

TECHNICAL NOTE

7. Troubleshooting for normal phase chromatography

Q 1 : How can I convert from reversed phase mode to normal phase mode or vice versus using the same HPLC equipment?

A 1 : To convert from reversed phase mode to normal phase mode, or vice versus, flush the equipment with a solvent that is miscible with both the current mobile phase and the intended mobile phase. Connect the HPLC pump directly with the detector, and replace the solvents according to following instructions.

Solvent conversion from reversed phase to normal phase

① To convert a mobile phase without buffer solution in reversed phase to normal phase, replace solvents according to the following steps :

- 1) Flush the equipment with a solvent for reversed phase. For example, methanol/H₂O (v/v=50/50).
- 2) Flush the equipment with a solvent miscible to both mobile phases. For example, tetrahydrofuran, ethanol.
- 3) Flush the equipment with a solvent for normal phase. For example, hexane/ethyl acetate

② To convert a mobile phase with buffer solution in reversed phase to normal phase, replace solvents according to the following steps :

- 1) Flush the equipment with a solvent with buffer solution for reversed phase. For example, methanol/phosphate buffer (v/v=50/50).
- 2) Flush the equipment with a solvent with the composition same as 1) and without salt, for example methanol/H₂O (v/v=50/50).
- 3) Flush the equipment with a solvent miscible to both mobile phases. For example, tetrahydrofuran, ethanol.
- 4) Flush the equipment with a solvent for normal phase. For example, hexane/ethyl acetate.

Solvent conversion from normal phase to reversed phase

① To convert from normal phase to a mobile phase without buffer solution in reversed phase, replace solvents according to following steps :

- 1) Flush the equipment with a solvent for normal phase. For example, hexane/ethyl acetate.
- 2) Flush the equipment with a solvent miscible to both mobile phases. For example tetrahydrofuran, ethanol.
- 3) Flush the equipment with a solvent for reversed phase. For example, methanol/H₂O (v/v=50/50).

② To convert from normal phase to a mobile phase with buffer solution in reversed phase, replace solvents according to following steps :

- 1) Flush the equipment with a solvent for normal phase. For example, hexane/ethyl acetate.
- 2) Flush the equipment with a solvent miscible to both mobile phases. For example tetrahydrofuran, ethanol.
- 3) Flush the equipment with a solvent with the composition same as 4) and without salt, for example methanol/H₂O (v/v=50/50).
- 4) Flush the equipment with a solvent for reversed phase. For example, methanol/phosphate buffer (v/v=50/50).

Q 2 : My flow rate is not stable. How can I troubleshoot?

A 2 : Possible causes for unstable flow rate can be a malfunctioning check valve or air in a mobile phase. Wash the check valve thoroughly by ultrasonic cleaner. Solvents with a low boiling point such as *n*-Hexane and *n*-Heptane generate air easily. To prevent air generation, degas the mobile phase sufficiently.

Q 3 : In spite of using the same condition, the retention time is different. How can I solve the problem?

A 3 : One possible cause is unstable flow rate. Please refer to Q2 section.

Another possible cause is variation of polar component in mobile phases. In normal phase chromatography, the retention time depends on the concentration of small amounts of very polar constituents in the mobile phase. This is especially true for water content in a mobile phase. In this case, always use fresh solvents in a mobile phase. If sample solvent includes water, change to a solvent without water or decrease injection volume. If a column contains water, remove water from the column by washing it with ethanol.

Q 4 How can I wash the COSMOSIL SL-II column?

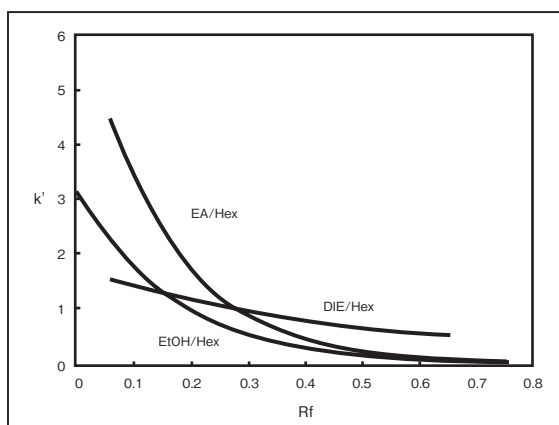
A 4 The SL-II column can be washed with tetrahydrofuran, methanol, ethanol, methylene chloride, *n*-Hexane or *n*-Heptane.

Q 5 : How can I store the COSMOSIL SL-II column?

A 5 : Store the SL-II column with the shipping screw tighten in order to prevent the solvent in the column from volatilization. In case where a solvent containing halogens is used, replace the solvent in the column with a solvent without halogens such as *n*-Heptane before storing.

Q 6 : Could I choose a mobile phase based on TLC data?

A 6 : The retention times of HPLC can be deduced from the R_f values of TLC. Refer to the figure below.



Q 7 : I get peak tailing in my run. What can I do about it?

A 7 : ① In case where the sample contains acidic compounds, add approx. 0.5% of acetic acid to the mobile phase.
② In case where the sample contains basic compounds, add approx. 0.5% of triethylamine to the mobile phase.

Q 8 : I get no peaks. How can I troubleshoot?

A 8 : First off, make sure that there is no problem with the system. If the problems are with the sample, sample solvent or mobile phase, try following check list.

1. The analyte may not be eluted from a column because the retention of the analyte is too strong. In this case, use a stronger eluent (mobile phase).
2. The sample contains chelating compounds or basic compounds. They may be adsorbed to the packing materials. In this case, add 0.1% - 1% acid (trifluoroacetic acid or acetic acid) to the mobile phase.