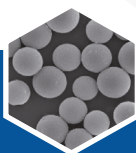


SOLUTIONS FOR
ANALYTICAL AND PREPARATIVE
CHROMATOGRAPHY





Founded in 1995, SiliCycle is specialized in the development, manufacturing and commercialization of high value silica gels and specialty products for chromatography, purification and synthesis.



Solutions for Analytical & Preparative Chromatography

| | |
|---|----|
| SiliaSphere™ Spherical Silica Gels | 3 |
| Bare Spherical Silica Gels | 3 |
| Functionalized Spherical Silica Gels | 5 |
| SiliaSphere Bare Spherical Silica Gels Ordering Information | 8 |
| SiliaChrom® HPLC Columns..... | 9 |
| Portfolio | 10 |
| SiliaChrom® Plus and dt Family | 11 |
| Ordering Information | 15 |
| Resource Center | 21 |



SiliaSphere Spherical Silica Gels

SiliaSphere, the right choice for:

- Chromatographic performance
- Loading capacity
- Reproducibility
- Chemical and physical stability

SiliaSphere™ Bare Spherical Silicas

With pore diameters ranging from 60 to 1,000 Å and particle sizes from 3 to 25 µm, we offer products to meet all your separation requirements. This is one of the most reliable portfolios of spherical silica gels for high pressure chromatography. SiliaSphere are ideal for both analytical and preparative chromatography, from laboratory to pilot-plant processes and production scales. Furthermore, the excellent properties of SiliaSphere make them the packing of choice for high performance liquid chromatography (HPLC), supercritical fluid chromatography (SFC), simulated moving bed (SMB), and dynamic axial compression (DAC) applications.

Features and Benefits of SiliaSphere Spherical Silica Gels

- | | |
|--|--|
| • High purity silica gels Consistency, reliability, and reproducibility | • Strong mechanical stability Low back-pressure without surface abrasion |
| • Perfect spherical shape, free of any cavities or cracks Ease of column packing and high resolution | • Same well controlled processes for all SiliaSphere Easy scalability |
| • Exceptional narrow particle size distribution Optimal separation and resolution | • Availability in bulk quantities at affordable price On-time delivery |

SiliaSphere as a Silica Matrix

SiliCycle has a strong know-how and expertise in silica gel manufacturing. To support the increasing demand on our spherical silicas, we have developed an optimized and highly controlled large-scale production process for all of our SiliaSphere products, without decreasing the quality of the silica.

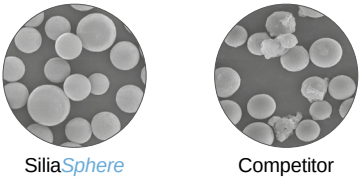
Particle shape, pore and particle size distributions, silica gel purity and surface properties, all have their influence on chromatographic performance. Therefore, in order to develop the most efficient process, all these parameters need to be evaluated and optimized to ensure batch-to-batch reproducibility.

SiliaSphere is manufactured from an organic form of silicon (alkoxydes). This ensures very low metal content as the starting material is purified by distillation. Deionized water is used to hydrolyze the silicon alkoxydes. Careful monitoring and control of the parameters that induce precipitation provide spherical silica gels with the desired characteristics. SiliaSphere products are characterized by a very low metal content and exceptional stability. Furthermore, our manufacturing process ensures quality and reproducibility in pore size, surface area, particle size, and morphology for all SiliaSphere products.

Perfectly Spherical Particle Shape

The perfectly spherical shape of SiliaSphere silicas, combined to their smooth surfaces free of any cracks, cavities, and fines make them the packing of choice for chromatography.

The SiliaSphere sphericity compares favorably to well-known brands in spherical silica gel as demonstrated by the scanning electron microscope (SEM) pictures.



Scalability

SiliaSphere are available in bulk for a wide range of HPLC purification, from laboratory to plant scale. All SiliaSphere products are manufactured under tightly controlled manufacturing processes, and stringent quality control ensures the highest quality and reproducibility. Scaling-up is extremely straight-forward with SiliaSphere silicas and performance will remain the same throughout the range of particle sizes.

Narrow Pore Size Distribution

The right pore diameter to use is related to the type of molecules present in the sample to be purified. Typically, for small molecules, a 100 Å pore size is recommended.

However, if higher resolution is required, then 60 - 80 Å will be more suitable.

For large molecules, such as peptides and proteins, 300 Å or higher is recommended.

Tight Particle Size Distribution

The importance of particle size distribution varies depending on the type of chromatography being done.

For instance, it is very important when using HPLC that the particle size distribution of the spherical particles used is very narrow. Tight particle size distribution yields greater column performance (*separation*), better peak shape, lower back-pressure as well as higher packing stability.

SiliaSphere offers one of the narrowest particle size distributions available on the market. To evaluate our particle size distribution, we use the D90/D10 ratio. The closer the ratio is to 1, the tighter the particle distribution is.

High Surface Area and Pore Volume

Our optimized manufacturing process ensures high specific surface area for greater loading capacity with a uniform and reproducible surface coverage.

Low Trace Metal Content & High Purity

SiliCycle's proprietary technology generates a silica gel with one of the lowest trace metal content on the market today. Our low trace metal content ensures you will get optimal performance chromatography. Tight control of trace metals in every batch also improves reproducibility and reduces risks of interaction between metals and analytes. Low metal content limits any unwanted metal ion solute interactions, providing symmetrical peaks with little or no tailing.

To probe low metal content in SiliaSphere silicas, we ran the following chromatographic test and we compared our products with a well-known competitor. With SiliaSphere, you can be assured that peak tailing or missing peaks are not coming from our silica.

- 1. Toluene**
Column Packing Efficiency ► Neutral solute
- 2. Acetoacetanilide**
Metal Content ► Can interact with heavy metals, resulting in tailing or missing peaks
- 3. Catechol**
Metal Content ► Can interact with heavy metals, resulting in tailing or missing peaks
- 4. 3-Pyridyl-acetonitrile**
Silica's Acidic Character ► Strongly binds to acidic sites

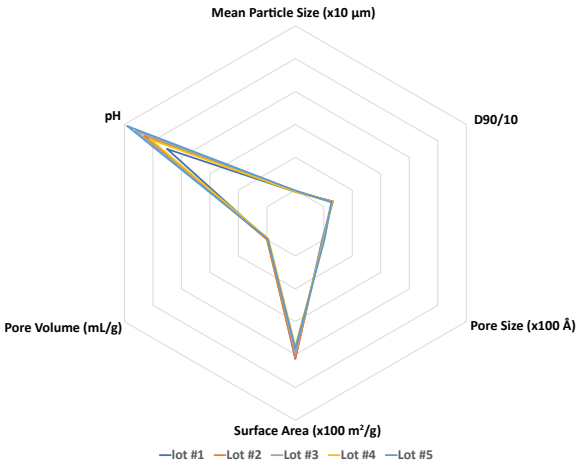
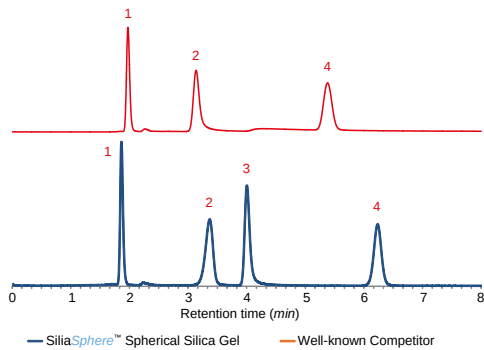
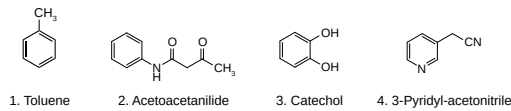
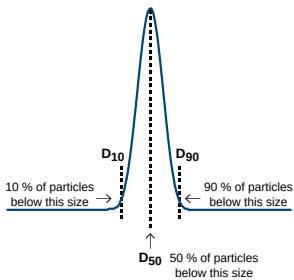
Lot-to-lot Reproducibility

SiliCycle is committed to high quality standards and always strives to provide defect-free products and lot-to-lot reproducibility. In doing so, all products are subjected to a stringent quality control, in an ISO 9001:2015 compliant facility. Every lot needs to meet the established specifications to be released. This ensures reliable & reproducible chromatographic performances.

The graph at right presents physical properties obtained for 5 consecutive lots of SiliaSphere 10 µm, 100 Å. It shows high product manufacturing reproducibility.



Video: Understanding particle size distribution



SiliaSphere Functionalized Spherical Silicas

For over 25 years, SiliCycle has been dedicated to the development of silica-based products and has acquired extensive experience in grafting technology. This expertise, combined with tightly controlled proprietary functionalization processes, allows SiliCycle to be a supplier of choice for all chromatographic applications.

Grafting Process

Silica surface possesses active silanols (*Si-OH, free OH groups of the silica*), which permits the modification of the surface chemistry by grafting silane moieties. This property allows control of the surface polarity, useful in separation techniques. Various types of silanes can be grafted on the surface to afford monomeric or polymeric bonded phases, where both have pros and cons as described below.

Main Differences Between Monomeric and Polymeric Bonded Phases

Monomeric Functionalization

By grafting a monofunctional alkylsilane reagent (*chlorodimethylsilane*), only one bond can be formed with the silica surface. This type of grafting is called monomeric. The dimethyl groups help to protect the surface by steric hindrance, but also prevent from reaching the highest possible silane density. The residual silanol groups are inhibited by grafting a small molecule, trimethylsilane: $\text{Si}(\text{CH}_3)_3$. This small reagent is called a capping agent, and this technique is called endcapping. Even after endcapping, a small portion of the initial silanols are still present, unable to react due to steric hindrance and hence isolated from the mobile phase and analytes present.

Benefits: Monomeric phases present a very high stability, batch-to-batch reproducibility, and good hydrophobic properties.

Drawbacks: The fact that the silane possesses only one bond with the surface makes this phase less stable at low pH, which may lead to silane hydrolysis and consequently leaching. So, for low pH, a polymeric phase is preferred.

Polymeric Functionalization

By grafting a trichlorosilane, it is possible to form multiple bonds in three dimensions with the surface and also between silane molecules. This grafting method is called polymeric functionalization.

Benefits: The silica surface is more hydrophobic, has greater stability in strong acidic condition (*pH 2-3*) and has a longer lifetime.

Drawbacks: Polymeric phases present lower homogeneous surface coverage due to cross-polymerization reactions, poorer batch-to-batch reproducibility leading to variation in retention, even for the same molecule.

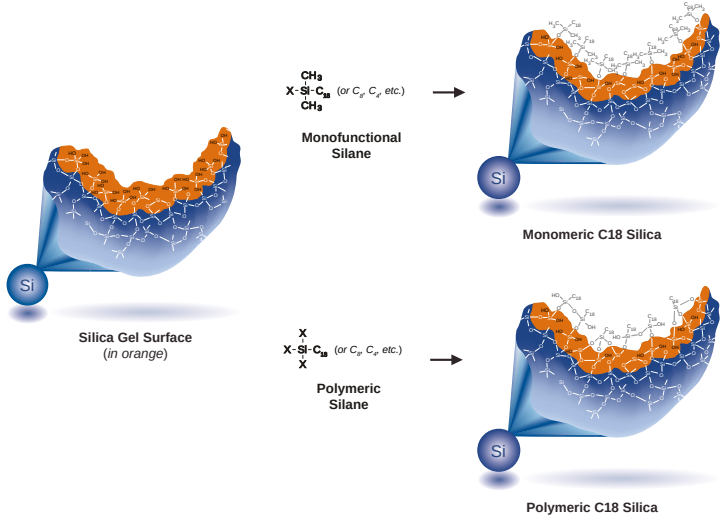
Homogeneous Surface Coverage

SiliCycle developed a new and innovative grafting technique, characterized by a homogeneous coverage of the alkyl chains on the surface. This proprietary process can be used with all silane types and ensures greater chemical stability as well as better performance due to the greater homogeneity of the surface coverage.

Endcapping

When functionalizing silica, it is impossible to react with every silanol group, so endcapping technology is often used to prevent peak tailing caused by non-specific interactions, and thus improve separations. Furthermore, more sophisticated methods lead to strong layer protection offering very high sorbent durability in harsh conditions.

The endcapping step can be done using various methodologies. The easiest way is to treat the surface with a small silylating agent, such as trimethylchlorosilane (*TMSCl*). However, at SiliCycle, we always try to improve and control this critical step to afford highly deactivated silanol phases. For some phases, we use the conventional single endcapping step technique, for others we use our proprietary endcapping processes which can include multistep methods, use of specific silylating agents or other special treatments.



Most Popular Bonded Phases

In liquid chromatography, there are various modes of operation possible, based on the interaction mechanism of the solute with the stationary phase (*sorbent*). Most known separation modes are summarized in the table below.

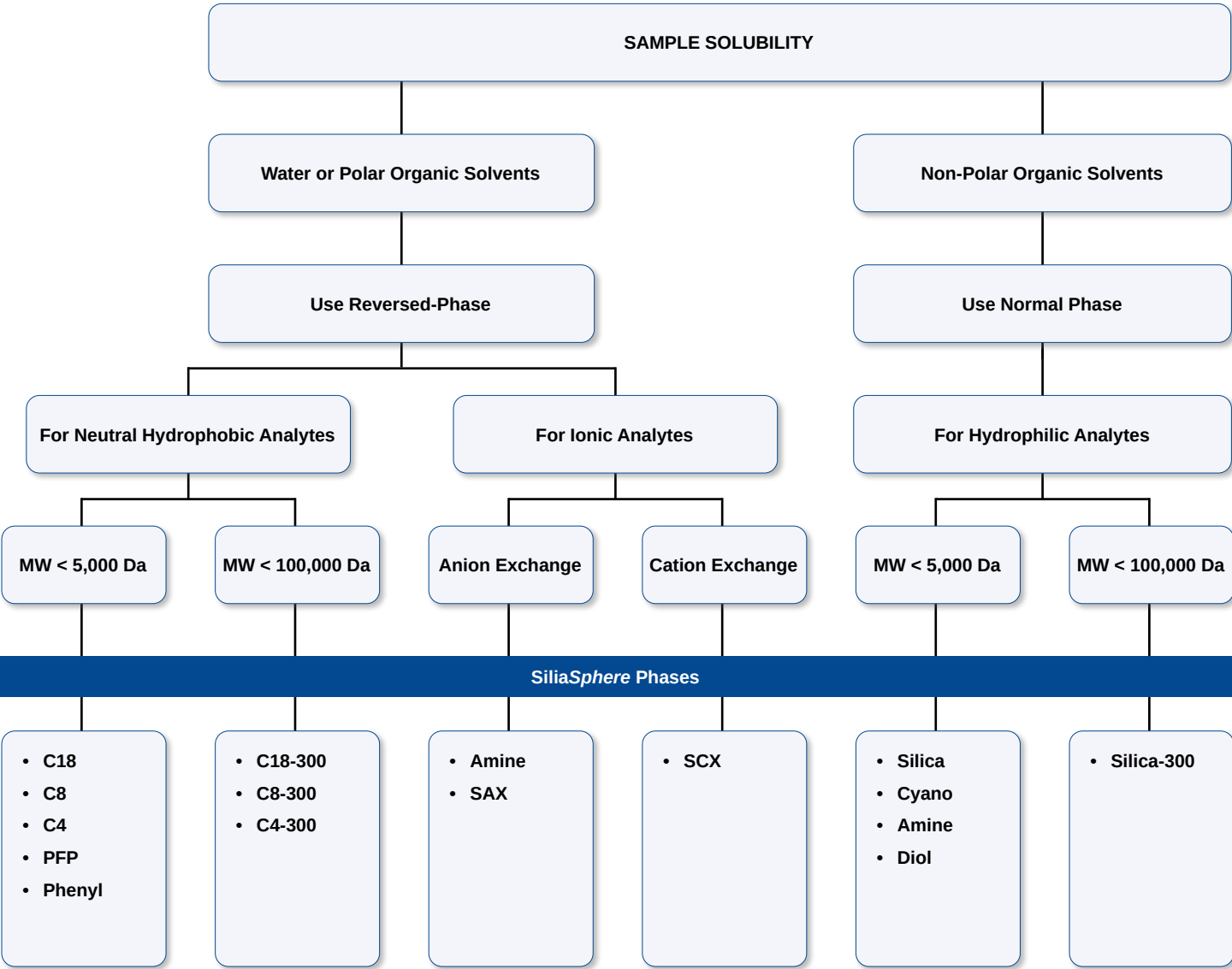
| Siliasphere Most Popular Bonded Phases | | | |
|--|--|--|---|
| Mode | Normal Phase (NP) | Reversed-Phase (RP) | Ion Exchange Phase (IEX) |
| Mode Mechanism | Polar or hydrophilic | Non-polar or lipophilic / hydrophobic | Ionic |
| Typical Stationary Phase | Bare silica or polar functionalized silica (<i>Amine, Cyano or Diol</i>) | Functionalized silica (<i>mostly C18, C8, C4, Cyano, Phenyl and PFP</i>) | Ionic functionalized silica (<i>SAX, SCX, WAX, WCX</i>) |
| Stationary Phase Polarity | Polar | Non-polar | Anionic or cationic exchanger |
| Typical Mobile Phase | Non-polar organic solvents such as hexane, EtOAc, dichloromethane, THF | Mixtures of water or aqueous buffers and organic solvents (<i>mostly ACN, MeOH, THF</i>), ion pairing agents can also be added | Water, buffers, acid, base |
| Mobile Phase Polarity | Non-polar | Polar | Buffer or ionic |

Typical Applications of Most Common Bonded Phases


| Typical Applications of Most Common Bonded Phases | | | | | |
|---|------------------|------|----|-----|---|
| Sorbent Phase | Functional Group | Mode | | | Typical Applications |
| | | NP | RP | IEX | |
| C18 | | | ✓ | | Great start for method development. Presents the maximum retention of non-polar compounds. Typically used for peptides, pesticides, PCBs, PAHs, drugs, etc. |
| C8 | | | ✓ | | Presents less retention compared to C18. Mainly used for highly hydrophobic pesticides, small peptides and heavy drugs. |
| C4 | | | ✓ | | Presents less retention compared to C18 and C8. Widely used for molecules with large hydrophobic regions such as peptides, proteins and zwitterions (<i>in 300 Å</i>). |
| Phenyl (PHE) | | | ✓ | | Moderate non-polar sorbent with different selectivity for aromatic compounds compared to other non-polar sorbents. |
| Pentafluorophenyl (PFP) | | | ✓ | | For a new selectivity approach or the purification of conjugated compounds (<i>isomers</i>). |
| Cyano (CN) | | ✓ | ✓ | | In reversed-phase: moderate non-polar sorbent with less hydrophobicity than C18 or C8. Purification of cyclosporine and carbohydrates. In normal phase: less polar sorbent compared to silica, used for the purification of polar organic compounds. |
| Diol | | ✓ | | | Moderate polar sorbent with a neutral character. Used to extract polar compounds. Alternative to silica when acidic character is problematic. |
| Silica | | ✓ | | | Most polar sorbent with a slight acidic character. Used for purification of polar and non-ionic compounds. |
| Amine (NH ₂ , WAX) | | ✓ | | ✓ | In normal phase: polar sorbent with a basic character, with less retention and a different selectivity for acidic / basic compounds compared to silica. In ion exchange mode: a weak anion exchanger with pK _a of 9.8. At pH 7.8 or below, the functional groups are positively charged. It facilitates the rapid release of very strong anions (<i>such as sulfonic acids</i>) that may be retained irreversibly on SAX. |
| Tosic Acid (SCX) | | | | ✓ | Due to the very low pK _a (< 1), this silica is a strong cation exchanger. The most common use is likely for catch and release purification of weak cations. |
| TMA Chloride (SAX) | | | | ✓ | The quaternary amine is permanently positively charged, and commonly used for the extraction of weak anions that may not bind strongly enough to weaker anion exchangers (WAX). |
| TMA Acetate (SAX-2) | | | | ✓ | The acetate counter ion is easier to exchange compared to the chloride ion. It is used for compounds with pK _a < 5, such as carboxylic acids, or to selectively purify acidic compounds or remove acidic impurities from reaction mixtures. |

SiliaSphere Phase Selection

The phase selection table below can be a starting point for choosing the right phase (*and HPLC column*) for your application. Use this decision tree to narrow down your options.



SiliaSphere Bare Spherical Silica Gels Ordering Information



| SiliaSphere Monodispersed Typical Characteristics | | | | |
|---|-------------|----------|-------------|---------|
| Pore Diameter | 60 Å | 100 Å | 300 Å | 1,000 Å |
| Specific Surface Area (m²/g) | ≥ 450 | ≥ 400 | ≥ 80 | ≥ 20 |
| Pore Volume (mL/g) | 0.85 - 1.15 | | 0.75 - 1.05 | |
| pH (5 % w/w) | 1.5 - 7 | | | |
| Available Particle Sizes (µm) | 3, 5, 10 | 3, 5, 10 | 3, 5, 10 | 10 |

| SiliaSphere Bare Monodispersed Spherical Silicas Ordering Information | | | | |
|---|---------------|-----------|---------|---------|
| Particle Size | Pore Diameter | | | |
| | 60 Å | 100 Å | 300 Å | 1,000 Å |
| 3 µm | S10003B | S10003E-A | S10003M | N/A |
| 5 µm | S10005B | S10005E-A | S10005M | S10005T |
| 10 µm | S10007B | S10007E-A | S10007M | S10007T |

| SiliaSphere PC Bare Spherical Silicas Ordering Information | | | | | | | |
|--|---------------|-----------|---------|---------|---------|---------|---------|
| Particle Size | Pore Diameter | | | | | | |
| | 70 Å | 90 Å | 100 Å | 300 Å | 500 Å | 800 Å | 1,000 Å |
| 25 µm | N/A | S10095D-A | N/A | N/A | N/A | N/A | N/A |
| 20 - 45 µm | S10020C | N/A | S10020E | S10020M | S10020P | S10020S | S10020T |

SiliaSphere Bonded Spherical Silica Gels Ordering Information

The table below presents the most popular SiliaSphere bonded phases available from SiliCycle.

To build your own product number, just add the Particle and Pore Size Code to the Phase Code: [Phase]-[Particle & Pore]
Example: S03205E-A for a C18 silica gel, 5 µm, 100 Å

Phase Codes

| SiliaSphere Phase Codes | |
|-------------------------------|------------|
| Phase | Phase Code |
| C18 | S032 |
| C8 | S308 |
| C4 | S327 |
| Phenyl | S340 |
| PFP | S675 |
| Cyano | S380 |
| Diol | S350 |
| Silica | S100 |
| Amine (NH ₂ , WAX) | S520 |
| Tosic Acid (SCX) | S605 |
| TMA Chloride (SAX) | S665 |
| TMA Acetate (SAX-2) | S664 |

Particle and Pore Size Codes

| SiliaSphere Particle and Pore Size Codes | | | | |
|--|---------------|-------|-------|---------|
| Particle Size | Pore Diameter | | | |
| | 60 Å | 100 Å | 300 Å | 1,000 Å |
| 3 µm | 03B | 03E-A | 03M | - |
| 5 µm | 05B | 05E-A | 05M | - |
| 10 µm | 07B | 07E-A | 07M | 07T |



HPLC Columns

- Excellent efficiency and column-to-column reproducibility
- Long lifetime
- Broad pH range from 1.5 to 9
- Compatibility with 100 % aqueous and organic mobile phases
- High surface coverage presenting no bleeding for LC/MS applications

SiliaChrom® Analytical and Preparative HPLC Columns

SiliCycle offers a wide range of chromatographic selectivities: reversed-phase, normal phase and ion exchange phase columns for analysis of acidic, neutral and basic compounds. We also have solutions for biochromatography of large molecules and analysis by SFC (Supercritical Fluid Chromatography).

Our R&D group works to continually enhance our portfolio to suit customer’s requirements. Whether you need stability with 100 % aqueous or organic mobile phases or low bleed material for LC/MS applications: we have the solution for you.

All our columns are available in 3, 5 & 10 µm, with internal diameters from 4.6 to 50 mm.

Both our raw materials and finished HPLC columns are QC-validated in our ISO 9001:2015 registered manufacturing facilities.

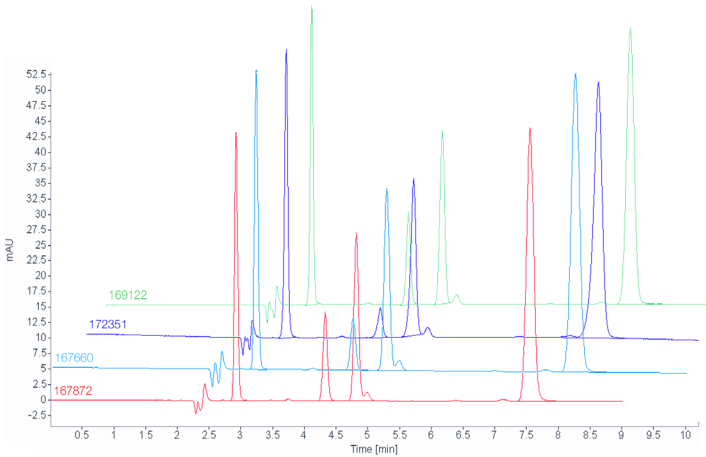
Manufacturing is done following strict SOPs to guarantee:

- Extremely pure silica
- Complete endcapping (meaning reduced silanol activity)
- Controlled surface coverage
- High surface area & loading capacity
- Exceptional chemical and mechanical stability
- Uniform column bed
- Enhanced chromatographic resolution
- Very good peak symmetry
- Robust columns with extended lifetime
- Lot-to-lot & column-to-column reproducibility



Video: HPLC analysis in a nutshell

Lot-to-Lot Reproducibility



| Chromatographic Conditions | |
|----------------------------|---|
| Parameter | Value |
| Column | SiliaChrom Plus C18, 3 µm, 100 Å |
| Dimensions | 4.6 x 150 mm |
| Part number | HPLC-S03203E-A-N150 |
| Mobile phase | Methanol:water (90:10) |
| Temperature | 25°C |
| Flow rate | 0.6 mL/min |
| Detector | UV at 254 nm |
| Injection volume | 1.0 µL |
| Sample | Peaks from left to right: acetophenone, toluene, naphthalene and anthracene |

Portfolio

| HPLC Columns Portfolio | | |
|--|--|--|
| SiliaChrom Plus | | SiliaChrom dt |
| For Your Everyday Separations | | 100 % Aqueous Compatible |
| <div><div><div>C18 & C18-300 (USP L1)</div><div>C8 & C8-300 (USP L7)</div><div>PFP (USP L43)</div><div>Phenyl (USP L11)</div></div><div><div>Cyano (USP L10)</div><div>Amine (USP L8)</div><div>Silica & Silica-300 (USP L3)</div></div></div> | | <div><div>C18 (USP L1)</div></div> |
| Main Characteristics | | |
| <div><div>Wide range of selectivities</div><div>Ultra pure metal-free silica (99.999 % purity)</div><div>High column performance and resolution</div><div>Enhanced batch-to-batch reproducibility</div><div>Extended column lifetime</div><div>Reduced silanol activity, better peak symmetry</div><div>Extremely low bleeding for LC/MS applications</div><div>Easy scale-up to preparative formats</div></div> | | <div><div>Ultra pure metal-free silica (99.999 % purity)</div><div>High sensitivity for LC/MS</div><div>Stable from 100 % aqueous to 100 % organic mobile phase</div><div>Universal: acidic, neutral, and basic analysis</div><div>Enhanced retention of hydrophilic molecules</div><div>Inertness for acidic and basic analytes</div></div> |

Selection Guide by USP Code

The table below will help you select the right column for different United States Pharmacopeia (USP) codes.

| Selection Guide by USP Code | | | |
|-----------------------------|--|---|--|
| USP Code | Packing Type | Description | SiliCycle Phase |
| L1 | Bonding: Octadecyl (C18) Particle size: 1.5 - 10 µm (silica) | Octadecyl silane chemically bonded to porous silica or ceramic micro-particles, 1.5 to 10 µm in diameter, or a monolithic rod. | SiliaChrom Plus C18 SiliaChrom dt C18 |
| L3 | Bonding: Silica Particle size: 1.5 - 10 µm (silica) | Porous silica particles, 1.5 to 10 µm in diameter, or a monolithic silica rod. | SiliaChrom Plus Silica |
| L7 | Bonding: Octyl (C8) Particle size: 1.5 - 10 µm (silica) | Octylsilane chemically bonded to totally or superficially porous silica particles, 1.5 to 10 µm in diameter, or a monolithic rod. | SiliaChrom Plus C8 |
| L8 | Bonding: Amine (NH ₂) Particle size: 1.5 - 10 µm (silica) | An essentially monomolecular layer of aminopropylsilane chemically bonded to totally porous silica gel support, 1.5 to 10 µm in diameter. | SiliaChrom Plus Amine |
| L10 | Bonding: Nitrile (CN) Particle size: 1.5 - 10 µm (silica) | Nitrile groups chemically bonded to porous silica particles, 1.5 to 10 µm in diameter. | SiliaChrom Plus Cyano |
| L11 | Bonding: Phenyl Particle size: 1.5 - 10 µm (silica) | Phenyl groups chemically bonded to porous silica particles, 1.5 to 10 µm in diameter. | SiliaChrom Plus Phenyl |
| L12 | Bonding: Strong anion exchange Particle size: 30 - 50 µm (silica) | A strong anion-exchange packing made by chemically bonding a quaternary amine to silica particles, 30 to 50 µm in diameter. | SiliaSphere PC SAX (bulk) |
| L27 | Bonding: Silica Particle size: 30 - 50 µm (silica) | Porous silica particles, 30 to 50 µm in diameter. | SiliaSphere PC Silica (bulk) |
| L43 | Bonding: Pentafluorophenyl (PFP) Particle size: 3 - 10 µm (silica) | Pentafluorophenyl groups chemically bonded to silica particles by a propyl spacer, 1.5 to 10 µm in diameter. | SiliaChrom Plus PFP |

SiliaChrom Plus and dt Family

SiliaChrom Plus and dt phases were designed to help you with your everyday analyses, requiring improved performance and resolution. The perfectly controlled particle size, pore size and bonding coverage of these phases allow for better reproducibility and scalability of your methods. All SiliaChrom Plus and dt columns are available in 3, 5 & 10 µm.

Sorbent Characteristics

| SiliaChrom Plus and dt Sorbent Characteristics | | | | | | | | |
|--|--|------|------------|---------------|---------------------|-----------|------------------|----------------------|
| SiliaChrom Phase | Description | % C* | Endcapping | Pore Size (Å) | Surface Area (m²/g) | pH Range | Temp. Limit (°C) | Pressure Limit (psi) |
| Reversed-Phases | | | | | | | | |
| SiliaChrom Plus C18 | 2 levels of hydrophobicity for almost all your everyday analytes. | 17 | Yes | 100 | 370 - 430 | 2.0 - 8.0 | 60 | 5,500 |
| SiliaChrom Plus C8 | | 10 | Yes | 100 | 370 - 430 | 2.0 - 8.0 | 60 | |
| SiliaChrom Plus C18-300 | 2 levels of hydrophobicity to separate your larger analytes. | 8 | Yes | 300 | 80 - 120 | 2.0 - 8.0 | 60 | 4,000 |
| SiliaChrom Plus C8-300 | | 5 | Yes | 300 | 80 - 120 | 2.0 - 8.0 | 60 | |
| SiliaChrom Plus PFP | Highly retentive phase for aromatic and polar compounds. | 11 | Yes | 120 | 320 - 360 | 2.0 - 8.0 | 60 | 5,500 |
| SiliaChrom Plus Phenyl | Highly retentive phase for aromatic and unsaturated compounds. | 9 | Yes | 100 | 370 - 430 | 2.0 - 8.0 | 60 | 5,500 |
| Normal Phases | | | | | | | | |
| SiliaChrom Plus Silica | Designed for normal phase conditions, to analyse small polar compounds. | - | - | 100 | 370 - 430 | 2.0 - 8.0 | 60 | 5,500 |
| SiliaChrom Plus Silica-300 | Designed for normal phase conditions, to analyse larger polar compounds. | - | - | 300 | 80 - 120 | 2.0 - 8.0 | 60 | 4,000 |
| SiliaChrom Plus Cyano | For small polar analytes, works in normal and reversed-phase conditions. | 7 | Yes | 100 | 370 - 430 | 2.0 - 8.0 | 60 | 5,500 |
| SiliaChrom Plus Amine | Recommended for normal phase analysis, especially for sugar analysis. | 8 | Yes | 100 | 370 - 430 | 2.0 - 8.0 | 60 | 5,500 |
| dt Phase | | | | | | | | |
| SiliaChrom dt C18 | Separation of hydrophobic molecules in aqueous or organic conditions. Compatible with 100 % aqueous and 100 % organic mobile phases. | 18 | Yes | 100 | 410 - 440 | 1.5 - 9.0 | 60 | 5,000 |

* Typical value



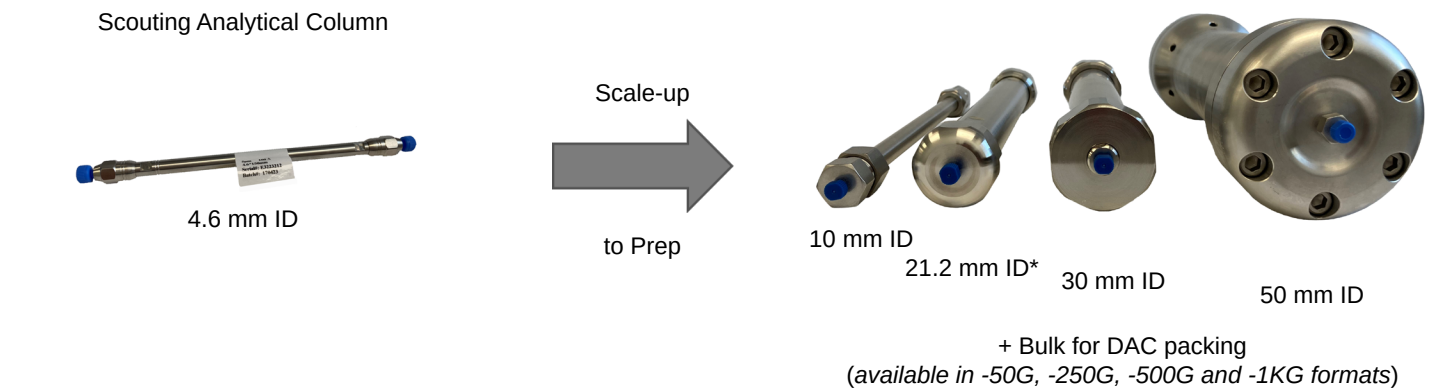
Scaling up Using SiliaChrom Plus Preparative HPLC Columns

All our HPLC phases are also offered in preparative formats (up to 50 mm internal diameter and 250 mm length).

Screen and Scout

In large scale purification using high pressure chromatography, it is paramount to first screen & scout analytical columns and then be able to scale-up using prep columns of the same silica (phase and particle size).

SiliCycle addresses this market reality by staying both flexible and readily available to respond to customers' needs and meet their expectations. Columns are available in analytical dimensions (4.6 mm ID) and preparative dimensions (10, 21.2*, 30 and 50 mm ID). In addition, bulk silica is also offered to allow DAC packing, giving the purification scientist even more options.



* 20 mm ID for the dt phase

Scaling Up and Scaling Down Theory

When your experimental conditions are well optimized to get the most suitable purification, it is possible to scale up / down your method by keeping the same particle size and sorbent using these two equations:

Adjustment of Sample Load:

x2 = (x1 x r2^2 x CL) / r1^2 where [CL = L2 / L1]

Where:

- x1 = maximum sample load on initial column
- x2 = maximum sample load on final column
- L1 = length of initial column
- L2 = length of final column
- r1 = radius of initial column
- r2 = radius of final column

Adjustment of Flow Rate:

V2 = (V1 x r2^2) / r1^2

- V1 = flow rate used with initial column
- V2 = flow rate used with final column

To help you choose the right dimensions for your project, here is a chart giving you typical sample loadings and flow rates.

| Choosing the Right SiliaChrom Preparative Column | | | | | |
|--|---------|---------|--------------|-----------|-------------|
| Length | 4.6 mm | 10 mm | 20 - 21.2 mm | 30 mm | 50 mm |
| 50 mm | 0.5 - 2 | 2 - 10 | 10 - 45 | 20 - 90 | 50 - 250 |
| 100 mm | 1 - 5 | 4 - 25 | 20 - 105 | 40 - 210 | 120 - 600 |
| 150 mm | 2 - 7 | 10 - 35 | 30 - 160 | 70 - 300 | 200 - 850 |
| 250 mm | 3 - 10 | 12 - 45 | 60 - 220 | 120 - 430 | 320 - 1,200 |
| Typical Flow Rate (mL/min) | | | | | |
| | 1 - 1.5 | 4 - 10 | 20 - 30 | 40 - 70 | 110 - 250 |

Phases for Supercritical Fluid Chromatography (SFC)

Supercritical Fluid Chromatography (SFC) is a green chromatographic technique for the separation of complex mixtures. Because there is no need for solvent disposal in SFC, ecologically concerned laboratories have considered SFC as an interesting alternative for analytical and purification applications.

The principles of SFC are extremely similar to those of normal phase chromatography but it uses CO2 as the main mobile phase or supercritical fluid. In fact, both techniques use the ability of the mobile phase to solvate the analytes. Changing from a liquid mobile phase to carbon dioxide greatly increases the resolution. Modifiers used in the mobile phase interact with the stationary phase creating a final surface changing the analyte selectivities.

For decades, SFC has been considered the preferred technique for preparative chromatography. The recent advances in preparative and analytical equipment for SFC coupled with the industry demand for reliable rapid analysis chromatography has created the need for a dependable source of SFC columns. The use of carbon dioxide based mobile phases enables the use of high performance preparative columns (10 to 50 mm ID), with a variety of particle sizes from 3 to 10 µm. The most commonly used particle size is 5 µm because it is small enough to give high performances and resolution (smaller particles have a tendency to reduce permeability and increase the column back-pressure).

Many SFC separations use stationary phases from normal phase HPLC (such as unmodified silica, amine and cyano), without the need for special packing techniques or hardware. The low viscosity of supercritical CO2 allows separations to occur 3 to 5 times faster with 70 - 90 % less in solvent usage than those of normal phase HPLC. All these considerations have made SFC a desirable preparative chromatographic technique for purifying chemical mixtures.

| SiliaChrom Plus Phases for Supercritical Fluid Chromatography | | | |
|---|-----------|------|---------------|
| SiliaChrom Plus Phase | Pore Size | % C | Particle Size |
| SiliaChrom Plus Silica | 100 Å | - | 3, 5, 10 µm |
| SiliaChrom Plus Cyano | 100 Å | 7 % | 3, 5, 10 µm |
| SiliaChrom Plus Amine | 100 Å | 8 % | 3, 5, 10 µm |
| SiliaChrom Plus PFP | 120 Å | 11 % | 3, 5, 10 µm |

SiliaChrom Plus HPLC Columns for Biochromatography

Separation and characterization of proteins, peptides and nucleic acids can be done through different chromatographic techniques. This section highlights the SiliaChrom Plus HPLC columns available for biochromatography.

SiliaChrom Plus Reversed-Phases for Biomolecules (MW < 5,000 Da)

| SiliaChrom Plus Reversed-Phases for Biochromatography (MW < 5,000 Da) | | | |
|---|-----------|------|---|
| SiliaChrom Plus Phase | Pore Size | % C | Characteristics |
| SiliaChrom Plus C18 | 100 Å | 17 % | Most popular C18 for almost all your everyday purifications |

SiliaChrom Plus Reversed-Phases for Large Biomolecules (MW < 100,000 Da)

| SiliaChrom Plus Reversed-Phases for Biochromatography (MW < 100,000 Da) | | | |
|---|-----------|-----|---|
| SiliaChrom Plus Phase | Pore Size | % C | Characteristics |
| SiliaChrom Plus C18-300 | 300 Å | 8 % | C18 phase, with wide pore size, specially designed for peptide & protein separation |
| SiliaChrom Plus C8-300 | 300 Å | 5 % | C8 phase, with wide pore size, presenting lower hydrophobicity than C18 |

SiliaChrom Plus Guard Cartridges and Holders

SiliaChrom Plus HPLC Guard Cartridges are designed to effectively protect both analytical and preparative HPLC columns. The use of these cartridges is highly recommended to protect and prolong column lifetime. While saving you time and money, it has limited influence on your chromatography.

SiliaChrom Plus Guard Cartridges are extremely cost effective and easy to use as a pre-filter to remove contaminants prior to your injections. Contaminants have an extremely high impact and can cause the following:

- Higher back-pressure
- Loss of resolution
- Peak shape alterations (tailing or peak splitting)
- Irreversible damages (column and / or system)
- Baseline noise or drift

Packing Material and Dimensions Recommendations

For optimal results and maximal protection, it is highly recommended to always use a guard cartridge packed with the same material than the HPLC column, or at least with the same chemistry. Particle size should match the HPLC column one, but it can also be chosen smaller. Never use a guard cartridge with bigger particles than the HPLC column (efficiency loss).

SiliaChrom Plus Guard Cartridges are available in different lengths (10 and 20 mm) and various internal diameters. 20 mm length will be useful when samples contain important quantity of impurities. The guard cartridge internal diameter should be the same as the HPLC column, or slightly smaller. Never use a guard cartridge with a larger ID than the HPLC column (efficiency loss).

Installation Procedure

- If a new capillary tubing has been installed, flush the lines free of particulate before connecting the guard system.
- Insert the stainless cartridge with the PEEK encapsulated SS frits into the metallic guard cartridge holder.
- Tighten both parts of the holder together using 2 wrenches. Take care not to overtighten to avoid cold welding together the 2 threaded metallic parts.
- Connect the assembled SiliaChrom Plus Guard Holder into the male fitting of the HPLC tuning system.
- Connect the outlet to the detector and start pumping the mobile phase at a low flow rate to equilibrate the system.
- Gradually increase the flow rate to working conditions and check for leaks. If the leak persists at the guard column level, retighten the fittings until the leak stops.



Guard Cartridges Holder Selection Guide

| How to Choose your SiliaChrom Plus Guard Holder | | | | |
|--|--------|-------|---------------|-------------|
| 1) SiliaChrom Plus HPLC Column Internal Diameter (ID): | 4.6 mm | 10 mm | 20 to 21.2 mm | 30 to 50 mm |
| 2) SiliaChrom Plus Guard Cartridge adequate ID to use: | 4.0 mm | 10 mm | 21.2 mm | 30 mm |
| 3) SiliaChrom Plus Guard Holder adequate PN to order: | | | | |
| HPH-N010 (10 mm length) | ✓ | | | |
| HPH-N020 (20 mm length) | ✓ | | | |
| HPH-Q010 (10 mm length) | | ✓ | | |
| HPH-T010 (10 mm length) | | | ✓ | |
| HPH-V010 (10 mm length) | | | | ✓ |

SiliaChrom Ordering Information

This section will show you how to build your HPLC column product number.

SiliaChrom Plus and dt HPLC Columns Ordering Information

How to build your PN for HPLC Column: **HPLC-[Phase]-[Format]***
Example: HPLC-S38003E-A-N150 for a SiliaChrom Plus Cyano 3 µm, 4.6 x 150 mm HPLC column*

* Note: for dt C18 you just need to write **[Phase]-[Format]**
Example: H141805E-N250 for a SiliaChrom dt C18 5 µm, 4.6 x 250 mm HPLC column

Phase Codes

| SiliaChrom Plus and dt Phase Codes | | | |
|------------------------------------|---------------|-----------|-----------|
| Phase | Particle Size | | |
| | 3 µm | 5 µm | 10 µm |
| C18 | S03203E-A | S03205E-A | S03207E-A |
| C18-300 | S03203M | S03205M | S03207M |
| C8 | S30803E-A | S30805E-A | S30807E-A |
| C8-300 | S30803M | S30805M | S30807M |
| PFP | S67503G-A | S67505G-A | S67507G-A |
| Phenyl | S34003E-A | S34005E-A | S34007E-A |
| Silica | S10003E-A | S10005E-A | S10007E-A |
| Silica-300 | S10003M | S10005M | S10007M |
| Cyano | S38003E-A | S38005E-A | S38007E-A |
| Amine | S52003E-A | S52005E-A | S52007E-A |
| dt C18 | H141803E | H141805E | H141807E |

Format Codes

| HPLC Column Format Codes | | | | | |
|--------------------------|---------|-------|------|------|-------|
| Dimension | Qty/box | Code | 3 µm | 5 µm | 10 µm |
| 4.6 x 50 mm | 1 | N050 | ✓ | ✓ | |
| 4.6 x 100 mm | 1 | N100 | ✓ | ✓ | |
| 4.6 x 150 mm | 1 | N150 | ✓ | ✓ | ✓ |
| 4.6 x 250 mm | 1 | N250 | ✓ | ✓ | ✓ |
| 10 x 150 mm | 1 | Q150 | | ✓ | ✓ |
| 10 x 250 mm | 1 | Q250 | | ✓ | ✓ |
| 20 x 150 mm* | 1 | Y150* | | ✓ | ✓ |
| 20 x 250 mm* | 1 | Y250* | | ✓ | ✓ |
| 21.2 x 150 mm | 1 | T150 | | ✓ | ✓ |
| 21.2 x 250 mm | 1 | T250 | | ✓ | ✓ |
| 30 x 150 mm | 1 | V150 | | ✓ | ✓ |
| 30 x 250 mm | 1 | V250 | | ✓ | ✓ |
| 50 x 150 mm | 1 | W150 | | | ✓ |
| 50 x 250 mm | 1 | W250 | | | ✓ |

* Only available with dt C18 phase.

SiliaChrom Plus Guard Cartridges Ordering Information

How to build your PN: **HPLG-[Phase]-[Format]**
Example: HPLG-S30807E-A-N010 for a SiliaChrom Plus C8 10 µm, 4.0 x 10 mm guard cartridge

Format Codes

| SiliaChrom Plus and dt Guard Cartridges Format Codes | | | | | |
|--|---------|------|------|------|-------|
| Dimension | Qty/box | Code | 3 µm | 5 µm | 10 µm |
| 4.0 x 10 mm | 4 | N010 | ✓ | ✓ | ✓ |
| 4.0 x 20 mm | 4 | N020 | ✓ | ✓ | ✓ |
| 10 x 10 mm | 2 | Q010 | | ✓ | ✓ |
| 21.2 x 10 mm | 1 | T010 | | ✓ | ✓ |
| 30 x 10 mm | 1 | V010 | | ✓ | ✓ |



HPLC Columns Selection & Operation

Important HPLC Definitions and Equations

- Capacity Factor or Retention Factor (*k'*) is measured by the retention time of the analyte compared to an unretained peak (*void volume marker*) using the following equation:

$$k' = \frac{(T_R - T_0)}{T_0}$$
Where T_R = retention time of the analyte
 T_0 = retention time of the unretained product

- Efficiency (*N*) is usually measured by the plate count (*also called theoretical plates number*) using various equations:

By USP (United States Pharmacopeia)
N = 16 x [T/W]^2
Where: N = number of theoretical plates
T = retention time of the analyte
W = width at the base of the analyte's peak

By DAB (German Pharmacopeia)
N = 5.54 x [T/W_0.5]^2
Where: N = number of theoretical plates
T = retention time of the analyte
W_0.5 = width-at-half-height of the analyte's peak

- Selectivity (α) is measured by the retention factor ratio between two similar compounds:

α = k'_1 / k'_2
Where: k'_1 = retention factor of product #1
k'_2 = retention factor of product #2

Separation's difficulty based on α value:
≥ 2 Easy separation
1.5 - 2 Possible separation (method adjustment could be required)
1.2 - 1.5 Difficult separation
≤ 1.2 Very difficult separation (selectivity optimization may be required)

- Resolution (R) can be expressed using the two following equations:

R = (sqrt(N)/4) x ((α-1)/α) x (k/(k+1))
Where: N = theoretical plates number
α = selectivity
k = retention factor

R = (2(T_2 - T_1)/(W_2 + W_1))
Where: T_1 = retention time of product #1
T_2 = retention time of product #2
W_1 = width at the base of product #1's peak
W_2 = width at the base of product #2's peak

Influencing Factors in HPLC

To choose the most suitable HPLC column, various parameters need to be taken into account: desired selectivity, sample load, efficiency and resolution. All these parameters are influenced by different factors in HPLC, summarized in the table below.

Table with 4 columns: Properties, Typical Parameters, Affected Influencing Factors, Limitations. Rows include Chromatographic Conditions (Solvent, pH, Flow Rate), Packing Characteristics (Chemistry, Pore Size, Particle Size), and HPLC Column Dimensions (Internal Diameter, Length).

How to Select the Right SiliaChrom Plus HPLC Column

Read the section below to select the most appropriate SiliaChrom Plus HPLC column to try first in your method development. However, before going forward in the selection, you need to have an idea of the sample quantity you need to purify as well as the HPLC equipment available.

Remember: Resolution R = (sqrt(N)/4) x ((α-1)/α) x (k/(k+1))

Step 1. Find the desired selectivity by choosing the chemistry

When chosing an HPLC column, the most important factor to achieve optimal resolution is the selectivity. A good knowledge of the composition of the sample mixture is crucial to select the most suitable chromatography mode and maximize interactions between sorbent and compounds. Please refer to previous sections to choose the most suitable phases to get optimal separation results.

Step 2. Select the pore diameter

To select the right pore diameter to use, find out the molecular weight of the solute. For small molecules (molecular weights below 5,000 Da), 100 - 150 Å pore size is usually recommended. For larger molecules, such as peptides and proteins, 300 Å or higher is recommended.

Step 3. Find the desired efficiency & resolution

The goal now is to separate your sample with the shortest possible analysis time AND optimal efficiency.

Two factors can influence the efficiency of a chromatography:

- Particle size: influence on resolution and back-pressure
- Column dimensions (internal diameter & length): influence on resolution and sample load

Step 3.1. Select the particle size

For analytical applications, different particle sizes are available. The most common one is the 5 µm due to a good price / performance ratio. However, if you require a better separation and want to decrease analysis time, then 3 µm would be a better choice. Keep in mind that with a smaller particle size the back-pressure will be higher. For preparative applications, a larger particle size is usually chosen. The most frequently used is 10 µm.

Step 3.2 Select column dimensions

For analytical applications, the most recommended format for initial trial is 4.6 x 150 mm. Then, if you need more resolution, you can increase column length.

Step 3.2.1 Select the internal diameter (influence on sample load)

With smaller internal diameters, solvent consumption is reduced due to lower flow rate required, but analysis time is increased. Furthermore, loading capacity is decreased as the diameter decreases. The table below identifies typical applications associated with typical internal diameters used in HPLC.

Table with 5 columns: Type of Column, ID (mm), Typical Sample Load, Typical Flow Rate, Typical Applications. Rows include Analytical, Semi-Preparative, and Preparative columns with their respective specifications.

Step 3.2.2 Select the column length (influence on resolution)

The rule of thumb is that with the same packing, longer columns provide better resolution and efficiency over shorter ones, but also longer retention times and higher pressure. In general, it is preferable to try using the shortest column length possible. If resolution is not good enough, increase column length or use a smaller particle size with the same length. The table below presents the most suitable length / particle combinations:

Table with 3 columns: Length (mm), Most Suitable Particle Size (µm), Typical Applications. Rows include 50, 100 & 150, and 200 & 250 mm column lengths.

SiliaChrom Cleaning and Regeneration Procedures

If adequate care is taken, it is possible to maintain column efficiency over an extended period of time.

We usually make the assumption that, after a separation, all the sample injected in the column has been eluted. However, some impurities that are strongly retained on the column can accumulate at the inlet, if the mobile-phase composition is not strong enough to elute them during a regular run.

Some non-negligible problems can arise when this happens:

- loss of performance
- retention time shift
- back-pressure build up
- baseline drift
- peak tailing

Importance of Cleaning

To avoid these problems, it is highly recommended to perform regular **CLEANING** of the column before any of these symptoms occurs. This process is simple and does not require modification of the usual chromatographic set up. The more you run a cleaning procedure, the less rigorous conditions will be necessary.

When cleaning is not sufficient (*column seems clogged*) or prior to column storage, a more thorough treatment (**REGENERATION**) may be necessary. The flow rate is usually set lower than during the separation (*typically from 20 % to 50 % of the usual one*).

Suggested Cleaning and Regeneration Procedures

Column Volume (*packing's volume included*) in mL = π * [Column Radius in cm]² * [Column Length in cm]

| SiliaChrom Suggested Cleaning and Regeneration Procedures | | |
|--|---|--|
| SiliaChrom HPLC Column | CLEANING Procedure | REGENERATION Procedure |
| Suggested Procedure: | <ul style="list-style-type: none">• Set the flow rate (<i>20 to 50 % of the usual one</i>)• Rinse with 2 - 3 column volumes of each of the following solvents: | <ul style="list-style-type: none">• Backflush the column• Set the flow rate (<i>20 to 50 % of the usual one</i>)• Rinse with 10 - 20 column volumes of each of the following solvents: |
| Reversed-Phase Columns (C18, dt C18, C18-300, C8, C8-300, Phenyl, PFP, Amine, Cyano) | <ul style="list-style-type: none">• Water / methanol (50 / 50) to remove buffers• Methanol• Mobile phase used during the separation | <ul style="list-style-type: none">• Water / methanol (90 / 10)*• Methanol, isopropanol, methanol• Mobile phase used during the separation |
| Normal Phase Columns (Amine, Cyano, Silica) Note: Never use water | <ul style="list-style-type: none">• Isopropanol, hexane• Mobile phase used during the separation | <ul style="list-style-type: none">• Isopropanol, methanol, isopropanol• Hexane• Mobile phase used during the separation |

* For amine and cyano columns, use water / methanol (70 / 30)

Suggested Storage Conditions

When SiliaChrom HPLC Columns are not used for an extended period of time, do not allow high aqueous or high salt mobile phases to remain in the column. Remove aqueous buffers remaining in the column by following the regeneration procedure. Our columns are shipped with two removable column end plugs to prevent drying of the column bed. Always put these plugs back on before column storage.

| SiliaChrom Suggested Storage Conditions | | | |
|---|---|--|----------|
| SiliaChrom HPLC Columns | | Recommended Storage Solvent | |
| Reversed-Phase | <ul style="list-style-type: none">• SiliaChrom Plus C18 & C18-300• SiliaChrom dt C18• SiliaChrom Plus C8 & C8-300• SiliaChrom Plus PFP | <ul style="list-style-type: none">• SiliaChrom Plus Phenyl• SiliaChrom Plus Cyano• SiliaChrom Plus Amine | Methanol |
| Normal Phase | <ul style="list-style-type: none">• SiliaChrom Plus Silica & Silica-300 | <ul style="list-style-type: none">• SiliaChrom Plus Amine• SiliaChrom Plus Cyano | Hexane |

Acceptable Modifications to an HPLC Validated Method

Even if you are following a USP recommended method, some operating conditions can be adjusted if the modifications respect the acceptable changes proposed by Pharmacopeias*. A side-by-side comparison of both the original and the adjusted methods needs to be performed to demonstrate that method's accuracy and precision are not affected by these modifications.

| Acceptable Modifications to an HPLC Validated Method | | |
|--|--|---|
| Parameter | Allowable Modification | Examples of Possible Modifications |
| Mobile phase pH | ± 0.2 units | Validated pH: 7.0 Allowed pH range: 6.8 - 7.2 |
| Concentration of salts in buffer | ± 10 % (<i>if the permitted pH variation is met</i>) | Validated concentration: 20 mM Allowed concentration range: 18 - 22 mM |
| Ratio of components in mobile phase | <p>Only the minor components ≤ (100/<i>n</i>) %, <i>n</i> being the total number of components of the mobile phase, can be adjusted by ± 30 % relative, and cannot exceed ± 10 % absolute (<i>i.e.: in regards to the total mobile phase</i>).</p> <p>Then a sufficient quantity of the 1st component is used to give a total of 100 %.</p> <p>Adjustment can be made to one minor component only in a ternary mixture.</p> | <p>Binary mixtures (n=2): Validated ratio: 50 / 50 Allowed ratio: 40 / 60 to 60 / 40</p> <p>Validated ratio: 2 / 98 Allowed ratio: 1.4 / 98.6 to 2.6 / 97.4</p> <p>Ternary mixtures (n=3): Validated ratio: 70 / 25 / 5 Allowed % of the 2nd component: 17.5 - 32.5 % Allowed % of the 3rd component: 3.5 - 6.5 %</p> |
| Wavelength of UV detector | No modification allowed. | N/A |
| Column length | May be modified provided that the ratio of the column length (<i>L</i>) to the particle size (<i>dp</i>) remains constant or in the range between -25 % and +50 % of the prescribed L/ <i>dp</i> ratio. | <p>Validated length (<i>L</i>): 150 mm Validated particle size (<i>dp</i>): 5 µm So prescribed L/<i>dp</i> ratio = 30</p> <p>Allowed range for L/<i>dp</i> ratio: 22.5 ≤ L/<i>dp</i> ≤ 45 A column L = 100 mm and <i>dp</i> = 3 µm, for example, would be accepted (<i>L/dp ratio</i> = 33.3)</p> |
| Column inner diameter | In absence of a change in particle size and/or length, the internal diameter of the column may be adjusted. | N/A |
| Flow rate | <p>The flow rate is adjusted for both the change in column diameter and particle size (<i>smaller-particle columns will require higher linear velocities for the same performance</i>).</p> <p>For isocratic separations only: after an adjustment due to a change in column dimensions, an additional change in flow rate of ± 50 % is permitted.</p> | <p>Validated flow rate: 1.0 mL/min</p> <p>Allowed flow rate range for isocratic separations: 0.5 - 1.5 mL/min</p> |
| Injection volume | Can be adjusted as far as it is consistent with accepted precision, linearity and detection limits. | N/A |
| Particle size | May be modified provided that the ratio of the column length (<i>L</i>) to the particle size (<i>dp</i>) remains constant or in the range between -25 % and +50 % of the prescribed L/ <i>dp</i> ratio. | <p>Validated length (<i>L</i>): 150 mm Validated particle size (<i>dp</i>): 5 µm So prescribed L/<i>dp</i> ratio = 30</p> <p>Allowed range for L/<i>dp</i> ratio: 22.5 ≤ L/<i>dp</i> ≤ 45 A column L = 100 mm and <i>dp</i> = 3 µm, for example, would be accepted (<i>L/dp ratio</i> = 33.3)</p> |
| Column temperature | <p>For isocratic separations: ± 10°C For gradient separations: ± 5°C</p> | <p>Validated temperature: 23°C Allowed temperature range for isocratic separations: 13 - 33°C Allowed temperature range for gradient separations: 18 - 28°C</p> |

* First supplement to USP 40-NF35, chromatography <621> - System suitability [Link](#)

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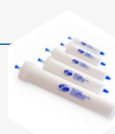
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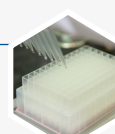
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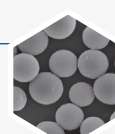
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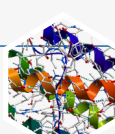
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