Dr. Maisch

Any Column, Any Size, Any Media

Column Care Guide

COLUMN CARE GUIDE

Content

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1 Quality Policy

Dr. Maisch's commitment to quality drives us to achieve the highest level of customer satisfaction with regard to our products and services. For this reason, we have set up an official Quality Management System (QMS). Both the management and all employees are responsible for maintaining and continuously improving our QMS. The performance of our QMS is monitored to ensure that we meet the requirements of our customers and that we continuously improve the effectiveness of the system and progress in achieving our goals. In addition, we regularly undergo the individual, strict audits of our customers.

2 General User Instructions

2.1 First steps and column installation

The proper setup of your LC system is very important to ensure column performance.

Your LC system is ready, when:

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

Flush LC system pump and line with mobile phase (HPLC grade and miscible with solvents column is shipped in).

Mobile phase starting conditions check list:

1. Ensure that HPLC grade mobile phase is well mixed, filtered, and degassed prior to use.
2. Check the shipping solvent of the column (mentioned on the certificate of analysis). NOTE: Most Reprosil Chiral columns are shipped with normal phase shipping solvent. For some products there are special part numbers. These are shipped with reversed phase shipping solvent.
3. Ensure that column shipping solvent, remaining solvent in LC system, and mobile phase solvents are miscible.

Set flow rate to 0.1 ml/min (for 2.1 - 4.6 mm ID) and install the column. Make sure that the arrow is in flow direction. Then increase the flow rate to 0.2 ml/min (2.1 mm ID) or 1.0 ml/min (4.6 mm ID) for 5-10 minutes. Collect solvent in a small beaker.

Stop flow and wipe outlet end. Remove any particulates before connecting to detector. Install fitting /tubing into outlet end and run minimum 10 column volumes at low flow (approx. 0.2 ml/min) while monitoring the back pressure.

1. A steady pressure should indicate a constant flow, while pressure fluctuation will indicate air in the system.

2. Wide fluctuations in pressure may shock and damage the column. So, it is important to monitor the pressure.

Monitor pressure as well as signal from the detector. When both are steady, the column is ready for use.

2.2 Mobile Phase

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

2.3 pH stability, temperature and back pressure

You can adjust physical parameters like pH, 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 to ensure an optimal performance of the column. For more specific information on any particular stationary phase please consult our website. Table 2 and Table 3 display the typical back pressure and flow rates of RP and NP columns. However, it is important to read the CoA carefully which is supplied with every single column. There you find the parameters to be used (e. g. maximum pressure).

Table 1. pH range and temperature conditions of selected Dr. Maisch phases.

Phases	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

Table 2. Typical* back pressure and flow rates of RP columns (Mobile Phase: MeOH:H_2O 85:15).

Particle size (µm)	ID (mm)	Typical* flow rate (ml/min)	Typical* back pressure (bar)	
			150 mm column length	250 mm column length
< 2.5 µm	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 µm	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 µm	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 µm	2.0	0.2	15	25
	3.0	0.4	15	30
	4.0	0.7	15	25
	4.6	1.0	15	25

*the table is meant as a guideline only. Please make sure on the CoA which parameters to use.

Table 3. Typical* back pressure and flow rates of NP columns. (Mobile Phase: n-Hexane:i-Propanol 9:1)

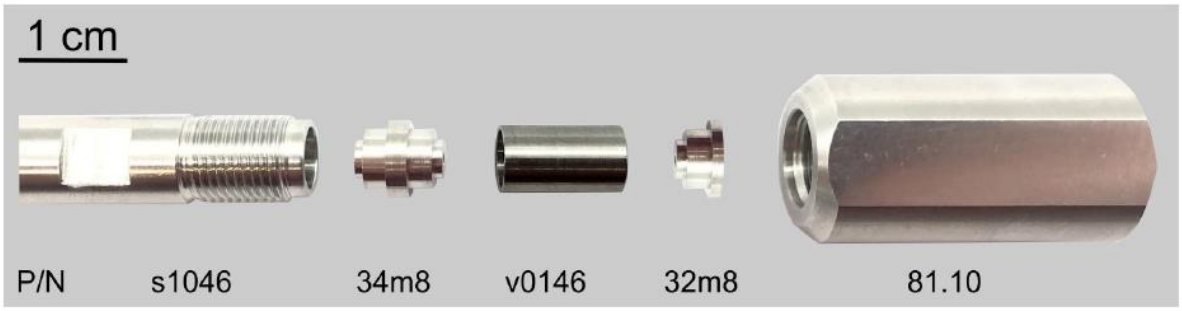
Particle size (µm)	ID (mm)	Typical* flow rate (ml/min)	Typical* back pressure (bar)	
			150 mm column length	250 mm column length
< 2.5 µm	2.0	0.2	120	190
	3.0	0.4	100	170
	4.0	0.7	100	170
	4.6	1.0	110	180
3 µm	2.0	0.2	75	120
	3.0	0.4	65	110
	4.0	0.7	65	100
	4.6	1.0	70	120
5 µm	2.0	0.2	25	45
	3.0	0.4	25	40
	4.0	0.7	25	40
	4.6	1.0	25	40
10 µm	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

*the table is meant as a guide line only. Please make sure on the CoA which parameters to use.

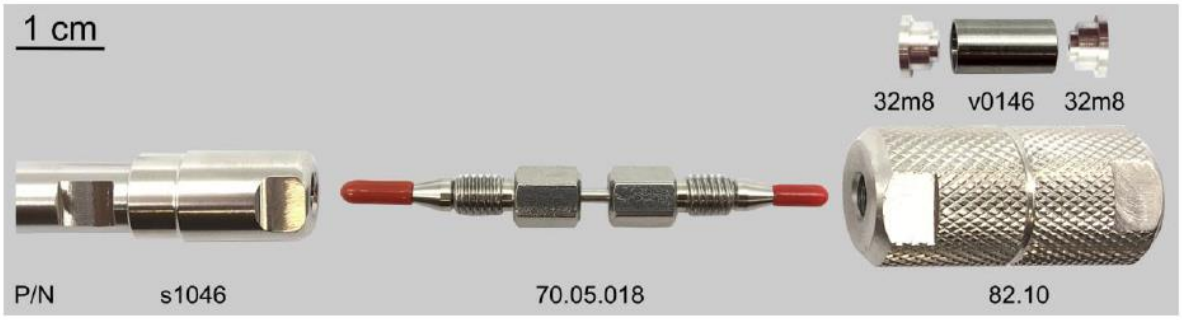
2.4 Guard Columns

Using guard columns might prolong the lifetime of your column. Dr. Maisch offers two different systems for guard columns as indicated in Figure 1. The direct guard column holder 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.



Direct guard holder



Indirect guard holder (stand-alone) with column coupler

Figure 1. The two available Dr. Maisch guard systems with direct or indirect guard holder.

2.5 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 / additives. If you plan to store the column for a longer period of time, use Table 4 as a guideline for the different modes of HPLC.

Table 4. Storage conditions for different modes of HPLC.

Separation mode	Phases*	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	MeCN:H_2O (85:15)
Normal Phase (NP)	Silica, CN, NH_2, Diol	n-Hexane
	Chiral	n-Hexane:i-Propanol (90:10)
Hydrophilic Interaction (HILIC)	CN, NH2, Diol or e. g. Reprosil Star ZIK HILIC	MeOH:H_2O (90:10) or MeCN:H_2O (80:20)
Ion exchange (IC)	SAX, SCX, WAX, WCX	MeOH:H_2O (10:90)
Size exclusion (SEC)	Diol	0.05 % NaN_3 in H_2O or 10 % MeOH
SFC	2-/4-EP, PEI, Chiral	Methanol

*valid for all of the following brands: Reprosil, Reprospher, etc

2.6 Washing procedure

If the back pressure 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 mode you use, you can follow the following cleaning steps thoroughly.

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Unbonded Silica Phase	
Hexane	10 CV
Dichloromethane	10 CV
Isopropanol	10 CV
Dichloromethane	10 CV
Mobile Phase	10 CV

Bonded Normal Phase (CN, NH2, Diol)	
Chloroform	10 CV
Isopropanol	10 CV
Dichloromethane	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

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

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

Ion-exchange (SAX, SCX, WAX, WCX)	
500 mM Phosphate Buffer pH7	10 CV
10 % CH_3COOH (Aq)	10 CV
H_2O	5 CV
Phosphate Buffer pH7	10 CV
H_2O	5 CV

MeOH	10 CV
H_2O	10 CV

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

3 Limited Liability

We have to reject complaints which concern the following reasons:

- Mishandling of the column by the user (not according to this Column Care Guide)
- The certificate of analysis shows *in spec* parameters and there is no sign of a malfunctioning column

If you experience a malfunctioning column and have evidence that the reason can be traced back to the column, contact our Technical Service (section 5.3).

4 Special Instructions

4.1 Repromer Columns (Polymer-based columns)

Table 5. Conditions for Repromer Columns.

	Repromer H, Na, Ca, K, Pb, Ag	Repromer Carbohydrate ES
Max Pressure (bar)	50	
Max flow rate (ml/min)	1	1
Max temperature (°C)	85	50
Mobile Phase	H_2O	MeCN:H_2O (75:25)
pH range	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:

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 liquid chromatography, but can also be used to analyse negatively charged compounds by ion-exchange chromatography. 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 Column permits the selection of both organic and aqueous mobile phases. The pH of the aqueous mobile phase may range from pH 2 to pH 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 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 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, 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.

4.2 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
- DMSO
- DMF
- N-methyl formamide
- Toluene
- Ketones
- Dimethylacetamide
- 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 of Reprosil Chiral columns.

Normal Phase	Polar Organic	Reversed Phase
Mixtures of hexane or heptane with alcohols (EtOH, IPA) = 80:20. Vary % alcohol to adjust retention time and selectivity	Mixtures of MeCN / IPA (95/5) or MeOH / IPA (90/10) or MeCN	MeCN 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 – 1 N Perchlorate or 0.1 % TFA for basic compounds
		Add $HClO_4$/$NaClO_4$ buffer for acidic compounds together with MeCN

4.2.1 Solvent Switching

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

1) Normal Phase to Polar Organic or Reversed Phase

To safely transfer a column from normal phase to polar organic or reversed phase conditions, use the following procedure:

1. Set the flow rate to 0.5 ml/min.
2. Flush the column with 10 column volumes of 2-propanol/ethanol (90:10 v/v) e.g. 25 ml for 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 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 or polar organic mobile phase.

2) Reversed Phase to Normal Phase

Once a Reprosil Chiral column is in reversed phase mode, it is not recommended switching from reversed phase back to normal phase mode.

3) Polar Organic to Normal Phase

Once a Reprosil Chiral column is in polar organic mode, it is not recommended switching to normal phase mode.

4.2.2 SFC Column Installation

1. Install the column in the SFC instrument oven compartment.
2. Set SFC instrument back pressure 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 choice as a SFC mobile phase is CO_2/MeOH or CO_2/EtOH (80:20, v/v) with or without additives.
4. Optimal flow rate for 4.6 mm ID columns is between 3 and 6 ml/min. We recommend increasing flow rate gradually to 3 ml/min to prevent back pressure to go above 300 bar (4300 psi).

5 Appendix

5.1 Empty Column Volumes

In Table 7 you can find the void volumes for different column dimensions. For example, if the protocol states *wash with 10 CV,* then you can calculate from the values below. If the column of the required column is not included in the table, you can calculate the volume yourself using the formula: $V = \pi r^2 \cdot l$ (V = volume, r = radius, l = length).

Table 7. Empty column volumes of different dimensions.

	void column 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

5.2 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 8 you can find the corresponding parameters.

Table 8. 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

Column ID (mm)	Flow rate (ml/min)
4.6	1
8	3
10	5
20	23

5.3 Contact of Technical Support

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