HPLC · SMB · Osmometry

KNAUER

FAQ

► Eurocel® chiral columns

1. What kind of chiral columns are Eurocel® columns?

The KNAUER Eurocel® materials are coated polysaccharide chiral stationary phases (CSPs) made with a spherical high quality silica gel. Due to the coated nature of the Eurocel® stationary phases, solvents should be carefully selected.

2. Which solvents can I use with the polysaccharide-based chiral columns from KNAUER?

Some of the most popular eluents for HPLC (such as acetone, chloroform, DMF, DMSO, MEK, toluene, dioxane, ethyl acetate, methylene chloride, pyridine and THF) which may be remaining in your HPLC system from previous analyses, can destroy Eurocel® CSPs, even in small concentrations. In the worst case, this can cause solubilization of the polysaccharide coating at the head of the column and result in a plugged column.
It is highly recommended that the HPLC system be flushed with appropriate eluents before the chiral column is installed. The instruction sheet shipped with your column lists solvents which can be used. Mixtures of three solvents should be avoided.

3. Can Eurocel® columns be used in normal phase and reversed phase mode?

Eurocel® columns are designed to be used either in normal phase mode, in polar organic mode or in reversed phase mode.

4. Which solvent should be used for switching between normal phase and reversed phase mode?

Please pay special attention to the miscibility of the eluents. Use 2-propanol in between eluents of different polarities or when switching between normal phase and reversed phase modes.

5. Which kind of solvents and modifiers can I use in normal phase mode?

Suitable mobile phases including hexane, heptane, 2-propanol and ethanol in different mixtures can be used in normal phase mode. Recommended mobile phase: hexane/2-propanol (90/10, v/v).
Modifiers: N,N-diethylamine for basic samples, trifluoroacetic acid for acidic samples. (NOTE: minimize use of a modifier; typical use is 0.1 %; maximum 0.5%.).
In NP mode, retention time is generally shorter with higher alcohol content. Ethanol will shorten the retention time compared to 2-propanol.

6. Which kind of solvents and modifiers can I use in reversed phase mode?

Suitable neutral mobile phases: water/acetonitrile or water/methanol in different mixtures.
Suitable basic mobile phases: 0.1 M aqueous salt/acetonitrile (methanol) in different mixtures (recommended salts: PF_6^-, ClO_4^-, NO_3^-, I^-, Br^-, SO_4^{2-}, $CH_3CO_2^-$, F^-).
Suitable acidic mobile phases: aqueous (limit pH 2)/acetonitrile (methanol) in different mixtures (use TFA or phosphoric acid).

7. What kind of solvents and modifiers can I use in reversed phase mode?

Suitable mobile phases including 2-propanol, ethanol, methanol and acetonitrile.
Modifiers include N,N-diethylamine (DEA) for basic samples, trifluoroacetic acid (TFA) for acidic samples. It is recommendable to minimize use of a modifier, typical use is 0.1 %, and maximum concentration should not exceed 0.5%.

8. Are there pH limitations for Eurocel® chiral columns?

Due to the material being based on silica, it is recommendable to work in a pH range of 2-8. Strongly basic compounds must be avoided, because they are likely to damage the silica gel used in this column type. A pH value lower than 2 can irreversible destroy the silica modification.

9. What kind of solvent should be used for column storage?

The column should preferably be stored in hexane/2-propanol (90/10, v/v) when stored for more than one week. Please pay special attention to the miscibility of the eluents. Use 2-propanol in between eluents of different polarities.

10. What kind of sample solvents can I use?

It is generally recommendable to dissolve the sample in mobile phase. A sample concentration of 1 mg/ml (or even less for analytical purposes) is sufficient. If the sample is not soluble in the mobile phase, polar solvents such as 100% methanol, ethanol, 2-propanol or acetonitrile can be tested. If the sample is soluble in aqueous solvents, a separation in reversed phase mode can be helpful.

If your sample is an acid salt of a base, the addition of 0.1 % DEA may helpful by converting the compound into a free base. The same principle can be helpful for a salt of an acid. The addition of 0.1% TFA can improve the solubility. In general, organic solvents such as acetone, chloroform, DMF, DMSO, MEK, toluene, dioxane, ethyl acetate, methylene chloride, pyridine and THF should be avoided. Even small amounts of these solvents can irreversibly destroy the column coating and dramatically shorten the column life time.

11. What kind of sample concentration should be used?

For analytical purposes, high sample concentrations are usually not necessary. A sample concentration of 1 mg/ml or less in mobile phase is usually sufficient.

12. How can the peak shape be improved?

Strong basic or acidic compounds can adsorb on the most active sides of the silica basis resulting in peak broadening and peak tailing. To avoid this problem, basic or acidic compounds are added to the mobile phase to adsorb on the most active sites and displace the analytes. Common additives are trifluoroacetic acid (TFA) for acidic compounds and diethylamine (DEA) for basic compounds.

13. Is a chiral precolumn needed or can a silica precolumn be combined with a chiral column?

It is highly recommended to use a precolumn packed with the same material as the chiral column. Due to the different physical properties of silica material and chiral column material, a silica precolumn would not be compatible. KNAUER offers a suitable precolumn with 5 x 4 mm dimensions for all of our chiral columns.

14. Is it possible to run a gradient with Eurocel® chiral columns?

A comprehensive chiral screening using fast HPLC gradient methods can be realized to reduce the optimization time for chiral method development, although in general, gradients are best run over a limited eluent range to avoid long equilibration times. For chiral enantioseparation in semipreparative and preparative mode, an isocratic HPLC method is the most suitable.

15. How can the right column performance be obtained?

In general, if a column problem is suspected, the column should first be thoroughly flushed (please refer to the column's operating instructions) and then tested under the QC conditions used. The column test certificate is shipped with every column. The results of this test can usually help to identify the problem. Most loss in efficiency problems are due either to partial blockage of the inlet filter or to the adsorption of material at the head of the column. This can be corrected by changing or cleaning the inlet filter unit. It is sometimes difficult to remove the inlet filter and the procedure always has the danger of disturbing the column bed. One easy experiment is to reverse the flow direction through the column in the hope that the foreign matter will be washed from the filter unit. The use of and regular replacement of a guard cartridge can prevent such kinds of problems.

16. How do I carry out upscaling from an analytical to a semi-preparative column?

The most important question is: What is the maximum loading capacity limit for the analytical column used? The separation has to first be optimized by analytical HPLC. The analytical column must then be investigated with respect to its loading capacity. The relative loading capacity (LCR) on a 250 x 4.6 mm analytical column is fixed to be "1." For different semi-preparative and preparative columns, the relative loading capacity and associated flow rates can be determined from the following table.

Column dimensions (length x ID)	Packing (g)	Loading capacity (relative)	Flow rate (ml/min)
250 x 4.6 mm	2.50	1	1.0
250 x 8 mm	7.60	3	3
250 x 20 mm	47.30	18.9	18.9
250 x 32 mm	121.00	48.4	48.4
250 x 40 mm	189	75.6	75.6

Thus, if the typical load on an analytical column is 1-10 mg, then a typical load on a 250 x 20 mm semi-preparative column is 25 –250 mg/injection. This parameter strongly depends on the relative retention and can be quite higher for values higher than 1.4.

17. Can I use Eurocel® columns in SFC mode?

Carbon dioxide as a mobile phase in SFC mode can be used alone or with suitable modifiers in combination with Eurocel® chiral columns. Due to the higher efficiency in SFC mode, the resolution can be better compared to HPLC. The selectivity is normally comparable in HPLC and SFC mode.

18. What is the pressure limit of Eurocel® columns?

Don't operate your KNAUER Eurocel® column above the recommended maximum pressure limit of 300 bar.

19. Additional recommendations:

1. Follow the "Column Care and Use" instructions supplied with the chiral Eurocel® columns carefully.

2. Use only those solvents which are recommended for your column.

3. Try to use simple mobile phases of HPLC quality.

4. To avoid effects caused by the sample diluent, try to dissolve the sample in mobile phase.

5. Avoid using strong basic or acidic modifier. Please keep in mind the pH limit of chiral phases (pH 2-8).

6. Flush the HPLC system carefully with the appropriate solvent before the column will be installed.

7. After solvent replacement, equilibrate the system to a stable baseline. At a flow rate of 1 ml/min, an equilibration time of nearly 30 min is needed.

8. It is recommendable to use a guard column to prevent contamination of the separation column.

9. Samples should be free of insoluble particles. Use syringe filtration to avoid plugging the column sieves and frits.

10. To recognize achiral impurities of the enantiomers, run the chiral separation at multiple wavelengths or use different types of detectors.

11. When the chiral analysis has been completed, flush the column with the appropriate storage solvent. If the column will not be used for several days it is recommendable to flush the column and HPLC system with mobile phase that does not contain modifiers or buffer. A suitable storage solvent can be hexane/2-propanol mixture for chiral columns used in normal phase mode. Chiral columns used in different mode (normal phase, polar organic mode or reversed phase mode) should be stored with 2-propanol.

Physical properties of Eurocel®

Stationary phase	Eurocel® 01, Eurocel® 02, Eurocel® 03, Eurocel® 04
USP code	L40
Pore size	1000 Å
Particle size	3 µm, 5 µm, 10 µm, 20 µm
Form	spherical