Shodex HPLC Column Selection Guide

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Shodex™

HPLC Columns

Content

Introduction

Selecting the right HPLC column is the most crucial decision in order to get the best and a maximum separation out of your HPLC measurements.

There are in principal 5 different starting points in your selection which will be described in the following sections.

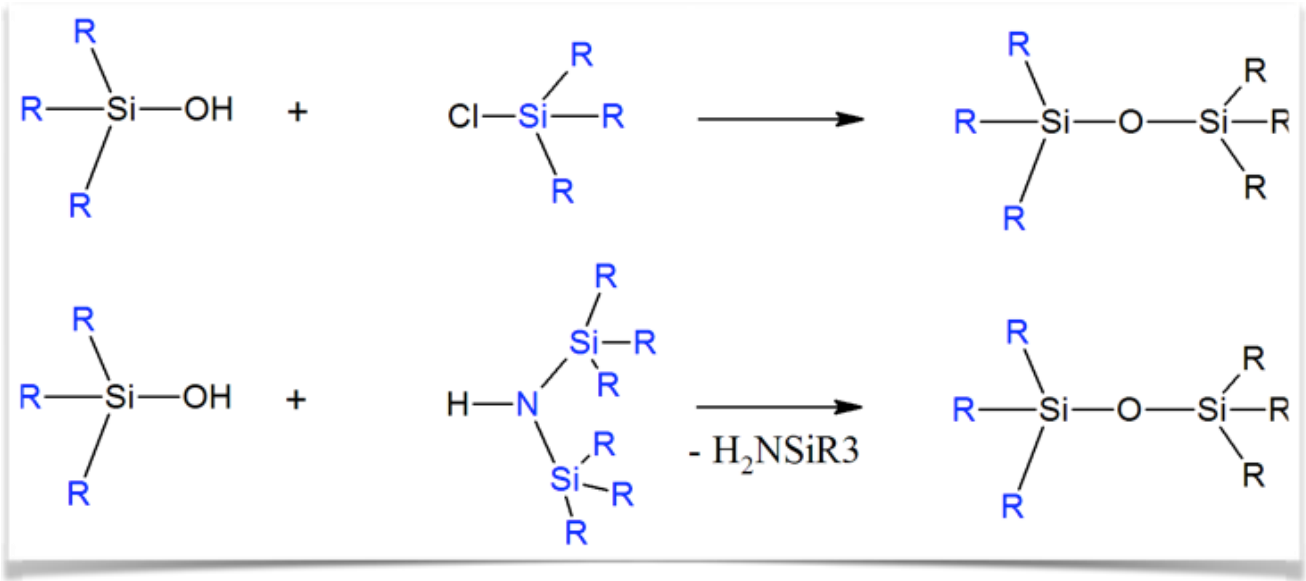
1) Selection by Base Material
2) Selection by HPLC Separation Mode
3) Selection by Functional group
4) Selection by Area of Application
5) Selection by Analyte

1. Selection by Base Material

In principal 2 major base materials for selection can be used. Silica based and polymer based resins.

a) Silica based material

The surface of silica based material is composed by silanol groups (Si-OH) and siloxane bridges (Si-O-Si). Under low and neutral pH conditions the silanol groups are protonated and stable. The situation is changing dramatically if higher pH values in the running solvent are used during HPLC. Under these conditions the silanol group is deprotonating and starts to dissolve. This will cause a fast aging of the used silica based column. In order to protect the free silanol groups better the process of end-capping is being applied. For endcapping several reagents can be used. Chlorsilane or silazanes are used.



Reaction of silanol groups with chlorsilane or silazanes for endcapping protection

Many Silica HPLC producers are claiming the wording “fully endcapped”. This means due to the sterically hindrance of endcapping procedure, only around 50 % of the free silanol groups can be bonded and protected. The free (unprotected) silanol groups are still able to interact with solvent (e.g. high pH values and dissolving) or with analytes. Interacting analytes usually create higher adsorption rate on the silica surface. This will age the column faster and it also has a negative influence on peak shape (symmetry) and base line separation.

In order to answer the question if a silica based HPLC is suitable for the analyte it is crucial to know which adsorptive interaction can happen. Amines or basic substances or also very polar substances show bad performance due to that reason on silica based HPLC columns. In addition if a higher pH value (>7) needs to be used Silica is not the best choice as a base material.

b) Polymer based material

The described negative aspects of Silica as base material can be overcame by using a chemical inert Polymer, where silanol groups are not present at all and therefore an end-capping process is not needed. The used cross-linked polymers are also stable under almost full pH range from 2-13 in many cases.

Available polymeric resins for HPLC columns are mainly:

- Styrene divinylbenzene Copolymer
- Polyhydroxymethacrylate
- Polyvinyl alcohol
- Polymethacrylate

The polarity varies from almost non polar for Styrene divinylbenzene Copolymer as base material to Polyhydroxymethacrylate which is quite polar. The different possibilities as base materials can also create several effects in separation, e.g. reversed phase separation only due to difference in polarity between stationary phase and analyzed sample. Several surface effects can be created as well with functional groups known well from Silica based columns e.g. C18 or amino groups.

2. Selection by HPLC Separation Mode

Liquid chromatography (LC) uses liquid as mobile phase (eluent). It is an analytical method that separates a mixture of compounds based on their physical and chemical differences. High

performance liquid chromatography (HPLC) is a method that introduces the mobile phase under high-pressure conditions resulting in rapid and high-performance separations. The various interactions between the analyte, stationary phase (packing material), and mobile phase are the key factors for the separation. A wide variety of separation modes can be achieved by using particular combinations of stationary and mobile phases. The following table gives an overview about most common used separation techniques and a short explanation of those.

Separation mode	Characteristics
Reversed Phase Chromatography (RPC)	•Separation is based on the partition equilibrium between stationary phase and mobile phase. •The polarity of the stationary phase is lower than that of the mobile phase. •Typically the mobile phase contains a mixture of organic solvents (methanol, acetonitrile, or THF) and aqueous solvents (water or buffer). •Using the lower polarity mobile phase causes a faster elution.
Hydrophilic Interaction Chromatography (HILIC)	•Separation is based on hydrophilic interaction. •A high polarity stationary phase is used. •Typically the mobile phase contains a mixture of organic solvents such as acetonitrile and aqueous solvents (water or buffer). •Using the higher polarity mobile phase causes a faster elution. •Applicable for the analysis of high polar substances.
Normal Phase Chromatography (NPC)	•Separation is based on the partition equilibrium between the stationary phase and the mobile phase. •The polarity of the stationary phase is higher than that of the mobile phase. •Typically the mobile phase contains a mixture of organic solvents with different polarities such as hexane and isopropanol.
Ligand Exchange Chromatography (LEX)	•Separation is based on differences in analytes' coordination complex. •Stationary phase modified with metal sulfonate complex ion. •Works in combination with size exclusion or HILIC modes.
Ion Exclusion Chromatography (IEX)	•Separation is based on electrostatic interaction (repulsion) between the ion exchanger and ionic solutes. •Dissociated ionic molecules elute faster than non-dissociated forms. •Used mainly for the analysis of organic acids.
Ion Chromatography (IC)	•Separation is based on electrostatic interaction (bonding) between the ion exchanger and ionic solutes. •Has a relatively small ion exchange capacity. •Electrical conductivity detector can be used with low-salt concentration mobile phase. •Used mainly for the analysis of inorganic compounds.
Size Exclusion Chromatography (SEC)	•Network or pores on the surface of the packing material works as molecular sieve to separate molecules based on their sizes. •To separate molecules solely based on their sizes, it requires an analytical condition without any analyte and packing gel interaction. •The bigger the molecule size, the faster the elution sequence. •Used for molecular weight or molecular distribution determination of macromolecules and qualification of oligomers.
Ion Exchange Chromatography (IEC)	•Separation is based on electrostatic interactions between the ion exchanger and ionic solutes. •The mobile phase of choice should have a sufficient buffering capacity at the pH that produces the largest charge differences between the analyte of interest. •The elution position is optimized by varying the pH, salt concentration, and/or ionic strength of the mobile phase.
Hydrophobic Interaction Chromatography (HIC)	•Separation is based on hydrophobic interaction. •Hydrophobic functional group is modified on the stationary phase. •Adsorption of analytes generally occurs at a high salt concentration and they are released by lowering the salt concentration. •Used mainly for the analysis of proteins.
Affinity Chromatography (AFC)	•Separation is based on adsorption of the analyte to the specific biologically derived ligand pair. •Highly selective. •A buffer solution with the appropriate pH and ionic strength is selected based on the type of ligand, analytes, and their interaction. •Used mainly for the purification and concentration of biological active substances.
Chiral Separation Chromatography (CS)	•Separation of optical isomers using chiral selectors. •Highly selective.
Multimode Chromatography	•Separation is based on the combination of different modes.

For further information a good recommendation would be the following tutorial videos:

a) **Reversed Phase** https://www.youtube.com/watch?v=DHt0gSDr5_Q

b) **HILIC** https://www.youtube.com/watch?v=jhZ8XcHTMjA

c) **Ligand Exchange** https://www.youtube.com/watch?v=DuiLKsZZhPM

d) **IC** https://www.youtube.com/watch?v=n6-97063OOI

e) **SEC** https://www.youtube.com/watch?v=2o0out1Gghg

3. Selection by Functional group

In HPLC columns many very different functional groups as a surface modification are used. Some examples are shown below.

Functional group	Mainly used in
Octadecyl (C18)	Reversed Phase
Octyl (C8)	Reversed Phase
Butyl (C4)	Reversed Phase
Sulfo	Reversed Phase + Cation Exchange
Quarternary Ammonium	Reversed Phase + Anion Exchange + HILIC
Prim. Amino	HILIC
Tert. Amino	HILIC
Cyanopropyl	Reversed Phase
Nitrophenylethyl	Reversed Phase
Sulfo (Ca^{2+}) Calcium as counter cation	Ligand Exchange
Sulfo (Pb^{2+}) Lead as counter cation	Ligand Exchange
Sulfo (Na^{+}) Sodium as counter cation	Ligand Exchange
Sulfo (Zn^{2+}) Zink as counter cation	Ligand Exchange
Carboxyl	IC
Sulfo	IC
Diethylaminoethyl	IC
Sulfopropyl	IEX
Carboxymethyl	IEX
Phenyl	HIC
L-amino acid derivatives	Chiral
ß-Cyclodextrin derivatives	Chiral

All functional groups fulfill different options, like spacer functionality, reversing the polarity, ligand exchange and many more. The mainly used separation technique is also given in the table above.

It is important to know the behavior of the sample in a selected separation mode in order to know which ligand is better for a specific sample e.g. if more polarity is needed in order to separate a sample with RP mode a change from a C18 spacer to C4 could improve the separation. The same effect could be reached by changing from a less polar stationary phase to a more polar stationary phase.

4. Selection by Area of Application

The Area of application is huge and a possible selection criteria or break down could be:

a) Pharmaceuticals & Cosmetics

Sample classification	Sample characteristics	Separation mode
Pharmaceuticals Metabolites Additive	Hydrophobic substances	RPC
	Hydrophilic substances	HILIC
		IEC+RPC
		LEX+SEC
	Substances in bio-fluid (serum/plasma/urine)	RPC
		SEC+RPC
	Polymer	SEC
Moisturizers	Polyalcohols	RPC
		LEX+SEC
		LEX+HILIC
		SEC
	Protein hydrolysates	RPC
		SEC
	Mucopolysaccharides	SEC
Emulsifiers	Surfactants	SEC+RPC
		SEC
Preservatives	Paraben Dehydroacetic acid	RPC

Optical active materials		CS

b) Foods

Sample classification	Sample characteristics	Separation mode
Nutritional ingredients	Monosaccharides Disaccharides Sugar alcohols	HILIC
		LEX+SEC
		LEX+HILIC
	Oligosaccharides	HILIC
		LEX+HILIC
		SEC
	Low molecular water-soluble dietary fiber	SEC
	Polysaccharides	SEC
	Organic acids	RPC
		IEX+RPC
		IC
	Water-soluble vitamins	RPC
		IEC+RPC
		HILIC
	Fat-soluble vitamins	RPC
		NPC
		SEC
	Fatty acids	RPC
		SEC
	Nucleic acids (umami)	IEC+SEC
	Amino acids	IEC+IEX+RPC
		IC
		IEC
Food safety	Food additives	RPC
		HILIC
	Pesticides	RPC
		IEC+RPC
		HILIC
		IC
	Mycotoxin	RPC

	Pretreatment of residual pesticides	SEC GPC (Clean up)

c) New Materials

Sample classification	Sample characteristics	Separation mode
Synthetic polymers	Organic solvent soluble	SEC
	Polar organic solvent soluble	
	High temperature/ Ultra high temperature	
	Water-soluble	
Additives oligomers		RPC
	Organic solvent soluble	SEC
	Polar organic solvent soluble	
	Water-soluble	

d) Biotechnology

Sample classification	Sample characteristics	Separation mode
Genomics	Nucleobases	RPC
	Nucleotides	IEC+SEC
	Nucleosides	IEC
	Oligo nucleic acids	RPC
		IEC+SEC
		IEC

	DNS/RNS	SEC
Proteomics	Amino acids	RPC
		IEC+IEX+RPC
		IEC
		IEC+SEC
	Peptides, proteins	RPC
		SEC
		IEC
		HIC
Glycomics	Glycoproteins	RPC
		SEC
		IEC
		HIC
		AFC
	Sugar chains	HILIC
		AFC
	Monosaccharides	HILIC
		LEX+SEC
		LEX+HILIC
	Sialic acids Uronic acids Aldonic acids	IEX+RPC
Hormones	Amines	RPC
		IEC
	Steroids	RPC
		HILIC
		SEC
Lipids	Phospholipids	NPC
		SEC
	Lipoproteins	SEC
		AFC

e) Environment

Sample classification	Sample characteristics	Separation mode
Water quality	Anions	IC
	Oxyhalides	IC
		IEC+HILIC

	Cyanide Cyanogen chloride	IEX
	Cations	IC
	Surfactants	RPC
		SEC+RPC
	Perchloric acids	IC
		IEC+HILIC
	Pesticides	RPC
		IEC+RPC
		HILIC
		IC
Soil	Anions	IC
	Heavy metals	IC
	Humic substances	SEC
	Organic arsenic	IEX+RPC
	Pesticides	RPC
		IEC+RPC
		HILIC
		IC
Environmental hormones	Pretreatment of Phthalates PCBs Benzo [a] pyrene	SEC GPC (Clean up)
Bioethanols	Monosaccharides Oligosaccharides	HILIC
		LEX+SEC
	Oligosaccharides Alcohols Furfural	LEX+SEC
	Saccharides Organic acids Alcohols Furfural	IEX+RPC+SEC
	Hemicelluloses Celluloses	SEC
Biodiesels	Cations	IC
	Fatty acid glycerides	SEC

	Fatty acid methyl esters	RPC
	Organic acids	IC

Shortcut	Full name separate mode
RPC	Reversed Phase Chromatography
HILIC	Hydrophilic Interaction Chromatography
NPC	Normal Phase Chromatography
LEX	Ligand Exchange Chromatography
IEX	Ion Exclusion Chromatography
IC	Ion Chromatography
SEC	Size Exclusion Chromatography
IEC	Ion Exchange Chromatography
HIC	Hydrophobic Interaction Chromatography
AFC	Affinity Chromatography
CS	Chiral Separation Chromatography

5. Selection by Analyte

If the only available information is the analyte it is rather difficult to give a general selection guide. In such a case it is best to contact our technical experts in Munich in order to break down your analyte either by its chemical composition and/or chemical behavior and your intended aim what you want to have as result. Furthermore a huge application database which is available at www.shodex.de is helpful to find many analytes already measured with ideal preconditions and sample preparations.

To know the main aim is a very crucial part to choose the right column and separation mode. A good example is to separate a Protein as analyte. Here it is important what is the aim of the analysis. If the molar mass and / or the separation from monomeric to dimeric compounds is important SEC as separation mode for aqueous environment is most suitable. If the separation from e.g. small molecules from the protein a RP or IEX mode could be interesting. Always keep in mind, that for very complicated analyte so called multi mode columns are available. These columns combine 2 or 3 different separation modes such as RPC, IEX and SEC in one columns and can achieve best results if one separation mode fails to separate your samples.