Chromatography Solutions

LC Tips #002

Column regeneration for Partisil/Partisphere ion-exchange columns

GENERAL CONSIDERATIONS

Column efficiency will diminish with use. Sample and/or mobile phase impurities can accumulate at the head of the column. A change in back pressure, lower column efficiency and sometimes a change in selectivity can indicate that a column requires regeneration. The procedure outlined overleaf may restore column performance but is not guaranteed.

Throughout column use, the following everyday practices can be employed to enhance the lifetime of a column:

Use only high-purity HPLC solvents and buffers. This will not only help to preserve the lifetime of the column, but also prevent unknown chromatographic peaks due to impurities.

Use freshly prepared mobile phases and buffers to prevent bacterial growth, particularly for low buffer concentrations and mobile phases around pH 7.

Filter mobile phases to remove particulates or use in-line filters.

Use appropriate sample clean-up procedures. This can prevent particulates reaching the column and also remove sample components that may become strongly bound to the column.

Use a guard column or pre-column filter to protect the column from particulates and other detrimental sample components.

When setting the flow rate, begin at a low flow rate and gradually increase the flow to the desired level. This minimises the physical shock to the column.

Always work within the pressure and flow rate limitations of the column. These are specified on the reverse of the QC chromatogram accompanying the column.

After use, wash buffers from the column and store on the solvent recommended on the test chromatogram.

COLUMN REGENERATION

For Partisil/Partisphere SAX, Partisil/Partisphere SCX and Partisphere WAX and WCX:

To remove strongly retained sample components, the column should be disconnected from the detector and the column outlet directed to waste using suitable tubing.

Ion-exchange columns are regenerated by passing 20 column volumes (see table 1) of each of the following mobile phases through the column:

1. Buffer wash – 5x as concentrated as that used previously
2. Distilled water
3. Acid wash - 0.5 M H_3PO_4, 0.01 M H_2SO_4, or 0.01 M HNO_3
4. Distilled water
5. Chelating wash - 0.1 M disodium EDTA
6. Distilled water
7. Methanol wash - to remove adsorbed organics from the bonded phase
8. Distilled water
9. Buffer for separation

Not all of these wash steps are required for every column clean-up. Some chromatographers require only a combination of 1, 2, 7, 8 and 9. Other washes can also be used, with the exception of any with basic pH's (7-14), which must be avoided to prevent damage to the Partisil silica backbone.

The water wash before and after the methanol wash is important. It prevents possible clogging due to the insolubility of the buffer salts in methanol.

For extended column life, please do not allow buffer solutions to remain on an ion-exchange column with no flow. Always flush these buffers off the column before storage.

Table 1: Approximate column volumes in mL for common column dimensions (fully-porous silica).

		Column length (mm)					
		50	75	100	125	150	250
Column ID (mm)	1.0	0.025	0.037	0.049	0.062	0.074	0.124
	2.1	0.109	0.164	0.218	0.273	0.327	0.546
	3.0	0.223	0.334	0.445	0.557	0.668	1.113
	4.6	0.523	0.785	1.047	1.309	1.570	2.617