosorb® HPLC Columns

Irregular shaped silica sorbent

LiChrosorb® is one of the most successful and reliable packing materials, used in HPLC for decades and documented in the literature in the form of several thousand applications. The totally porous, irregular particles are linely graded in the 5 and 10 µm range.

LiChrosorb® packing materials offer non-polar (RP-8, RP-18, RP-select B) and polar modifications (Si 60 and Si 100). In addition to the analytical cartridges and columns, such as LiChroCART® 250-4 or Hibar® RT 250-4, we offer semi-preparative cartridges LiChroCART® 250-10 as well as Hibar® RT columns 250-10, packed on request with various LiChrosorb® packing materials.

LiChrosorb® HPLC column can be easily replaced with LiChrospher® spherical fully porous particulate columns. For further improvements in resolution, column efficiency and peak symmetry as well as for fast and UHPLC applications, we recommend alternatives based on more advanced column technology such as fully porous Type B silica particles, Superficially porous particles or monolithic columns.

Ordering Information

LiChrosorb®

Column dimension								
Length (mm)		I.D. (mm)	RP-18 (5 µm)	RP-18 (10 µm)	RP-8 (5 µm)	RP-8 (10 µm)	Si 60 (5 µm)	Si 100 (5 µm)
LiChroCART® HPLC Cartridge [1 unit]								
125	x	4	1.51349.0001	on request	1.51345.0001	on request	on request	1.51343.0001
250	x	4	1.51355.0001	1.51356.0001	1.51353.0001	1.51354.0001	on request	1.51351.0001

The LiChroCART® columns (125, and 250 mm length) in the list on the previous page (4 mm I.D.) require part number **1.51486.0001** manu-CART® cartridge column holder, which can be used to hold one cartridge column with or without a 4-4 mm guard column. Additional dimensions and validation kit available as customized packings see page 126.

Hibar® RT HPLC Column [1 unit]								
125	x	4	1.50433.0001	on request	1.50432.0001	on request	on request	on request
125	x	4.6	on request	on request	on request	on request	on request	on request
250	x	4	1.50333.0001	1.50334.0001	1.50332.0001	1.50318.0001	1.50388.0001	on request
250	x	3	on request	on request	on request	on request	on request	on request
250	x	4.6	1.51902.0001	on request	1.51903.0001	on request	on request	on request

The Hibar® columns are complete with endittings. When using a guard column with a Hibar® column, we recommend part number **1.51487.0001** guard column cartridge holder for 4-4 mm guard column cartridges LiChroCART®. Additional dimensions available as customized packings see page 126.

LiChrosorb® - Bulk Sorbents								
10 g Sorbent in glass bottle			1.09333.0010		1.09318.0010			1.09309.0010
Stainless steel ready to use HPLC Column								
150	x	3.2	54952					
125	x	4						
100	x	4.6	50124-U					
150	x	4.6	54951		54955-U			
200	x	4.6				50125-U		
250	x	4.6	54949		54953-U			
SupelGuard								
10	x	4.6	54965-U		54966			

HPLC Columns "on request" are available as Custom Product. Please see page 126/127

HPLC Columns

SUPELCOSIL™ HPLC Columns

Our SUPELCOSIL™ silica-based HPLC column line includes many phase chemistries, in a range of particle sizes and column configurations from microbore to preparative scale. This product line is the original high quality Supelco® product line referenced in many USP methods.

For improvements in resolution, column efficiency and peak symmetry as well as for fast and UHPLC applications, we recommend alternatives based on more advanced column technology such as Type B silica, Superficially Porous Particles or monolithic columns.

Phase Bonding	USP Designation	Bonding Chemistry	Chromatographic Properties / Use		Particle Size (µm)	Pore Size (Å)	Surface Area (m^2/g)	Carbon Load (%)	Surface Coverage ($\mu mol/m^2$)	pH Stability	Max Temperature	Endcapped
LC-18	L1	Octadecyl	General-purpose hydrophobic alkyl phase suitable for a variety of compounds.		3, 5	120	170	11	3.1	2 - 7.5	70	yes
LC-18-DB	L1	Octadecyl	C18-DB phases are specially deactivated for the separation of basic compounds providing improved peak shape.		3, 5	120	170	11	3.1	2 - 7.5	70	yes
LC-18-T	L1	Octadecyl	C-18-T columns feature an octadecylsilane bonded phase and a special surface treatment for efficient separations of nucleotides.		3, 5	120	170	12.3	3.1	2 - 7.5	70	yes
LC-18-S	L1	Octadecyl	C-18-S columns are designed for reliable separations of deoxyribonucleosides and ribonucleosides.		5	120	170	11	3.1	2 - 7.5	70	yes
LC-8	L7	Octyl	C8 phases are less hydrophobic than C18 and povides less retention of both polar and non-polar compounds than C18.		3, 5	120	170	6	3.2	2 - 7.5	70	yes
LC-8-DB	L7	Octyl	C8-DB phases are specially deactivated for basic compounds providing improved peak shape.		3, 5	120	170	6	3.2	2 - 7.5	70	yes
LC-1 Methyl	L13	Methyl	Due to a mixed retention mechanism, selectivity differences for polar groups are more pronounced than on C8 and C18 columns.		5	120	170	2	3.4	2 - 7.5	70	yes
LC-DP Diphenyl	L11	Diphenyl	Diphenyl bonded phase, which gives greater selectivity for aromatic groups compared to alkyl-type bonded phases.		5	120	170	6	2.4	2 - 7.5	70	yes
LC-ABZ	L60	Alkylamide	This deactivated phase provides enhenced reversed-phase performance for basic compounds, as well as those that are acidic, polar neutral, and non-polar.		5	120	170	12	3.4	2 - 7.5	70	yes
ABZ+Plus	L60	Alkylamide	SUPELCOSIL™ ABZ+Plus HPLC columns offer both high deactivation and unique selectivity allowing the use of low ionic strength buffers without having to add an ion-suppressing modifier.		3, 5	120	170	12	3.4	2 - 7.5	70	yes
Suplex pKb-100	L68	Alkylamide	Suplex pKb-100 columns are not end-capped, but feature the same bonded phase functionality as SUPELCOSIL™ LC-ABZ columns. The absence of end-capping reagent results in better performance for the strongest basic compounds, while LC-ABZ is preferred when the sample also contains acids and zwitterions.		5	120	170	12.5	3.4	2 - 7.5	70	Yes
LC-CN	L10	Cyano	The LC-CN phases are suitable for operation under reversed-phase conditions (HILIC) as well as under normal phase conditions.		3, 5	120	170	4	3.5	2 - 7.5	70	yes
LC-Diol	L20	Diol	LC-Diol columns can be used to separate proteins by gel filtration chromatography. They are suitable for operation under reversed-phase conditions (HILIC) as well as under normal phase conditions.		5	120	170	3.5	3.8	2 - 7.5	70	no
LC-NH2	L8	Amino	The amino column is most often employed for the separation of mono- and disaccharides. As a normal-phase application, amino columns are used in the petroleum industry.		3, 5	120	170	3	5.1	2 - 7.5	70	no
LC-Si	L3	Silica	Non-polar compounds elute first on a normal phase silica column, while polar compounds elute late. LC-Si columns can operate in normal phase mode as well as in HILIC mode.		3, 5	120	170	n.a.	n.a.	2 - 7.5	70	n.a.
LC-SCX	L52	Sulfonic acid; strong cation exchanger	The LC-SCX cation-exchange columns have strongly acidic propylsulfonic acid groups and are used for separating cations.		5	120	170	n.a.	n.a.	2 - 7.5	70	n.a.
SAX1	L14	Propyltrimethylammonium phase	SAX1 HPLC Column is typically employed as an anion exchange column with strongly basic quaternary aminopropyl phase and is used for separating anions.		5	120	170	12	n.a.	2 - 7.5	70	n.a.

Ordering Information

SUPELCOSIL™ (5 µm)

Length (mm)		I.D. (mm)	LC-18	LC-18-DB	LC-18-T	LC-18-S	LC-8	LC-8-DB	LC-1 Methyl		LC-DP Diphenyl	LC-ABZ Alkylamide	ABZ+Plus Alkylamide	Suplex pKb-100 Alkylamide	LC-CN	LC-Diol	LC-NH2	LC-Si	LC-SCX	SAX1
50	x	2.1	on request	on request	on request	on request	on request	on request	on request		on request	on request	on request	on request	on request	on request	on request	on request	on request	on request
100	x	2.1	on request	on request	on request	on request	on request	on request	on request		on request	on request	on request	on request	on request	on request	on request	on request	on request	on request
150	x	2.1	57934	on request	on request	on request	on request	on request	on request		on request	on request	on request	on request	on request	on request	on request	on request	on request	on request
250	x	2.1	57935	57940	on request	57939	on request	on request	on request		on request	on request	on request	on request	on request	on request	on request	57930-U	on request	on request
50	x	3	on request	on request	on request	on request	on request	on request	on request		on request	on request	on request	on request	on request	on request	on request	on request	on request	on request
100	x	3	on request	on request	on request	on request	on request	on request	on request		on request	on request	on request	on request	on request	on request	on request	on request	on request	on request
150	x	3	58230C30	on request	on request	on request	on request	on request	on request		on request	on request	on request	on request	on request	on request	on request	on request	on request	on request
250	x	3	on request	on request	on request	on request	on request	on request	on request		on request	59142C30	59197C30	58934C30	on request	58201C30	58338C30	on request	58997C30	on request
30	x	4	on request	on request	on request	on request	on request	on request	on request		59167	on request	on request	on request	on request	on request	on request	on request	on request	on request
50	x	4	58239C40	on request	on request	on request	on request	on request	on request		on request	on request	on request	on request	on request	on request	on request	on request	on request	on request
150	x	4	58230C40	58348C40	on request	on request	58220C40	on request	on request		on request	on request	on request	on request	on request	on request	on request	on request	on request	on request
250	x	4	58298C40	on request	on request	on request	on request	58354C40	on request		on request	59142C40	59197C40	58934C40	on request	58201C40	58338C40	on request	on request	on request
300	x	4	59165	59164	on request	on request	on request	on request	on request		on request	on request	on request	on request	on request	on request	on request	on request	on request	on request
50	x	4.6	58239	58345	on request	on request	58238	58344	on request		on request	59141	on request	on request	on request	on request	on request	on request	on request	on request
100	x	4.6	59209	on request	on request	on request	on request	on request	on request		59211	on request	on request	on request	on request	on request	on request	on request	on request	on request
150	x	4.6	58230-U	58348	on request	58931	58220-U	58347	on request		59150-U	59140-U	59196	58932	58221-U	on request	on request	58200-U	on request	on request
250	x	4.6	58298	58355-U	58971	58928-U	58297	58354	58296		58842	59142	59197	58934	58231	58201	58338	on request	58997	59138
250	x	10	58368	58358	on request	on request	58367	on request	on request		on request	on request	59179	59172	on request	on request	on request	on request	on request	on request
100	x	21.2	on request	on request	on request	on request	on request	on request	on request		on request	on request	on request	on request	on request	on request	on request	on request	on request	on request
250	x	21.2	57935	on request	on request	on request	on request	on request	on request		on request	on request	54855	on request	on request	on request	on request	on request	on request	on request
SUPELCOSIL™ Supelguard Guard Cartridge (2 pack)																				
20	x	2.1	59613	59617	on request	59162	59615	on request	on request		on request	59611	59605	on request	on request	on request	on request	on request	on request	on request
20	x	3	59564C30	on request	59621C30	on request	on request	59563C30	on request		on request	on request	on request	on request	on request	on request	59568C30	on request	on request	on request
20	x	4	59564	59565	59621	59630	59562	59563	59561		59566	59545-U	59535-U	59541-U	59567	59569	59568	on request	59519	on request
SUPELCOSIL™ Supelguard Guard Kit (Guard cartridge, stand-alone holder, tubing, 2 nuts and ferrules)																				
20	x	4	59554	59555	59620	59629	59552	59553	on request		59556	59544-U	59534-U	59531-U	59557	59559	59558	on request	59509	on request

HPLC Columns "on request" are available as Custom Product. Please see page 126/127

SUPELCOSIL™ (3 µm)

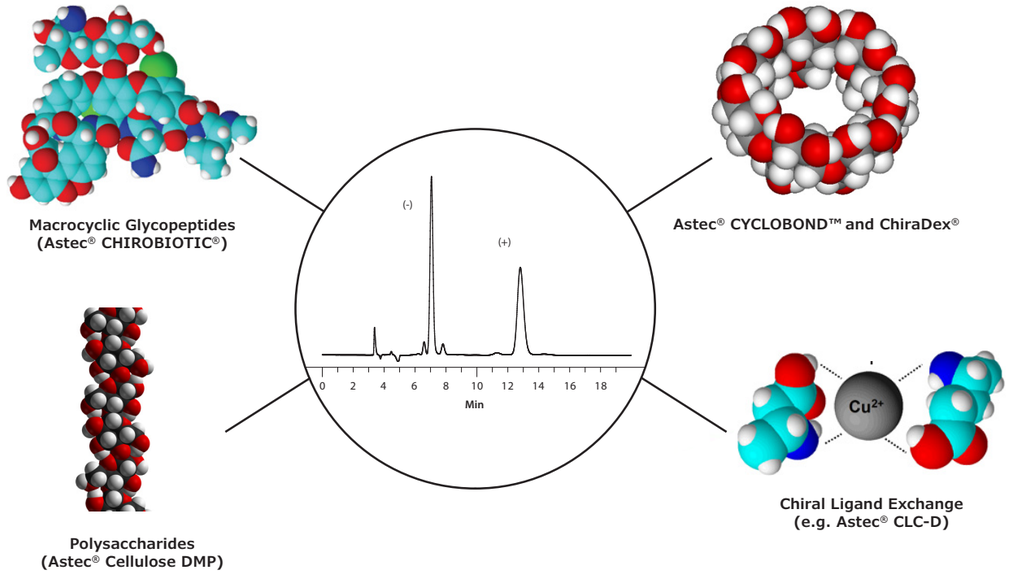
Length (mm)		I.D. (mm)	LC-18	LC-18-DB	LC-18-T	LC-8	LC-8-DB	ABZ+Plus Alkylamide	LC-CN	LC-NH2	LC-Si
33	x	2.1	on request	57943	on request	on request	58149-U	on request	on request	on request	on request
100	x	2.1	on request	on request	on request	on request	on request	57917	on request	on request	on request
250	x	2.1	on request	on request	on request	on request	on request	on request	on request	on request	on request
33	x	3	on request	58978C30	on request	on request	on request	on request	58979C30	on request	on request
50	x	3	on request	on request	on request	on request	on request	on request	on request	on request	on request
75	x	3	on request	on request	on request	on request	on request	on request	58986C30	on request	on request
150	x	3	58985C30	58993C30	58970C30	on request	on request	59194C30	on request	58989C30	58981C30
75	x	4	58984C40	on request	on request	on request	on request	on request	on request	on request	on request
150	x	4	on request	on request	on request	on request	58991C40	on request	on request	on request	on request
33	x	4.6	58977	58978	on request	58975	58976	on request	58979	on request	on request
50	x	4.6	58973	on request	on request	on request	on request	on request	on request	on request	on request
75	x	4.6	58984	58992	on request	58982	58990-U	on request	58986	58988	on request
150	x	4.6	58985	58993	58970-U	58983	58991	59194	on request	58989	58981

HPLC Columns "on request" are available as Custom Product. Please see page 126/127

HPLC Columns

HPLC Columns

Chiral HPLC Columns



Chirality belongs to the discipline of stereochemistry, which is the study of the three-dimensional structure of molecules. Chiral compounds are optically active, that means they rotate polarized light to the left or to the right depending on their configuration. The word comes from the Greek stem “chir-” meaning hand, for handedness. Chiral molecules are like left and right hands – they are mirror images. With no amount of rotation you can make the two images or molecules overlap. A chiral compound will rotate the plane of polarized light; the degree to which it does this is called the specific rotation or optical rotation.

Besides the fact that one enantiomer is often safer and more efficacious than the other enantiomer, there are other arguments for having optically pure compounds. (1) Dosing is lower. If the product contains unwanted or inactive enantiomer, then they need to dose twice as much than they would if clinicians had only the pure active enantiomer. (2) No interference of the desired activity by the unwanted enantiomer. In many cases, the unwanted enantiomer will have different biological activity (e.g. toxic or harmful) and will interfere with the performance of the intended enantiomer. (3) Time savings in testing. If the product contains more than one enantiomer, the biological activity of each isomer plus the racemate needs to be checked. This is three times the work than testing the pure enantiomer! These arguments are true for other industries besides pharmaceutical, for example agrochemicals. This aspect has environmental implications as it can affect the total amount of chemical applied to the crop.

The Supelco® line of chiral columns offers a broad portfolio of columns that can be used in reversed-phase mode, normal phase mode, polar organic mode, and polar ionic mode. In addition, these columns are economically priced compared to other vendors’ chiral columns and we offer complete scalability from analytical to prep.

HPLC Columns

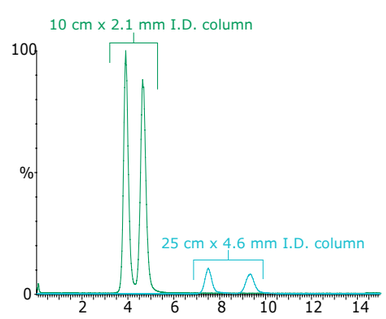
CHIROBIOTIC® Chiral HPLC Columns

CHIROBIOTIC® phases are based on covalently bonding macrocyclic glycoproteins to a high purity 5 µm silica gel in such a way as to establish its stability while retaining essential components for chiral recognition. CHIROBIOTIC® V and V2 are based on bonding Vancomycin, which contains 18 chiral centers surrounding three pockets or cavities. Five aromatic ring structures bridge these strategic cavities. Hydrogen donor acceptor sites are readily available close to the ring structures. CHIROBIOTIC® V has demonstrated selectivity similar to glycoprotein phases except it is stable from 0-100% organic modifier and exhibits high sample capacity.

For CHIROBIOTIC® V2, changes to the linkage chemistry and silica offer improvements for preparative LC and for more demanding chiral separations. CHIROBIOTIC® T, T2, and TAG are based on bonding the amphoteric glycopeptide, Teicoplanin, which contains 23 chiral centers surrounding four pockets or cavities. For CHIROBIOTIC® T2, changes to the linkage chemistry and silica offer improvements for preparative LC and for more demanding chiral separations. CHIROBIOTIC® TAG has the sugars removed from the macrocyclic glycopeptide to produce an aglycone structure as a variant of CHIROBIOTIC® T. CHIROBIOTIC® R is based on bonding Ristocetin A to high purity 5 µm silica.

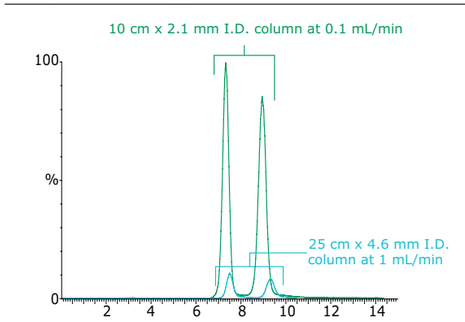
LC-MS of Fluoxetine Enantiomers on CHIROBIOTIC® V2: Fast, Sensitive Analysis on Short, Narrow I.D. Columns

Column:	CHIROBIOTIC® V2, 25 cm x 4.6 mm or 10 cm x 2.1 mm I.D. 5 µm
Mobile Phase:	10 mM ammonium formate in 10:90 v/v water:methanol
Flow Rate:	1.0 or 0.2 mL/min
Temperature:	ambient
Detection:	+ESI, m/z 310
Injection:	2 µL
Sample:	5 µg/mL in methanol



LC-MS of Fluoxetine Enantiomers on CHIROBIOTIC® V2: Use Column Dimensions and Flow Rate to Enhance Sensitivity without Loss of Resolution

Column:	CHIROBIOTIC® V2, 25 cm x 4.6 mm or 10 cm x 2.1 mm I.D., 5 µm
Mobile Phase:	10 mM ammonium formate in 10:90 v/v water:methanol
Flow Rate:	1.0 or 0.1 mL/min
Temperature:	ambient
Detection:	+ESI, m/z 310
Injection:	2 µL
Sample:	5 µg/mL in methanol



CHIROBIOTIC® (5 µm)

Length (mm)		I.D. (mm)	V	V2	T	T2	TAG	R
100	x	2.1	11018AST	15018AST	12018AST	16018AST	14018AST	on request
150	x	2.1	11019AST	15019AST	12019AST	16019AST	14019AST	13019AST
250	x	2.1	11020AST	15020AST	12020AST	on request	14020AST	13020AST
100	x	3.0	on request	on request	12010AST	on request	on request	on request
50	x	4.6	on request	on request	12021AST	on request	on request	on request
100	x	4.6	11022AST	15022AST	12022AST	on request	14022AST	13022AST
150	x	4.6	11023AST	15023AST	12023AST	16023AST	14023AST	13023AST
250	x	4.6	11024AST	15024AST	12024AST	16024AST	14024AST	13024AST
250	x	10	11034AST	on request	12034AST	on request	14034AST	on request
250	x	21.2	11044AST	15044AST	on request	on request	14044AST	on request
Guard 20	x	1.0	on request	on request	on request	on request	on request	on request
Guard 20	x	4.0	11100AST	15100AST	12100AST	on request	14100AST	on request
Guard Holder	x		21150AST					

HPLC Columns

CYCLOBOND™ Chiral HPLC Columns

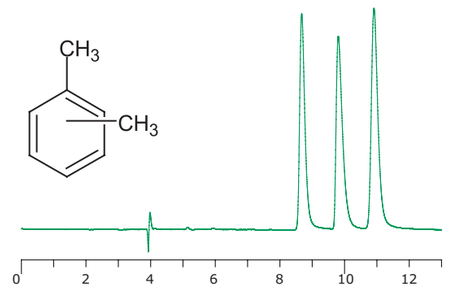
CYCLOBOND™ is the name given to Supelco® technology for bonding cyclodextrins to a high purity silica gel through a stable, ether linkage. Introduced in 1983, this patented, stationary phase has the ability to form inclusion complexes for a wide variety of organic molecules into the cyclodextrin cavities leading to numerous chiral separations. CYCLOBOND™ I are bonded β-cyclodextrins and CYCLOBOND™ II are bonded γ-cyclodextrins.

CYCLOBOND™ I 2000 series of HPLC columns is specially formulated to meet today's stringent requirements for analysis in the pharmaceutical industry, and for small analytes of general interest in chemical and environmental areas. We have focused on the need to accurately and reproducibly separate enantiomers. The result is a high performance range of chiral separation phases with guaranteed batch to batch reproducibility, greater stability and improved selectivity and resolution. Based on the original CYCLOBOND™ I (β-cyclodextrin) columns, the CYCLOBOND™ I 2000 series are second-generation products. The native β-cyclodextrin and eight β-cyclodextrin derivatives are in the CYCLOBOND™ I 2000 series.

CYCLOBOND™ II series columns are excellent chiral selectors for multi-ring structures such as those based on anthracene, chrysene or pyrene. These are γ-cyclodextrin bonded phases, and consist of eight glucopyranose units arranged in the same truncated cone shape. Applications include steroids, porphorins, and FMOC amino acids.

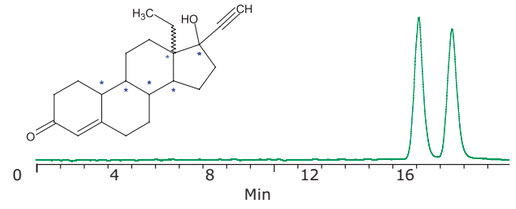
Positional Isomers (Xylenes) on CYCLOBOND™ I 2000

Column:	CYCLOBOND™ I 2000, 25 cm x 4.6 mm I.D., 5 µm (20024AST)
Mobile phase	[A] Acetonitrile; [B] water
Mobile phase ratio:	15:85 (A:B)
Flow rate:	0.8 mL/min
Temp.:	45 °C
Det.:	UV, 230 nm
Injection:	3 µL
Sample:	each compound, 0.1 mg/mL in acetonitrile:water (50:50)
Elution order:	m-, o-, p-xylene



Norgestrel on CYCLOBOND™ II

Column:	CYCLOBOND™ II, 25 cm x 4.6 mm I.D., 5 µm (41020AST)
Mobile phase	[A] water; [B] acetonitrile
Mobile phase ratio:	70:30 (A:B)
Flow rate:	0.8 mL/min
Temp.:	42 °C
Det.:	UV, 254 nm
Injection:	1 µL
Sample:	norgestrel, 1 mg/mL in methanol



CYCLOBOND™ (5.0 µm)

Length (mm)		I.D. (mm)	I 2000	I 2000 AC	I 2000 SP	I 2000 RSP	I 2000 HP RSP	I 2000 DMP	II
100	x	2.1	20018AST	on request	on request	on request	on request	on request	on request
150	x	2.1	20019AST	on request	on request	on request	on request	on request	on request
100	x	4.6	on request	on request	on request	on request	on request	20722AST	on request
150	x	4.6	on request	20123AST	on request	on request	24023AST	on request	46023AST
250	x	4.6	20024AST	20124AST	20224AST	20324AST	24024AST	20724AST	41020AST
250	x	10.0	20034AST	on request	on request	on request	on request	20734AST	on request
250	x	21.2	on request	on request	on request	on request	on request	20744AST	on request
Guard 20	x	4.0	on request	on request	on request	21103AST	on request	on request	on request
Guard Holder			21150AST						

HPLC Columns

Cellulose DMP Chiral HPLC Columns

Cellulose DMP is a chiral stationary phase (CSP) comprising spherical, high-purity porous silica coated with DMPC (3,5-dimethylphenyl carbamate)-derivatized cellulose, and packed in analytical to preparative size HPLC columns. This technology separates a wide range of chiral compounds under normal phase, polar organic, and SFC conditions, with high efficiency, high loading capacity, and excellent column lifetime. Performance is comparable to other DMPC-derivatized, cellulose CSPs.

Key Features and Application Areas:.

- Classic DMPC-cellulose chiral selectivity
- Efficient, rugged, reproducible, and scalable
- Low backpressure
- Ideal for chiral analysis in the pharmaceutical industry and for small analytes in chemical and environmental areas
- Routine chiral column method development screening protocols
- Approximately half of the cost of most other DMPC-cellulose columns

Cellulose DMP is complementary to the other CSPs, including CHIROBIOTIC® and CYCLOBOND™ product lines, and a must-have for every chiral HPLC or SFC screening protocol.

Cellulose DMP (5.0 µm)

Length (mm)		I.D. (mm)	SKU
150	x	2.1	51100AST
100	x	4.6	on request
150	x	4.6	51098AST
250	x	4.6	51099AST
Guard 20	x	2.1	51104AST
Guard 20	x	4.0	on request

Copper Ligand Exchange (CLC) Chiral HPLC Columns

The CLC phases are based on coupling an enantiomeric form of an amine to a proprietary derivative to create an appropriate distance for copper coupling. Using the copper ligand concept, this phase resolves hydroxy acids like lactic, malic, tartaric and mandelic. This phase can also resolve amino acids and other amines by the same mechanism. It has been reported that, in addition to amino acids, other bifunctional racemates like amino alcohols can be resolved. In theory, any analyte that can complete the coordination with the copper ion can be resolved. For the CLC-D column, the L enantiomer generally elutes before D with the exception of tartaric acid where the D elutes first. The CLC-L column has the opposite elution order and the D enantiomer elutes before L.

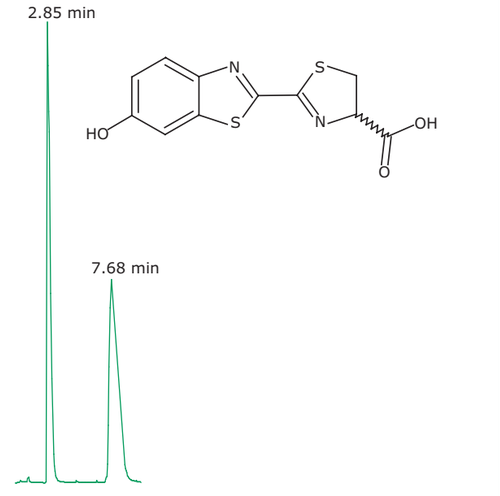
Copper Ligand Exchange (5.0 µm)

Length (mm)		I.D. (mm)	CLC-D	CLC-L
150	x	4.6	53023AST	53123AST

Protein-Based Chiral HPLC Columns

Hermansson described the use of natural proteins immobilized onto a silica support for chiral separations in 1983. Proteins contain a large number of chiral centers of one configuration, and many other sites that contribute to the general retention process. We offer three CSPs with proteins as the chiral selectors, CHIRALPAK® AGP (α1-acid glycoprotein), CHIRALPAK® CBH (cellobiohydrolase) and CHIRALPAK® HSA (human serum albumin). All are manufactured by DAICEL Corporation. These columns are typically used in reversed-phase mode, and perform a wide variety of chiral separations. CHIRALPAK® HSA is also used for drug-binding studies. Solutes are retained by three types of interactions: ionic (for charged solutes), hydrophobic and hydrogen bonding. The relative contribution of the different forces to solute retention depends on the nature of the analyte.

- **CHIRALPAK® AGP:** Extremely broad applicability. First choice when developing methods on protein-CSPs.
- **CHIRALPAK® HSA:** Analytes are typically hydrophilic acids.
- **CHIRALPAK® CBH:** Analytes are typically hydrophilic amines and amino alcohols.



Column:	CHIRALPAK® AGP, 10 cm x 4 mm I.D., 5 µm (58150AST)
Column temp.:	25 °C
Mobile phase:	10 mM sodium phosphate, pH 6.0
Flow rate:	0.9 mL/min
Sample:	luciferin
Detector:	UV, 225 nm

HPLC Columns

CHIRALPAK® (5.0 µm)

Length (mm)		I.D. (mm)	AGP	CBH	HSA
50	x	2.0	on request	on request	on request
100	x	2.0	58130AST	58530AST	58430AST
150	x	2.0	58131AST	on request	on request
50	x	3.0	58169AST	on request	58469AST
100	x	3.0	58170AST	58570AST	58470AST
150	x	3.0	58171AST	58571AST	on request
50	x	4.0	on request	on request	58449AST

Length (mm)		I.D. (mm)	AGP	CBH	HSA
100	x	4.0	58150AST	58550AST	58450AST
150	x	4.0	58151AST	58551AST	58451AST
Guard 10	x	3.0	58158AST	58558AST	on request
Guard 10	x	4.0	58188AST	58588AST	on request
Coupler for Legacy Guard Column Holder: 54986					
Guard Column Holder: 58159AST					

ChiraDex® and ChiraDex® HR HPLC Columns

ChiraDex® HR HPLC columns can be used for the separation of enantiomers of numerous different compounds. ChiraDex® columns are based on a beta-cyclodextrin covalently linked to spherical particles of silica and is well suited for chiral separations of hydrocarbons, steroids, phenyl esters, aromatic amines, and heterocycles with 5-membered rings to 7-membered rings. Simply composed reversed phase-eluents can be used in most separations.

ChiraDex® (5.0 µm)

Length (mm)		I.D. (mm)	ChiraDex®	ChiraDex® HR
4	x	4	1.50117	on request
250	x	4	1.51333	on request
250	x	4	on request	1.51000

Complementary Chiral Columns

Astec® HPLC CSPs include CHIROBIOTIC®, CYCLOBOND™, CLC-L, and CLC-D. Astec® CHIRALDEX and Supelco® DEX™ columns are the market leaders for chiral GC separations. The HPLC CSPs are complementary to each other in terms of selectivity and mobile phase compatibility. This fact means:

- It is likely that at least one Astec® CSP will give the necessary selectivity. Incorporating Astec® Cellulose DMP, CHIROBIOTIC® and CYCLOBOND™ in the HPLC or SFC screening protocol will give at least 90% success rate.
- Multiple Astec® CSPs may provide the necessary enantioselectivity, but one may operate in a preferred mobile phase system, one that is more compatible with the detection mode, or provides better analyte solubility or shorter retention time, or many other considerations. For example, polar ionic CHIROBIOTIC® mobile phases are ideal for LC/ESI-MS.
- Different CSPs may provide reversal of elution order, a useful attribute for prep and for low-level detection of the presence of an unwanted enantiomer in large excess of the opposite enantiomer (trace analysis).

The wide choice of CSPs in the Astec® line means they cover many different areas of interest within chiral chromatography. Some of these areas are captured in the below table.

Techniques, Applications, and Fields of Use for Astec® Chiral Phases

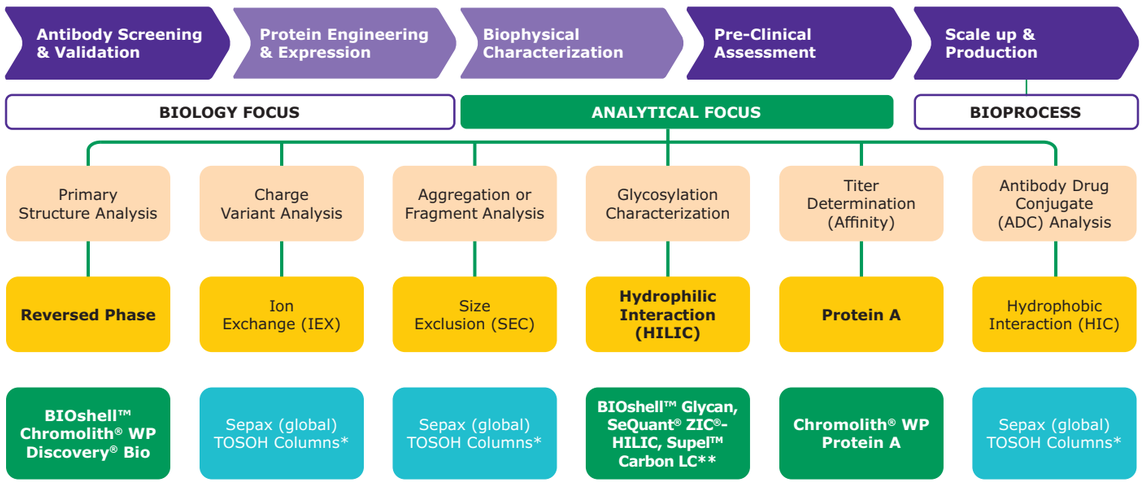
	Astec® Cellulose DMP	Astec® CHIROBIOTIC™	Astec® CYCLOBOND™	Astec® CLC	Astec® CHIRALDEX	Supelco® DEX™
Routine Chiral Column Screening	■	■	▒		■	■
Normal Phase HPLC	■	▒	▒			
SFC	■	▒	▒			
Reversed-Phase HPLC		■	■	▒		
Hydrophilic Interaction HPLC (HILIC)		■				
Polar Organic Mode (HPLC)	■	■	▒			
Polar Ionic Mode (HPLC)		■				
Ligand Exchange Mode HPLC				■		
Gas Chromatography (GC)					■	■
Prep (LC and/or SFC)	■	■	▒			
Polar/Ionic Analytes	▒	■	▒			
Amino Acids, Peptides		■		▒		
Non-Aromatic Organic Acids			▒	■		
Mass Spec (LC/ESI)	▒	■	▒			
Mass Spec (LC/APCI)	■	■	▒			
Mass Spec (GC/MS)					■	■
Bioanalysis (drugs in biological fluids)	▒		▒		▒	▒

■ Highly suitable

▒ Marginally suitable, or limited to specific applications

□ Not suitable nor recommended

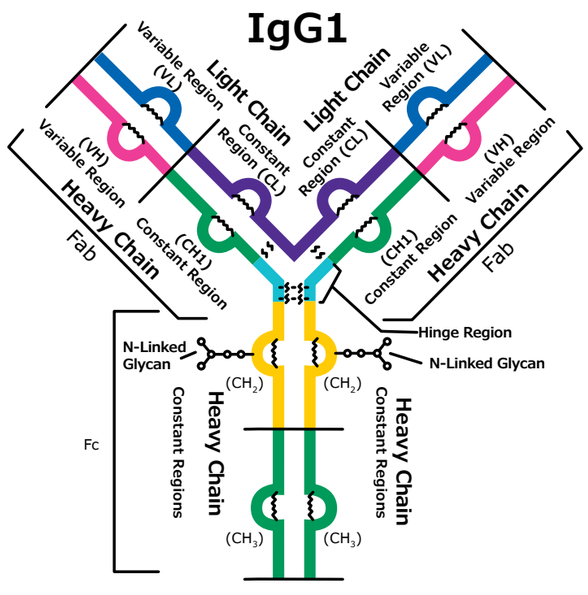
Biomacromolecule Characterization: A Multipronged Separation Challenge



* Tosoh Bioscience columns are available in select countries. For a list, please go to the page 109 of this brochure.
**Supel™ Carbon LC columns are not HILIC columns but can retain and resolve polar compounds like glycans.

Biomacromolecules (in particular monoclonal antibodies (mAbs)) have seen a renewed interest in the pharmaceutical and biotechnology industry. The reason for this high level of interest resides in the number of benefits these biological molecules have for patients including, but not limited to, high efficacy in treating an illness, high specificity for a target receptor or antigen, wide therapeutic range, and limited, undesirable side effects. However, due to the fact that these molecules are complex and are often produced in host cell lines, bacteria, or fermentation reactors, these potential therapeutics exhibit significant heterogeneity which needs to be evaluated and characterized using various analytical techniques. The complexity of such biomacromolecules can be easily illustrated by examining the structure of a typical mAb as depicted on the right.

mAbs are large, tetrameric immunoglobulin G (IgG) molecules with a molecular weight of approximately 150 kDa (150,000 g/mol). These molecules form a Y-shape composed of four peptide chains: two identical light (L) chains, with a molecular weight of approximately 25 kDa each, and two identical heavy (H) chains, with a molecular weight of approximately 50 kDa each. To form the Y-shape, these four polypeptide chains associate with each other through the creation of inter- and intra-chain disulfide bonds.



Due to the inherent complexity of biomacromolecules, multiple, orthogonal modes of chromatography like reversed-phase chromatography and HILIC are required to fully characterize these molecules.

Size Exclusion Chromatography

Size Exclusion Chromatography (SEC) is a mode of chromatography that separates molecules by their size (i.e. hydrodynamic radius). This mode of chromatography does not rely on the interaction of the analytes with a stationary phase ligand; it is an entropic process meaning that it relies on the random flow of the analytes through the stationary phase particles. For most practical purposes, this can be envisioned as analytes with a higher molecular weight will elute earlier in the run, since these analytes are fully or partially excluded from the pores of the stationary phase particles, while lower molecular weight analytes will elute later in the run, since these analytes will spend more time navigating the tortuous path through the particle.

Hydrophobic Interaction Chromatography

Hydrophobic Interaction Chromatography (HIC) is a mode of chromatography that separates analytes based on the degree of interaction between hydrophobic moieties on the analyte and hydrophobic ligands on the stationary phase. Under conditions of high concentrations of salt, the hydration layer around a protein may be disrupted enough such that it becomes entropically favorable for hydrophobic regions of the protein's surface to interface with the non-polar stationary phase. This phenomenon is not unlike the classical biochemical technique of protein salting out, but in HIC's case, the interactions are between protein-stationary phase ligand, not between different protein molecules. Due to the lower molecular weight and lower propensity for folding, HIC is usually not employed for separating peptides. Salt selection in HIC is dictated by the Hofmeister series, which classifies cations and anions in terms of their ability to disrupt the hydration layer around a protein (chaotropic) or promote the formation of a hydration layer (kosmotropic). Typical salts in HIC are ammonium sulfate, potassium sulfate, and sodium sulfate. Elution is achieved by gradients with decreasing salt concentration.

Ion Exchange Chromatography

Ion Exchange Chromatography (IEX) is a mode of chromatography that separates analytes by charge. Proteins and peptides are amphoteric, which means that they exhibit both acidic and basic functionalities. The acidic portions of a protein are provided by aspartic acid, glutamic acid, cysteine, tyrosine, and the α-carboxylate on the C-terminus. The basic portions of a protein are provided by arginine, histidine, lysine, and the α-amine on the N-terminus. Charge variants of a biotherapeutic, another critical quality attribute (CQA) that regulatory bodies require manufacturers to monitor, can be detected and resolved by IEX. These charge variants can arise from mistranslation of messenger RNA (mRNA) transcripts and/or post-translational modifications such as deamidation, oxidation, or glycosylation, among others.

Affinity Chromatography

Affinity chromatography is a mode of chromatography that relies on a specific interaction between the analyte of interest and the stationary phase ligand. Ideally, no other component of the sample would interact with the ligand, thus only the analyte of interest interacts with the stationary phase. Afterwards, a different mobile phase is passed through the column that breaks this interaction, thus eluting the analyte.

Reversed Phase Chromatography

Reversed-Phase Chromatography (RPC) is a mode of chromatography that separates analytes based primarily on hydrophobicity. Unlike HIC, RPC employs a water/organic mixture for the mobile phase. Typically, this mobile phase combination is supplemented with an ion pairing reagent like trifluoroacetic acid (TFA), formic acid, or difluoroacetic acid (DFA) to mask the secondary interactions between exposed silanols on the silica stationary phase and H-bonding donor groups on analytes. Common applications for characterizing biomolecules by RPC include peptide mapping, where a protease, like trypsin, cleaves a protein at defined sites into characteristic peptide fragments, and middle-up analyses, which include reducing a protein into larger fragments for easier characterization.

Sepax Technologies: HPLC Columns for Bioanalysis

Size Exclusion Chromatography (SEC)

Unix™ SEC-200 and SEC-300 UHPLC Columns

Utilizing proprietary surface technologies, Unix™ SEC-200 and SEC-300 phases are made of uniform, hydrophilic, and neutral nanometer thick films chemically bonded to high purity and mechanically stabilized silica with a particle size of 1.8 µm. The combination of small particle size and large pore volume of Unix™ SEC-300 renders the highest separation efficiency and resolution of analytes. The well-controlled surface chemistry results in excellent lot-to-lot reproducibility. The unique bonding chemistry, coupled with the maximized bonding density, allows Unix™ SEC-300 to provide high stability and negligible non-specific interactions. Typical applications for Unix™ SEC-300 columns include separation and analysis of biological molecules and water-soluble polymers.

Unix™ (1.8 µm)

Length (mm)		I.D. (mm)	SEC-200	SEC-300
150	x	4.6	Z777303	Z777300
300	x	4.6	Z777304	Z777302
Guard 10	x	4.0	Z777305	Z777301

SRT® and SRT®-C HPLC Columns

SRT® size exclusion columns are prepared from high purity, porous silica that is treated to provide the mechanical stability required for high performance HPLC analysis of biopolymers. The particles are derivatized with a uniform, hydrophilic bonded layer to minimize interaction of sample molecules with the silica surface. The proprietary surface technology results in excellent column-to-column reproducibility, while the composition of the bonded phase and the maximized bonding density impart excellent chemical stability and negligible non-specific interactions. In addition to the high pore volume per unit column volume, SRT® columns are available in six different pore sizes from 100 Å to 2000 Å, allowing the user to select the pore size that best matches the dynamic radius of the biopolymer, be it a peptide, protein, virus, or vaccine. SRT®-C SEC columns are packed with high purity and mechanically stable, 5 µm, silica particles which are derivatized with a uniform, chemically neutral, hydrophilic bonded phase that effectively shields the sample from interacting with the underlying silica. Since SRT®-C columns can be operated up to 3500 psi pressure, they allow faster analysis and higher throughput than competitor columns. SRT®-C columns are the preferred choice for relatively hydrophobic sample types such as insulin, membrane proteins and derivatized monoclonal antibodies.

SRT® (5.0 µm)

Length (mm)		I.D. (mm)	Column Hardware	SEC-100	SEC-150	SEC-300	SEC-500	SEC-1000	SEC-2000
300	x	4.6	Stainless Steel	Z777037	Z777043	Z777049	Z777055	Z777061	Z777067
300	x	4.6	PEEK	Z777041	Z777047	Z777053	Z777059	Z777065	Z777071
300	x	7.8	Stainless Steel	Z777039	Z777045	Z777051	Z777057	Z777063	Z777069
Guard 50	x	4.6	Stainless Steel	Z777036	Z777042	Z777048	Z777054	Z777060	Z777066
Guard 50	x	4.6	PEEK	Z777040	Z777046	Z777052	Z777058	Z777064	Z777070
Guard 50	x	7.8	Stainless Steel	Z777038	Z777044	Z777050	Z777056	Z777062	Z777068

SRT®-C (5.0 µm)

Length (mm)		I.D. (mm)	Column Hardware	SEC-100	SEC-150	SEC-300	SEC-500	SEC-1000	SEC-2000
300	x	4.6	Stainless Steel	Z777096	Z777102	Z777108	Z777114	Z777120	Z777126
300	x	4.6	PEEK	Z777100	Z777106	Z777112	Z777118	Z777124	Z777130
300	x	7.8	Stainless Steel	Z777098	Z777104	Z777110	Z777116	Z777122	Z777128
Guard 50	x	4.6	Stainless Steel	Z777095	Z777101	Z777107	Z777113	Z777119	Z777125
Guard 50	x	4.6	PEEK	Z777099	Z777105	Z777111	Z777117	Z777123	Z777129
Guard 50	x	7.8	Stainless Steel	Z777097	Z777103	Z777109	Z777115	Z777121	Z777127

HPLC Columns

Zenix® and Zenix®-C HPLC Columns

Use Zenix® SEC high performance gel filtration columns to analyze hydrophilic polymers including proteins and other water soluble polymers. Prepared from spherical 3 µm silica particles, Zenix® SEC columns represent a breakthrough technology for high performance size exclusion chromatography of biopolymers. The combination of 3 µm silica particle size and a proprietary surface technology provides the highest separation efficiency and resolution for biological molecules and water soluble polymers. Zenix® columns are available in 100, 150 and 300 Å pore sizes, and are packed in stainless steel or PEEK hardware. Since Zenix® columns can be operated up to 3500 psi pressure, they allow fast analysis and high sample throughput. Zenix®-C SEC columns are the preferred gel filtration columns to analyze relatively hydrophobic sample types such as insulin, membrane proteins and derivatized monoclonal antibodies. Zenix®-C SEC columns are packed with high purity and mechanically stable, 3 µm, silica particles that are chemically modified with a uniform, chemically neutral, hydrophilic bonded phase that effectively shields the sample from interacting with the underlying silica. Since Zenix®-C columns can be operated up to 3500 psi pressure, they allow fast analysis and high sample throughput. Zenix®-C columns are available in 100, 150 and 300 Å pore sizes, and are packed in stainless steel or PEEK hardware.

Zenix® (3.0 µm)

Length (mm)		I.D. (mm)	Column Hardware	SEC-100	SEC-150	SEC-300
300	x	1.0	Stainless Steel	Z777002	Z777012	Z777022
300	x	2.1	Stainless Steel	Z777004	Z777014	Z777024
150	x	4.6	Stainless Steel			Z777026
250	x	4.6	Stainless Steel			Z777027
300	x	4.6	Stainless Steel	Z777006	Z777016	Z777028
300	x	4.6	PEEK	Z777010	Z777020	Z777035
150	x	7.8	Stainless Steel			Z777030
200	x	7.8	Stainless Steel			Z777031
250	x	7.8	Stainless Steel			Z777032
300	x	7.8	Stainless Steel	Z777008	Z777018	Z777033
Guard 50	x	1.0	Stainless Steel	Z777001	Z777011	Z777021
Guard 50	x	2.1	Stainless Steel	Z777003	Z777013	Z777023
Guard 50	x	4.6	Stainless Steel	Z777005	Z777015	Z777025
Guard 50	x	4.6	PEEK	Z777009	Z777019	Z777034
Guard 50	x	7.8	Stainless Steel	Z777007	Z777017	Z777029

Zenix®-C (3.0 µm)

Length (mm)		I.D. (mm)	Column Hardware	SEC-100	SEC-150	SEC-300
150	x	4.6	Stainless Steel			Z777085
250	x	4.6	Stainless Steel			Z777086
300	x	4.6	Stainless Steel	Z777073	Z777079	Z777087
300	x	4.6	PEEK	Z777077	Z777083	Z777094
150	x	7.8	Stainless Steel			Z777089
200	x	7.8	Stainless Steel			Z777090
250	x	7.8	Stainless Steel			Z777091
300	x	7.8	Stainless Steel	Z777075	Z777081	Z777092
Guard 50	x	4.6	Stainless Steel	Z777072	Z777078	Z777084
Guard 50	x	4.6	PEEK	Z777076	Z777082	Z777093
Guard 50	x	7.8	Stainless Steel	Z777074	Z777080	Z777088

HPLC Columns

Ion Exchange Chromatography

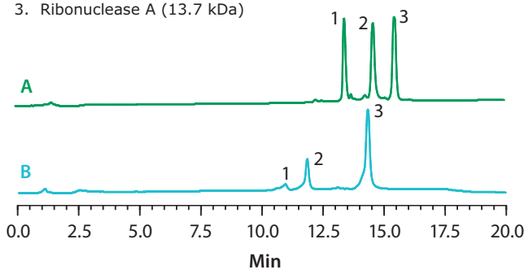
Antibodix™ U/HPLC Columns

Antibodix™ columns are specially designed for high resolution, high efficiency and high recovery separations of antibodies. These columns are packed with spherical, non-porous, particles that consist of highly cross-linked poly(styrene divinylbenzene) (PS/DVB). Antibodix™ columns are available packed with 1.7, 3, 5 or 10 µm particles that can withstand pressures varying from 4,000 psi (10 µm) up to 10,000 psi (1.7 µm). The hydrophobic PS/DVB resin surface is shielded by a hydrophilic, neutral polymer to which a dense layer of weak cation-exchange functional groups are attached, thus combining the benefits of minimal secondary interaction of antibodies with the base matrix and high dynamic binding capacity.

A. **Antibodix™ WCX-NP10**
25 cm × 4.6 mm I.D., 10 µm (non-porous)

B. **Vendor D WCX**
25 cm × 4.0 mm I.D., 10 µm (non-porous)

1. Cytochrome c (12.2 kDa)
2. Lysozyme (14.3 kDa)
3. Ribonuclease A (13.7 kDa)



Column:	Antibodix™ WCX-NP10, 25 cm x 4.6 mm I.D., 10 µm (Z777272)
Column temp.:	25 °C
Mobile phase:	(A) 10 mM sodium phosphate buffer, 6.0; (B) A + 1.0 M sodium chloride
Gradient:	0 to 100% B in 42 min
Flow rate:	1.0 mL/min
Sample:	1 mg/mL each
Injection:	5 µL
Detector:	UV, 214 nm

Antibodix™ (1.7 µm)

Length (mm)		I.D. (mm)	Column Hardware	Cat. No.
30	x	2.1	Stainless Steel	Z777278
50	x	4.6	Stainless Steel	Z777280
Guard 10	x	2.0	Stainless Steel	Z777277
Guard 10	x	4.0	Stainless Steel	Z777279

Antibodix™ (3.0 µm)

Length (mm)		I.D. (mm)	Column Hardware	Cat. No.
30	x	2.1	Stainless Steel	Z777282
50	x	2.1	Stainless Steel	Z777283
50	x	2.1	PEEK	Z777287
50	x	4.6	Stainless Steel	Z777285
50	x	4.6	PEEK	Z777288
150	x	4.6	Stainless Steel	Z777286
Guard 10	x	2.0	Stainless Steel	Z777281
Guard 10	x	4.0	Stainless Steel	Z777284

Antibodix™ (5.0 µm)

Length (mm)		I.D. (mm)	Column Hardware	Cat. No.
50	x	2.1	Stainless Steel	Z777290
50	x	2.1	PEEK	Z777295
50	x	4.6	Stainless Steel	Z777292
50	x	4.6	PEEK	Z777296
150	x	4.6	Stainless Steel	Z777293
250	x	4.6	Stainless Steel	Z777294
250	x	4.6	PEEK	Z777297
Guard 10	x	2.0	Stainless Steel	Z777289
Guard 10	x	4.0	Stainless Steel	Z777291

Antibodix™ (10.0 µm)

Length (mm)		I.D. (mm)	Column Hardware	Cat. No.
50	x	2.1	Stainless Steel	Z777268
50	x	2.1	PEEK	Z777273
250	x	2.1	Stainless Steel	Z777269
250	x	2.1	PEEK	Z777274
50	x	4.6	Stainless Steel	Z777271
50	x	4.6	PEEK	Z777275
250	x	4.6	Stainless Steel	Z777272
250	x	4.6	PEEK	Z777276
Guard 10	x	2.0	Stainless Steel	Z777267
Guard 10	x	4.0	Stainless Steel	Z777270

HPLC Columns

Proteomix® U/HPLC Columns

Based on non-porous particles, the Proteomix® ion-exchange column line from Sepax Technologies was designed to achieve high recovery of peptides, proteins, oligonucleotides, polysaccharides, cell lysates, nanoparticles and nanotubes when analyzed under HPLC or UHPLC conditions. Proteomix® columns are packed with spherical, non-porous, poly(styrene divinylbenzene) (PS/DVB) particles that are encapsulated with a hydrophilic, neutral polymer layer to eliminate non-specific binding. Using proprietary, coupling chemistry, weak (WAX and WCX) and strong (SAX and SCX) anion and cation exchange functional groups are attached to the hydrophilic bonded phase to obtain a high capacity ion-exchange layer. Proteomix® columns are available packed with 1.7, 3, 5 or 10 µm particles that can withstand pressures varying from 4,000 psi (10 µm) up to 10,000 psi (1.7 µm). Non-porous Proteomix® ion-exchange columns are unique as they combine increased ion exchange capacity with high efficiency, recovery and throughput.

Proteomix® Anion Exchange (1.7 µm)

Length (mm)		I.D. (mm)	Column Hardware	Quaternary Ammonium	Tertiary Amine
30	x	2.1	Stainless Steel	Z777210	Z777244
30	x	4.6	Stainless Steel	Z777213	Z777247
50	x	2.1	Stainless Steel	Z777211	Z777245
50	x	4.6	Stainless Steel	Z777214	Z777248
Guard 10	x	2.0	Stainless Steel	Z777209	Z777243
Guard 10	x	4.0	Stainless Steel	Z777212	Z777246

Proteomix® Anion Exchange (3.0 µm)

Length (mm)		I.D. (mm)	Column Hardware	Quaternary Ammonium	Tertiary Amine
30	x	2.1	Stainless Steel	Z777216	Z777250
50	x	2.1	Stainless Steel	Z777217	Z777251
50	x	2.1	PEEK	Z777221	Z777255
50	x	4.6	Stainless Steel	Z777219	Z777253
50	x	4.6	PEEK	Z777222	Z777256
150	x	4.6	Stainless Steel	Z777220	Z777254
Guard 10	x	2.0	Stainless Steel	Z777215	Z777249
Guard 10	x	4.0	Stainless Steel	Z777218	Z777252

Proteomix® Anion Exchange (5.0 µm)

Length (mm)		I.D. (mm)	Column Hardware	Quaternary Ammonium	Tertiary Amine
50	x	2.1	Stainless Steel	Z777224	Z777258
50	x	2.1	PEEK	Z777230	Z777264
150	x	2.1	Stainless Steel	Z777225	Z777259
50	x	4.6	Stainless Steel	Z777227	Z777261
50	x	4.6	PEEK	Z777231	Z777265
150	x	4.6	Stainless Steel	Z777228	Z777262
250	x	4.6	Stainless Steel	Z777229	Z777263
250	x	4.6	PEEK	Z777232	Z777266
Guard 10	x	2.0	Stainless Steel	Z777223	Z777257
Guard 10	x	4.0	Stainless Steel	Z777226	Z777260

Proteomix® Anion Exchange (10.0 µm)

Length (mm)		I.D. (mm)	Column Hardware	Quaternary Ammonium	Tertiary Amine
50	x	2.1	Stainless Steel	Z777200	Z777234
50	x	2.1	PEEK	Z777205	Z777239
250	x	2.1	Stainless Steel	Z777201	Z777235
250	x	2.1	PEEK	Z777206	Z777240
50	x	4.6	Stainless Steel	Z777203	Z777237
50	x	4.6	PEEK	Z777207	Z777241
250	x	4.6	Stainless Steel	Z777204	Z777238
250	x	4.6	PEEK	Z777208	Z777242
Guard 10	x	2.0	Stainless Steel	Z777199	Z777233
Guard 10	x	4.0	Stainless Steel	Z777202	Z777236

HPLC Columns

Proteomix® Cation Exchange (1.7 µm)

Length (mm)		I.D. (mm)	Column Hardware	Sulfonate	Carboxylate
30	x	2.1	Stainless Steel	Z777142	Z777176
30	x	4.6	Stainless Steel	Z777145	Z777179
50	x	2.1	Stainless Steel	Z777143	Z777177
50	x	4.6	Stainless Steel	Z777146	Z777180
Guard 10	x	2.0	Stainless Steel	Z777141	Z777175
Guard 10	x	4.0	Stainless Steel	Z777144	Z777178

Proteomix® Cation Exchange (5.0 µm)

Length (mm)		I.D. (mm)	Column Hardware	Sulfonate	Carboxylate
50	x	2.1	Stainless Steel	Z777156	Z777190
50	x	2.1	PEEK	Z777162	Z777196
150	x	2.1	Stainless Steel	Z777157	Z777191
50	x	4.6	Stainless Steel	Z777159	Z777193
50	x	4.6	PEEK	Z777163	Z777197
150	x	4.6	Stainless Steel	Z777160	Z777194
250	x	4.6	Stainless Steel	Z777161	Z777195
250	x	4.6	PEEK	Z777164	Z777198
Guard 10	x	2.0	Stainless Steel	Z777155	Z777189
Guard 10	x	4.0	Stainless Steel	Z777158	Z777192

Proteomix® Cation Exchange (3.0 µm)

Length (mm)		I.D. (mm)	Column Hardware	Sulfonate	Carboxylate
30	x	2.1	Stainless Steel	Z777148	Z777182
50	x	2.1	Stainless Steel	Z777149	Z777183
50	x	2.1	PEEK	Z777153	Z777187
50	x	4.6	Stainless Steel	Z777151	Z777185
50	x	4.6	PEEK	Z777154	Z777188
150	x	4.6	Stainless Steel	Z777152	Z777186
Guard 10	x	2.0	Stainless Steel	Z777147	Z777181
Guard 10	x	4.0	Stainless Steel	Z777150	Z777184

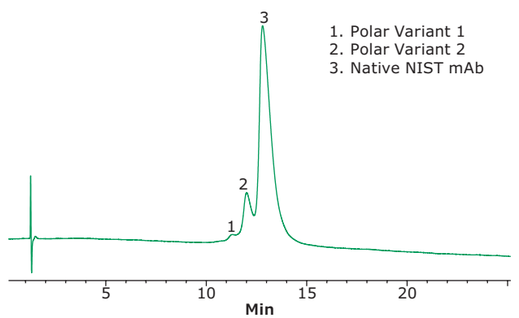
Proteomix® Cation Exchange (10.0 µm)

Length (mm)		I.D. (mm)	Column Hardware	Sulfonate	Carboxylate
50	x	2.1	Stainless Steel	Z777132	Z777166
50	x	2.1	PEEK		Z777171
250	x	2.1	Stainless Steel	Z777133	Z777167
250	x	2.1	PEEK	Z777138	Z777172
50	x	4.6	Stainless Steel	Z777135	Z777169
50	x	4.6	PEEK	Z777139	Z777173
250	x	4.6	Stainless Steel	Z777136	Z777170
250	x	4.6	PEEK	Z777140	Z777174
Guard 10	x	2.0	Stainless Steel	Z777131	Z777165
Guard 10	x	4.0	Stainless Steel	Z777134	Z777168

Hydrophobic Interaction Chromatography (HIC)

Proteomix® HIC U/HPLC Columns

Proteomix® HIC columns are specially designed for high resolution and high efficiency separations of proteins and oligonucleotides. Utilizing proprietary surface technologies, Proteomix® HIC-NP resin is made of non-porous polystyrenedivinylbenzene (PS/DVB) beads with narrow-dispersed particle size distribution. The PS/DVB bead is modified with alkyl groups or aryl group that provides hydrophobic interaction with analytes. Proteomix® HIC-NP resin is highly rigid and mechanically stable. In comparison to silica-based HIC phase media, Proteomix® HIC-NP phases have advantages for biomolecule separations with wide pH range (2-12) and high thermal stability. The nonporous structure and narrow particle size distribution offer special selectivity, high resolution separation of proteins such as mAb (monoclonal antibody), ADC (antibody drug conjugate) and related protein fragments, DNA and oligonucleotides. Proteomix® HIC-NP media is applicable at laboratory discovery, laboratory-scale purification and process chromatography for the production of a few mgs to kilogram of proteins.



Column:	Proteomix® HIC Butyl-NP5, 10 cm x 4.6 mm I.D., 5 µm
Mobile phase:	[A] 2 M ammonium sulfate in 100 mM sodium phosphate, pH 7.0; [B] 100 mM sodium phosphate, pH 7.0
Gradient:	55% B to 75% B in 25 min
Flow rate :	0.5 mL/min
Column temp.:	30 °C
Detector:	UV, 280 nm
Injection:	2.0 µL
Sample:	NIST mAb, 10 mg/mL, 12.5 mM histidine, pH 6.0

HPLC Columns

Proteomix® HIC (5.0 µm)

Length (mm)		I.D. (mm)	Butyl	Ethyl	Phenyl	Propyl
50	x	2.1	61862-U			
35	x	4.6		61869-U	61874-U	61881-U
100	x	4.6	61865-U	61870-U	61876-U	61883-U

Length (mm)		I.D. (mm)	Butyl	Ethyl	Phenyl	Propyl
150	x	4.6	61866-U			
50	x	7.8	61867-U	61871-U	61878-U	61884-U
Guard 10	x	4.0	61863-U	61868-U	61873-U	61879-U

Tosoh* Bioscience: HPLC Columns for Bioanalysis

Size Exclusion Chromatography (SEC)

TSKgel® UP-SW2000 and UP-SW3000 UHPLC Columns

TSKgel® UP-SW3000 and UP-SW2000, 2 µm UHPLC columns with 25 nm and 12.5 nm pore sizes, respectively, are high efficiency additions to the renowned TSKgel® SW series. TSKgel® UP-SW represents the fifth generation of high performance gel filtration columns. These columns feature the same pore size as the well-established TSKgel® G3000SW$_{XL}$ and SuperSW2000 columns and facilitate method transfer from conventional gel filtration to UHPLC technology. The 15 cm column is used to shorten analysis time while maintaining resolution. The 30 cm column delivers dramatically increased resolution between fragments, monomers, and aggregates.

TSKgel® SW$_{XL}$ Type and QC-PAK HPLC Columns

TSKgel® SW$_{XL}$ columns, introduced in 1987, represent the second generation of high performance Gel Filtration columns that have become synonymous with analyzing protein molecular weights in the emerging field of biotechnology. TSKgel® SW$_{XL}$-type columns contain smaller particles than TSKgel® SW-type columns; 5 and 8 µm versus 10 and 13 µm. As the TSKgel® SW-type columns, TSKgel® SW$_{XL}$ columns feature highly porous silica particles with a surface functionalized with diol groups to prevent any interaction with proteins. TSKgel® SW$_{XL}$-type columns distinguish themselves from other silica- or polymer-based high performance size exclusion columns by virtue of their large pore volumes. These columns are commonly used in the quality control of monoclonal antibodies and other biopharma products. TSKgel® BIOassist columns are packed in PEEK hardware.

TSKgel® UP-SW (2.0 µm)

Length (mm)		I.D. (mm)	SW3000	SW2000
150	x	4.6	80023449	823515
300	x	4.6	80023448	823514
Direct Connect Guard 20	x	4.6	80023451	823517
Guard 20	x	4.6	80023450	823516

TSKgel® SW$_{XL}$ (5.0 µm)

Length (mm)		I.D. (mm)	G2000	G3000	G4000	QC-PAK 200	QC-PAK 300	SW$_{XL}$-125	SW$_{XL}$-250	SW$_{XL}$-450
150	x	7.8				816215	816049			
300	x	7.8	808540	808541				820027	820026	

TSKgel® SW$_{XL}$ (7.0 µm)

Length (mm)		I.D. (mm)	G2000	G3000	G4000	QC-PAK 200	QC-PAK 300	SW$_{XL}$-125	SW$_{XL}$-250	SW$_{XL}$-450
Guard 40	x	6.0						818008	808543	

TSKgel® SW$_{XL}$ (8.0 µm)

Length (mm)		I.D. (mm)	G2000	G3000	G4000	QC-PAK 200	QC-PAK 300	SW$_{XL}$-125	SW$_{XL}$-250	SW$_{XL}$-450
300	x	7.8			808542					820025

*Tosoh Bioscience columns are available in the following countries: Argentina, Albania, Andorra, Armenia, Austria, Azerbaijan, Afghanistan, Algeria, Albania, Armenia, Azerbaijan, Bolivia, Brazil, Belarus, Belgium, Bosnia and Herzegovina, Bulgaria, Bahrain, Bosnia-Herzegovina, Botswana, Burkina-Faso, Chile, Colombia, Costa Rica, Cuba, Caribbean Islands, Canada, Croatia, Cyprus, Czechia, Cameron, Central Afr. Rep, Chad, Congo, Congo dem. Rep., Croatia, Denmark, Dominican Republic, Djibouti, Ecuador, El Salvador, Egypt, Eritrea, Ethiopia, Estonia, French Guiana, Finland, France, Georgia, Germany, Greece, Gabon, Georgia, Ghana, Guatemala, Guyana, Haiti, Honduras, Hungary, Iceland, Ireland, Italy, Iraq, Israel, Kazakhstan, Kosovo, Kenya, Kosovo, Kuwait, Kyrgyzstan, Lebanon, Libya, Latvia, Liechtenstein, Lithuania, Luxembourg, Malta, Moldova, Monaco, Montenegro, Macedonia, Madagascar, Malawi, Mauretania, Mauritius, Moldavia, Mongolia, Morocco, Mozambique, Mexico, Nicaragua, Nigeria, Netherlands, North Macedonia (formerly Macedonia), Norway, Oman, Poland, Portugal, Panama, Paraguay, Peru, Puerto Rico, Qatar, Rwanda, Romania, Russia, San Marino, Serbia, Slovakia, Slovenia, Spain, Sweden, Suriname Switzerland, Saudi Arabia, Senegal, Serbia-Montenegro, South Africa, Tadzhikistan, Tanzania, Togo, Tunisia, Turkey, Turkmenistan, Ukraine, United Kingdom (UK), Uruguay, United States of America, Uganda, Utd.Arab.Emir., Uzbekistan, Vatican City, Venezuela, Yemen, Zambia, Zimbabwe, Belize

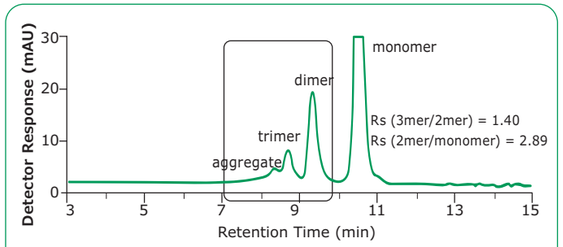
HPLC Columns

TSKgel® SuperSW, SuperSW mAb and UltraSW Aggregate HPLC Columns

TSKgel® SuperSW columns, introduced in 1997, represent the third generation of high performance Gel Filtration columns that have become synonymous with analyzing protein molecular weights in the emerging field of biotechnology. TSKgel® SuperSW-type columns contain smaller particles than TSKgel® SW_{xl}-type columns: 4 µm versus 5 µm. In addition, the column internal diameter has been reduced from 7.8 mm I.D. to 4.6 mm I.D. to provide higher sensitivity in sample-limited cases and to cut down on solvent use; in addition to 4.6 mm I.D., TSKgel® SuperSW3000 columns are also available in 2 mm and 1 mm I.D. column formats. It is important to employ an HPLC system that is optimized with regards to extra-column band broadening to take full advantage of the high column efficiency that can be obtained on TSKgel® SuperSW columns. The TSKgel® SW mAb product line consist of three specialized columns designed for the separation and analysis of monoclonal antibodies (mAb). While mAbs can be analyzed using many different modes of HPLC, size exclusion is best to determine aggregation, dimer, and fragmentation, making it the best method for heterogeneity studies. The TSKgel® SW mAb columns include:

- TSKgel® SuperSW mAb HR column is best suited for high resolution analysis of mAb monomer and dimer
- TSKgel® SuperSW mAb HTP column is optimized for high throughput analysis of mAb monomer and dimer (UHPLC compatible)

TSKgel® UltraSW Aggregate column provides the best solution for the analysis of mAb aggregates. Compared to competitive columns, these stainless steel, silica-based TSKgel® columns offer reduced lot-to-lot variation, long column life, reduction of unspecified adsorption, and superior recovery of aggregates. The TSKgel® SW mAb columns utilize a unique, pore-controlled technology, which produces a shallow calibration curve in the molecular weight region of a typical monoclonal antibody. The calibration curve for the TSKgel® SuperSW mAb HR column is similar to that of the TSKgel® G3000SWxl column and has a shallower slope than the TSKgel® UltraSW Aggregate column around the molecular weight range of gamma-globulin. This shallow calibration curve produces high resolution separations. The TSKgel® UltraSW Aggregate calibration curve shows a separation range up to around 2 million Da, which implies better resolution of aggregate/multimer of a monoclonal antibody.



Column:	TSKgel® UltraSW Aggregate, 30 cm x 7.8 mm I.D., 3 µm particles
Mobile phase:	0.2 M phosphate buffer, pH 6.7 + 0.05% sodium azide
Flow rate:	0.8 mL/min
Sample:	Monoclonal antibody 2 (mouse-human chimeric IfG, Erbitux)
Injection:	10 µL
Detector:	UV, 280 nm

TSKgel® SuperSW (4.0 µm) and UltraSW (3.0 µm)

Length (mm)		I.D. (mm)	mAb-HR	mAb-HTP	2000	3000	UltraSW-Aggregate
300	x	1.0				821845	
300	x	2.0				821485	
150	x	4.6		822855			
300	x	4.6			818674	818675	
300	x	7.8	822854				822856
Guard 30	x	3.0		822858			
Guard 35	x	4.6			818762		
Guard 40	x	6.0	822857				822859

TSKgel® SW Type HPLC Columns

TSKgel® SW columns, introduced in 1977, were the first of a long line of high performance Gel Filtration columns that have become synonymous with analyzing protein molecular weights in the emerging field of biotechnology. TSKgel® SW-type columns are based on highly porous silica particles with a surface functionalized with diol groups to prevent any interaction with proteins. TSKgel® SW-type columns distinguish themselves from other silica- or polymer-based high performance size exclusion columns by virtue of their large pore volumes.

TSKgel® SW (10.0 µm)

Length (mm)		I.D. (mm)	G2000	G3000
300	x	7.5	805788	805789
600	x	7.5	805102	805103
300	x	8.0		808800
Guard 75	x	7.5	805371	
Guard 40	x	8.0	808805	

TSKgel® SW (13.0 µm)

Length (mm)		I.D. (mm)	G2000	G3000	G4000
300	x	7.5			805790
600	x	7.5			805104
300	x	8.0			808801
300	x	21.5	806727	806728	
600	x	21.5	805146	805147	
Guard 75	x	21.5	805758		

TSKgel® SW (17.0 µm)

Length (mm)		I.D. (mm)	G4000
300	x	21.5	806729
600	x	21.5	805148

Ion Exchange Chromatography

Ion exchange chromatography separates molecules based on differences in the net charge of the molecules. In anion exchange, the analyte is negatively charged (anion), while the chromatographic material is positively charged (cation). Ion exchange is commonly used for protein purification, but it may be used for purification of oligonucleotides, peptides, or other charged molecules. For interaction to occur, the protein of interest must have a charge opposite to that of the functional group of the sorbent particle. Because the interaction is ionic, binding must take place under low ionic strength conditions. Elution is achieved by increasing the ionic strength of the mobile phase to reduce ionic attractions, or by changing the pH of the mobile phase to alter the ionization state of the protein.

TSKgel® columns are highly efficient for sample purification with excellent recovery. Anion- and cation-exchangers are available on porous polymer-based and silica-based particles. Proteins and nucleic acids can be analyzed faster on a TSKgel® non-porous resin column, although STAT and NPR columns have lower capacity than particles with large pore sizes. TSKgel® BioAssist columns are constructed from PEEK (polyether ether ketone).

Anion Exchange HPLC Columns:

TSKgel® 5PW Anion Exchange (10.0 µm)

Length (mm)		I.D. (mm)	DEAE	SuperQ
75	x	2.0	818757	
50	x	5.0	813061	
75	x	7.5	807164	818257
75	x	8.0	808802	818386

TSKgel® 5PW Anion Exchange (13.0 µm)

Length (mm)		I.D. (mm)	DEAE	SuperQ
150	x	20	814016	
150	x	21.5	807574	818387
Guard 20	x	20	814466	

TSKgel® 5PW Anion Exchange (20.0 µm)

Length (mm)		I.D. (mm)	DEAE	SuperQ
Guard 25	x	6.0	807210	818388
Guard 10	x	8.0	808806	
Guard 35	x	10.0	816092	

TSKgel® NPR

Length (mm)		I.D. (mm)	2.5 µm	5.0 µm
35	x	4.6	813075	
75	x	4.6	818249	
Guard 5	x	4.6	818253	817088

TSKgel® BioAssist Q

Length (mm)		I.D. (mm)	10 µm	13 µm
50 mm	x	4.6 mm	819685	
100 mm	x	10 mm		821410

TSKgel® STAT

Length (mm)		I.D. (mm)	5.0 µm	7.0 µm	10.0 µm
35	x	3.0			821960
100	x	4.6	821962	821961	

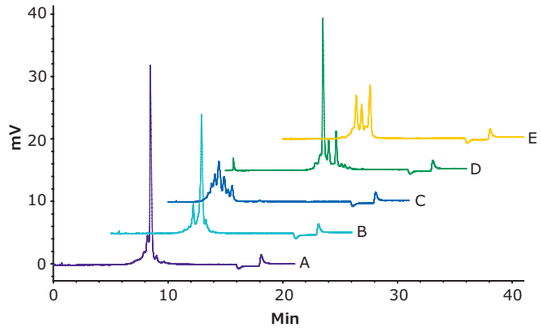
Silica-based TSKgel® DEAE Anion Exchange

Length (mm)		I.D. (mm)	5.0 µm	10.0 µm	20.0 µm
250	x	2.0	818761		
250	x	4.6	807168		
75	x	7.5		807163	

Silica-based TSKgel® QAE Anion Exchange

Length (mm)		I.D. (mm)	SKU
250	x	4.6	807166
Guard 25	x	6.0	807646

Cation Exchange HPLC Columns:



Column:	TSKgel® CM-STAT, 10 cm x 4.6 mm I.D., 7 µm
Column temp.:	25 °C
Mobile phase:	[A] 20 mM MES buffer, pH 6.0; [B] 0.5 M sodium chloride in buffer A, pH 6.0
Gradient:	0% B (0 min), 30% B (15 min), 100% B (15 min), 0% B (17 min), 10% B (17 min), 10% B (21 min)
Flow rate:	1 mL/min
Sample:	monoclonal antibodies (mAb A through E)
Injection:	20 µL
Detector:	UV, 280 nm

TSKgel® 5PW Cation Exchange (10.0 µm)

Length (mm)		I.D. (mm)	CM	SP
75	x	2.0		818758
50	x	5.0		813062
75	x	7.5	813068	807161
75	x	8.0		808803

TSKgel® 5PW Cation Exchange (13.0 µm)

Length (mm)		I.D. (mm)	SP
150	x	20	814017
150	x	21.5	807575

TSKgel® 5PW Cation Exchange (20.0 µm)

Length (mm)		I.D. (mm)	SP
Guard 25	x	6.0	807211
Guard 10	x	8.0	808807
Guard 35	x	10.0	816093

TSKgel® BioAssist S

Length (mm)		I.D. (mm)	7.0 µm	13.0 µm
50	x	4.6	819686	
100	x	10		821411

TSKgel® NPR Cation Exchange (2.5 µm)

Length (mm)		I.D. (mm)	SKU
35	x	4.6	813076

TSKgel® STAT Carboxymethyl

Length (mm)		I.D. (mm)	7.0 µm	10.0 µm
35	x	3.0		821965
100	x	4.6	821966	

TSKgel® STAT Sulfonic Acid

Length (mm)		I.D. (mm)	7.0 µm	10.0 µm
35	x	3.0		821963
100	x	4.6	821964	

Silica-based TSKgel® Carboxymethyl Cation Exchange

Length (mm)		I.D. (mm)	5.0 µm	10.0 µm
250	x	4.6	807167	
75	x	7.5		807162
Guard 25	x	6.0		807650

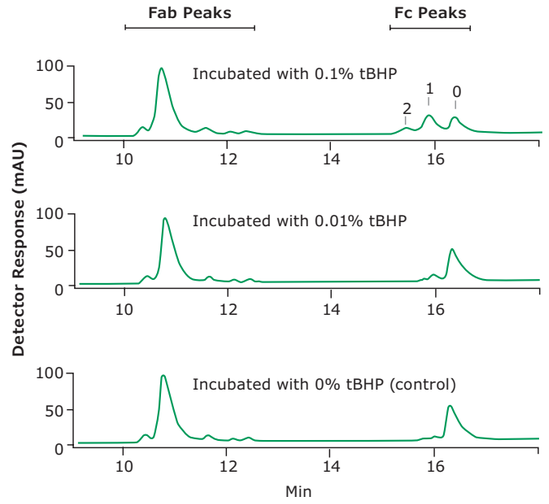
Silica-based TSKgel® Sulfonic Acid Cation Exchange

Length (mm)		I.D. (mm)	5.0 µm
250	x	4.6	807165
Guard 25	x	6.0	807644

Hydrophobic Interaction Chromatography (HIC)

Hydrophobic Interaction Chromatography (HIC) is a gentle technique compared to reversed-phase LC for the binding and desorption of hydrophobic proteins. The use of aqueous mobile phases in HIC is less likely to disturb protein conformation and results in better activity and recovery. Biomolecules adsorb to a weak, hydrophobic surface at high salt concentrations and are eluted by a decreasing salt gradient. As a result, hydrophobic interaction chromatography combines the gentleness of salt precipitation with the precision of chromatography for excellent recovery of protein activity.

TSKgel® BioAssist Phenyl columns are constructed from PEEK (polyether ether ketone).



Column:	TSKgel® Butyl-NPR, 3.5 cm x 4.6 mm I.D., 2.5 µm (814947)
Column temp.:	30 °C
Mobile phase:	[A] 2 M ammonium sulfate, 20 mM Tris, pH 7; [B] 20 mM Tris, pH 7
Gradient:	10 to 100% B in 34 min
Flow rate:	1 mL/min

TSKgel® Butyl NPR

Length (mm)		I.D. (mm)	SKU
35	x	4.6	814947
100	x	4.6	842168

TSKgel® Phenyl-5PW

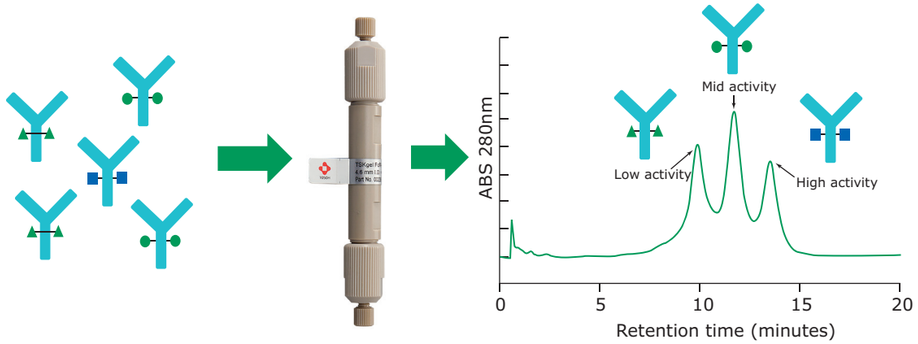
Length (mm)		I.D. (mm)	10 µm	13 µm	20 µm
75	x	2.0	818759		
50	x	5.0	813063		
75	x	7.5	807573		
50	x	7.8	820023		
75	x	8.0	808804		
150	x	21.5		807656	
Guard 25	x	6.0			807652
Guard 35	x	10.0			816095

TSKgel® Ether-5PW

Length (mm)		I.D. (mm)	10.0 µm	20.0 µm
75	x	2.0	818760	
50	x	5.0	814013	
75	x	7.5	808641	
10	x	8.0		814025
75	x	8.0	814014	

Affinity Chromatography

Affinity chromatography allows purification of biomolecules on the basis of biological function or three-dimensional structure. The molecule to be purified is specifically and reversibly adsorbed by a complementary binding ligand immobilized on a matrix. The natural specificities of the interacting molecules offer high selectivities that can greatly reduce the time needed to purify the molecule. High-efficiency, resin-based TSKgel® affinity columns separate or purify many enzymes and other proteins.



Chromatographic Conditions	
Column:	TSKgel® FcR-IIIA-NPR, 7.5 cm x 4.6 mm I.D., 5 µm, PEEK (823513)
Mobile phase:	[A] 50 mM Citrate, pH 6.5 ; [B] 50 mM Citrate, pH 4.5
Flow rate:	1 mL/min
Column temp.:	25 °C
Detector:	UV, 260 nm
Injection:	30 µL
Sample:	Rituximab, 1 µg/µL, Rituximab kindly provided by Rentschler Biopharma

TSKgel® Iminodiacetic Acid

Length (mm)		I.D. (mm)	10 µm	13 µm	20 µm
50	x	5.0	814440		
75	x	7.5	808645		
50	x	7.8	820022		
150	x	21.5		808646	
Guard 25	x	6.0			808647

TSKgel® Boronate

Length (mm)		I.D. (mm)	10 µm	20 µm
50	x	5.0	814449	
75	x	7.5	813066	
Guard 25	x	6.0		813125
Guard 10	x	8.0		814451

TSKgel® Tresyl (10 µm)

Length (mm)		I.D. (mm)	SKU
40	x	6.0	814455

TSKgel® FcR (5.0 µm)

Length (mm)		I.D. (mm)	SKU
75	x	4.6	823513

Chromolith® Protein A monolithic columns for Affinity Chromatography, please see page 59

HPLC Columns

Protein Purification

You have a diverse set of molecules that need to be purified. We have a full suite of industry-trusted and proven chromatography resins and membranes to help you to tackle them all from lab to process scale.

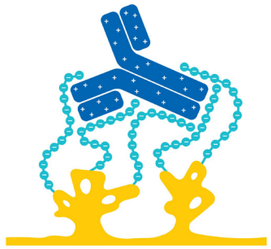
- Membrane Chromatography
- Affinity Chromatography
- Ion Exchange Chromatography
- Prepacked Columns
- Process Chemicals
- Multi-use Systems
- Single-use Systems

Chromatography Resins

Our portfolio of reliable, trusted chromatography solutions has been optimized for your molecule across its entire life cycle, from early-phase development through commercial manufacturing.

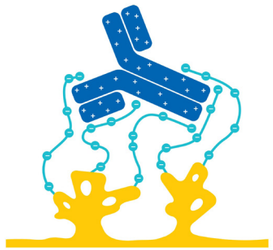
Our proven, tentacle technology offers a number of advantages compared to conventional resins due to increased accessibility of functional groups and binding of target molecules. This unique feature allows reliable purification and efficiency for our process with high selectivity, excellent yield and purity. We optimized the tentacular surface chemistry of our resins for different applications.

Eshmuno® CP-FT resin



Eshmuno® CPX resin

Eshmuno® CPS resin



For more information on protein purification products, please visit

SigmaAldrich.com/safc/bioprocess/purification

HPLC Columns

Synthetic Carbons for Chromatographic Applications

Our portfolio includes many different carbon adsorbents, tailored to meet customer needs. These particles can be designed with:

- The desired shape – either spherical or granular
- No pores, or more or less of the desired pores (micro-, meso-, macro-) to meet the application of interest
- Tapered pores, which increase thermodynamic and kinetic efficiency
- A through-pore or a closed-pore structure, which influences microporous strength and kinetic effectiveness
- Surface pH adjustments, from 2.5 to 10.5

These carbons can be used in gas chromatography (GC), liquid chromatography (LC), solid phase extraction (SPE), and bulk scale applications. A sampling of these carbon particles includes:

Carbosieve – These are spherical, non-friable, highly porous particles and used for an analyte size relative to the C2-C5 n-alkanes. These particles have non-tapered pores and very high adsorptive strength. These particles are effective for small, volatile analytes that most adsorbents have trouble retaining.

Carboxen® – These particles are carbon molecular sieves (CMS), much like the Carbosieve materials, but include an expanded selection of physical characteristics. Many of the carboxen materials include a combination of micro-, meso-, and macropores to suit customer needs.

Graphsphere™ – Graphsphere™ particles are spherical, graphitized, polymer carbon (SGPC) with a porous or non-porous core, and a graphitized shell of controlled thickness. This shell imparts a variable amount of surface area and capacity. These carbons are used for an analyte size relative to the C5-C12 n-alkanes. Graphsphere™ particles generally have a weaker adsorbent strength as compared to the carbon molecular sieves discussed above.

Carbotrap® and Carbopack™ – These materials are graphitized carbon blacks (GCB) which can be porous or non-porous and are generally granular and friable.

These particles are used for an analyte size relative to C3-C20+ n-alkanes. These also have lower adsorbent strength than the CMS products.

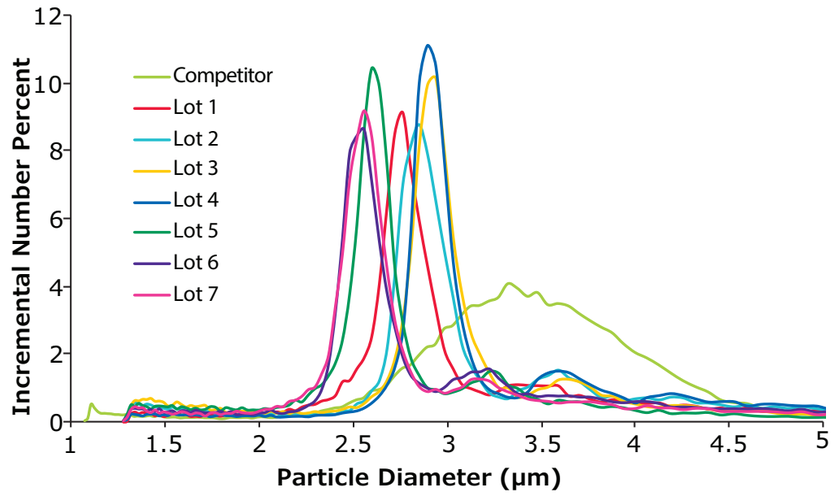
Supel™ Carbon LC – These innovative, porous sub-3 µm particles are used in HPLC applications, and exhibit high pressure stability, elevated temperature stability, unique retention mechanisms, and are compatible with any solvent system. The figure on the next page highlights the repeatability and narrow particle size distribution of this material.

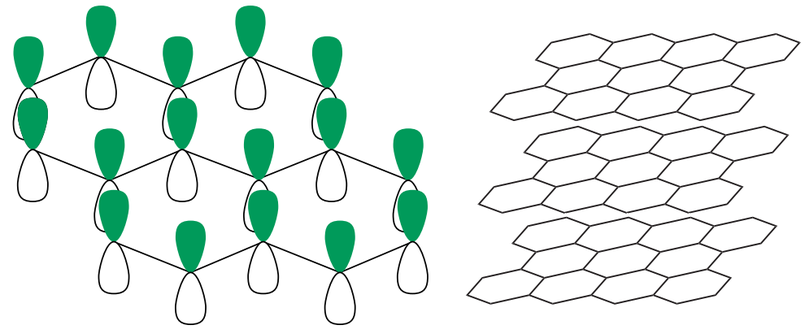
About Supel™ Carbon LC HPLC Particles

Supel™ Carbon LC particles are a fully porous graphitic carbon-based material designed specifically for HPLC and UHPLC applications. Supel™ Carbon LC is the first commercially available sub-3 µm particle. This new, patented, porous graphitic carbon (PGC) exhibits much narrower particle size distributions compared to pre-existing technology, as the plots on the next page indicate. PGC materials are similar to graphitized carbon blacks (GCBs), but PGC has a spongy structure which is able to withstand the shearing forces occuring in HPLC. As a packing for HPLC, PGC can act as a strong, hydrophobic adsorbent behaving similar to reversed-phase (RP) chromatography. However, where PGC differs to RP chromatography is in its ability to retain more polar analytes that tend to elute too early by RP. In addition, planar (especially aromatic) compounds, due to their shape, have intense interactions with the surface resulting in strong retention as consequence of the graphitic nature of the particles. There are no alkyl ligands off the support, but, the top layer has flat, hexagonally arranged carbon atoms that are all covalently bonded to three carbon atoms. The remaining electron on each carbon, needed for bonding, is transposed perpendicular to the plane in p-orbitals which subsequently hybridize together to form a continuous pi orbital allowing the delocalized electrons to freely roam across the plane. For chromatography, this “sea” of electrons is believed to allow for electrostatic interactions with the pi-cloud of graphite.

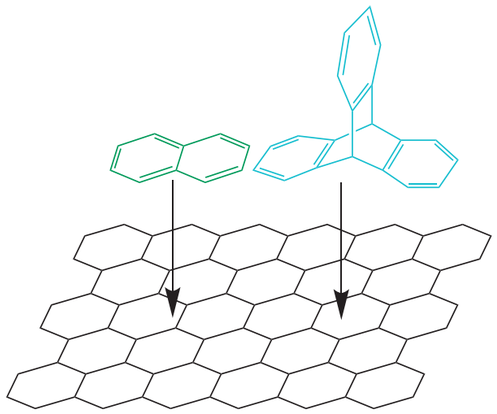
SigmaAldrich.com/carboxen

Particle size distribution of Supel™ Carbon LC HPLC lots vs. Competitor





(Right) Simplified view of graphite; (Left) P-orbitals on a section of graphite. This quality gives the surface not only conductive properties, but also the ability for electronic interactions at the surface



An example of compound alignment with the graphitic surface. Left: Naphthalene is planar and can align well against the graphitic plane. Right: Triptycene is rigid and not able to align completely flat against the surface . This better alignment of naphthalene results in a stronger interaction with the surface and increased retention.

Supel™ Carbon LC HPLC Columns

Porous graphitic carbon (PGC) particles, designed by a unique and patented synthetic process, constitute the packing of Supel™ Carbon LC U/HPLC columns. The key advantages of using PGC columns over silica particle-packed columns include:

Elevated Temperature Stability: Columns can be readily operated at temperatures up to 250 °C, thereby allowing faster and more efficient separations.

pH Stability: Compatible with mobile phases in the pH range of 1 – 14 at any temperature without causing a decline in the column lifespan.

Unique Retention Mechanism: The Polar Retention Effect on Graphite (PREG) mechanism allows for the retention of polar or charged compounds without hydrophilic interaction liquid chromatography (HILIC) conditions. The mechanism also allows the resolution of geometric isomers.

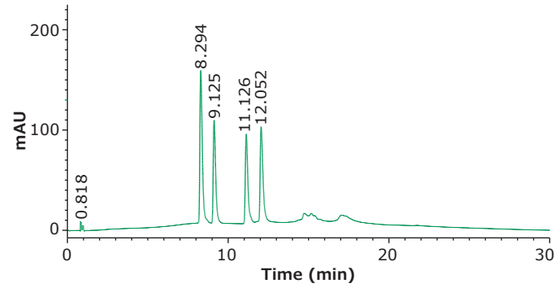
Compatibility with Any Solvent: Any polar or non-polar solvent can be used for the resolution of an analyte of interest.

Pressure Stability: Up to 700 bar.

Supel™ Carbon LC Specifications

Phase Bonding	Bonding Chemistry	Particle Size (µm)	Pore Size (Å)	Surface Area (m^2/g)	pH Stability	Max Temperature	Endcapped	Shipping Solvent
n.a. (porous graphitic carbon)	N/A	2.7	200	155	1 to 14	250 °C	No	Acetonitrile/ Water

Peaks	Compound	Ret. Time (min)
1	3-epi-25-hydroxyvitamin D3 (50 µg/mL)	8.294
2	25-hydroxyvitamin D3 (25 µg/mL)	9.125
3	3-epi-25-hydroxyvitamin D2 (50 µg/mL)	11.126
4	25-hydroxyvitamin D2 (100 µg/mL)	12.052

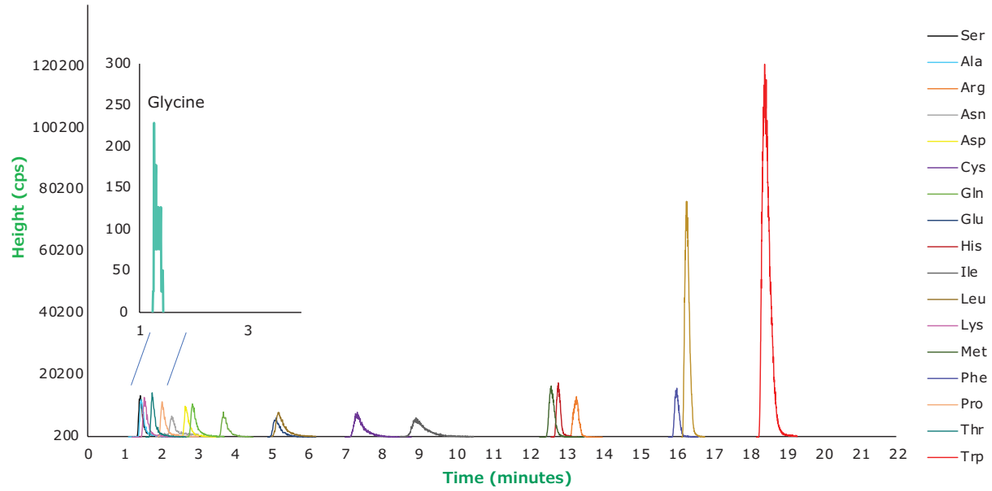


Chromatographic separation of Vitamin D2 and D3 metabolites on Supel™ Carbon LC

Chromatographic Conditions	
Column:	Supel™ Carbon LC, 10 cm x 2.1 mm I.D., 2.7 µm
Mobile phase:	[A] 2-Propanol; [B] Tetrahydrofuran
Gradient	0% B to 70% B in 15 min; hold at 70% B for 5 min
Flow rate:	0.3 mL/min
Column temp.:	25 °C
Detector:	UV, 275 nm
Injection:	2.0 µL
Sample:	Vitamin D2 and D3 metabolites mix, varied concentration in ethanol

HPLC Columns

Supel™ Carbon LC column - 20 Underivatized Amino Acids



Separation of 20 underivatized amino acids by LC-MS/MS. Conditions: Column: Supel™ Carbon LC, 10 cm x 2.1 mm I.D., 2.7 µm; Mobile Phase: [A] Water (0.1% (v/v) DFA); [B] Acetonitrile (0.1% (v/v) DFA); Gradient: Hold at 0% B for 7 min; 0% B to 5% B in 5 min; 5% B to 100% B in 10 min; Flow Rate: 0.2 mL/min; Column temp.: 12 °C; Detector: MSD; Injection: 1.0 µL; Sample: Amino Acid Mix, varied concentration, water (0.1% (v/v) DFA)

Elution Order	Compound	Retention Time (min)
1	Glycine	1.27
2	Serine	1.43
3	Alanine	1.45
4	Lysine	1.54
5	Threonine	1.75
6	Proline	2.03
7	Asparagine	2.29
8	Aspartic Acid	2.65
9	Valine	2.85
10	Glutamine	3.69

Elution Order	Compound	Retention Time (min)
11	Glutamic Acid	5.13
12	Leucine	5.18
13	Cystine	7.34
14	Isoleucine	8.93
15	Methionine	12.61
16	Histidine	12.81
17	Arginine	13.30
18	Phenylalanine	16.03
19	Tyrosine	16.29
20	Tryptophan	18.42

Supel™ Carbon LC

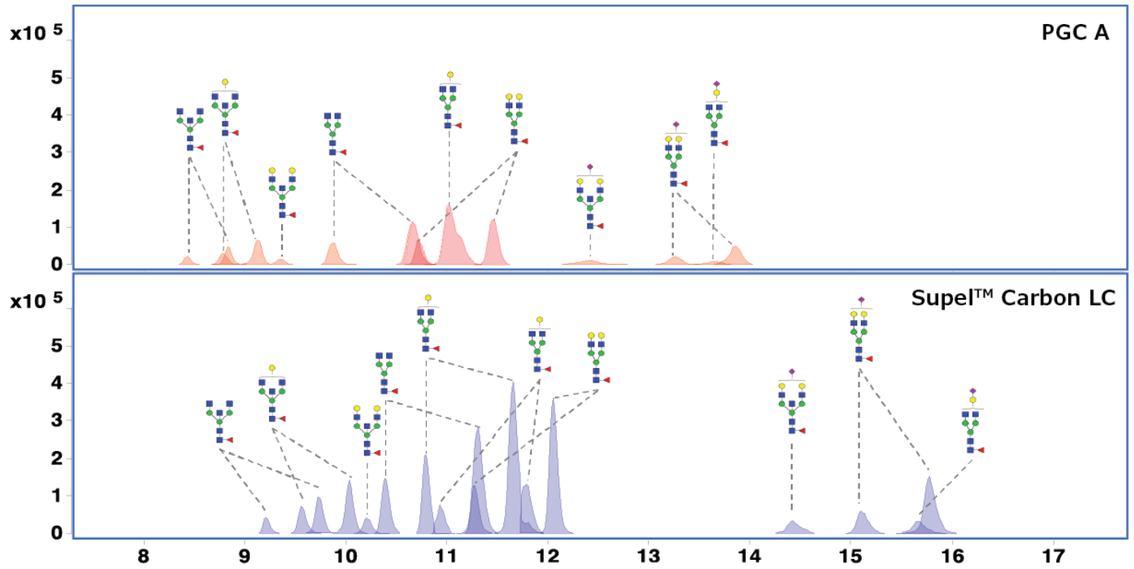
Length (mm)		I.D. (mm)	SKU
50	x	2.1	59984-U
100	x	2.1	59986-U
150	x	2.1	59987-U
50	x	3.0	59991-U
100	x	3.0	59993-U
150	x	3.0	59994-U
50	x	4.6	59997-U
100	x	4.6	59998-U
Guard 3pk	x	2.1	59981-U
Guard 3pk	x	3.0	59988-U
Guard 3pk	x	4.0	59995-U
Guard Kit	x	2.1	59982-U
Guard Kit	x	3.0	59989-U
Guard Kit	x	4.0	59996-U
Guard Holder	x	N/A	59999-U

Supel™ Carbon LC Columns
HPLC Columns

UHPLC-MS Analysis of Released N-Glycans from Human IgG on a Supel™ Carbon LC Column

Glycans are molecules consisting of monosaccharides linked through glycosidic bonds that are attached to proteins through certain amino acids. From a biotherapeutic perspective, glycans play an important role in ensuring that the protein interacts with the appropriate cell to elicit its therapeutic effect. The glycan profile of a biotherapeutic is a critical quality attribute that must be reported to regulatory agencies prior to the drug being authorized for use.

Since glycans are polar in nature, it is challenging to analyze these compounds by reversed-phase chromatography; therefore, hydrophilic interaction liquid chromatography (HILIC) with derivatization has been the method of choice for years. This application demonstrates the use of Supel™ Carbon LC, a column packed with porous graphitic carbon (PGC) particles, to resolve released glycans from human IgG under reversed-phase conditions without derivatization. Supel™ Carbon LC is also compared to an alternative PGC column, demonstrating higher efficiencies over the competing column.



Data courtesy of Prof. Hyun Joo An, Chungnam National University, South Korea

Conditions

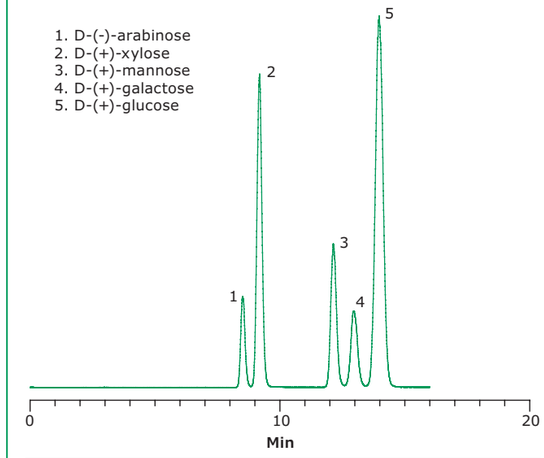
Column	Supel™ Carbon LC, 10 cm x 2.1 mm I.D., 2.7 µm with guard cartridge, 2 cm x 2.1 mm I.D., 2.7 µm
Mobile Phase	[A] 97:3 Water (0.1% (v/v) Formic acid): Acetonitrile (0.1% (v/v) Formic acid); [B] 10:90 Water (0.1% (v/v) Formic acid): Acetonitrile (0.1% (v/v) Formic acid)
Gradient	Hold at 3% B for 2 min; 3% B to 16% B in 8 min; 16% B to 40% B in 8 min; 40% B to 60% B in 2 min; 60% B to 100% B in 2 min; hold at 100% B for 8 min
Flow Rate	0.3 mL/min
Column Temp.	40 °C
Detector	MSD
Injection	1.0 µL
Sample	Released N-glycans from human IgG, 3 µg on column, water

HPLC Columns

apHera™ HPLC Columns

apHera™ reversed phase columns were developed specifically to provide the superior advantages of both silica and polystyrene columns, without the disadvantages of either. This trait was accomplished using a vinyl alcohol copolymer base that keeps the surface wetted even with high carbon loads. The columns are packed with butyl (C4), octyl (C8) or octadecyl (C18) packings obtained by the introduction of the alkyl function on the hydroxyl groups of vinyl alcohol copolymers. The porous structure has an average pore diameter large enough to produce ideal results for small analytes, peptides and small proteins. These columns have comparable efficiency to silica-based columns with organic solvents but provide efficiency with buffered and alkaline solutions not possible on silica. Shrinkage and swelling are minimal in a broad range of solvents, and the high NTP values of these columns are practically unaffected by differing solvent polarities, unlike polystyrene based polymer columns. One of the most significant features is the logical elution order of alkylated bases where retention increases proportionately with increasing chain length.

apHera™ amino columns are based on covalently bonded polyamine specifically optimized for the separation of mono- and oligosaccharides. The elution order mono-, di-, tri-saccharide shows increased elution volume with increased acetonitrile concentration and complete stability for both acidic and alkaline eluates. The small, robust PVA copolymer bead provides mechanical and chemical strength as well as high column efficiency. Conventional amino columns based on silica do not show long column life, perhaps due to hydrolysis of the silica particle by the basic amino group. Since apHera™ uses a strong alkaline compatible polymer, these problems are eliminated. Stable retention time and long column lifetime are also characteristics of the column.



Column:	apHera™ NH_2, 15 cm x 4.6 mm I.D., 5 µm
Column temp.:	25 °C
Mobile phase:	[A] Water: [B] Acetonitrile; (20:80, A:B)
Flow rate:	1.0 mL/min
Sample:	500 µg/mL in 30:70, water: acetonitrile
Injection:	10 µL
Detector:	ELSD, 45 °C, 3.5 psi nitrogen

apHera™ (5.0 µm)

Length (mm)		I.D. (mm)	C18	C8	C4	NH2
150	x	2.0	56100AST			56400AST
150	x	4.6	56102AST		56302AST	56401AST
250	x	4.6	56103AST			56403AST
250	x	10.0		56208AST	56308AST	
Guard 10	x	2.0	56129AST			56429AST
Guard 10	x	4.6	56130AST	56230AST		56430AST
Guard 50	x	7.5			56332AST	

HPLC Columns

SUPELCOGEL™ Ion Exclusion Columns

We offer several types of polymeric based columns, SUPELCOGEL™, for the analysis of carbohydrates, organic acids, and alcohols. The optimum choice of column is typically a combination of peak resolution, analysis time and value. Below is summary of the different types of column choices: SUPELCOGEL™ columns are packed with sulfonated (strong cation) polystyrene-divinylbenzene co-polymers. These PS-DVB based columns are chemically stable and extremely reliable. To maximize the separation of the different types of samples common to the industries we serve, SUPELCOGEL™ columns utilize two variations of the PS-DVB co-polymer (6% and 8% cross-linked). These two base polymers are functionalized into different ionic forms to further enhance the separation of specific samples.

SUPELCOGEL™ (6% XL PS-DVB):

Molecular Weight Exclusion Limit: 1200 Da

Minimum Wet Capacity meq/mL: 1.5 – 1.7

Flow Rate Recommendation: 0.4 – 0.8 mL/min

SUPELCOGEL™ Column Series (manufactured with 6% XL PS-DVB):

Advantages: Due to a higher molecular weight exclusion limit than 8% polymers, columns packed with 6% polymer can separate larger carbohydrate molecules (up to DP6). 6% polymer gives superior selectivity for some mixtures of carbohydrates and organic acids compared to the 8% polymer.

Disadvantages: Due to the lower cross-linkage compared to 8% polymer, the 6% polymer is more pressure sensitive and, therefore, has a lower flow rate threshold. For some samples with comparable resolution between the 6% and 8% columns, it makes sense to use the more durable 8% polymer for faster analysis times

SUPELCOGEL™ 8 (8% XL PS-DVB):

Molecular Weight Exclusion Limit: 1000 Da

Minimum Wet Capacity meq/mL: 1.7 – 1.9

Flow Rate Recommendation: 0.4 – 1.2 mL/min

SUPELCOGEL™ 8 Column Series (manufactured with 8% XL PS-DVB):

Most columns on the market for the analysis of carbohydrates, organic acids, and alcohols utilize an 8% XL PS-DVB. The 8% polymer offers excellent separation of many common samples.

Advantages: 8% polymers combine durability and good resolution. Due to higher cross-linking than 6% XL polymers, columns packed with 8% polymer can be used at higher flow rates therefore decreasing analysis times.

Disadvantages: 8% polymers are limited in separating carbohydrates larger than trisaccharides due to lower molecular weight exclusion limits. Compared to the 6% XL polymer, for some types of carbohydrates and organic acid mixtures, the 8% shows less resolution due to selectivity differences.

SUPELCOGEL™ Column “Chemistries”

Carbohydrates elute in descending order of molecular size, monosaccharides last, from the resin-based SUPELCOGEL™ columns described below. The pores in the resins exclude polysaccharides and larger oligosaccharides, which elute first. Smaller di- and monosaccharides enter the pores, interact with the counterions, and are more strongly retained.

SUPELCOGEL™ Ca Columns

The SUPELCOGEL™ Ca column contains a polystyrene-divinylbenzene cross-linked resin in the calcium form. The column separates oligo-, tri-, and disaccharides by class, using a mixed size exclusion/ion exchange mode, with the largest molecules eluting first. The true separating power, however, is in the column’s chromatography of monosaccharides and sugar alcohols – a variety of monosaccharides can be separated using only water as the mobile phase. The column can be operated at low temperatures, but separations are best at elevated temperatures.

SUPELCOGEL™ C-610H Columns

This column contains a polystyrene resin in the hydrogen form. The column is ideal for separating mixtures of organic acids, fermentation products (e.g., alcohols), and carbohydrates. Such mixtures commonly occur in fruits, vegetables, and beverages. Larger and acidic analytes elute before smaller analytes. The column is stable between pH 1 and 13, but results are best at low pH. A simple mobile phase containing 0.1% phosphoric acid is suitable for a wide variety of analyses.

SUPELCOGEL™ Pb Columns

The lead-form resin in SUPELCOGEL™ Pb columns provides the highest resolution and best selectivity for monosaccharides. SUPELCOGEL™ Pb columns provide excellent separation of xylose, galactose, and mannose, which are not completely resolved on calcium-form resin columns.

SUPELCOGEL™ H Columns

SUPELCOGEL™ H columns have the same particle composition, retention mechanism, performance, sensitivity, and applications as SUPELCOGEL™ C-610H columns. However, particle improvements have made it possible to pack the SUPELCOGEL™ H packing material efficiently into conventional 4.6 mm I.D. columns to improve detection and reduce solvent consumption relative to 7.8 mm I.D. columns.

How to Choose the Right SUPELCOGEL™ Column To choose the best column for carbohydrates analysis, you should find the compounds of interest in the table on the next page and note their retention times on each column. Using this information, select the column that will separate the compounds of interest with at least 1 minute between any pair.

Typical Retention Times On SUPELCOGEL™ HPLC Columns

	SUPELCOGEL™ Columns				
	Ca	**C-610H**	**H**	**H**	**Pb**
Cat. No.	59305-U	59320-U	59304-U	59346	59343
Dimensions (mm)	300 x 7.8	300 x 7.8	300 x 7.8	250 x 4.6	300 x 7.8
Column Temp. (°C)	80	30	30	30	85
Mobile Phase	Water	0.1% Phosphoric Acid	0.1% Phosphoric Acid	0.1% Phosphoric Acid	Water
Flow Rate (mL/min)	0.5	0.5	0.5	0.17	0.5
Detector	Refractive Index				
Compound	**Retention Time (min)**				
Arabinose	15.3	13.9	14.3	13.8	19.2
Arabitol	19.8	14.1	14.9	14.3	32.3
Betaine	ND	ND	ND	ND	NR
Dulcitol	22.3	13.4	14.2	13.7	43.4
Erythritol	17.7	15	15.6	14.8	24.5
Ethanol	19.4	25.6	ND	ND	ND
Fructose	14.9	13.1	13.3	12.9	20.8
Galactose	13.4	12.9	13	12.6	17.6
Glucose	12	12.1	11.9	11.7	14.9
Glycerol	18.7	16.8	17.6	16.6	23.8
Inositol	14.9	12.6	12.7	12.4	24.5
Isomaltose	9.6	10.3	ND	ND	ND
Isomaltotriose	8.5	9.5	ND	ND	ND
Lactitol	ND	ND	11.1	11	26.5
Lactose	10.2	10.8	10.2	10.2	13.5
Maltitol	13.6	11	10.7	10.7	23.8
Maltoheptaose	7.5	8.8	7.6	7.9	9.2
Maltohexaose	7.7	8.9	7.7	8.1	9.7
Maltopentaose	7.9	9.1	7.9	8.2	10.5
Maltose	9.8	10.5	9.9	9.9	13
Maltotetraose	8.3	9.3	8.2	8.5	11.2
Maltotriose	8.8	9.7	8.8	9	12
Mannitol	19.2	13.2	13.7	13.2	32.5
Mannose	13.7	12.8	12.9	12.5	19.8
Melezitose	8.7	9.7	8.8	9	10.8
Psicose	22.5	13.4	14.5	13.9	36.5
Raffinose	8.7	9.7	8.7	8.9	11.2
Ribitol	16.7	13.7	14.2	13.6	25.1
Ribose	24.3	14.2	15.8	15	40.7
Sorbitol	23.4	13.4	14.4	13.9	46.9
Stachyose	8.1	9.3	8.1	8.4	10.4
Sucrose	9.8	10.6	9.9	9.9	12.2
Xylitol	23.3	14.4	15.7	15	42.1
Xylose	13.2	12.8	12.8	12.6	16.1

NR - Not recommended
ND - No data available
For optimal separations, allow at least 1 min between compounds.

Ordering Information

Length (mm)		I.D. (mm)	C-610H	Pb	Ca	H	8Ca	8H	8Pb
300	x	7.8	59320-U	59343	59305-U	59304-U	59247-U	59246-U	59248-U
250	x	4.6				59346			
100	x	7.8		59335-U					
Guard 50	x	4.6	59319	59345	59306-U	59319		59251-U	59253-U

Note: SUPELCOGEL™ C-610H uses SUPELCOGEL™ H guard columns.

Customized Packings

On top of the extensive column assortment, Supelco® offers, customized packed columns for high flexibility and professional solutions are available. The sorbents and the packed HPLC columns are tested before delivering. Each finished column is provided with a Certificate of Analysis.

Easy Ordering: Please combine the ordering number of the column hardware (LiChroCART®, Hibar® RT or Hibar® HR) and the sorbent number:

Example:

Customized packing ordering number of Hibar® RT 250-4.6		1.00424.
Sorbent Number of Purospher® STAR Si, 5 µm		7180
Ordering Number of Hibar® RT 250-4.6 Purospher® STAR Si, 5 µm		1.00424.7180

Length (mm)		I.D. (mm)	LiChroCART®	Hibar® RT	Hibar® HR
30	x	2	150229		
50	x	2		151928	
55	x	2	150234		
100	x	2	151939	151929	
125	x	2	150195	151930	
150	x	2	151940	151931	
250	x	2		151932	
30	x	2.1			151934
50	x	2.1			151935
100	x	2.1			151936
150	x	2.1			151937
250	x	2.1			151938
30	x	3	150233		
50	x	3		151923	
55	x	3	150236		
100	x	3	151941	151924	
125	x	3	150175	151925	
150	x	3	151942	151926	
250	x	3	150177	100423	
300	x	3.9	151943	151933	

Length (mm)		I.D. (mm)	LiChroCART®	Hibar® RT	Hibar® HR
25	x	4	150172		
30	x	4	150302	151196	
50	x	4		151927	
55	x	4	150228		
75	x	4	150171		
125	x	4	150170	150181	
250	x	4	150174	150182	
100	x	4.6	151448	150013	
125	x	4.6	151442	150012	
150	x	4.6	151432	150009	
250	x	4.6	151431	100424	
75	x	10	151449		
100	x	10	151445		
125	x	10	151443		
150	x	10	151444		
250	x	10	150179	150183	
75	x	25		151449	
Guard Cartridge					
10	x	2	150201		
4	x	4	150173		
10	x	10	150178		

Sorbent Code	Packing Material
Purospher® STAR	
7174	Purospher® STAR SI 3µm
7175	Purospher® STAR SI 5µm
7177	Purospher® STAR NH2 5µm
7184	Purospher® STAR RP-18e 3µm
7185	Purospher® STAR RP-18e 5µm
7194	Purospher® STAR RP-8e 5µm
7220	Purospher® STAR RP-8e 3µm
7232	Purospher® STAR Phenyl 2µm
7234	Purospher® STAR Phenyl 3µm
7235	Purospher® STAR Phenyl 5µm
7236	Purospher® STAR RP-18e 2µm
7237	Purospher® STAR RP-8e 2µm
Purospher®	
7127	Purospher® RP-18 5µm
7130	Purospher® RP-18e 5µm
7180	Purospher® Si 5µm

Sorbent Code	Packing Material
Superspher®	
7137	Superspher® 100 RP-18 4 µm
7138	Superspher® 100 RP-18e 4 µm
7139	Superspher® 60 RP-8 4 µm
7140	Superspher® 60 RP-8e 4 µm
7141	Superspher® 60 RP select B 4 µm
7142	Superspher® 60 Si 4 µm
7143	Superspher® 100 Si 4 µm
LiChrospher®	
7070	LiChrospher® 100 CN 10µm
7071	LiChrospher® 100 CN 5µm
7075	LiChrospher® 100 DIOL 5µm
7076	LiChrospher® 100 NH2 5µm
7077	LiChrospher® 100 NH2 10µm
7078	LiChrospher® PAH 5µm
7079	LiChrospher® 100 RP-18 5µm
7081	LiChrospher® 100 RP-18 10µm

Sorbent Code	Packing Material
7084	LiChrospher® 100 RP-18e 10µm
7085	LiChrospher® 100 RP-18e 5µm
7087	LiChrospher® 100 RP-8 5µm
7088	LiChrospher® 100 RP-8 10µm
7091	LiChrospher® 100 RP-8e 10µm
7092	LiChrospher® 100 RP-8e 5µm
7093	LiChrospher® 60 RP select B 5µm
7094	LiChrospher® 60 RP select B, 10 µm
7104	LiChrospher® 60 Si 10µm
7109	LiChrospher® 60 Si 5µm
ChiraDex®	
7004	ChiraDex® 5µm
7222	ChiraDex® HR 5µm

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Frankfurter Strasse 250
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