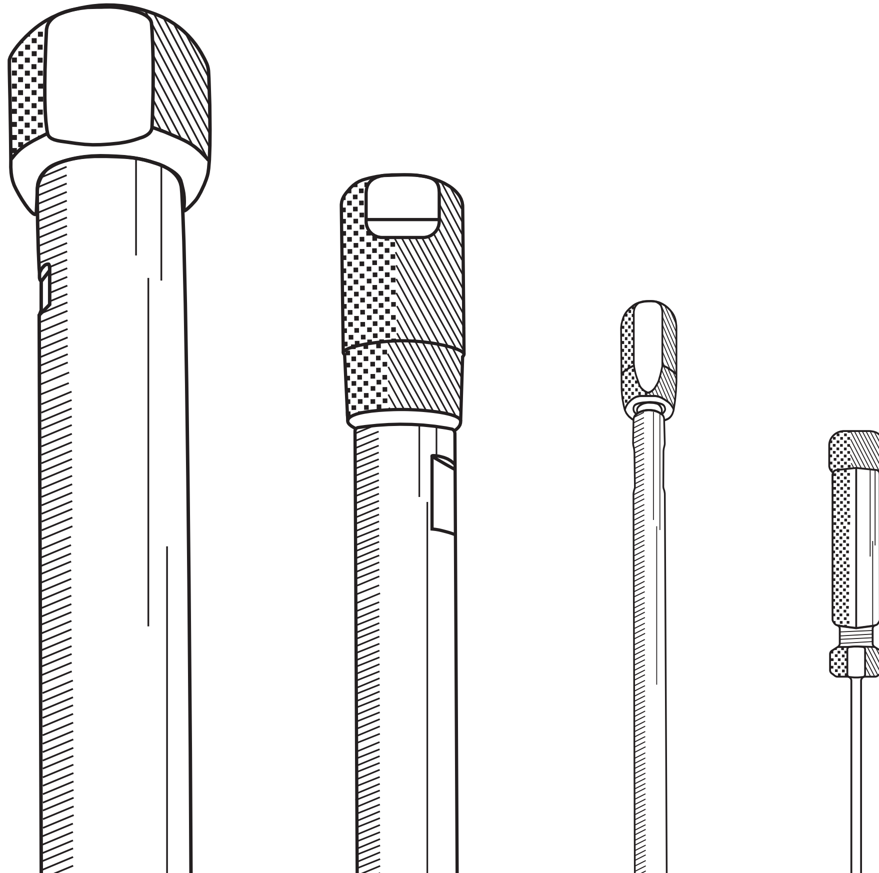
Dr. Maisch

Any Column, Any Size, Any Media



COLUMN CARE GUIDE

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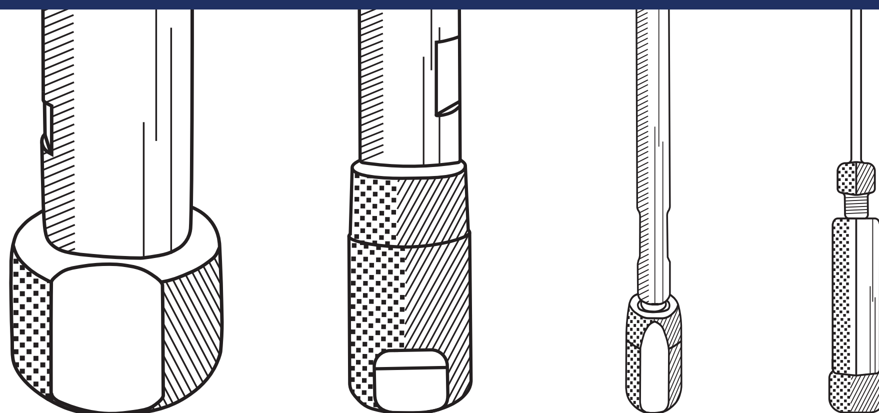

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1 General Instructions for Use

1.1 First Steps and Column Installation

The proper setup of your liquid chromatography (LC) system is essential to ensure the optimal column performance.

Your LC system is ready, when:

1. Seals, lines, injector are clean.
2. Lines are primed (no dry lines or bubbles).
3. Backpressure is consistent.
4. Detector: Baseline is steady.

Flush LC system pump and lines with a mobile phase (HPLC grade and miscible with the used shipping solvent).

Checklist for Initial Mobile Phase Conditions:

1. Ensure that the HPLC grade mobile phase is thoroughly mixed, properly filtered, and degassed before use.
2. Check the shipping solvent of the column (mentioned on the Certificate of Analysis (CoA)).
3. Ensure that shipping solvent of the column, the remaining solvent in the LC system, and solvents used as mobile phase are miscible.

Column Installation:

1. Install the column in the LC system and ensure that the arrow on the column label is in flow direction.
2. Set the flow rate to 0.1 ml/min (for 2.1 - 4.6 mm Inner Diameter (ID)).
3. Increase the flow rate to 0.2 ml/min (for 2.1 mm ID) or 1.0 ml/min (for 4.6 mm ID) for 5-10 minutes. Collect the solvent in a small beaker.
4. Stop the flow and wipe the outlet end. Remove any particles before connecting to detector.
5. Install the fitting/tubing into the outlet end and run minimum 10 column volumes at low flow (approx. 0.2 ml/min) while monitoring the backpressure.

A steady pressure indicates a constant flow, whereas pressure fluctuations typically indicate the presence of air in the system.

Wide fluctuations in pressure may shock and damage the column. Therefore, pressure monitoring is essential. Monitor the pressure as well as the signal from the detector. When both are steady, the column is ready for use.

1.2 Mobile Phase

Generally, it is mandatory to use ultra-pure, HPLC-grade solvents.

1.3 pH Stability, Temperature and Backpressure

You can adjust physical parameters like pH value, temperature and pressure and influence the selectivity of the column by changing these. However, the lifetime of a column is not infinite. By treating the column with care and according to the following suggested conditions you can prolong the lifetime and get the maximal performance out of your column.

Table 1 shows typical pH ranges and maximum temperatures recommended by Dr. Maisch HPLC GmbH to ensure an optimal performance of the column. For more specific information on any particular stationary phase please consult our website (www.dr-maisch.com). **Table 2** and **Table 3** display the typical backpressure and flow rates of Reversed-Phase (RP) and Normal-Phase (NP) columns. However, it is important to read the Certificate of Analysis (CoA) carefully which is supplied with every single column. There you can find the parameters to be used (e. g. maximum pressure).

Table 1: pH Range and Temperature Conditions of selected Dr. Maisch Phases.

Dr. Maisch Phase	pH Range	Max. Temperature [°C]
Silica-based Media (ReproSil-XR/Pur/Gold/etc.)	2-8	50
Polymer-based Media (Repromer)	5-9	40
Repromer H	1-3	
ReproSil pHoenix	1-12	50

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Table 2: Typical[1] Backpressure and Flow Rates of RP Columns (Mobile Phase: MeOH/H_2O 85:15).

Particle Size [µm]	Inner Diameter (ID) [mm]	Flow Rate [ml/min]	Backpressure [bar] Column Length 150 mm	250 mm
< 2.5	2.0	0.2	270	460
	3.0	0.4	250	400
	4.0	0.7	240	400
	4.6	1.0	260	430
3	2.0	0.2	180	290
	3.0	0.4	160	320
	4.0	0.7	150	260
	4.6	1.0	160	280
5	2.0	0.2	65	100
	3.0	0.4	55	110
	4.0	0.7	55	90
	4.6	1.0	60	100
10	2.0	0.2	15	25
	3.0	0.4	15	30
	4.0	0.7	15	25
	4.6	1.0	15	25

[1] The table is meant as a guideline only. The suggested parameters are documented on the specific Certificate of Analysis (CoA).

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Table 3: Typical[1)] Backpressure and Flow Rates of NP Columns. (Mobile Phase: n-Hexane/IPA 9:1)

Particle Size [µm]	Inner Diameter (ID) [mm]	Flow Rate [ml/min]	Backpressure [bar] Column Length	
			150 mm	250 mm
< 2.5	2.0	0.2	120	190
	3.0	0.4	100	170
	4.0	0.7	100	170
	4.6	1.0	110	180
3	2.0	0.2	75	120
	3.0	0.4	65	110
	4.0	0.7	65	100
	4.6	1.0	70	120
5	2.0	0.2	25	45
	3.0	0.4	25	40
	4.0	0.7	25	40
	4.6	1.0	25	40
10	2.0	0.2	N/A	10
	3.0	0.4	N/A	10
	4.0	0.7	N/A	10
	4.6	1.0	N/A	10

[1)] The table is meant as a guideline only. The suggested parameters are documented on the specific Certificate of Analysis (CoA).

1.4 Guard Columns

Using Guard Columns might prolong the lifetime of your column. Dr. Maisch HPLC GmbH offers two different systems for Guard Columns as indicated in **Figure** and **Figure 2**.

The Direct Guard Column Holder (**Figure 1**) is assembled directly to the column and replaces the column head. If you need help installing it, please check out our YouTube channel! (https://www.youtube.com/watch?v=TGHI0VRpy04).

The Indirect Guard Holder is simply connected to column using a column coupler (**Figure 2**).

Figure 1: Direct Guard Holder.

Figure 2: Indirect Guard Holder.

1.5 Storage

For short-term storage (1-2 days), the columns can be stored in the mobile phase, however, remove any buffers or additives prior to storage by flushing the column with the mobile phase composition omitting the buffer or additives. If you plan to store the column for a longer period of time, use **Table 4** as a guideline for the different Separation Modes.

Table 4: Solvents for long-term Storage for different Separation Modes.

Separation Mode	Phases[1)]	Solvents for long-term Storage
Reversed-Phase (RP)	C1, C2, C3, C6, C8, C12, C18 (ODS), C18-AQ, (Bi-, Di-) Phenyl, Phenyl-Hexyl, PFP	$MeOH/H_2O$ 50:50
	Chiral	ACN/H_2O 85:15
Normal-Phase (NP)	Silica, CN, NH_2, Diol	*n*-Hexane
	Chiral	*n*-Hexane/Isopropanol 90:10
Hydrophilic Interaction Liquid Chromatography (HILIC)	CN, NH2, Diol or e.g. ReproSil Star ZIK HILIC	$MeOH/H_2O$ 90:10 or ACN/H_2O 80:20
Ion Exchange Chromatography (IEX)	SAX, SCX, WAX, WCX	$MeOH/H_2O$ 10:90
Size Exclusion Chromatography (SEC)	Diol	0.05% NaN_3 in H_2O or 10% MeOH
Supercritical Fluid Chromatography (SFC)	2-/4-EP, PEI, Chiral	MeOH

[1)]Valid for all of the following brands: ReproSil, Reprospher, etc.

1.6 Washing Procedure

If the backpressure increases and reaches a certain level where the system cannot work properly anymore, certain washing protocols might help in this situation. However, before setting up the protocol, it is important to follow the following steps beforehand.

Reverse the column and apply the flow in the opposite flow direction. Make sure that the column is connected directly to the waste (without using the detector!). Always set the pumps to a low flow rate in order not to harm the column. As a rule of thumb, you can use 10% of the usual flow rate.

Depending on the used Mode, you can follow the Washing Procedures listed in **Table 5**.

Table 5: Washing Procedures for different Modes.

Coated Silica Phase	
n-Hexane	10 Column Volume (CV)
DCM	10 CV
IPA	10 CV
DCM	10 CV
Mobile Phase	10 CV

Bonded Normal-Phase (CN, NH_2, Diol)	
Chloroform	10 CV
IPA	10 CV
DCM	10 CV
Mobile Phase	10 CV

Reversed-Phase (C18, C8, C4, C2, Phenyl, PFP)	
H_2O/ACN 95:5	10 CV
THF	10 CV
H_2O/ACN 95:5	10 CV
Mobile Phase	10 CV

Hydrophilic Interaction Liquid Chromatography (HILIC)	
H_2O/ACN 95:5	10 CV
100 mM NH_4COOH pH5.8/ACN 95:5	10 CV
H_2O/ACN 95:5	10 CV
Mobile Phase	10 CV

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Reversed-Phase (RP) Protein/Peptide		
Mobile Phase (w/o Buffer)		20 CV
A)	0.1% TFA in H_2O	
B)	0.1% TFA in ACN/IPA 1:2	
25% B to 100% B for 30 min, 2 times!		
Mobile Phase		10 CV

Ion Exchange Chromatography (IEX): SAX, SCX, WAX, WCX	
500 mM Phosphate Buffer pH 7	10 CV
10% CH_3COOH (aq)	10 CV
H_2O	5 CV
Phosphate Buffer pH 7	10 CV
H_2O	5 CV
MeOH	10 CV
H_2O	10 CV

Supercritical Fluid Chromatography (SFC)	
Flush with ACN/IPA 50:50 and 100% IPA.	
Re-equilibration: Mobile Phase	10 CV

2 Special Instructions for Use

2.1 Repromer Columns (Polymer-based Columns)

Table 5: Conditions for Repromer Columns.

	Repromer H, Na, Ca, K, Pb, Ag	Repromer Carbohydrate ES
Max Pressure [bar]	150	
Max. Flow Rate [ml/min]	1	1
Max. Temperature [°C]	85	50
Mobile Phase	H_2O	ACN/H_2O 75:25
pH Range	H 1-3 Na, Ca, K, Pb, Ag 5-9	2-13
Regeneration Solvent	Corresponding to each Phase: H 25 mM H_2SO_4, Na 0.1 M $NaNO_3$, Ca 0.1 M $Ca(NO_3)_2$, K 25 mM KNO_3, Pb 0.1 M $Pb(NO_3)_2$, Ag 0.1 M $AgNO_3$	0.01 M HNO_3, 0.01 M NaOH

Specific Information on Repromer Carbohydrate ES Columns:

Repromer Carbohydrate ES Columns are packed with a rugged, hydrophilic polymeric gel giving high efficiency, excellent stability without column bleed, good reproducibility, and long column lifetime. The columns are versatile. They are predominantly used to analyse mono- and oligosaccharides by Normal-Phase (NP) Liquid Chromatography (LC), but can also be used to analyse negatively charged compounds by Ion Exchange Chromatography (IEX). Under the chromatographic conditions generally used for sugar analysis, Repromer Carbohydrate ES Columns provide equal resolution to and greater reproducibility than competitive Silica-based Columns. Because of its superior gel stability, the Repromer Carbohydrate ES Columns permit the selection of both

organic and aqueous mobile phases. The pH value of the aqueous mobile phase may range from pH 2 to 13, allowing a variety of buffers for mobile phase optimization and chromatographic efficiency. Repromer Carbohydrate ES Columns reach their full potential when used with the Evaporative Light Scattering Detector (ELSD). This combination of column/detector yields excellent sensitivity, total gradient compatibility, and stable baselines free from noise and drift.

Column Cleaning

Long-term, repeated use of the column may cause considerable change in the elution characteristics of saccharides due to accumulation of micro-adsorbents from the sample solution. In these and other cases the column may be cleaned in the following manner. Pass aqueous 0.01N nitric acid (HNO_3) totalling 10 to 20 times the column volume through the column at the normal flow rate or lower. Purge all nitric acid from the column with distilled water and then pass aqueous 0.01N sodium hydroxide (NaOH) totalling 10 to 20 times the column volume at the normal flow rate or lower. Purge all sodium hydroxide from the column with distilled water.

Column Handling and Storage

When not in use, the column may be left in the LC system without flushing for up to several days, so long as no corrosive agent or propagating bacteria are present. It is essential to ensure that no part of the flow path in the LC system or column becomes dry at any time while not in use. If any possibility of contamination or drying is present, thoroughly purge the column and LC system with 30-80% aqueous acetonitrile (ACN), disconnect, and stopper the column. Disconnected columns should be stored in an area free from large temperature changes (preferably in a constant temperature room) with both ends tightly stoppered to prevent internal drying. Storage in an area exposed to direct sunlight or large temperature changes may cause column degradation.

2.2 Chiral Columns

ReproSil Chiral OM, CM, JM, ZM, BM, AM, AMS, ZA and YM chiral stationary phase are prepared by coating the silica with a polysaccharide derivative. Therefore, any solvent that can dissolve the polysaccharide derivative, such as those mentioned below, must be avoided even in trace amounts:

- Ethers incl. THF
- Acetone
- Chlorinated solvents
- Ethyl acetate
- Dimethyl Sulfoxide (DMSO)
- Dimethylformamide (DMF)
- N-Methyl formamide (NMF)
- Toluene
- Ketones
- Dimethylacetamide (DMAc)
- IPA > 50%

The immobilized stationary phases ReproSil Chiral MIA, MIF, MID, MIB, MIC, MIX, MIZ and MOF with greatly increased column robustness tolerate strong organic solvents such as DMSO, DCM, Ethyl acetate, MTBE, and THF to be injected onto the column both as an injection solvent or part of the eluent.

ReproSil Chiral Columns will deliver consistent results when operated with mobile phases containing additives at the concentration levels specified below. However, limited decrease in column efficiency may occur when a column is used in combination with these additives. Therefore, we advise to dedicate columns to mobile phases containing basic additives.

For basic samples or acidic chiral compounds, it may be necessary to use an appropriate mobile phase modifier in order to achieve chiral resolution and to ensure proper peak shapes.

Table 6: Three different Modes for ReproSil Chiral Columns.

Normal-Phase (NP)	Polar Organic	Reversed-Phase (RP)
Mixtures of *n*-Hexane or *n*-Heptane with alcohols EtOH/IPA 80:20. Vary % alcohol to adjust retention time and selectivity.	Mixtures of ACN/IPA 95:5 or MeOH/IPA 90:10 or ACN.	ACN or MeOH or EtOH / water mixtures.
Add 0.1-0.5% TFA or Acetic acid for acidic analytes.	Add 0.1-0.5% DEA or TEA for basic analytes.	Water content must be < 85%.
Add 0.1–0.5% diethylamine or triethylamine for basic compounds.	Add 0.1-0.5% TFA or EtOH for acidic analytes.	Add 0.5-1N Perchlorate or 0.1% TFA for basic compounds. Add $HClO_4$/$NaClO_4$ buffer for acidic compounds together with ACN.

2.2.1 Solvent Switching

Chiral Columns can be used either in NP Mode, RP Mode, SFC Mode or with Polar Organic solvents. To switch between Modes, please follow the rules for solvent switching below:

1) Normal-Phase (NP) to Polar Organic or Reversed-Phase (RP)

To safely transfer a column from Normal-Phase (NP) to Polar Organic or Reversed-Phase (RP) conditions, use the following procedure:

1. Set the flow rate to 0.5 ml/min.
2. Flush the column with 10 column volumes of IPA/EtOH 90:10 e.g. 25 ml for a 250 x 4.6 mm column.
3. Condition the column with at least 10 column volumes of the new mobile phase.

If the salts of your Reversed-Phase (RP) Mobile Phase buffer are insoluble in methanol and/or ethanol, flush column briefly with water following methanol/ethanol step before conditioning with 10 column volumes of Reversed-Phase (RP) or Polar Organic Mobile Phase.

2) Reversed-Phase (RP) to Normal-Phase (NP)

Once a ReproSil Chiral column is in Reversed-Phase (RP) Mode, it is not recommended switching from Reversed-Phase (RP) Mode back to Normal-Phase (NP) Mode.

3) Polar Organic to Normal-Phase (NP)

Once a ReproSil Chiral Column is in Polar Organic Mode, it is not recommended switching from to Polar Organic Mode back to Normal-Phase (NP) Mode.

2.2.2 SFC Column Installation

1. Install the column in the SFC instrument oven compartment.
2. Set SFC instrument backpressure regulator between 80-100 bar and equilibrate the column with a minimum of ten column volumes of the SFC mobile phase prior to use.
3. A good starting point for an SFC mobile phase is CO_2/MeOH or CO_2/EtOH 80:20 with or without additives.
4. Optimal flow rate for 4.6 mm ID columns is between 3 and 6 ml/min. We recommend gradually increasing the flow rate to 3 ml/min to prevent the backpressure from exceeding 300 bar (4300 psi).

2.3 SEC/GPC Columns

Table 7: Parameters for SEC/GPC Columns.

	Dimension [mm]: 300 x 4.6	Dimension [mm]: 300 x 8
Sample Load [µg]	Max.: 100 Optimal: 10-40	Max.: 200-300 Optimal: 20-100
Injection Volume [µl]	< 10	1-20
Flow Rate [ml/min]	0.4	1
Temperature [°C]	Max.: 50	
Flow Rate [ml/min]	Max.: 1.5 (if backpressure is < max. backpressure)	
pH Range	2.5-7.5	
Backpressure	Column pressure must not exceed the maximum packing pressure (see QC sheet)	
Organic Modifier	Up to 100% ACN 10% DMSO 500 mM Mercaptoethanol	
Buffer Concentration	High ionic strength buffers are recommended for most protein applications. A neutral salt is often added to increase ionic strength. Maximum Salt Concentration: 1 M	
Detergents	In General: Detergent stick on the SEC-media surface affecting column lifetime and the future use of the column. If SEC method requires denaturing conditions (SDS) or formulations detergents (Tween 20, Triton): ReproSil SEC columns can be operated under these conditions but with reduced lifetime. Use of guard columns is recommended. SEC columns that were in "contact" with denaturing should be used later on ONLY with detergent containing eluents.	

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Arginine	In very rare cases proteins bind to the SEC media and lead to a compromised protein elution and peak resolution which results in incorrect determination of the size of the eluted proteins and the amount of the aggregated species. In such cases arginine (e.g. 0.2 M Arginine-HCl) can be added to the mobile phase instead. This mobile phase reduces non-specific protein binding while not affecting the protein structure.	
Cleaning Procedure	General Protein Removal:	Wash with 30 ml of 0.1 M NaH_2PO_4, pH 3.0
	Hydrophobic Compounds:	Use ACN gradient.
	Charged Contaminants:	0.5 mol/l salt (NaCl, Na_2SO_4) at pH 3
	Strongly Absorbed Proteins:	Wash with 6 M Guanidine Thiocyanate or 10% DMSO.
Column Storage	Overnight: Run mobile phase at 0.2 ml/min. Prolonged Storage: use 0.05% NaN_3 in H_2O or 20% MeOH in H_2O.	

3 Liability

We have to reject complaints which concern the following reasons:

- Mishandling of the column by the user (usage of the column not according to this Column Care Guide).
- The parameters listed on the Certificate of Analysis (CoA) are within our specifications.

If you have a malfunction within your system and you have the objective evidence that the issue originates from the column itself, please contact our Technical Support (see Section 4.3).

4 Appendix

4.1 Void Volumes

In **Table** you can find the Void Volumes (Column Volume (CV)) for different column dimensions. For example, if the protocol states "wash with 10 CV", then you can calculate the required washing volume using the values listed below. If the Void Volume of the required column is not included in **Table 8**, you can calculate the Void Volume yourself using the formula: $V = \pi r^2 \cdot l$ (V = Volume, r = Radius, l = Length).

Table 8: Void Volumes of different Column Dimensions.

	Void Volume CV [ml]	
Column ID [mm]	Column Length 150 mm	Column Length 250 mm
1.0	0.1	0.2
2.0 (2.1)	0.5	0.8
3.0	1	1.8
4.0	1.9	3.1
4.6	2.5	4.1
8	7.5	12.5

4.2 Flow Rates

For Up- (or Down-) Scaling of your established methods you might need to change the column ID. Therefore, you need to adjust the flow rate as well as the injection volume and the detector sensitivity. In **Table** you can find the corresponding parameters.

Table 9: Typical Flow Rates for Columns with different IDs.

Column ID [mm]	Flow Rate [ml/min]
1.0	0.05
2.0 (2.1)	0.2
3.0	0.4
4.0	0.7
4.6	1
8	3
10	5
20	23

4.3 Contact of Technical Support

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