High Performance Liquid Chromatographic Columns

High performance liquid chromatography (HPLC) is a type of liquid chromatography that uses a liquid moblie phase. The same basic principals from gas chromatography are applied to liquid chromatography. There are three basic types of liquid chromatographic columns: liquid-liquid, liquid-solid, and ion-exchange. Liquid-liquid chromatographic columns have the liquid stationary phase bonded or absorbed to the surface of the column, or packed material. liquid-liquid chromatographic columns are not as popular because they have limited stability and they are inconvenient. Partitioning occurs between the two different liquids of the mobile and stationary phases. In liquid-solid chromatographic columns the stationary phase is a solid and the analyte absorbs onto the stationary phase which separates the components of the mixture. In ion-exchange chromatographic columns the stationary phase is an ion-exchange resin and partitioning occurs with ion exchanges that occur between the analyte and stationary phase

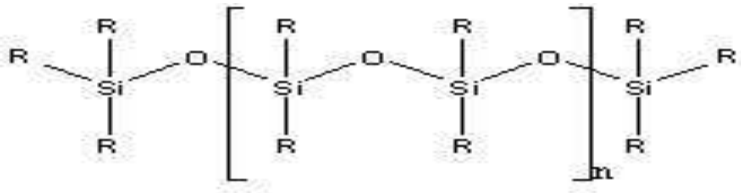
Usually HPLC has a guard column ahead of the analytical column to protect and extend the life of the analytical column. The guard column removes particulate matter, contaminants, and molecules that bind irreversibly to the column. The guard column has a stationary phase similar to the analytical column.

The most common HPLC columns are made from stainless steel, but they can be also made out of thick glass, polymers such as polyetherethelketone, a combination of stainless steel and glass, or a combination of stainless steel and polymers. Typical HPLC analytical columns are between 3 and 25 cm long and have a diameter of 1 to 5 mm. The columns are usually straight unlike GC columns. Particles that pack the columns have a typical diameter between 3 to 5 µm. Liquid chromatographic columns will increase in efficiency when the diameter of the packed particles inside the column decreases.

Packing Material

HPLC columns are usually packed with pellicular, or porous particles. Pellicular particles are made from polymer, or glass beads. Pellicular particles are surrounded by a thin uniform layer of silica, polystyrene-divinyl-benzene synthetic resin, alumina, or other type of ion-exchange resin. The diameter of the pellicular beads is between 30 and 40 µm. Porous particles are more commonly used and have diameters between 3 to 10 µm. Porous particles are made up silica, polystyrene-divinyl-benzene synthetic resin, alumina, or other type of ion-exchange resin. Silica is the most common type of porous particle packing material.

Partition HPLC uses liquid bonded phase columns, where the liquid stationary phase is chemically bonded to the packing material. The packing material is usually hydrolyzed silica which reacts with the bond-phase coating. Common bond phase coatings are siloxanes. The relative structure of the siloxane is shown



Reverse and Normal Phase HPLC

A polar stationary phase and a non-polar mobile phase are used for normal phase HPLC. In normal phase, the most common R groups attached to the siloxane are: diol, amino, cyano, inorganic oxides, and dimethylamino. Normal phase is also a form of liquid-solid chromatography. The most non-polar compounds will elute first when doing normal phase HPLC.

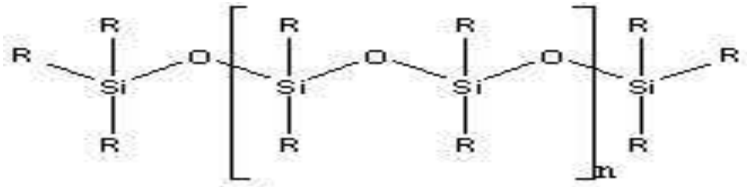


Figure 11: Basic structure of a siloxane. The R groups can be varied depending on the type of column and analyte being analyzed. This figure was created with ChemBioDraw Ultra 12.0.

Reverse phase HPLC uses a polar mobile phase and a non-polar stationary phase. Reverse phase HPLC is the most common liquid chromatography method used. The R groups usually attached to the siloxane for reverse phase HPLC are: C_8, C_{18},or any hydrocarbon. Reverse phase can also use water as the mobile phase, which is advantageous because water is cheap, nontoxic, and invisible in the UV region. The most polar compounds will elute first when performing reverse phase HPLC. Check the animation on the principle of reversed-phase chromatography to understand its principle.

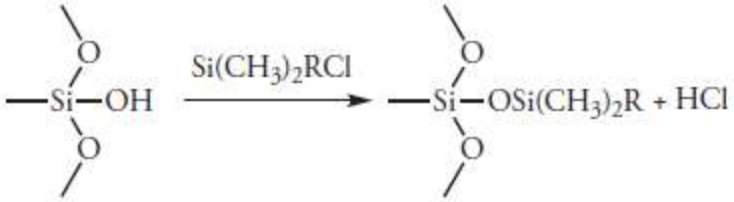
Properties of Stationary Phases

stationary phase, in analytical chemistry, **the phase over which the mobile phase passes in the** technique of chromatography. ... Typically, the stationary phase is a porous solid (e.g., glass, silica, or alumina) that is packed into a glass or metal tube or that constitutes the walls of an open-tube capillary.

The combination of a polar stationary phase and a **nonpolar** mobile phase is called normal-phase chromatography. In reversed-phase chromatography, which is the more common form of HPLC, the stationary phase is nonpolar and the mobile phase is polar.

Examples of polar stationary phases include those where R contains a cyano ($–C_2H_4CN$), **a diol ($–C_3H_6OCH_2CHOHCH_2OH$)**, or an amino ($–C_3H_6NH_2$) functional group. Because the stationary phase is polar, the mobile phase is a nonpolar or moderately polar solvent.

In liquid–liquid chromatography the stationary phase is a liquid film coated on a packing material, typically 3–10 µm porous silica particles. Because the stationary phase may be partially soluble in the mobile phase, it may elute, or bleed from the column over time. To prevent the loss of stationary phase, which shortens the column's lifetime, it is covalently bound to the silica particles. **Bonded stationary phases** are created by reacting the silica particles with an organochlorosilane of the general form $Si(CH_3)_2RCl$, where R is an alkyl, or substituted alkyl group.



To prevent unwanted interactions between the solutes and any remaining –SiOH groups, $Si(CH_3)_3Cl$ is added, converting the unreacted sites to $–SiOSi(CH_3)_3$; such columns are designated as end-capped.

The properties of a stationary phase depend on the organosilane's alkyl group. If R is a polar functional group, then the stationary phase is polar. Examples of polar stationary phases include those where R contains a cyano ($–C_2H_4CN$), a diol ($–C_3H_6OCH_2CHOHCH_2OH$), or an amino ($–C_3H_6NH_2$) functional group. Because the stationary phase is polar, the mobile phase is a nonpolar or moderately polar solvent. The combination of a polar stationary phase and a nonpolar mobile phase is called **normal-phase chromatography**.

In **reversed-phase chromatography**, which is the more common form of HPLC, the stationary phase is nonpolar and the mobile phase is polar. The most common nonpolar stationary phases use an organochlorosilane where the R group is an *n*-octyl (C_8) or *n*-octyldecyl (C_{18}) hydrocarbon chain. Most reversed-phase separations are carried out using a buffered aqueous solution as a polar mobile phase, or with other polar solvents, such as methanol and acetonitrile. Because the silica substrate may undergo hydrolysis in basic solutions, the pH of the mobile phase must be less than 7.5.

Column care and Regenration

Increased back-pressure, changes in retention times/loss of column performance are all common symptoms of deposits on the column inlet frit, in the column or on the surface of the stationary phase. Most of the times, these problems can be overcome by the use of a correctly applied washing procedure. The sooner you use the column regeneration procedures the more chances you have to recover your column original performance.

Strongly adsorbed species are collected at the solvent-inlet end of the column and in many cases it is a benefit to use a reversed flow during washing. Contrary to popular belief reversing the flow will never harm a column that is well packed. The mentioned flow direction is usually the direction in which the column was originally packed. Please note that *the irregular/amorphous original silicas nevertheless represent a possible problem when reversing the flow as possible restructuration of the packed bed is then possible. Extreme care should always be taken when reversing the flow on these irregular silicas.*

Please check our suggested regeneration procedures for the different family of phases (*RP, NP, Ion Exchange etc.*) and perform the CoA column test after these procedures, should the original pressure be restored, in order to assess the column performance/peak symmetry.

Silia *Chrom* Cleaning and Regeneration Procedures

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

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 of the initial solvent mixture before starting a new separation. However, some impurities that are strongly retained on the column will accumulate at the intlet, if the mobile-phase composition is not strong enough to elute them during a regular run. Some non-negligible problems can arise when this happen: 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 of these symptoms occurs. 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.

Suggested Cleaning and Regeneration Procedures

The more you use a cleaning procedure, the less rigorous conditions be necessary. Cleaning should be performed after running a known *"dirty"* sample. Regeneration procedure should be performed prior to column storage, or when a column seems clogged. The flow rate is usually set lower than during the separation (*typically from 20% to 50%*).

Silia*Chrom* Suggested Cleaning and Regeneration Procedures		
Column	**Suggested Cleaning Procedure**	**Suggested Regeneration Procedure**
Silia*Chrom* HPLC Column	- Set the flow rate (*20 to 50% of the usual one*) - Rinse with 2-3 column volumes of each of the following solvents	- Backflush the column - Set the flow rate (*20 to 50% of the usual one*) - Rinse with 10 column volumes of each of the following solvents
Reversed-Phase Columns (*C18, C8, C4, C1, Amine, Cyano, Phenyl, PFP, etc.*)	- Water/ACN (95/5) to remove buffer - Water/ACN (5/95) - Mobile phase used during the separation	- Water/ACN (95/5) - THF - Water/ACN (5/95) - Mobile phase used during the separation
Normal Phase Columns (*Amine, Cyano, Diol, etc.*) Note: Never use water.	- MeOH/$CHCl_3$ (50/50) - Ethyl Acetate - Mobile phase used during the separation	- MeOH/$CHCl_3$ (50/50) - Isopropanol and ethyl acetate - Mobile phase used during the separation
Unbonded Silica Columns (*Silica*)	- Hexane, isopropanol and dichloromethane - Mobile phase used during the separation	- Hexane, isopropanol and dichloromethane - Mobile phase used during the separation
Ion Exchange Columns (*SCX, SAX, etc.*)	- 5 mM Phosphate Buffer pH 7.00 - Acetic Acid/Water (10/90) - Water, methanol and water	- 5 mM Phosphate Buffer pH 7.00 - Acetic Acid/Water (10/90) - Water, methanol and water