Technical Note | USP <621> L8:
Amino-propyl-modified stationary phases

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General Care: USP <621> L8 stationary-phases

HPLC-columns by MZ-Analysentechnik with stationary phase providing -NH_2/Amino-groups for interaction with analytes as retention mechanism may be used in both normal phase and reversed phase mode. They feature stable and predictable separations with high column efficiencies over and over again - as long as reaction or deactivation of the amino-groups is avoided. The following technical note will help users to get the maximum out of the columns and to extend the lifetime to its maximum.

General Care

To extend column lifetime and prevent contamination please consider the following suggestions:

1. NH2/Amino columns are usually shipped in n-heptane/2-propanol 97/3. The eluent is written on the column quality certificate, which is shipped together with the column.
2. **Avoid acetone as a solvent!** Ketones react with amino-groups to form Schiff bases, and damage stationary phase irreversibly.
3. Column equilibration varies with the desired type of mobile phase / or condition the column is used for:

   Normal Phase Separations:
   - No special preparation is needed, directly use desired mobile phase and equilibrate as usual until stable baseline is achieved (usually 10 column volumes)

   Reversed Phase Separations:
   - Flush the column with 20 column volumes of 2-propanole prior to usual equilibration with desired mobile phase
4. Storage: all HPLC columns need to be stored in an appropriate solvent and capped tightly to prevent draining. Store column in a cool (not cold) place, free from vibration. Used a special column storage cabinet or use the shipping box. Flush columns after use with pH-neutral solvent to remove all possible contaminants and tag the column with the used storage solvent for later use. We recommend as storage solvent 2-propanol, which is compatible with both Normal Phase and Reversed Phase Chromatography.
5. Use: only use analytical grade solvents, filtered through a 0.2–0.45 µm membrane and degassed.
6. If possible, filter samples through a 0.2–0.45 µm syringe filter before injection and/or use guard column if contaminated samples have to be analyzed.

Column regeneration

Contamination of the column by impurities from the sample or mobile phase can cause changes in peak shape, peak splitting, shifts in retention or result in high back-pressure. It is important, to locate the source of contamination before using the column again for the analysis of samples. If a guard column is used high back-pressure is observed, try to exchange the guard column first. If the problem consists, please follow the next steps to try to regenerate the column:

Normal Phase Conditions :

1. Flush the column with 20 column volumes of heptane
2. Flush the column with 5 column volumes of 2-propanol
3. Flush the column with 20 column volumes of acetonitrile
4. Flush the column with 20 column volumes of water
5. Flush the column with 20 column volumes of acetonitrile
6. Flush the column with 5 column volumes of 2-propanol
7. Flush the column with 20 column volumes of heptane
8. Re-equilibrate the column with the required mobile phase

Reversed Phase Conditions :

1. Flush the column with 20 column volumes of water
2. Flush the column with 20 column volumes of acetonitrile
3. Flush the column with 5 column volumes of 2-propanol
4. Flush the column with 20 column volumes of heptane
5. Flush the column with 5 column volumes of 2-propanol
6. Flush the column with 20 column volumes of acetonitrile
7. Re-equilibrate the column with the required mobile phase