Application Note #H1.0

Column Cleaning and Regeneration

If adequate care is taken, it is possible to maintain column efficiency and reliability over an extended period of time. This document is intended to give information on the different procedures to help extend HPLC column lifetime. This document will also cover column storage.

Difference between cleaning and regeneration

We usually make the assumption that, after a separation, all the material initially present in the column or cartridge has been eluted. After a run, the column is simply washed with 2-3 column volumes with the initial solvent mixture before starting a new separation. Impurities are compounds present in the sample that are of no interest to the analyst and may have varying affinities for the stationary phase. Impurities that are strongly retained on the column will accumulate at its head if the mobile-phase composition is not strong enough to elute them during a regular run. The build up may cause loss of performance, back-pressure build up, peak tailing, retention time shift, or baseline drift. To avoid this, it is highly recommended to perform regular cleaning of the column before any symptoms appear. This process is simple and does not require modification of the usual chromatographic set up. When cleaning is not sufficient, a more thorough treatment, i.e. regeneration, may be necessary to avoid discarding the column.

Cleaning

The more frequent cleaning is performed, the less rigorous conditions are necessary. Cleaning should also be performed: after running a known "dirty" sample and prior to column storage or dormancy.

Table 1 : Column volumes of various analytical columns

Column Size (mm x mm)	Column Volume (mL)
250 x 4.6	2.5
150 x 1.5	1.5
150 x 3.0	0.64
150 x 2.1	0.28
50 x 4.6	0.50
30 x 4.6	0.30
15 x 4.6	0.15

Regeneration

If problems do not resolve with cleaning, it is then necessary to use more aggressive, but also lengthy washing methods. This procedure consists in washing the column with solvents of gradual polarity, going from the weakest to the strongest and backwards to return to the initial mobile phase composition. Since most of the impurities are retained at the head of the column, reversing the column direction will reduce the distance impurities have to travel to exit the column. Pressure used to pack the column is much higher than normal operating pressures so packing should not be disturbed by this operation. During regeneration, disconnect the column from the detector to avoid contamination of the detector cell. Also, reduce flow rate to Ð its typical value: some solvents or combination of solvents have higher viscosity than that of the normal mobile phase. If a buffer is normally used, replace it with water during the procedure. Washing directly with 100 % of an organic solvent may cause buffer precipitation, hence creating even bigger problems. Table 2 shows a typical procedure for regeneration of reversed and normal phase sorbents. Between 10 and 20 column volumes of each solvent should be passed through the column. These volumes should be double for the normal phase procedure.

Table 2 : Typical regeneration procedures

For Reversed Phase	For Normal Phase
•Disconnect and reconnect the column in reverse direction; •Flush buffer out by replacing it with water in the mobile phase; •100 % MeOH; •100 % Isopropanol; •100 % DCM; •100 % Hexane; •100 % DCM; •100 % Isopropanol; •Reconnect the column in the proper direction; •Mobile phase with buffer; •Check performance with a standard sample.	•Disconnect and reconnect the column in reverse direction; •50:50 MeOH/CHCl3; •100 % Ethyl Acetate; •Reconnect the column in the proper direction; •Mobile phase; •Check performance with a standard sample.

Metals

If metal ions are part of the contaminants, it may be useful to wash the column with 0.05M ethylenediaminetetraacetic acid (EDTA) to help to solublize them. Afterwards, wash thoroughly with water before going back to the initial composition of the mobile phase.

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pH

If ionizable compounds are present, changing the pH of a water-organic solvent may increase their mobility. If working in reversed phase, it would mean ionizing them to increase their solubility in the mobile phase. For example, if an amine is strongly retained, adjusting the pH to 3 or less will ensure complete protonation, hence increasing its solubility in the aqueous mobile phase. If working in normal phase, the reverse would be true, i.e. acidic wash for acidic compounds. However care must be taken not to work outside the column stability pH range.

Storage

When a column is no longer needed, it is possible to store it under certain conditions that will allow it to retain its full efficiency. The first thing to consider is the storage solvent. It should be exempt of buffers or salts to avoid precipitation or damage to the stationary phase. For reversed phase, the organic content must be > 50 %. If water is present in larger proportion, a biocide such as 0.05 % NaN_3 in water must be used to prevent bacterial growth. It is desirable to use a storage solution that is miscible with the typical mobile phase. Table 3 lists the suggested storage solvents.

Table 3 : Recommended storage solvents

Column Type	Solvent
Reversed Phase	> 50 % organic in water
Normal Phase	Hexane or isopropanol
Ion-Exchange	> 50 % organic in water
Diol	0.05 % NaN_3 in water

Cartridges

The same procedures may be adapted for cartridges although lifetime will be much shorter than HPLC analytical columns. They should be reusable up to 10 times.