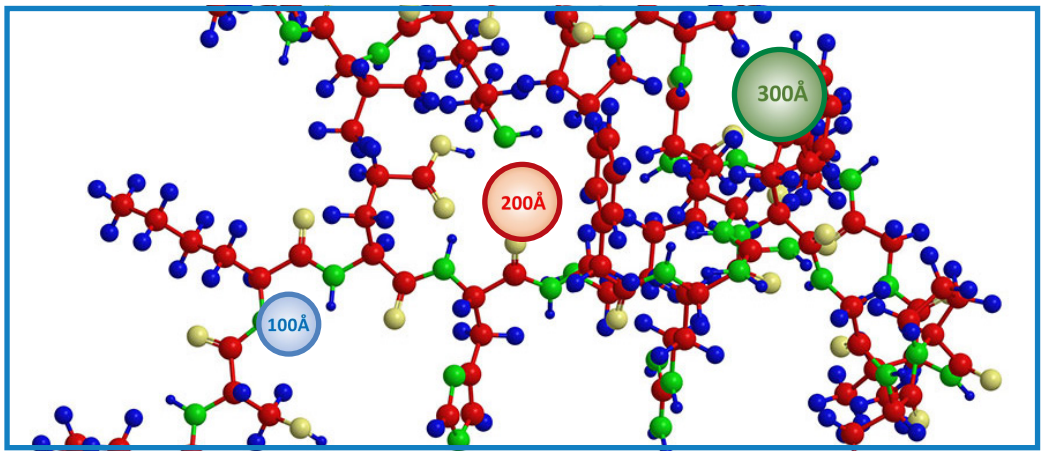
SepaChrom — Your Specialists in Chromatography

VYdamas®



HPLC & Columns and Media for Large Molecules Separation and Purification

Your Specialists in Chromatography

Company Profile

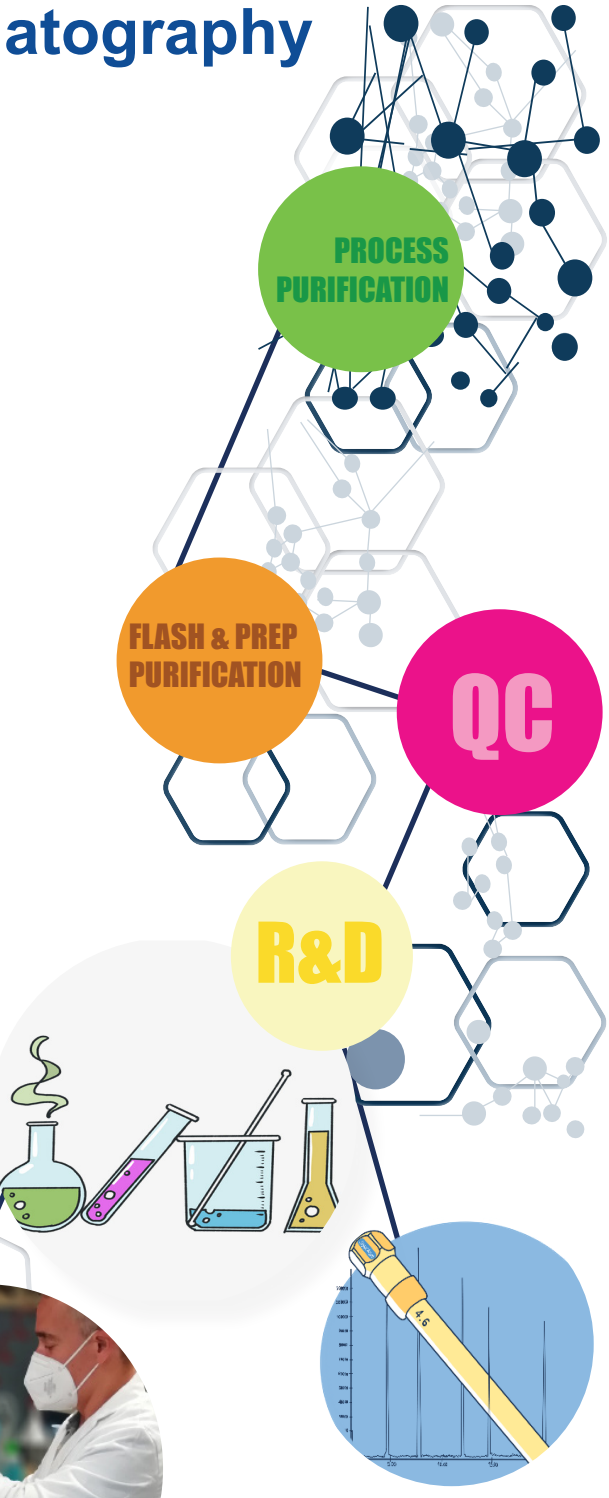
SepaChrom is the brainchild of the founders to create a dedicated reality, unique and able to support the **Chromatography users** optimizing their challenges.

Our Core competence is the manufacturing and trading of ***High-Quality*** products for **Chromatography**.

SepaChrom product portfolio includes a wide range of in-house manufactured ***HPLC*** Columns in both ***Analytical*** and ***Preparative*** scale, ***Flash*** cartridges & Instruments, and ***Process*** scale purification.

Our offer of products for Chromatography includes consumables and accessories, for both **HPLC** and **GC** techniques.

Our brands **Robusta®, Adamas®, Vydamas®, TMC®, Purezza®**, **Sepa-Bulk®** are only few of the product lines we propose to the **Chromatographers**.



Our Mission

Decades of experience of our team, combined with a range of High Quality selected products and the most efficient technological solutions, allows ***SepaChrom*** to be a reference to :

- **Pharma,**
- **Biotech,**
- **Chemical,**
- **Food and Beverage,**
- **Cosmetic,**
- **Environmental,**
- **Clinical**
- **Petrolchemical**

industries, at **R&D** department as well **QC** laboratories and **Production**.

Our commitment is to provide the Highest Technical Support that Chromatographers expect from

Your Specialist in Chromatography

Customers in Mind

The success of ***SepaChrom*** depends by the complete ***satisfaction*** of our customers, and consequently by their success.

SepaChrom expertise result in a High-Quality support ***pre & after*** sales to the Chromatographic Users.

A world-wide Distributor Network will assure the users the best in class technical and commercial support to properly approach their Chromatography challenges.

This include a ***fast delivery*** of your products from our warehouse to everywhere.

Chosing the Right HPLC Column

Choosing the right column for your application is very important and can be a difficult exercise. However following some simple steps will help you to make the correct choice and positively impact your chromatographic results. Here are some tips :

1. Set Your Separation Goals.

Do you need **High Resolution** or **Maximum Sensitivity**? And is our **Analysis** Time crucial? These are the main questions an HPLC user should consider in the development of a method. You also need to determine wheter long column life, low operating cost, or other factors are important.

2. Packing Material.

The choice of the most appropriate media depends on the nature of your compounds and on your goals. The **Right Selectivity** of your packing to obtain a good separation in a relatively short analysis time is the base on which to select the media.

3. Column Format.

Analytical, Semi-Prep or Prep format choice depends on your application and your goals. Inner Diameter and Length will also impact the result of your separation.

Base Material

Polymer-based media such as Polystyrene DVB or Methacrylate offer higher pH stability (pH 1-14) than Silica-based material, so columns packed with these packings can be thoroughly cleaned with strong acids or bases.
However these packings are compressible and may shrink or swell with certain solvents, and they do not offer the same resolution when compared to Silica-based packings.

Silica-based media are physically much stronger and will not shrink or swell. They offer higher resolution and provide sharper peaks compared to Polymer-based material. Silica-based media are also available with a wide range of bonded phases to ensure the widest selectivity for almost any application.
Silica-Based media are compatible with a broad range of polar and non-polar mobile phases and they can be stable to a wide pH range.

Particle Shape

Silica-based media particles can be **Irregular, Spheroidal** or **Spherical** in shape.

Most modern HPLC packings are spherical. A **Spherical** shape particle offers lower back pressure, much higher performance, stability and reproducibility than irregular particles.

Irregular particles have a larger surface area, higher loadibility and they are relatively less expensive. These are the reasons why they are still commonly used in prep and process scale purifications.

Spherical

Irregular

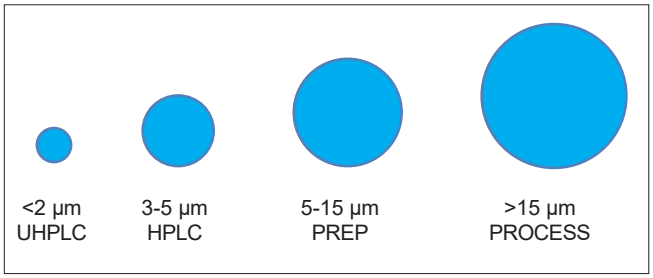
Particle Size

Smaller particle sizes give **Higher Efficiency and Resolution** than larger particle sizes but create higher back-pressure.

Larger particle sizes offer faster flow rates and lower back-pressure.

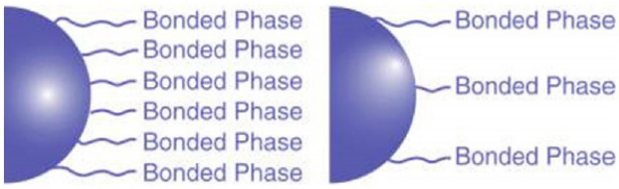
In analytical applications the typical particle sizes range is from 1.5µm to 10µm diameter, however most of the applications are performed with **3µm and 5µm**, which represent the best compromise between efficiency and back-pressure.

In Preparative applications larger particle sizes are commonly used (10µm to 30µm).



Carbon Load

For **Silica-based Reversed-Phase** packings, a carbon load percentage indicates the amount of functional bonded phase attached to the Silica-base material.
Lower amount of carbon load means that packings are more weakly hydrophobic, which may reduce retention times compared to phases with higher carbon load.
However, a higher carbon load will give higher capacity and often greater separation, especially for compounds of similar hydrophobicity.

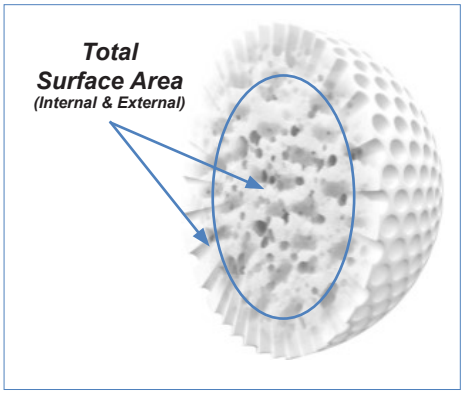


High Carbon Load **Low Carbon Load**

Pore Size & Surface Area

Pore Size
Packing materials having smaller pore sizes have higher surface area and consequently a higher capacity than packings with larger pore sizes.
To maximize the interaction between the target molecules and the packing a correct choice of the Pore Size is critical.
In general a 100Å material provide great results for small molecule analysis. For large molecules, such as Proteins and Peptides a 300Å media is typically used.

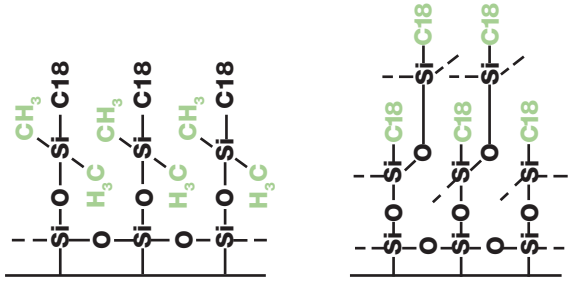
Surface Area
The Surface Area is the total available surface, most of which is inside the pores, for interaction with the target molecules.
Typically, Small pores means a larger surface area and
Large pores means a smaller surface area.



Bonding

Most commercially available reverse phase HPLC packing materials are Monomeric or Polymeric bonded phases.
When a monofunctional alkylsilane reagent is used to prepare the packing material, the functional chains have a single attachment point to the silica media. These are called **Monomeric** bonded phases.
If di- or trifunctional alkylsilane reagents are used, the bonded phases have functional chains bound to the base silica particle at multiple attachment points and can involve cross-linking between chains.
These are called **Polymeric** bonded phases.
New high-purity silica phases are very stable, whether monomerically or polymerically bonded, however they differ in their selectivity.

Monomeric **Polymeric**



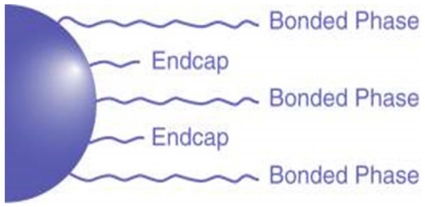
Endcapping

After the bonding procedures to obtain Silica-based reversed-phase packings, a certain amount of residual free silanol groups can remain unreacted on the silica surface.

These groups will interact with polar compounds. Endcapping the bonded phase minimizes these secondary interactions.

Partial or Total endcapping procedures are used to reduce the residual silanols on the silica surface.

Higher endcapping means less interactions with polar compounds while non-endcapped phases mean enhanced polar selectivity, for stronger retention of polar organic compounds.



Endcapping

HPLC Analytical Column Formats

Custom Made Columns Available

Choosing the right column format is critical to obtain the best performance during your analysis or purification.

Analytical Columns Format

Column Length

When starting a new HPLC method development, the user has to consider the complexity of their sample and the desired run time, in order to find the best column length for their application.

Shorter column length provides faster run times and solvent saving. Usually smaller particle size media is used in shorter column which achieves good resolution in a shorter run time, however complex samples may still need longer columns, even when using smaller particle sizes.

Column I.D.

Smaller internal diameter columns provide better mass sensitivity, require smaller sample size injection, and reduce solvent consumption.

Wider internal diameter columns allow for larger sample sizes and minimize the negative effects of your system's dead volume due to the higher flowrates.

2.1mmID columns work best with a microbore flow cell at your detector and an internal loop injector otherwise you have to tolerate some loss in efficiency and resolution due to system dead volumes.

Column Lengths Available	
20-30-50mm Column Length	Fast Separations Work best with 3 µm Particle Size
75-100-125-150mm Column Length	Standard & Hi-Resolution Separations Work best with 3-5 µm Particle Size
200-250-300mm Column Length	Standard & Hi-Resolution Separations Work best with 5-10 µm Particle Size

Analytical Column I.D. Available	
2.1mm Column I.D.	High Sensitiivity and Low Low Sample Volume Best use with Microbore Cell and Internal Sample Loop Valve.
3.0mm Column I.D.	High Sensitivity and ideal to reduce sovent consumption Work with standard HPLC instrumentation
4.0mm Column I.D.	Standard Separations Work with standard HPLC instrumentation
4.6mm Column I.D.	Standard Separations Work with standard HPLC instrumentation

Replaceable Frit

In most well-known and popular HPLC Columns, when a backpressure increase occurs, whatever the reason, you have to replace the entire expensive column.
With all ***SepaChrom*** HPLC Columns you can replace the frit and significantly extend its lifetime.

Full-Guard Cartridges

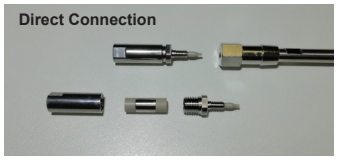
How can I best protect my HPLC column?

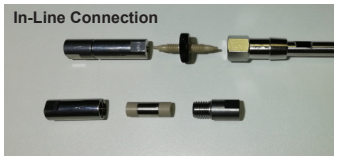
Full-Guard is the convenient protection system for your HPLC column and allows you to change the Guard Cartridge in seconds.

Select the suitable reusable Holder (In-Line or Direct Connect). They work with all Full-Guard Cartridges with following IDs :

2.1 - 3.0 - 4.0 - 4.6 mm ID

Full-Guard Holder	
CD0100	Full-Guard Holder - Direct Connection
CD0101	Full-Guard Holder - In-Line Connection





HPLC Column Selection

A Comparison of Reversed - Phase Columns

Typically, chromatographers choose HPLC columns by comparing physical characteristics, such as surface area and carbon load, however quite often this does not provide enough information for adequate column selection.

In the late 1990's Dr. Lloyd Snyder initiated working on what is known as Hydrophobic Subtraction Model (HSM) which then evolved, thanks to others expertise as Dr. John Dolan, Dr. Uwe Neue, Prof. Peter Carr and Prof. Dan Marchand, in a broader understanding of selectivity in Reversed-Phase HPLC (RPLC).

The Hydrophobic Subtraction Model (HSM) has been developed to quantitatively describe the chromatographic selectivity of reversed-phase (RP) HPLC columns. Upon characterization of a given Reversed Phase packing, the HS model provide quantitative values for five parameters including the phase hydrophobicity (H), its resistance to penetration by a solute molecule (S*), the hydrogen-bond acidity & basicity (A & B) and its interaction with ionized solute molecules (C).

These parameters describe the physico-chemical nature of the stationary phase.

This chart lists some of the parameters: Hydrophobicity (H), Hydrogen-bond Acidicty (A) (A) & Interaction with ionized soluted molecules (C) (at pH 7.0) (C)

Manufacturer	Column name	Hydrogen-bond acidity value A	Interaction with ionized soluted molecules value C	Hydrophobicity
Advanced Materials Technology	Halo 5 C18			1,15
Restek	Allure C18			1,13
Supelco	Ascentis Express C18			1,13
Advanced Materials Technology	Halo C18			1,10
Thermo/Hypersil	Accucore C18			1,09
Agilent Technologies	Zorbax Extend C18			1,09
Thermo/Hypersil	Accucore XL C18			1,09
Shimadzu	Shim-pack XR-ODS II			1,09
Agilent Technologies	Zorbax C18			1,08
Hichrom	Ultrasphere ODS			1,08
Grace/Alltech (Formerly)	Alltima HP C18 High Load			1,08
Waters	Cortecs C18			1,08
Agilent Technologies	Zorbax Rx-18			1,07
Supelco	Ascentis C18			1,07
Agilent Technologies	Zorbax Eclipse XDB-C18			1,07
SepaChrom	***Robusta C18***			1,06
SepaChrom	***Adamas C18-Extreme***			1,05
Grace/Vydac (Formerly)	Denali 120A C18			1,05
Grace/Grom (Formerly)	GROM Saphire 110 C18			1,05
Waters	Symmetry C18			1,05
Kromasil by Nouryon	Kromasil 100 5 C18			1,05
Thermo/Hypersil	Hypersil 100 C18			1,04
Waters	Nova-Pak C18			1,04
ACT	ACE 5 C18-HL			1,04
SepaChrom	***Adamas C18-X-Bond***			1,04
Waters	Cortecs C18+			1,04
Waters	Sunfire C18			1,03
Merck KGaA (EMD Millipore)	Superspher 100 RP-18e			1,03
Restek	Pinnacle II C18			1,03
Agilent Technologies	Zorbax Eclipse Plus C18			1,03
Nacalai Tesque	COSMOSIL C18-MS-II			1,03
Grace/Grom (Formerly)	GROM-SIL 120 ODS-3 CP			1,02
Waters	DeltaPak C18 100A			1,02
Waters	HSS C18			1,02
Phenomenex	Prodigy ODS(3)			1,02
Supelco	Supelcosil LC-18			1,01
Nacalai Tesque	COSMOSIL C18-AR-II			1,01
Phenomenex	Luna C18			1,01
Shiseido	Capcell Pak C18 MGII			1,01
Restek	Pinnacle DB C18			1,01
GL Sciences	InertSustain C18			1,01

HPLC Column Selection
A Comparison of Reversed - Phase Columns

Manufacturer	Column name	Hydrogen-bond acidity value (A)	Interaction with ionized soluted molecules value (C)	Hydrophobicity
Shimadzu	Shim-pack XR-ODS	0,22	0,79	1,01
Phenomenex	Kinetex EVO C18	0,19	0,79	1,01
SepaChrom	***Adamas C18-Classic***	0,22	0,79	1,01
Advanced Materials Technology	Halo AQ-C18	0,25	0,88	1,00
Grace/Alltech (Formerly)	Allsphere ODS2	0,28	0,93	1,00
Merck KGaA (EMD Millipore)	LiChrospher 100 RP-18	0,26	0,88	1,00
Grace/Jones (Formerly)	Genesis C18 120A	0,23	0,79	1,00
GL Sciences	Inertsil ODS-2	0,22	0,83	1,00
Waters	XBridge C18	0,22	0,80	1,00
ACT	ACE 5 C18	0,22	0,79	1,00
Phenomenex	Luna C18(2)	0,21	0,76	1,00
Waters	Acquity UPLC BEH C18	0,17	0,79	1,00
Agilent Technologies	Zorbax StableBond 80A C18	0,28	0,90	0,99
Grace/Alltech (Formerly)	Alltima C18	0,24	0,83	0,99
Thermo/Hypersil	Hypersil BDS C18	0,22	0,81	0,99
Phenomenex	Prodigy ODS(2)	0,22	0,81	0,99
Nomura	Develosil ODS-UG-5	0,20	0,80	0,99
GL Sciences	Inertsil ODS-3	0,20	0,74	0,99
Thermo/Hypersil	Hypersil ODS-2	0,26	0,83	0,98
Grace/Alltech (Formerly)	Adsorbosphere C18	0,24	0,93	0,98
Phenomenex	Synergi Max-RP	0,23	0,77	0,98
Grace/Alltech (Formerly)	Alltima HP C18	0,23	0,80	0,98
Supelco	Discovery C18	0,21	0,80	0,98
Waters	XTerra MS C18	0,20	0,78	0,98
Phenomenex	Luna Omega C18	0,19	0,78	0,98
Waters	Spherisorb S5 ODSB	0,28	0,93	0,97
Tosoh Bioscience	TSKgel ODS-120T	0,25	0,81	0,97
Supelco	Supelcosil LC-18-DB	0,24	0,84	0,97
Phenomenex	Kinetex XB-C18	0,22	0,81	0,97
Bischoff	ProntoSIL 120 C18-AQ	0,21	0,81	0,97
Thermo/Hypersil	Hypersil ODS	0,21	0,89	0,97
ES Industries	Chromegabond WR C18	0,20	0,81	0,97
Tosoh Bioscience	TSKgel ODS-80T	0,26	0,84	0,96
Waters	Spherisorb ODS-2	0,24	0,93	0,96
Phenomenex	Gemini C18 110A	0,23	0,80	0,96
Phenomenex	Kinetex C18 100A	0,21	0,79	0,96
YMC	YMC-Pack ODS-AQ	0,21	0,79	0,96
Fortis Technologies	Fortis C18	0,19	0,79	0,96
Agilent Technologies	Poroshell 120 SB-C18	0,27	0,81	0,96
Shiseido	Capcell Pak C18 MG III	0,22	0,78	0,95
Shiseido	Capcell Pak C18 IF	0,19	0,78	0,95
SepaChrom	***Adamas C18-Select***	0,29	0,92	0,95
YMC	YMC-Triart C18	0,19	0,76	0,92
Thermo/Hypersil	Hypersil GOLD aQ	0,22	0,85	0,91
Waters	Atlantis dC18	0,19	0,79	0,91
GL Sciences	Inertsil ODS-4	0,18	0,76	0,91
Merck KGaA (EMD Millipore)	LiChrosorb RP-18	0,26	0,89	0,90
Macherey Nagel	Nucleosil C18	0,23	0,86	0,90
ACT	Ace 5 C18-PFP	0,18	0,66	0,90
Tosoh Bioscience	TSKgel ODS-120A	0,28	0,89	0,89
Grace/Alltech (Formerly)	Prevail C18	0,29	0,92	0,88
Grace/Alltech (Formerly)	Alltima C18 AQ	0,29	0,91	0,88
Thermo/Hypersil	Hypersil GOLD	0,23	0,83	0,88

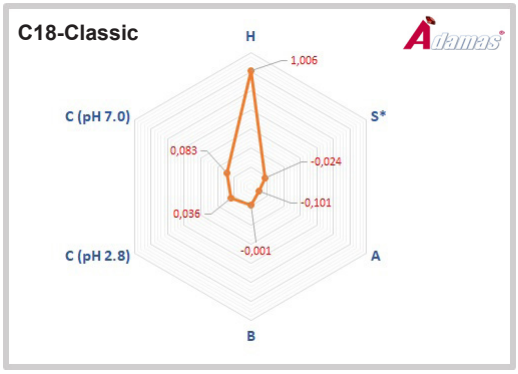
HPLC Columns Introduction

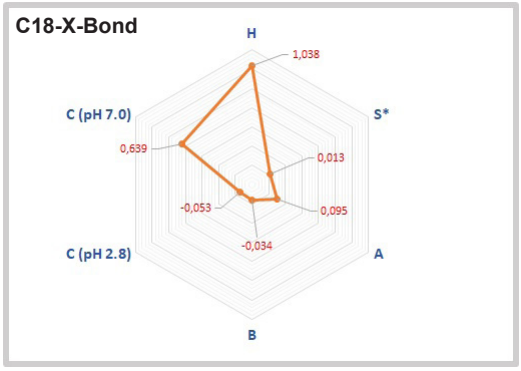
HPLC Column Selection
A Comparison of Reversed - Phase Columns

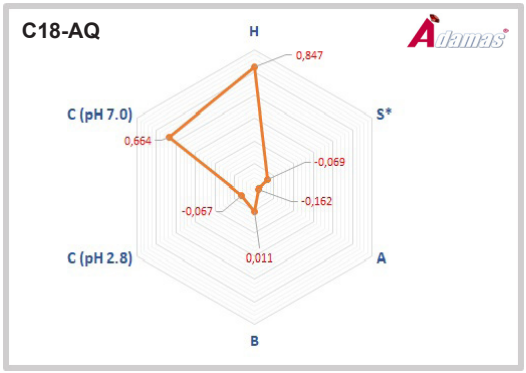
Manufacturer	Column name	Hydrogen-bond acidity value (A)	Interaction with ionized soluted molecules value	Hydrophobicity
SepaChrom	***Adamas C18-AQ***	A	C	0,85
Merck KGaA (EMD Millipore)	Purospher RP-18	A	C	0,84
Grace/Alltech (Formerly)	GraceSmart RP 18	A	C	0,83
Grace/Alltech (Formerly)	Econosphere C18	A	C	0,81
Phenomenex	Partisil ODS(3)	A	C	0,81
Waters	MicroBondapak C18	A	C	0,79
Grace/Alltech (Formerly)	Platinum C18	A	C	0,78
Grace/Alltech (Formerly)	VisionHT C18	A	C	0,78
Grace/Alltech (Formerly)	Alltima C18-LL	A	C	0,78
Waters	Spherisorb ODS-1	A	C	0,68
Grace/Alltech (Formerly)	Platinum EPS C18	A	C	0,61
Agilent Technologies	Zorbax SB-AQ	A	C	0,59

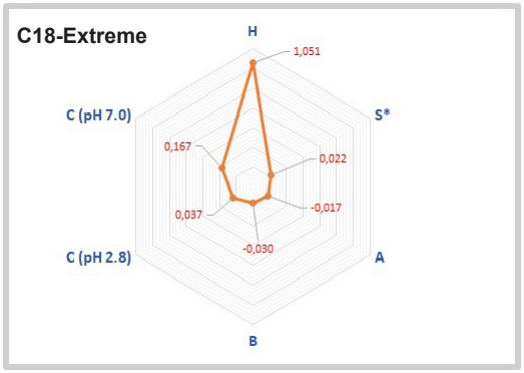
Hydrophobic Subtraction Model (HSM) chart

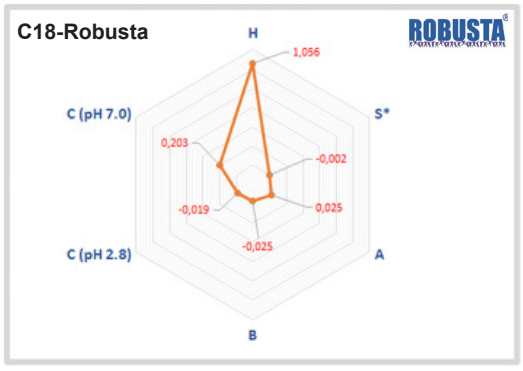
H= Hydrophobicity
S*= Resistance to penetration by a solute molecule
A= Hydrogen-bond acidity
B= Hydrogen-bond basicity
C(pH2.8)= interaction with ionized solute molecules
C(pH7.0)= interaction with ionized solute molecules

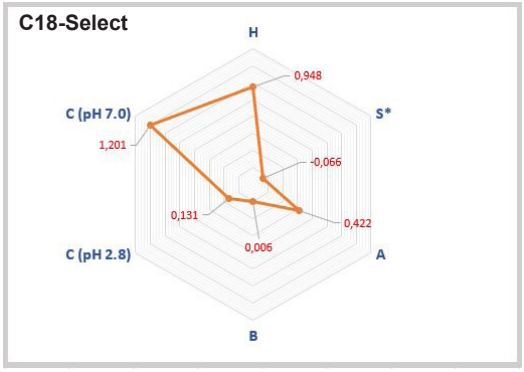








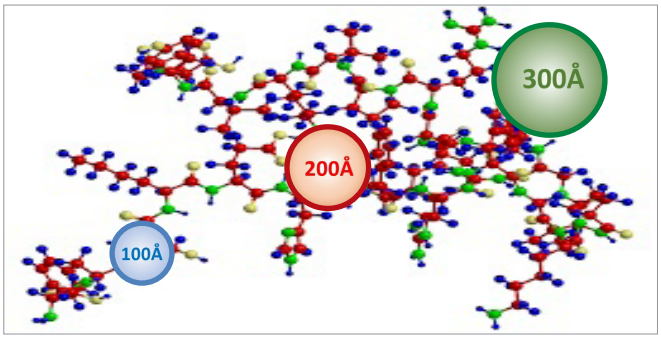




Analysis of Large Molecules Introduction

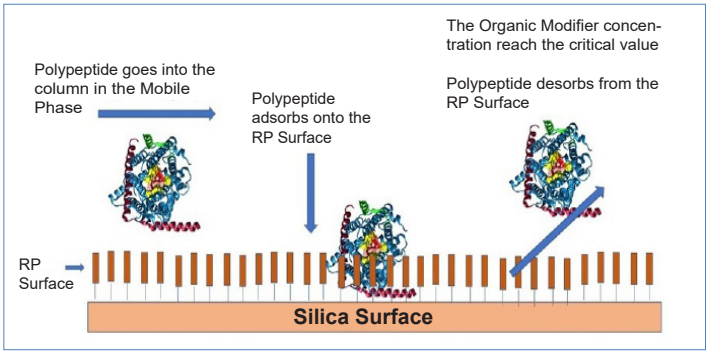
Analysis of Peptides and Proteins by Reversed-Phase HPLC

(RP-HPLC) High Performance Liquid Chromatography in Reversed-Phase mode is the preferred choice for the analysis and purification of biomolecules. The key reason why it is the most popular choice to analyze and purify proteins and peptides is the resolution.
RP-HPLC is able to separate polypeptides of nearly identical sequences, both small peptides as much larger proteins. Polypeptides with single amino acid residue difference can often be separated by RP-HPLC. Preparative RP-HPLC is often used for the purification of peptides from milligram to multigram quantities.



Mechanism of Interaction between RP-HPLC Columns and Polypeptides

The separation of small molecules involves continuous partitioning of the molecules between the mobile phase and the hydrophobic stationary phase. As Polypeptides are too large, during the chromatographic run they adsorb to the hydrophobic surface and remain adsorbed until the change of organic modifier concentration cause their desorption.They then interact only slightly with the surface when eluting out of the column. Polypeptides have only a small part of their molecule in contact with the RP surface, commonly known as “hydrophobic foot”; large part of the molecule are exposed to the mobile phase. RP-HPLC separates polypeptides based on subtle differences in the “hydrophobic foot” of the polypeptide being separated.

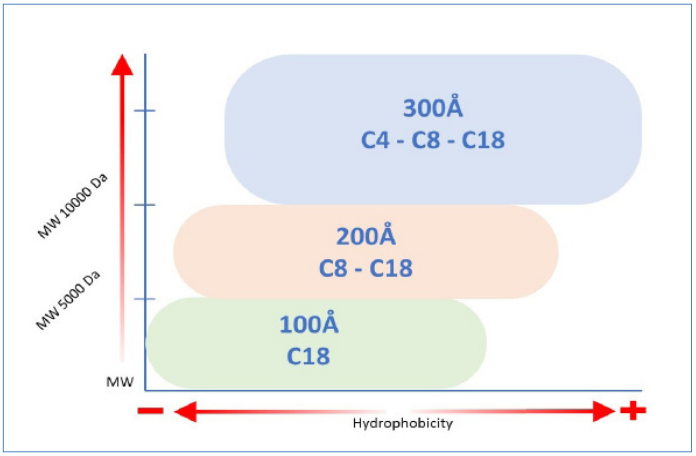


Important aspects of the adsorption/desorption mechanism of interactions between polypeptides and the hydrophobic phase.

The quantity of organic modifier required to desorb a polypeptide is very precise and consequently the desorption takes place within a very narrow range of organic modifier concentration. The sensitivity of polypeptide desorption to very precise concentrations of organic modifier explain the selectivity of RP-HPLC for the analysis of these molecules.
Sharp peaks are the result of the sudden desorption of polypeptides when the critical organic concentration is reached.
The adsorption/desorption mechanism takes place only once while the polypeptide is on the column. After its desorption, the interaction between the polypeptide and the reversed-phase surface is very little and have little affect on the separation.
Gradient elution is usually preferred for RP-HPLC polypeptide separations, however it shall be very swallow and precise.

The RP-HPLC Column in the Peptides and Proteins Separations

The interactive surface of a Silica Base HPLC Media is inside its pores. Polypeptides must enter into the pores in order to be adsorbed and then desorbed. Our Ultra High Purity Spherical Silica s available with three different porosities to make the most of the possibilities according with the size of the molecules of interest.
100Å pore diameter is ideal for analysis and purification of small peptides with Molecular Weight till 5000 Dalton. For larger peptides with Molecular Weight range of 5000-10000 Dalton, our 200Å pore diameter silica is the most appropriate. 300Å is the most indicate for analysis and purification of polypeptides and proteins with Molecular Weight > 10000 Dalton, up to 100K Dalton.
The particle size depend by the application you are challenging. 3-5µm are use in analytical methods (up to 4.6mmID) while the 5-10µm are preferred for a preparative laboratory scale application (up to 21.2mmID). If you need a process scale purification the choice of the particle size should go to larger particle size (15µm or >).



Analysis of Large Molecules Introduction

Reversed-Phase Stationary Phases

Reversed-Phase HPLC adsorbents are manufactured by bonding a hydrocarbon chain to the silica base material. The hydrocarbon group forming the packing is a linear aliphatic hydrocarbon of eighteen (C18), eight (C8) or four (C4) carbons and determine the hydrophobicity of the phase. Some guidelines provide information about which packing could be the most effective for the separation of peptides and proteins.
C18 columns are usually suggested for peptides and small proteins with MW < 5000 daltons. For proteins and small polypeptides with MW range between 5000 - 10000 daltons, often a C8 or C18 columns is used, according with the hydrophobicity of the molecules.
For molecules larger than 10000 daltons and highly hydrophobic a C4 column is highly recommended, however C8 and C18 offer sometime the different selectivity which allow to separate your target compounds.
VYdamas® C8-P and VYdamas® C18-P are more suitable for polar/medium polar peptides.

VYdamas® - Ultra High Purity Silica for Large Molecules Separation						
Phase	**Porosity**	**Particle Size**	**Surface Area**	**Pore Volume**	**Carbon Load**	**Metal Content***
VYdamas® C4	100Å	3µ-5µ-10µ-15µ-20/45µ	320 m^2/g	0.80 mL/g	6 %	< 1mg/kg
	200Å	3µ-5µ-10µ-15µ-20/45µ	160 m^2/g	0.80 mL/g	4 %	< 1mg/kg
	300Å	3µ-5µ-10µ-15µ-20/45µ	120 m^2/g	0.80 mL/g	3 %	< 1mg/kg
VYdamas® C8	100Å	3µ-5µ-10µ-15µ-20/45µ	320 m^2/g	0.80 mL/g	10 %	< 1mg/kg
	200Å	3µ-5µ-10µ-15µ-20/45µ	160 m^2/g	0.80 mL/g	6 %	< 1mg/kg
	300Å	3µ-5µ-10µ-15µ-20/45µ	120 m^2/g	0.80 mL/g	5 %	< 1mg/kg
VYdamas® C8-P	100Å	3µ-5µ-10µ-15µ-20/45µ	320 m^2/g	0.80 mL/g	10 %	< 1mg/kg
VYdamas® C18	100Å	3µ-5µ-10µ-15µ-20/45µ	320 m^2/g	0.80 mL/g	17 %	< 1mg/kg
	200Å	3µ-5µ-10µ-15µ-20/45µ	160 m^2/g	0.80 mL/g	10 %	< 1mg/kg
	300Å	3µ-5µ-10µ-15µ-20/45µ	120 m^2/g	0.80 mL/g	7 %	< 1mg/kg
VYdamas® C18-P	100Å	3µ-5µ-10µ-15µ-20/45µ	320 m^2/g	0.80 mL/g	17 %	< 1mg/kg

* 3/pkg - Full-Guard Cartridges require Full-Guard Holder. Two versions available :
Part.No **CD0100** - **Direct Connection**
Part.No **CD0101** - **In-Line Connection**

Bonding

The most commercially available reverse phase HPLC packing materials are Monomeric or Polymeric bonded phase.
When a monofunctional alkylsilane reagent is used to prepare the packing material, the functional chains have a single attachment point to the base. These are called **Monomeric** bonded phases. If di- or trifunctional alkylsilane reagent are used, the polymerically bonded phases have functional chains bound to the base silica particle at multiple attachment points and can involve cross-linking between chains. These are called **Polymeric** bonded phase.
Our ultra high-purity silica phases for analysis and purification of peptides and proteins are polymerically bonded, however we can supply on request different type of bonding to offer a wide range of selectivity.

Mobile Phase

To desorb and elute large molecules from the RP-HPLC column the aqueous mobile phase contains an organic modifier and an ion-pair reahent or buffer. The polypeptide is solubilized and desorbed from the hydrophobic surface by the organic modifier, while the ion-pair reagent or buffer sets the pH to enhance the separation. Increasing the concentration of organic modifier during the run achieve the elution of the polypeptides.
The purpose of the organic modifier is to desorb the polypeptides from the hydrophobic surface.
These are most used organic modifiers :

Acetonitrile (ACN) : it is the most commonly used organic modifier. It is volatile, easily removed from fractions, low viscosity, minimize column back-pressure, little UV adsorption at low wavelengths, and a lot of references in the RP-HPLC polypeptide separations.
Isopropanol : it is commonly used for large proteins or very hydrophobic molecules, however o reduce Isopropanol viscosity it is often used mixed with Acetonitrile (50:50). In small percentage (<3%) t could it increase the protein recovery.
Ethanol : is often referred for process scale purifications for economical reason; it comply to most important regulatory authorities
Other solvents : other solvents including methanol do not offer advantages compare to the most commonly used solvents for polypeptide separation and purification.

The ion-pairing reagent or buffer sets the pH of the eluent; interacting with the molecules of interest it enhances the separation.
TFA : The most common ion-pairing reagent is trifluoroacetic acid (TFA) due to the same reason hy Acetonitrile is the preferred organic modifier. TFA is normally used at concentrations of about 0.1% (w/v). Higher concentration (up 0.5%) are used for large or high hydrophbic proteins. Lower concentration (<0.1%) are used for tryptic digest separations.
Formic Acid : TFA reduce the ion signal in the electrospay interface; so the use of alternative ion-pairing reagent is leading to the formic acid tilization instead of TFA. However it does not always give the same good resolution of TFA.
Acetic Acid : it is same of formic acid.

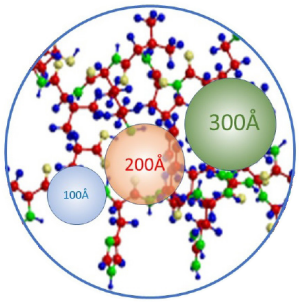
pH and Temperature

Separations of Peptides are often sensitive to the eluent pH because of protonation or deprotonation of acidic or basic side-chains. pH can have a important impact on peptide selectivity and t is a great tool usable to optimize the separations.
The temperature of the column affects solvent viscosity, back pressure, retention times and it may also affect peptide and protein selectivity. Temperature is an important parameter and should be optimized in any HPLC method development for eptide and protein separation.

VYdamas®

Ultra High Purity Spherical Silica

- 100Å & 200Å for Peptides Analysis and Purification
- 300Å for Proteins Analysis and Purification
- 3µ - 5µ for Analytical purpose
- 5µ - 10µ - 15µ for Preparative purpose
- 20/45µ for Flash Chromatography Purification



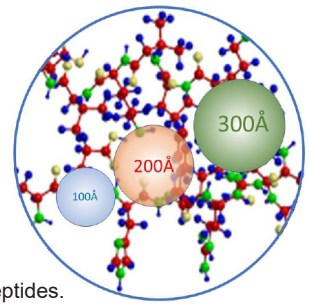
Ordering Information							
Phase	Porosity	Particle Size	Length / ID	4.6mm	4.0mm	3.0mm	2.1mm
VYdamas® C4	100Å	3µ	50mm	VD0016	VD0015	VD0014	VD0013
		3µ	100mm	VD0004	VD0003	VD0002	VD0001
		3µ	150mm	VD0012	VD0011	VD0010	VD0009
		Full-Guard - 3µ*	10mm	CD0267	CD0266	CD0265	CD0264
		5µ	100mm	VD0024	VD0023	VD0022	VD0021
		5µ	150mm	VD0032	VD0031	VD0030	VD0029
		5µ	250mm	VD0040	VD0039	VD0038	VD0037
		Full-Guard - 5µ*	10mm	CD0271	CD0270	CD0269	CD0268
	200Å	3µ	50mm	VD0060	VD0059	VD0058	VD0057
		3µ	100mm	VD0048	VD0047	VD0046	VD0045
		3µ	150mm	VD0056	VD0055	VD0054	VD0053
		Full-Guard - 3µ*	10mm	CD0275	CD0274	CD0273	CD0272
		5µ	100mm	VD0068	VD0067	VD0066	VD0065
		5µ	150mm	VD0076	VD0075	VD0074	VD0073
		5µ	250mm	VD0084	VD0083	VD0082	VD0081
		Full-Guard - 5µ*	10mm	CD0279	CD0278	CD0277	CD0276
	300Å	3µ	50mm	VD0104	VD0103	VD0102	VD0101
		3µ	100mm	VD0092	VD0091	VD0090	VD0089
		3µ	150mm	VD0100	VD0099	VD0098	VD0097
		Full-Guard - 3µ*	10mm	CD0283	CD0282	CD0281	CD0280
		5µ	100mm	VD0112	VD0111	VD0110	VD0109
		5µ	150mm	VD0120	VD0119	VD0118	VD0117
		5µ	250mm	VD0128	VD0127	VD0126	VD0125
		Full-Guard - 5µ*	10mm	CD0287	CD0286	CD0285	CD0284

* 3/pkg - Full-Guard Cartridges require Full-Guard Holder. Two versions available :
Part.No **CD0100** - **Direct Connection**
Part.No **CD0101** - **In-Line Connection**

VYdamas®

Ultra High Purity Spherical Silica

- 100Å & 200Å for Peptides Analysis and Purification
- 300Å for Proteins Analysis and Purification
- 3µ - 5µ for Analytical purpose
- 5µ - 10µ - 15µ for Preparative purpose
- 20/45µ for Flash Chromatography Purification



VYdamas® C8 is completely endcapped and it is more suitable for the separation and the purification of non polar peptides.

VYdamas® C8-P provide an higher hydrophilic interaction which enhance the separation and purification of polar/medium polar peptides.

Ordering Information							
Phase	Porosity	Particle Size	Length / ID	4.6mm	4.0mm	3.0mm	2.1mm
VYdamas® C8-P	100Å	3µ	50mm	VD0651	VD0650	VD0649	VD0648
		3µ	100mm	VD0643	VD0642	VD0641	VD0640
		3µ	150mm	VD0647	VD0646	VD0645	VD0644
		Full-Guard - 3µ*	10mm	CD0339	CD0338	CD0337	CD0336
		5µ	100mm	VD0655	VD0654	VD0653	VD0652
		5µ	150mm	VD0659	VD0658	VD0657	VD0656
		5µ	250mm	VD0663	VD0662	VD0661	VD0660
		Full-Guard - 5µ*	10mm	CD0343	CD0342	CD0341	CD0340
VYdamas® C8	100Å	3µ	50mm	VD0148	VD0147	VD0146	VD0145
		3µ	100mm	VD0136	VD0135	VD0134	VD0133
		3µ	150mm	VD0144	VD0143	VD0142	VD0141
		Full-Guard - 3µ*	10mm	CD0291	CD0290	CD0289	CD0288
		5µ	100mm	VD0156	VD0155	VD0154	VD0153
		5µ	150mm	VD0164	VD0163	VD0162	VD0161
		5µ	250mm	VD0172	VD0171	VD0170	VD0169
		Full-Guard - 5µ*	10mm	CD0295	CD0294	CD0293	CD0292
	200Å	3µ	50mm	VD0192	VD0191	VD0190	VD0189
		3µ	100mm	VD0180	VD0179	VD0178	VD0177
		3µ	150mm	VD0188	VD0187	VD0186	VD0185
		Full-Guard - 3µ*	10mm	CD0299	CD0298	CD0297	CD0296
		5µ	100mm	VD0200	VD0199	VD0198	VD0197
		5µ	150mm	VD0208	VD0207	VD0206	VD0205
		5µ	250mm	VD0216	VD0215	VD0214	VD0213
		Full-Guard - 5µ*	10mm	CD0303	CD0302	CD0301	CD0300
	300Å	3µ	50mm	VD0236	VD0235	VD0234	VD0233
		3µ	100mm	VD0224	VD0223	VD0222	VD0221
		3µ	150mm	VD0232	VD0231	VD0230	VD0229
		Full-Guard - 3µ*	10mm	CD0307	CD0306	CD0305	CD0304
		5µ	100mm	VD0244	VD0243	VD0242	VD0241
		5µ	150mm	VD0252	VD0251	VD0250	VD0249
		5µ	250mm	VD0260	VD0259	VD0258	VD0257
		Full-Guard - 5µ*	10mm	CD0311	CD0310	CD0309	CD0308

* 3/pkg - Full-Guard Cartridges require Full-Guard Holder. Two versions available :
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Part.No **CD0101** - **In-Line Connection**

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- 100Å & 200Å for Peptides Analysis and Purification
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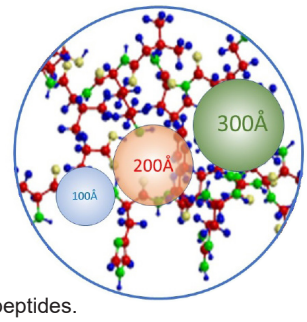

VYdamas® C18 is completely endcapped and it is more suitable for the separation and the purification of non polar peptides.

VYdamas® C18-P provide an higher hydrophilic interaction which enhance the separation and purification of polar/medium polar peptides.

Ordering Information							
Phase	Porosity	Particle Size	Length / ID	4.6mm	4.0mm	3.0mm	2.1mm
VYdamas® C18-P	100Å	3µ	50mm	VD0702	VD0701	VD0700	VD0699
		3µ	100mm	VD0694	VD0693	VD0692	VD0691
		3µ	150mm	VD0698	VD0697	VD0696	VD0695
		Full-Guard - 3µ*	10mm	CD0347	CD0346	CD0345	CD0344
		5µ	100mm	VD0706	VD0705	VD0704	VD0703
		5µ	150mm	VD0710	VD0709	VD0708	VD0707
		5µ	250mm	VD0714	VD0713	VD0712	VD0711
		Full-Guard - 5µ*	10mm	CD0351	CD0350	CD0349	CD0348
VYdamas® C18	100Å	3µ	50mm	VD0280	VD0279	VD0278	VD0277
		3µ	100mm	VD0268	VD0267	VD0266	VD0265
		3µ	150mm	VD0276	VD0275	VD0274	VD0273
		Full-Guard - 3µ*	10mm	CD0315	CD0314	CD0313	CD0312
		5µ	100mm	VD0288	VD0287	VD0286	VD0285
		5µ	150mm	VD0296	VD0295	VD0294	VD0293
		5µ	250mm	VD0304	VD0303	VD0302	VD0301
		Full-Guard - 5µ*	10mm	CD0319	CD0318	CD0317	CD0316
	200Å	3µ	50mm	VD0324	VD0323	VD0322	VD0321
		3µ	100mm	VD0312	VD0311	VD0310	VD0309
		3µ	150mm	VD0320	VD0319	VD0318	VD0317
		Full-Guard - 3µ*	10mm	CD0323	CD0322	CD0321	CD0320
		5µ	100mm	VD0332	VD0331	VD0330	VD0329
		5µ	150mm	VD0340	VD0339	VD0338	VD0337
		5µ	250mm	VD0348	VD0347	VD0346	VD0345
		Full-Guard - 5µ*	10mm	CD0327	CD0326	CD0325	CD0324
	300Å	3µ	50mm	VD0368	VD0367	VD0366	VD0365
		3µ	100mm	VD0356	VD0355	VD0354	VD0353
		3µ	150mm	VD0364	VD0363	VD0362	VD0361
		Full-Guard - 3µ*	10mm	CD0331	CD0330	CD0329	CD0328
		5µ	100mm	VD0376	VD0375	VD0374	VD0373
		5µ	150mm	VD0384	VD0383	VD0382	VD0381
		5µ	250mm	VD0392	VD0391	VD0390	VD0389
		Full-Guard - 5µ*	10mm	CD0335	CD0334	CD0333	CD0332

* 3/pkg - Full-Guard Cartridges require Full-Guard Holder. Two versions available :
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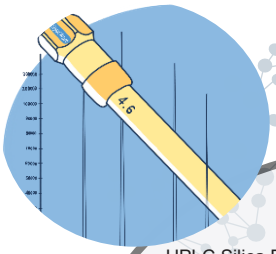
SepaChrom

Other products available from SepaChrom



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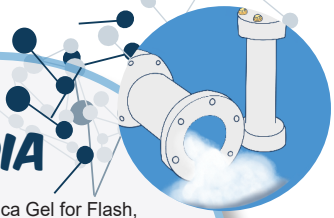

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Raw & Bonded Silica Gel for Any Application Wide Range Porosity (30Å- 2500Å) and Particle Size (10µ-200µ)

Polymer Based Resin for Reversed Phase and Ion Exchange Chromatography

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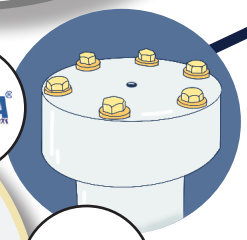

ROBUSTA

Adamas

10mm - 50mmID Packed Preparative Columns for Lab Scale Purification packed by SepaChrom

50mm - 2000mmID Process Scale Chromatography Columns & Systems, Flanged & DAC Technology

OEM Packed Preparative Columns

Scale-up Method Development & Custom Packing Service

VYdamas

FLASH

Instruments for Flash and Prep Chromatography up to 825mL/min & 400 bar pressure

Integrated ELSD & MS Simple Quad Detector for Flash Purification

TLC Plates and Accessories for Flash Chromatography

A Complete Range of Flash Columns for All Existing Flash Instruments

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Maxi-Clean™ Ion Chromatography SPE Cartridges

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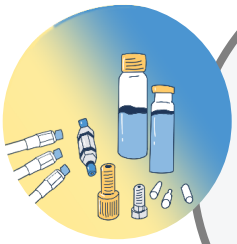
SepaChrom Nitrogen Generators for LC-MS

Automated Sample Evaporators for Lab Scale Purification

Interchim Advion CMS Compact Mass Spectrometer Detector

CONSUMABLE



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