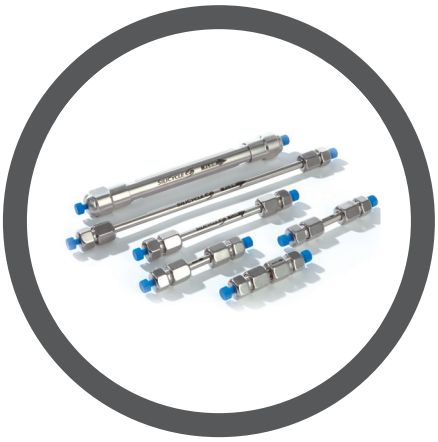
APPLICATION NOTE
#SC001-0

SiliCycle

SiliaChrom® Plus



HPLC Columns - Cleaning & Regeneration Procedures

If proper care is taken, it is possible to maintain column efficiency over an extended period of time. This Application Note shares different procedures used to help extend the lifetime of HPLC columns.

LEARN MORE about SiliaChrom Plus in our brochure "*Solutions for Analytical & Preparative Chromatography*."

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 and the column is simply washed with 2-3 column volumes of 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 is not strong enough to elute them during a regular run. The build-up may cause: loss of performance, an increase in back-pressure, peak tailing, retention time shift and/or baseline drift. To avoid this, it is highly recommended to regularly clean the column. This process is simple and does not require modification of the usual chromatographic set up. When cleaning is not sufficient (*the column seems clogged*) or prior to column storage, a more thorough treatment, i.e. regeneration, may be necessary to avoid discarding the column.

Column Volume (*packing's volume included*) in mL = π * [Column Radius in cm]^2 * [Column Length in cm]

Table 1: Column volumes of various analytical columns

Column Volumes of Various Analytical Columns					
Column Size (*mm*)	Column Volume (*mL*)	Column Size (*mm*)	Column Volume (*mL*)	Column Size (*mm*)	Column Volume (*mL*)
250 x 4.6	4.15	250 x 3.0	1.77	250 x 2.1	0.87
150 x 4.6	2.49	150 x 3.0	1.06	150 x 2.1	0.52
100 x 4.6	1.66	100 x 3.0	0.71	100 x 2.1	0.35
50 x 4.6	0.83	50 x 3.0	0.35	50 x 2.1	0.17

CLEANING

Frequent cleaning ensures that the process tends to be easier and quicker subsequently each time. Also, cleaning should always be performed after running a known "dirty" sample.

During the cleaning, the flow rate has to be reduced to about 20 - 50 % of its typical value because some solvents or combination of solvents have higher viscosities than that of the normal mobile phase. If a buffer solution is normally used, it must be replaced with water during the procedure. Washing directly with 100 % of an organic solvent may cause buffer precipitation, consequently creating even bigger problems.

Table 2 reveals typical procedures for cleaning of reversed and normal phases as well as ion exchange sorbents. To achieve this, 2 to 3 column volumes of each solvent should be passed through the column.

Table 2: Typical cleaning procedures

SiliaChrom Plus Suggested Cleaning Procedures	
Column Type	Step-by-Step Procedure
Reversed-Phase Columns (*C18, C18-300, C8, C8-300, C4, C4-300, Phenyl, PFP, Amine, Cyano*)	• Water / methanol (*50/50*) to remove buffers • Methanol • Mobile phase used during the separation
Normal Phase Columns (*Amine, Cyano, Diol, Silica*) **Note**: never use water	• Isopropanol, hexane • Mobile phase used during the separation
Ion Exchange Columns (*SCX, SAX*)	• Water / methanol (*50/50*) to remove buffers • Methanol • Mobile phase used during the separation

REGENERATION

If after the cleaning procedure some problems still persist, it is then necessary to use more aggressive and lengthy washing methods. Since most of the impurities are retained at the head of the column, reversing the column direction (*known as backflushing*) will reduce the distance that impurities have to travel to exit the column. During the regeneration procedure, the column must be disconnected from the detector to avoid the contamination of the detector cell. The flow rate also has to be reduced to about 20 - 50 % of its typical value because some solvents or combination of solvents have higher viscosities than the normal mobile phase. If a buffer solution is normally used, it must be replaced with water during the procedure. Washing directly with 100 % of an organic solvent may cause buffer precipitation, thereby creating even bigger problems.

Typical procedures for regeneration of reversed and normal phases as well as ion exchange sorbents are shared in Table 3. To achieve regeneration, between 10 and 20 column volumes of each solvent should be passed through the column.

Table 3: Typical regeneration procedures

Silia*Chrom* Plus Suggested Regeneration Procedures	
Column Type	**Step-by-Step Procedure**
Reversed-Phase Columns (*C18, C18-300, C8, C8-300, C4, C4-300, Phenyl, PFP, Amine, Cyano*)	• Water / methanol (*90/10*)* • Methanol, isopropanol, methanol • Mobile phase used during the separation
Normal Phase Columns (*Amine, Cyano, Diol, Silica*) **Note**: never use water	• Isopropanol, methanol, isopropanol • Hexane • Mobile phase used during the separation
Ion Exchange Columns (*SCX, SAX*)	• Mobile phase with buffer concentration doubled (*be careful for salt precipitation*) • Water / methanol (*90/10*) • Methanol, isopropanol, methanol and water / methanol (*90/10*) • Mobile phase used during the separation

* For amine and cyano columns, use water / methanol (*70/30*)

METALS

If metal ions are part of the contaminants, it may be useful to wash the column with 0.05 M EDTA to help in solubilizing them. Afterwards, the column must be washed thoroughly with water before going back to the initial composition of the mobile phase.

PH

If ionizable compounds are present, modifying the pH of a water-organic solvent may increase their mobility. In the reversed-phase mode, it would mean ionizing them and thus increasing their solubility in the mobile phase. For example, if an amine compound is strongly retained, adjusting the pH to 3 or lower will ensure the complete protonation of the amine function, hence increasing its solubility in the aqueous mobile phase. However, care must be taken not to work outside of the column pH stability range.

STORAGE

When Silia*Chrom* Plus HPLC Columns are not used for an extended period of time, do not leave high aqueous or high salt mobile phases in the column to avoid precipitation or damage to the stationary phase. Always start by following the regeneration procedure before storing a column.

Next, the storage solvent itself must be considered. It is always easier to use a storage solution miscible with the typical mobile phase. For reversed-phases, the organic content must always be > 50 %, 100 % methanol is recommended. For normal phases, 100 % hexane is always a good choice. Table 4 sums up these suggested storage solvents according to the type of phase.

Finally, our columns are shipped with two removable column end plugs to prevent drying of the column bed. Always put these plugs back on before storing the column.

Table 4: Recommended storage solvents

Recommended Storage Solvents	
Column Type	**Solvent**
Reversed-Phase	Methanol
Normal Phase	Hexane
Ion Exchange	Methanol

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