

COLUMN CARE GUIDE



Reprosil Chiral - Polysaccharide columns

Chiral-OM, -OM-R

Chiral-CM

Chiral-BM

Chiral-ZA

Chiral-MIB

Chiral-MOF

Chiral-MIG

Chiral-AM, -AM-R

Chiral-GM

Chiral-ZM

Chiral-YM

Chiral-MIC

Chiral-MIA

Chiral-AMS, AMS-R,

Chiral-JM,-JM-R

Chiral-XM

Chiral-MIX

Chiral-MIF

Chiral-MIZ

Chiral-MID

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Tips for Care and Use

Dr. Maisch LC columns are available globally. You can source these directly from our headquarters in Germany or through the Dr. Maisch distributor network. Each Reprisil Chiral column is manufactured by Dr. Maisch and is individually tested.

Each LC column is supplied with a “HPLC column certificate” which indicates testing conditions, operating parameters and column details.

Inspection

Upon receipt of column, please verify that the column you received is the one you ordered (dimension, silica modification, particle size).

Additionally, please check the column for any physical damage potentially caused during shipment.

Test the column immediately to verify performance and record the result of your test in your lab information system.

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Column Installation

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

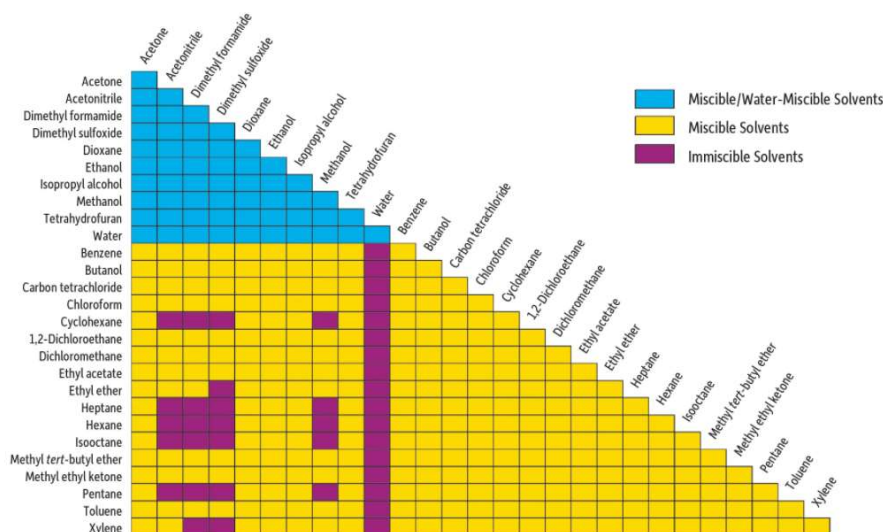
Your LC system is ready, when:

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.



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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.

Immobilized 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 for SFC mobile phase is CO₂ /Methanol or CO₂ /Ethanol (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).

Solvent Switching

An appropriate column washing procedure must be applied when changing from one mobile phase to another. The miscibility of the different mobile phase components must be carefully considered for this wash. Reprosil Chiral coated columns are recommended to be dedicated to either normal phase, reversed phase or polar organic. It is not recommended switching back and forth between different operating conditions.

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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 methanol/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.*

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.

Polar Organic to Normal Phase

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

Normal Phase or Polar Organic or Reversed Phase to SFC

1. Use methanol /ethanol (90:10 v/v) and set the flow to 0.5 ml /min.
2. Flush for at least 10 column volumes before switching to CO₂ .
3. When conditioning using SFC solvents, lower the flow rate to 0.3 ml /min until the methanol /ethanol flushed out.

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SFC to Normal Phase or Polar Organic or Reversed Phase

1. Use methanol /ethanol (90:10 v/v) and set the flow to 0.5 ml /min.
2. Flush for at least 10 column volumes until all the CO₂ has been purged.
3. Then condition for at least 10 column volumes with the new mobile phase.

Typical Flow Rate

Here are some typical values for common dimensions of Reprosil Chiral HPLC columns. These numbers are not absolute values as they can differ based on LC system, running parameters and sample analytes/matrix. The values below have been created using a solvent system of Hexane and IPA.

Internal Diameter (mm)	Particle size (µm)	Typical flow rate (ml/min)	Column length (mm)	Typical pressure (bar)	Typical pressure (psi)	Column length (mm)	Typical pressure (bar)	Typical pressure (psi)
2	3	0,2	150	40	600	250	n/a	n/a
3	3	0,4	150	50	725	250	n/a	n/a
4,6	3	0,5	150	30	420	250	30	550
4,6	5	0,5	150	16,5	240	250	16,5	300
10	5	2,5	150	40	580	250	40	780
20	5	20	150	30	435	250	30	670
30	5	40	150	41	600	250	41	830
50	5	50	150	62	900	250	62	1200

Max Back pressure

Suggested max pressure for Reprosil Chiral LC analytical columns is 4500 psi (310 bar). For preparative columns the applicable maximum pressure limit is mentioned on the column test chromatogram and depends on the column hardware type and column dimensions.

Max Temperature

Suggested max temperature for Reprosil Chiral LC columns is 50° C, however temperature limits are dependent on your running parameters and may vary.

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Mobile Phase Compatibility

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
- Dimethylacetamid
- 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.

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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

Column Storage

It is very important to make sure that your column is clean before storage. This includes removal of buffer, salts, sample and ion-pairing agents. The recommended storage conditions are:

- Reversed phase: Acetonitrile/H₂O (85:15 v/v), Methanol can be used in place of Acetonitrile.
- Normal phase: n-Hexane/2-Propanol (9:1 v/v)

Tips for Extending Column Lifetime

- To regenerate or remove potential contaminants after extended use of your Reprisil Chiral column, we recommend flushing the column with 100 % methanol or with ethanol for 2-3 hours at the appropriate flow rate. Back flush can also be used to clean the column.
- Utilize sample preparation techniques such as solid phase extraction (SPE) or accessories (syringe filter) to minimize the injection of unwanted contaminants onto your system and column.
- Use the correct guard column or guard cartridge system to help remove particulates before they foul your column.

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- Do not overload your column. Inject suitable sample concentrations and volumes. (See chart below).
- Work in the appropriate separation mode for the column. Please see column characteristic chart for typical modes each stationary phase is used for.
- Store your column in appropriate solvent(s).

Solvent switch correctly by slowly acclimating the phase from one miscible solvent to the other at a low flow: 0.1 ml /min for 2.1 mm ID and 0.5 ml /min for 4.6 mm ID.

ID (mm)	Description	Approx. Dead volume (ml)	Typical flow rate (ml)	Typical injection masses (mg)	Max injection masses (mg)	Typical injection volume (µl)	Max injection volume (µl)
0,32	Capillary (Fused silica)	0,0075	0,001-0,02	0,001	0,01	1	10
1	Microbore	0,07	0,02-0,1	0,01	0,1	5	25
4,6	Analytical	1,5	0,5-2	0,1	2,5	10	200
10	Semi-Prep	7,3	5.0-20	1	25	50	1000
20	Preparative	29,2	10-200	5	500	200	5000

Testing Column Performance

When testing column performance please use the manufacturer's recommended test conditions as mentioned in the respective column test chromatogram.

Column Warranties

- Dr. Maisch HPLC columns are warranted to meet the stated performance and quality and to be free of defects in material and workmanship.
- If you are unsatisfied for any reason, please give your Dr. Maisch HPLC Technical Representative a call. We'll do our best to solve the problem to your satisfaction.
- Should it become necessary to return the column, a Return Authorization must be obtained from Dr. Maisch HPLC first.

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Disclaimers

New columns should be tested with the manufacturers recommended test mix, and previously used columns should be tested with the same or a suitable test mix for the analysis. Remember to re-equilibrate the system when changing solvents. Never change from one solvent to another which is immiscible, without going through an intermediate solvent which is miscible with both. This will damage the column. Never change to (or from) a buffer/salt solution where the buffer/salt is not soluble in the second solvent. Again, this will damage the column. Never attempt to remove the column end fittings. This will void the warranty.

Column Shock

Handle columns with care. Do not drop or create physical shock. Do not start pump at high flow rates, instead ramp up gradually over a few minutes. Set your pump pressure limit to protect the column in event of blockage. This can create voids which will detrimentally affect the column's performance.

Column Questions and Support

If you have any additional questions, please reach out to our Technical team through:

E-mail: handlowcy_akcesoria@shim-pol.pl

For more information on Reprosil Chiral UHPLC, HPLC, and Preparative columns, please visit www.shim-pol.pl