Application Note

► FAQ´s concerning Chiral Columns

Category	Chiral Separation
Matrix	
Method	Chiral HPLC
Keywords	FAQ
Analytes	
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1. What kind of chiral columns are Eurocel and Europak columns?

The Knauer Eurocel and Europak material are coated polysaccharide chiral stationary phases made with a spherical high quality silica gel. Due to the coating nature of the Eurocel / Europak phases, solvents should be carefully selected.

2. What solvents may not be used 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, can destroy even in small concentrations this type of column. In worst case this can result in solubilizing the polysaccharide coating at the head of the column and in a plugged column.
It is highly recommended that the HPLC system be flushed with appropriate eluents before the installation of the chiral column is undertaken. The Instruction sheet shipped with your column refers to solvents that can be used. Mixture of three solvents should be avoided.

3. Can the Eurocel/Europak columns used in normal phase and reversed phase mode?

Eurocel/Europak 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 switching between normal phase and reversed phase mode.

5. What 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. Recommended mobile phase: hexane/2-propanol (90/10 v/v).
Modifier: includes N,N-diethylamine for basic samples; trifluoroacetic acid for acidic samples; (NOTE: minimize use of the 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.

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

Suitable mobile phases **-** neutral: water/acetonitrile or water/methanol in different mixtures; basic: 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^-)
acidic: 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. Modifier includes N,N-diethylamine (DEA) for basic samples; trifluoroacetic acid (TFA) for acidic samples. It is recommendable to minimize the use of the modifier, typical use is 0.1 %, and maximum concentration should not exceed 0.5%.

8. Are there pH limitations for the Eurocel/Europak chiral columns?

Due to the silica basis 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 types. 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 recommendable to solve the sample usually 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 mobile phase polar solvents like 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. Especially your sample is an acid salt of a base the addition of 0.1 % DEA may helpful by converting the compound in 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 like acetone, chloroform, DMF, DMSO, MEK, toluene, dioxane, ethyl acetate, methylene chloride, pyridine and THF should be avoided. Even small amounts of these solvents can irreversible 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 preparation of 1 mg/ml, or even less, in mobile phase is usually sufficient.

12. How can be improved the peak shape

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 displacing 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 combined with a chiral column?

It is highly recommendable to use a precolumn with the same chiral column material. Due to the different physical properties of the silica material and the chiral column material the precolumn is not compatible. We are offering for all chiral column material a suitable precolumn with 5 x 4 mm dimension.

14. Is it possible to run a gradient with the Eurocel/Europak chiral columns?

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

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15. How can be obtained the right column performance?

In general, if a column problem is suspected, it should first be thoroughly flushed (see Knauer 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 recognize the problem. Most loss in efficiency problems are due either to partial blockage of the inlet filter or are due 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 such removal always comes with 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 kind of problems.

16. How can I realize an upscaling from analytical column to semi-preparative column?

The most important question is: What is the maximum loading capacity limit for the used analytical column? The separation has to been first 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 Size (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 α and can be quite higher for values higher than 1.4.

17. Can I use the Eurocel/Europak columns in SFC mode?

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

18. How is the pressure limit?

Don't operate your Knauer Eurocel/Europak above the recommended maximum pressure limit of 300 bar.

19. Additional recommendations:

1. Read carefully the “Column Care and Use” for the chiral Eurocel and Europak columns.

2. Use only the recommended solvents.

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

4. To avoid injected solvents effects try to dissolve the sample in mobile phase.

5. Avoid to use strong basic or acidic modifier. Please keep in mind the pH limit of the chiral phases (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 a equilibration time of nearly 30 min is needed.

8. It is recommendable to use a guard column to prevent contaminations on the main column.

9. Samples should be free of insoluble particles. Use syringe filtration for avoiding plugging of column sieves and frits.

10. To recognize achiral impurities of the enantiomers running the chiral separation at multiple wavelength or use different types of detectors.

11. If the chiral analysis is 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 storage with 2-propanol.

Physical Properties of recommended Column

Knauer Eurocel/Europak material is a high-performance chiral stationary phase designed to cover a majority of chiral applications. Based on high quality spherical silica matrix the derivatized cellulose or amylose coating represents the Eurocel/Europak material with high loading capacity.

Stationary phase	Eurocel 01	Eurocel 02	Eurocel 03	Eurocel 04	Europak 01
USP code	L40				L 51
Pore size	-	1000 A	-		
Particle size	-	3 µm, 5 µm, 10 µm, 20 µm	-		
Form	-	spherical	-		
Dimensions	-	250 x 4.6 mm	-		
Order number	25EM370ESJ	25EM390ECJ	…..		25EM320EPJ