Column care and use

Column hardware

HPLC and UHPLC columns from Merck Millipore come in a variety of different column hardware formats and materials for different applications. All columns have 10-32 UNF female end fittings that connect to 1/16" capillary tubing. Note that removing pre-installed end fittings from HPLC columns might damage the column bed and reduce performance.

Particulate silica columns for reversed phase and normal phase HPLC are delivered in stainless steel column hardware; either as ready-to-use Hibar® columns or as the LiChroCART® cartridge system comprising separately ordered, re-usable end fittings (manu-CART®). Hibar® HR columns have extra-high pressure stability and extremely small internal dead volumes, making them especially suitable for use in UHPLC instruments. Both Hibar® and LiChroCART® columns have stainless steel frits to keep the stationary phase particles in place.

Chromolith® columns are cladded with a mechanically stable and chemically robust poly(ether-ether-ketone) polymer (PEEK). The end fittings are made of the same material. Chromolith® columns contain no frits.

Chromolith® CapROD® columns are of fused silica tubing and contain no frits. These columns are delivered without end fittings.

SeQuant® columns have different column hardware depending on the internal diameter. Analytical sizes (2.1, 4.6, and 7.5 mm i.d.) have PEEK hardware with PEEK frits for maximum inertness towards hydrophilic analytes. SeQuant® semi-preparative columns have stainless steel hardware and stainless steel frits. SeQuant® microbore and capillary columns (1.0 and 0.3 mm i.d.) are of glass-lined stainless steel and contain stainless steel frits. SeQuant® nano columns (100 µm and 75 µm i.d.) are of PEEK-sheeted fused silica tubing.

NB! The fused silica tubing column material of SeQuant® nano columns and Chromolith® CapROD® is brittle and should not be exposed to extensive bending as it might break.

NB! Columns in PEEK, i.e. Chromolith® and SeQuant® analytical columns, cannot be used with more than 50% tetrahydrofuran (THF), 5% dichloromethane (DCM) or 5% dimethylsulfoxide (DMSO). Such solvents can, however, be used at 100% as sample solvent.

Column hardware	Type	Body	Frit	Max. pressure	Solvent restrictions
Hibar® RT	Ready-to-use	Stainless steel	Stainless steel	400 bar	
Hibar® HR	Ready-to-use	Stainless steel	Stainless steel	600 bar	
LiChroCART®	Cartridge	Stainless steel	Stainless steel	250 bar	
Chromolith®	Ready-to-use	PEEK	-	200 bar	THF, DMSO, DCM
Chromolith® CapROD®	Ready-to-use	Fused silica	-	200 bar	
SeQuant® Analytical	Ready-to-use	PEEK	PEEK	350 bar*	THF, DMSO, DCM
SeQuant® Semi-prep	Ready-to-use	Stainless steel	Stainless steel	400 bar	
SeQuant® Capillary	Ready-to-use	Glass-lined stainless steel	Stainless steel	400 bar	
SeQuant® Nano	Ready-to-use	PEEK-sheeted fused silica	Stainless steel	400 bar	

*** The maximum pressure for SeQuant® ZIC®-*p*HILIC columns is 200 bar due to the polymer-based particles.**

Column installation

Merck Millipore HPLC and UHPLC columns are designed to fit any HPLC instrument; however, care should be taken at installation so as not to introduce dead volumes in the connections, which would reduce separation efficiency. Note that stainless steel tubing fittings are inflexible and cannot be adapted to different port designs after the first installation, whereas PEEK fittings can be adjusted for different columns several times. Also note that stainless steel fittings and ferrules can damage the end fittings of PEEK column hardware, especially if installed with excessive force by using wrench tools.

Merck Millipore columns should be installed with the flow arrow on the label pointing towards the detector. Before the column outlet is connected to the detector, it is wise to flush the column with mobile phase.

Column equilibration

Proper column equilibration is time well spent as it will give you more consistent results and reduce trouble-shooting. Verify that your mobile phase is miscible with the shipping solvent before starting to flush or equilibrate the column. Gradually increase the flow rate in small steps until it satisfies your conditions. Flush the column with your mobile phase until you obtain a stable baseline. Mobile phases with additives in low concentrations (e.g. ion-pair reagents) may require longer equilibration times.

Reversed phase columns (RP-18, RP-8) are shipped in acetonitrile/water. If the column has dried out during storage or shipping, thoroughly activate the packing by flushing with 10-20 column volumes of pure organic solvent (e.g. acetonitrile) before equilibrating the column with the mobile phase.

Normal phase columns (Si, NH_2, CN, Diol) are shipped with n-heptane/dioxane (99/1). If they are going to be used with aqueous eluents, flush the column with ethanol or 2-propanol before you equilibrate with the mobile phase.

HILIC columns (ZIC®) are shipped with acetonitrile/water (80/20) containing 5 mM ammonium acetate salt. In the event that the column has dried out, flush with 20 column volumes of water at a low flow rate before equilibrating the column with the mobile phase.

Validating column performance

Every HPLC column from Merck Millipore is delivered with a test certificate displaying its separation efficiency and selectivity at the time of manufacturing. Repeating the test periodically is a good way of following trends in performance change over time. Please note that the test instruments have been optimized so as not to be significantly affected by external sources of band broadening, and that things might be different in your system. For optimum separation efficiency minimize the injection volume, detector volume, capillary tubing length, internal diameter and detector response time.

Fast chromatographic peaks from Chromolith®, Purospher® STAR UHPLC and SeQuant® ZIC®-HILIC columns can be just a few seconds wide. Note that for accurate representation of a chromatographic peak the data system needs to enable approximately 20 data points to be acquired during the peak width time.

Mobile phases

Merck Millipore's silica-based particulate HPLC columns in stainless steel hardware are compatible with all organic solvents in pH range mentioned in the table below. However, a few restrictions on the use of THF, DCM and DMSO apply to columns in PEEK hardware (i.e. Chromolith® and SeQuant®), see table above.

For best results, high-quality solvents such as HPLC-grade LiChrosolv® should be used. All prepared buffers should be filtered through a 0.45 µm filter (0.22 µm for UHPLC columns) before use in the HPLC system. Always keep in mind that your column will collect any particulate material that enters the flow stream. The use of non-pure solvents will result in adsorption of impurities on the column head. These impurities block adsorption sites, change the selectivity of the column and lead to peak splitting in the chromatogram. In gradient elution, impure solvents may result in ghost peaks that always appear at the same position in the chromatogram.

Any type of buffer, organic modifier and paired-ion reagent will be compatible with Merck Millipore's HPLC columns as long as the appropriate pH range is not exceeded. Verify that solvents are miscible when changing mobile phases and that no buffer precipitation will occur. Ion-pair reagents are often difficult to flush completely from the column and columns used with these reagents should be dedicated to the particular analysis involved. Ion-pair reagents are also known to reduce sensitivity in mass spectrometry detection.

NB! Ion-pair reagents are not suitable for HILIC columns since they will make the stationary phase less polar and thus diminish retention.

NB! The limited solubility of some buffers (e.g. phosphate) in organic solvents may limit their use in HILIC separations and precautions should thus be taken to avoid precipitation problems.

Column lifetime

Column lifetime is highly dependent on the sample and conditions, and cannot be generalized upon; however, you can apply some general measures to increase the lifetime of the column.
Make sure that your sample and mobile phase are clean and particle-free. Always degas and filter mobile phases. Clean up your sample prior to analysis using filtration or more advanced sample preparations if your sample contains large amounts of contaminants. The use of guard columns is always recommended for real samples.

Pressure stability

Pressure limits for different column formats are listed in the table above. All stationary phases are specified to the same or higher pressures than the hardware except for polymer-based SeQuant® ZIC®-*p*HILIC, which is pressure-stable up to 200 bar.

pH stability

Silica-based stationary phases have a limited pH stability. A pH higher than the limit will dissolve the silica, creating voids in the column. A lower pH can strip away some of the bonded phase resulting in defects that will cause changes in retention times and loss of resolution. pH stability ranges for stationary phases from Merck Millipore are presented in the table below.

Do not use strong acids (e.g. hydrochloric, nitric, and sulfuric acids) in the column. Limit your use of strong bases (e.g. sodium, potassium, ammonium hydroxide) to amounts needed to adjust the pH of the mobile phase.
When measuring the pH of mobile phases, the measurement should be done in the aqueous media before mixing the eluent with organic solvents. Although this will not give the actual pH in the mixed aqueous-organic solvent, it will give more consistent results than a mixed mobile phase.

Stationary phase	pH stability range	Max. temperature
LiChrospher®	2-7.5	60°C
Superspher®	2-7.5	60°C
LiChrosorb®	2-7.5	60°C
Chromolith®	2-7.5	45°C
Purospher®	2-8	65°C
Purospher® STAR RP-18e and RP-8e	1.5-10.5	65°C
SeQuant® ZIC®-HILIC	2-8	70°C
SeQuant® ZIC®-*p*HILIC	2-12	50°C
Aluspher® RP-select B	2-12	30°C

Temperature stability

The maximum operating temperatures are stated in the table above. To avoid band broadening and loss of separation efficiency the mobile phase should always be kept at the same temperature as the column. This can be done either through the use of active heaters or by passive heating using a short piece of capillary tubing within the column oven.

Storing the column

For short-term storage (overnight), HPLC columns can be stored in the eluent. Always confirm that the column end plugs are firmly in place, regardless of how long the column will be stored. When columns are stored for several days or longer, reversed-phase columns should be stored in an organic solvent, preferably acetonitrile, containing less than 50% water and no buffer. Purospher® STAR RP-8 endcapped and Purospher® STAR RP-18 endcapped columns are best stored in 100% acetonitrile. If you are changing storage solvent and your last reversed-phase mobile phase contained buffer salt, flush the column with 10 column volumes of water before storing in organic eluent. Buffer salts might not be soluble in high concentrations of organic solvent and might precipitate and block the column or capillary tubing.

Separation mode	Phases	Short-term storage	Long-term storage
RP [reversed-phase]	• LiChrosorb® RP-8, RP-18, (Diol, CN, NH_2)* • LiChrospher® RP-8, RP-18, (Diol, CN, NH_2)* • Purospher® STAR RP-8e, RP-18e, (NH_2)*	Mobile phase	Acetonitrile or acetonitrile in water (<50%)
NP [normal phase]	• LiChrosorb® Si, Diol, CN, NH_2 • LiChrospher® Si, Diol, CN, NH_2 • Purospher® STAR Si, NH_2 • Chromolith® Si	Mobile phase	n-Heptane or similar organic solvent
HILIC [hydrophilic interaction]	• SeQuant® ZIC®-HILIC • SeQuant® ZIC®-*p*HILIC	Mobile phase	80% acetonitrile in water or dilute buffer

*** When used in RP mode.**

Column regeneration

Exposure of a column to samples or solvents containing highly adsorptive components will result in increased back-pressure and a change in selectivity. Often the column can be restored to original performance by suitable wash protocols. When performing solvent rinse regeneration, the column should be reversed and transferred from the analytical HPLC system to a simple, inexpensive pump. Alternatively, disconnect the column from the detector and rinse directly to waste. Each solvent should be rinsed with a minimum of 20, preferably 30, column volumes.

Separation mode	Phases	Wash sequence	Comments
RP [reversed-phase]	• LiChrosorb® RP-8, RP-18, (Diol, CN, NH_2)* • LiChrospher® RP-8, RP-18, (Diol, CN, NH_2)* • Purospher® STAR RP-8e, RP-18e, (NH_2)*	• Water • Acetonitrile • 2-Propanol + 0.1% formic acid • Heptane • 2-Propanol + 0.1% formic acid • Acetonitrile • Mobile phase	* When used in RP mode.
NP [normal phase]	• LiChrosorb® Si, Diol, CN, NH_2 • LiChrospher® Si, Diol, CN, NH_2 • Purospher® STAR Si, NH_2 • Chromolith® Si	• Heptane • Chloroform • Ethanol or 2-propanol • Chloroform • Heptane • Mobile phase	Sequence of dry solvents
HILIC [hydrophilic interaction]	• SeQuant® ZIC®-HILIC • SeQuant® ZIC®-*p*HILIC	• Water** • 0.5 M NaCl or another salt • Water • Mobile phase	** Double the initial water rinse

Calculation of column void time

Knowledge of the void time tm is important for the calculation of chromatographic parameters like k and u.
The void time may be calculated from the volume of the empty column V_{empty} the volume flow f_c and the porosity of the carrier material. The total porosity of a column is the volume fraction occupied by the mobile phase.

$e = V_m/V_{empty}$
$t_m = V_{empty}\ e/f_c$

For totally porous materials like silica and modified silica, e is between 0.7 and 0.8. The void time may also be determined by measuring the retention time of non-retarded sample substances. Suitable substances for measuring the void time are:

Determination of column void time

Reversed-phase: UV detection: thiourea. RI detection: D_2O, CD_3OH, CD_3CN, eluent itself.
Normal phase: UV detection: benzene, tetrachloroethylene; RI detection: cyclohexane, benzene. When using very weak solvents, benzene and tetrachloroethylene may also be retained.
HILIC: toluene or naphthalene

How to use the right column

The fundamental equation for chromatographic resolution (R_S) is an aid for the selection of a suitable combination of stationary phase and column size.

$$R_S = \frac{1}{4}\left(\frac{k}{1+k}\right)\left(\frac{\alpha-1}{1+k}\right)\sqrt{N}$$

The calculation of the individual contributions under different conditions shows what influence the different parameters exert. Below table shows that with the correct choice of chromatographic system, good separations can be achieved even at relative low plate numbers. On the other hand even with extremely high plate numbers a satisfactory separation can not be obtained with poor separation factors.

Individual contributions of the chromatographic resolution

$k\ \left(\frac{k}{1+k}\right)$;	$\alpha\ \left(\frac{\alpha-1}{\alpha}\right)$;	$N\ \left(\frac{\sqrt{N}}{4}\right)$	R_S for N = 1,000	R_S for N = 5,000	R_S for N = 10,000
1 (0.5)	1.05 (0.05)	1,000 (7.9)	0.20	0.4	0.6
3 (0.75)		5,000 (17.7)	0.30	0.7	0.9
5 (0.83)		10,000 (25.0)	0.33	0.7	1.0
10 (0.91)			0.36	0.8	1.1
1	1.1 (0.09)		0.36	0.8	1.1
3			0.50	1.2	1.7
5			0.60	1.3	1.9
10			0.65	1.4	2.0
1	1.2 (0.16)		0.60	1.4	2.0
3			0.95	2.1	3.0
5			1.00	2.3	3.3
10			1.10	2.6	3.6
1	1.3 (0.23)		0.90	2.0	2.9
3			1.40	3.0	4.3
5			1.50	3.4	4.8
10			1.60	3.7	5.2
1	1.5 (0.33)		1.30	2.9	4.1
3			1.90	4.4	6.2
5			2.20	4.8	6.8
10			2.40	5.3	7.5

Empty column volumes

Column [length x i.d.]	Volume	Washing volume [10 column volume]
125 x 2 mm	0.4 mL	4 mL
250 x 2 mm	0.8 mL	8 mL
125 x 3 mm	0.9 mL	9 mL
250 x 3 mm	1.8 mL	18 mL
100 x 4.6 mm	1.7 mL	17 mL
125 x 4 mm	1.6 mL	16 mL
150 x 4.6 mm	2.5 mL	25 mL
250 x 4 mm	3.2 mL	32 mL
250 x 4.6 mm	4 mL	40 mL