Packing, Cleaning and Storage of ZEOsphere® underivatized, DIOL, SH, NH2 and CN

Column packing

Always use clean or new frits. ZEOsphere® normal-phase materials should be packed using the so-called slurry method. 40-60g silica per 100 mL slurry solvent should be used. Typical slurry solvents are: toluene / iso-propanol (50/50) or acetone / iso-propanol (50/50). CN phases can be packed with anhydrous ethanol.

Column cleaning

It is advisable to reverse the column direction before cleaning (make sure that the inlet frit is small enough to avoid the loss of particles). Regular cleaning of the material with high polar solvent concentration should be performed to avoid large contamination of the bed. Weakly bonded impurities can be removed by the application of at least 20 empty column volumes (CV) of a polar solvent, e.g. isopropanol, ethyl acetate or chloroform. To avoid precipitation make sure that buffered solution are totally removed from the column before applying the organic solvent.

Material storage

The materials can be stored either dry or in an organic solvent. To store the material dry, flush the column with THF of acetone (for amino phases only THF should be used); unpack the column and dry the material in a suited oven (90°C for derivatized materials and 120°C for underivatized materials). To store the materials in the wet state, it is advisable to use the same solvent that will be used for repacking the material (see “column packing” section).

All ratios are intended as volume ratios.

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ZEOCHEM®

Packing, Cleaning and Storage of ZEOsphere® C18, C8 and C4

Column packing

Always use clean or new frits. ZEOsphere® reversed-phase materials should be packed using the so-called slurry method. 40-60g silica per 100 mL slurry solvent should be used. Typical slurry solvents are for C4, C8 derivatized materials: acetone / iso-propanol (50/50), anhydrous ethanol or methanol. C18 materials can be packed with methanol, THF / iso-propanol (50/50), toluene / iso-propanol (50/50) or chloroform / iso-propanol (50/50).

Column cleaning

It is advisable to reverse the column direction before cleaning (make sure that the inlet frit is small enough to avoid the loss of particles). Regular cleaning of the material with high organic modifier concentration should be performed to avoid large contamination of the bed. Weakly bonded impurities can be removed by the application of at least 20 empty column volumes (CV) of an organic solvent, e.g. methanol, THF or acetonitrile. To void precipitation make sure that buffered solution are totally removed from the column before applying the organic solvent. To remove stronger bound impurity, lipids or fats, the following wash cycle can be tried: methanol, acetonitrile, acetonitrile / iso-propanol (75/25), iso-propanol, methylene chloride and hexane. Before going back to buffer solutions, it is advisable to wash the column with iso-propanol and water. At least 10 CV of each solvent should be used. Proteinaceous material can be removed with acetic acid (1% in water), trifluoroacetic acid (1% in water), 0.1% trifluoroacetic acid / propanol (40/60) or triethylamine / propanol (40/60, adjust 0.25N phosphoric acid to pH=2.5 with triethylamine before mix). Urea and guanidine (5-8 M, adjust the pH to 6-8) or a mixture of dimethyl sulfoxide / water or dimethyl formamide / water (50/50) can also be used. 20 CV of the solvents should be used. Pay attention to possible increase in backpressure. After using urea and guanidine solutions flush the column with at least 40-50 CV of water. In case the above mentioned solution are unsuccessful, cleaning in place (CIP) can also be applied: purge the column with a solution of 0.1M NaOH aq / EtOH 50/50 for 2-3 CV (maximum contact time: 15 min) and then immediately lower the pH with an acid solution (e.g. EtOH/H2O/AcH: 10/90/0.2 weight). Depending on the application, some retention time shift can happen after the CIP treatment. Finally, in case of metal ions contamination, a 0.05 M EDTA solution can be used.

Material storage

The materials can be stored either dry or in an organic solvent. To store the material dry, flush the column with THF of acetone; unpack the column and dry the material at 90°C in a suited oven. To store the materials in the wet state, it is advisable to use the same solvent that will be used for repacking the material (see “column packing” section).

All ratios are intended as volume ratios.

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