

## Particulate analytical HPLC columns

(LiChrosorb®, Lichrospher®, Superspher®, Purospher® and Aluspher®)

### General information and guidelines for care and use

Thank you for choosing an analytical HPLC column supplied by Merck KGaA, Darmstadt, Germany. Each column is individually packed and tested to ensure superior performance documented in the certificate of analysis containing a test chromatogram, column number, sorbent Lot number and important performance data specific to your column.

Please examine your column for possible damage caused in transit. If damage has occurred, immediately notify your local representative and the delivery carrier.

### Column hardware and pressure stability

HPLC and UHPLC columns supplied by Merck KGaA, Darmstadt, Germany 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.

|             | Column type  | Holder needed   | Pressure stability |
|-------------|--------------|-----------------|--------------------|
| LiChroCART® | Cartridge    | yes (manuCART®) | 250 bar            |
| Hibar® RT   | HPLC Column  | no              | 400 bar            |
| Hibar® HR   | UHPLC Column | no              | 1000 bar           |

### Calculation of column void time

Knowledge of the void time  $t_m$  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  $f_v$  and the porosity of the carrier material. The total porosity of a column is the volume fraction occupied by the mobile phase.

$$e = V_m/V_{empty}$$

$$t_m = V_{empty} e/f_v$$

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.

### Determination of column void time

Adsorption chromatography on silica gel: UV detection: benzene, tetrachloroethylene; RI detection: cyclohexane, benzene. When using very weak solvents, benzene and tetrachloroethylene may also be retarded. Reversed-phase HPLC: UV detection: thiourea. RI detection: D<sub>2</sub>O, CD<sub>3</sub>OH, CD<sub>3</sub>CN, eluent itself.

### Column equilibration

Proper column equilibration is time well spent as it will give you more consistent results and reduce trouble-shooting. This is of special importance with LiChroCART® cartridges, because the columns are not closed and can dry out easily. LiChroCART® cartridges need to be flushed properly before use.

Verify that your mobile phase is miscible with the shipping solvent before starting to flush or equilibrate the column.

### Empty column volumes

| Column length (mm) | Column id (mm) | Column volume (mL) | Min. washing volume (mL) (10 column volume) |
|--------------------|----------------|--------------------|---|
| 125                | 2              | 0.4                | 4   |
| 250                | 2              | 0.8                | 8   |
| 30                 | 2.1            | 0.10               | 1   |
| 50                 | 2.1            | 0.17               | 1.7   |
| 100                | 2.1            | 0.35               | 3.5   |
| 125                | 3              | 0.9                | 9   |
| 250                | 3              | 1.8                | 18  |
| 125                | 4              | 1.6                | 16  |
| 250                | 4              | 3.2                | 32  |
| 100                | 4.6            | 1.7                | 17  |
| 150                | 4.6            | 2.5                | 25  |
| 250                | 4.6            | 4                  | 40  |

### Flow rates and sample size

The optimal flow rate and the sample size (sample amount and sample volume) depends on the column inner diameter. Typical flow rates and orientation values for the loading capacities for different inner diameter columns can be found in the table below:

| Column dimensions (mm) | Typical flow rates (mL/min) | Sample amount (mg) | Sample volume (µL) |
|------------------------|-----------------------------|--------------------|--------------------|
| 150-1                  | 0.06                        | ca. 0.05           | 0.05 - 1           |
| 250-2                  | 0.25                        | ca. 0.2            | 0.2 - 5            |
| 250-3                  | 0.6                         | ca. 1              | 1 - 20             |
| 250-4                  | 1                           | ca. 5              | 5 - 80             |
| 250-10                 | 6                           | ca. 30             | 30 - 500           |
| 250-25                 | 39                          | ca. 200            | 200 - 3000         |

### Column installation

These 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. These 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.

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. paired-ion 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<sub>2</sub>, CN, Diol) are shipped with nheptane/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.

### Column care

- Do not exceed the pH stability range of the sorbent
- Always avoid dramatic changes in running conditions
- Always degas and filter mobile phases
- Protect your column from contamination (filter through 0.45 µm for HPLC and 0.2 µm for UHPLC columns and use proper sample preparation)
- Store RP-columns in pure acetonitrile or a mixture of organic solvent (e.g. acetonitrile or methanol) and water (e.g. 50/50)

## Mobile phase

Use only high-quality HPLC grade solvents and HPLC grade buffers, freshly prepared, filtered (0.45 µm) and degassed before use. Degassing mobile phases prevents bubble formation.

Buffers, organic modifiers and ion pair reagents present no problems as long as the appropriate pH stability range is not exceeded. Ion pair reagents are often difficult to completely flush from the column. Therefore columns used with these reagents should be dedicated to the particular analysis involved. Do not exceed the pH stability range of the column. Higher pH values will dissolve the silica, creating voids in the column. Lower pH values can eventually strip away some of the bonded phase. These defects will cause changes in retention times and loss of resolution. Verify that solvents are miscible when changing mobile phases and that no buffer precipitation will occur.

Si (silica) columns are generally used with solvents such as n-heptane and dioxane, which are typical solvents for adsorption chromatography.

## 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 of columns supplied by Merck KGaA, Darmstadt, Germany 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.

| Phase                            | pH stability range | Max. temperature |
|----------------------------------|--------------------|------------------|
| LiChrospher®                     | 2–7.5              | 60 °C            |
| Superspher®                      | 2–7.5              | 60 °C            |
| LiChrosorb®                      | 2–7.5              | 60 °C            |
| Purospher®                       | 2–8                | 65 °C            |
| Purospher® STAR RP-18e and RP-8e | 1.5–10.5           | 65 °C            |
| Aluspher® RP-select B            | 2–12               | 60 °C            |

## Temperature

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.

## Column storage

Reversed phase columns should be stored in a pure acetonitrile or a mixture of organic solvent (e.g. acetonitrile or methanol) and water (e.g. 50/50).

Storing the column in buffers for a prolonged time will shorten the lifetime of the column. Before extended storage (i.e. over the weekend or long term storage), the columns should be thoroughly rinsed-out from buffer salts, or ion-pair reagents which can cause bacterial growth or precipitate in the stationary phase or the HPLC system. The following procedure is recommended: First flush the column with 10–20 column volumes of mobile phase minus buffer, then with 10–20 column volumes of the shipping solvent (acetonitrile/water, 75/25).

Alternatively, follow this protocol if the mobile phase contains a buffer: Flush with 5–10 column volumes water, then with 20–50 column volumes organic solvent/water (e.g. acetonitrile/water, 1/1). After flushing with 20 column volumes of the storage solvent (e.g. preferably acetonitrile), the column can be easily stored. By rinsing with acetonitrile, also aprotic impurities can be removed from the column.

## 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  |
|------------------------|---|--|
| RP<br>[reversed-phase] | <ul style="list-style-type: none"><li>• LiChrosorb® RP-8, RP-18, (Diol, CN, NH<sub>2</sub>)*</li><li>• LiChrospher® RP-8, RP-18, (Diol, CN, NH<sub>2</sub>)*</li><li>• Purospher® STAR RP-8e, RP-18e, (NH<sub>2</sub>)*</li></ul> | <ul style="list-style-type: none"><li>• Water</li><li>• Acetonitrile</li><li>• 2-Propanol + 0.1% formic acid</li><li>• Heptane</li><li>• 2-Propanol + 0.1% formic acid</li><li>• Acetonitrile</li><li>• Mobile phase</li></ul> |
| NP<br>[normal phase]   | <ul style="list-style-type: none"><li>• LiChrosorb® Si, Diol, CN, NH<sub>2</sub></li><li>• LiChrospher® Si, Diol, CN, NH<sub>2</sub></li><li>• Purospher® STAR Si, NH<sub>2</sub></li></ul>                                       | <ul style="list-style-type: none"><li>• Heptane</li><li>• Chloroform</li><li>• Ethanol or 2-propanol</li><li>• Chloroform</li><li>• Heptane</li><li>• Mobile phase</li></ul>   |

## Special recommendations for fast and ultra fast HPLC [UHPLC]

### Detector response time

Most HPLC detectors have a variable response time or time constant. In setting this parameter the „reaction speed“ of the detector can be controlled by an electronic damping device. If the response time is too slow, peaks may appear broad and tailing. UHPLC columns like Hibar® HR Purospher® STAR typically produce fast narrow peaks, particularly when run at flow rates faster than 1ml/min. Fast peaks on Hibar® HR Purospher® STAR columns require a fast time constant, such as 0.1 second. Please note – by reducing the time constant from 2 to 0.1 sec the plate count for Hibar® HR Purospher® STAR columns may improve up to 100%!

### Data system settings

Fast chromatographic peaks can be just a few seconds wide. For good integration of the peak area and good optical presentation of the chromatogram, the data system settings must enable approximately 20 data samples to be acquired during the peak width time. We recommend checking the data acquisition rate of the data system.

### Handling of LiChroCART® HPLC cartridges

LiChroCART® cartridges are used with re-usable endfittings (manuCART®) which fit different cartridge lengths (75 mm–250 mm) and inner diameters (4.6 mm, 4 mm, 3 mm or 2 mm). The endfittings (manuCART®) are designed to allow the cartridges to be hand-sealed at normal working pressures of 150 to 200 bar without the need for any tools. Only at higher pressures may further tightening with a wrench become necessary.

### CARTRIDGE EQUILIBRATION

LiChroCART® cartridges were flushed with acetonitrile/water. As they can dry out during stockings and shipping they have to be activated by purging 10–20 column volumes of pure organic component (e.g. acetonitrile, methanol) before equilibrating them with the mobile phase. Please increase the flow rate gradually stepwise from 0 ml/min to the desired value. If the eluent contains a buffer salt, you have to flush the cartridges with water after the activating and before the equilibrating step.

### Mounting the manuCART® to a LiChroCART® cartridge without precolumn

The manuCART® (Cat. No. 1.51486.0001) consists of the following pieces. Check that you have all of these pieces before you begin to assemble your column.

| No. | Description | Quantity |
|-----|-------------|----------|
| 1   | Cap nut     | 2        |
| 2   | Spin collet | 4        |
| 3   | End fitting | 2        |

The following procedure describes how to fit the pieces to just one end of your cartridge. Repeat steps 1–4 for the other end.

1. Insert the cartridge through the end fitting, so that the outer thread is at the outermost end.
2. Place 2 split collets around the neck of the cartridge as shown in Figure 1.
3. Push the end fitting up over the 2 split collets.
4. Screw the end cap over the assembled endfitting. Tighten finger tight.
5. When you install your column into the flow path of your LC, tighten the end fittings leak tight using wrenches.

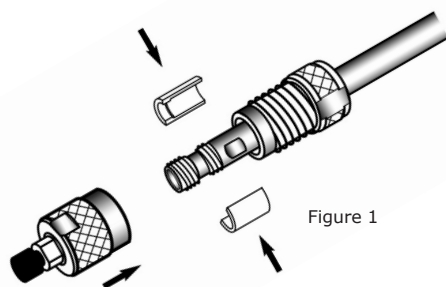


Figure 1

### Mounting the manuCART® to a LiChroCART® cartridge precolumn

1. Insert the cartridge through the end fitting, so that the outer thread is at the outermost end.
2. Place 2 split collets around the neck of the cartridge as shown in Figure 2.
3. Place the guard column cartridge inside the space at the end of the collets.
4. Push the end fitting up over the assembled guard column cartridge.
5. Screw the end cap over the end fitting. Tighten finger tight.
6. When you install your combined column and guard column into the flow path of your LC, tighten the end fittings leak-tight using wrenches.

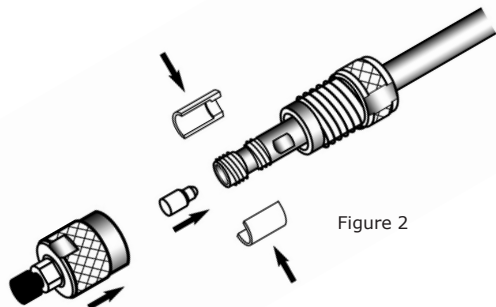


Figure 2

### Exchanging the sieve and glass fiber filter

Content of the tool for replacement of frits (Cat. No. 1.15576.0001):  
1 pin, 1 plastic tool, 1 centering sleeve

1. Using a wrench, loosen the cap nut from the end fitting.
2. Unscrew the cap nut, and remove the semicylindrical collets from the neck of the column cartridge. Put them aside for re-use later.
3. Using the pin, gently remove the sieve from the end of the column cartridge (Figure 3).
4. Using a small spatula, remove the remains of the glass fiber filter and any soiled packing material.
5. Fill the void with freshly prepared packing material and smooth-off the surface.
6. Place a new filter on the open end of the cartridge. Do not attempt to push it into place with your fingers – it may wrinkle.
7. Using the plastic tool, push the filter inside the cartridge (Figure 4).
8. Place the cylindrical sleeve over the open end of the cartridge.
9. Place a sieve inside the funnel.
10. Using the plastic tool, push the sieve firmly into place (Figure 5).

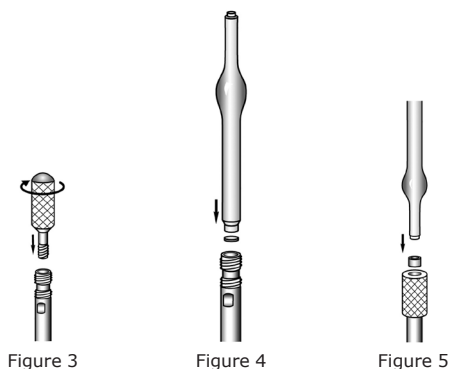


Figure 3

Figure 4

Figure 5

### Mounting of shorter LiChroCART® cartridges

LiChroCART® cartridges of 30mm or 55 mm length are mounted with a special manu-CART® – cartridge holder (either for 30 mm cartridges Cat. No. 1.50227.0001 or 55 mm cartridges Cat. No. 1.50226.0001) containing 1 tube (30 or 55 mm in length) and 2 endfittings. These cartridge holder fit for different inner diameter columns (2 mm, 3mm and 4 mm i.d.)

Push the tube over the cartridge and screw the endfittings on the ends of the tube.

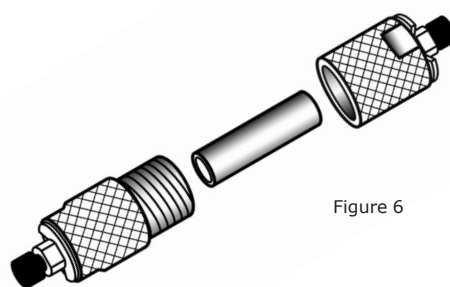


Figure 6

### Use of Hibar® HR UHPLC columns

Hibar® HR UHPLC are especially designed for use with UHPLC systems. They fit to all HPLC and UHPLC instruments without additionally tools.

To ensure best results, please follow these simple usage guidelines:

1. Install and run the column only in the flow direction marked on the column.
2. Use only solvents that are high quality, chromatography grade.
3. Filter all aqueous buffers and all samples through an appropriate 0.2 µm filter before use.
4. Replace bottles of mobile phase buffer every 24–48 hours – do not add mobile phase to the bottle; always use a fresh bottle.
5. Do not use a high buffer salt mobile phase (>50mM) in combination with high ACN concentrations due to possible precipitation.
6. An in-line filter is recommended to catch particulates and extend column life. Change the filter when the pressure increases by 10%.

### Handling of Hibar® RT HPLC columns

Hibar® RT HPLC columns are traditional "ready-to-use" columns including end-fittings. These columns are pressure stable up to 400 bar. A direct connection to all HPLC systems is possible with out any additional column holder.

The use of precolumns is recommended also for Hibar RT HPLC columns. For direct connection of the precolumn to the analytical HPLC column a special holder is necessary.

## Mounting of LiChroCART® 4-4 precolumn with direct connection to a Hibar® RT HPLC column

Precolumn holder for 4-4 LiChroCART® cartridges for direct coupling to Hibar® RT column (Cat. No. 1.51487.0001)

1. Remove the endfitting of the Hibar® RT column at the inlet side of the column (in front of flow direction).
2. Screw the holder with seat for the precolumn onto the Hibar® RT column and tighten with tool to avoid leaking.
3. Fit the LiChroCART® 4-4 precolumn into the holder seat.
4. Fit the endfitting of the holder and tighten with tool to avoid leaking.

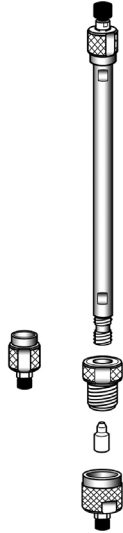


Figure 7

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