COLUMN GUIDE - Usage and maintenance of HPLC columns

1. HPLC

1.1 Introduction

The HPLC columns manufactured by VDS optilab Chromatographietechnik GmbH are, on the basis of modern technology, filled with highly stable and classified supporting materials. The absolutely stable column fillings guarantee, in the event of correct usage, a long service life for the separating column

The information and recommendations contained in this column guide were drawn up to show you how the HPLC column can be handled in a gentle manner; however, they should not be considered to be absolute. Please follow the instructions to attain the highest possible column performance and lifespan.

If you have questions, please contact VDS optilab directly. Alternatively, you can also contact your local VDS optilab retailer. We would be happy to accept your references, comments and criticisms pertaining to this manual.

First tasks after receipt of the column:

- Check whether the delivered column corresponds to the one that was ordered
- Check whether the column has been damaged, e.g. during transport
- If necessary, check the separating capacity

All VDS optilab HPLC columns are shipped in the test solvent (eluent) at delivery; otherwise it is specified on the column test report.

In order to guarantee a high level of quality, each VDS optilab HPLC column is produced and tested individually. The column is delivered along with a test chromatogram, which mentions the test conditions, the bulk material batch, the serial number and the manufacturing date.

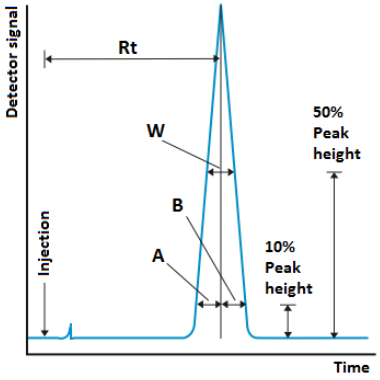
Please note: The warranty period begins with receipt of the HPLC column.

If you wish to test the column, please use the test conditions that are mentioned in the test chromatogram. Please note that the chromatographic performance depends on the entire HPLC system. The quality control procedure for the HPLC columns is carried out in conjunction with optimal HPLC conditions, in order to minimise band enlargement and 'extra column effects'.

Formulas used to calculate the theoretical plate numbers (column performance) and peak asymmetry:

Column performance: $N (0.5) = 5.54 (Rt/W0.5)^2$

Peak asymmetry: $As = B/A$



1.2 Capillaries and fittings

Tubing and fittings mostly add to the dead volume in the HPLC system:

→ Peak enlargement (band enlargement)
→ Poor peak shape

Tubing

The use of various tubing is determined on the basis of: Application, flow rate, back pressure

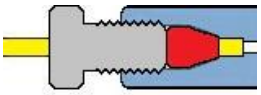
Chemical resistance:

Stainless steel (SS316):	Avoid high concentrations of acids and halogens
PEEK:	Avoid high acid concentrations, chlorinated solvents and 100% THF
Titan:	Be mindful of the electrochemical potential when it comes to combinations with other metals

In general, please be mindful of the chemical resistance of the materials that come into contact with the eluents!

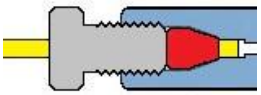
Fittings

All VDS optilab HPLC columns are equipped with a type 10-32 column head internal thread. They can be connected with all 1/16" AD tubing, and this can be done using a stainless steel or polymer fittings and a ferrule, or polymer fingertight fittings etc.

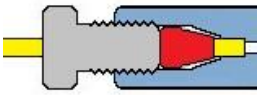


Correct screw connection

Please ensure that the ferrule is in the correct position. If you previously used columns belonging to another type, and if the ferrule has embedded itself in the tubing in a specific position, please shorten the tubing and replace the ferrule.



Tubing is too high / Dead volume



Ferrule is too high / Leak

A large selection of suitable tubing and fittings can be found in our catalogue of accessories.

1.3 Column installation

Ensure that the solvent in the column is miscible with the eluents that you have used. Rinse the system with the filtered and degassed eluents. Connect the column inlet to the injector. The column outlet is guided into a waste container. Please be mindful of the flow direction that is marked on the column. Now, rinse the column with a flow of 0.05 to 0.4 ml/min. (depending on the ID of the column) for ca. 3-5 min. Then connect the column outlet to the detector, and operate the column in conjunction with the normal flow rate. Rinse ca. 10 column volumes through the column. The base line should be stable, and it should not drift. The column is now ready for use.

Structure of the VDS optilab HPLC column (stainless steel)

2. Analytical HPLC columns based on silica gel

2.1 HPLC phases

	Normal phases			
	SiOH	Plain Silica	**OH**	Diol-modified silica
	CN	Cyano-modified silica	**NH2**	Amino-modified silica

	Reversed phases			
	C30	C30-modified silica	**C18**	C18-modified silica
	C8	C8-modified silica	**C6**	C6-modified silica
	C4	C4-modified silica	**C2**	C2-modified silica
	C1	C1-modified silica	**Phenyl**	Phenyl-modified silica
	Phenyl-Hexyl	Phenyl-Hexyl-modified silica	**CN-RP**	Cyano-modified silica
	NH2-RP	Amino-modified silica		

	Ion exchanger phases			
	NH2	Amino-modified silica	**PEI**	PolyEthylenImine-modified silica

	HILIC phases			
	HILIC	Plain Silica	**HILIC-OH**	Diol-modified silica
	HILIC-AM	Amino-modified silica		

2.2 Usage and handling of HPLC columns

The HPLC columns manufactured by VDS optilab Chromatographietechnik GmbH:
- are impervious to pressure and flow fluctuations and impacts.
- can be operated in any direction, so that the column can be back-flushed for column-switching and column regeneration operations.
- remain stable for a long time when they are used in a normal manner with organic-aqueous eluents, and they retain their separating capacity.

However, this only happens if the device is used as intended. This means that:
- the HPLC columns should only be used for the intended purpose.
- no dirt particles and/or irreversibly absorbable contaminants should reach the HPLC column.
- only high-quality and high-purity eluents should be used.

The service life of all separating columns based on silica gel are impaired and shortened through:
- the use of aqueous buffer solutions.
- the overshooting or undercutting of the permissible pH value of the mobile phase. Information regarding these ph-values can be found in the product information for the respective separation phases.
- the operation of normal phases with aqueous eluents.
- usage in temperatures that are > 60°C (phase is destroyed). This applies to all modified phases.
- the composition and purity of the injected samples.

Dirt particles are the enemy of all HPLC columns. These usually originate from the HPLC system, the eluent and the sample (remnants of the sample matrix). Even if the HPLC column is used for a short period of time without an eluent filter, a guard column between the HPLC pump and the injection valve and a pre-column, it (i.e. the HPLC column) can suffer significant damage. Matrix-induced colloidal dirt from the sample extraction quickly leads to a contamination of the pre-column or column inlet filter, which in turn raises the column's back pressure. The efforts taken with regard to the contamination of the HPLC column pay off in terms of the lifetime of the HPLC column.

This gives rise to the following principles for a secure analysis cycle:
- When possible, use eluent filters and guard columns between the HPLC pump and the injection valve.
- Always use a micro-filter to filter the samples.
- Always use a pre-column to protect the HPLC column. Replacing the pre-column on a frequent basis helps avoid problems.
- Always be mindful of the column back pressure and changes in it. Leaks cause a drop in pressure, while the presence of contaminants on the column inlet filter causes a rise in pressure.
- If the separation phase material associated with the RP phases is not inert (acidic centres that cannot be end-capped, metallic contaminations), it can result in the column being contaminated, which in turn leads to poor peak shapes. Use RP phases associated with high-purity silica gel.
- When it comes to the localisation of defects, you will be able to attain your objective quickly if you first check the eluent filter and the guard column for permeability, and then widen the examination to include the pre-column and the separation column.
- Condition the separation column vis-à-vis the separation-related problem that is to be solved. Good conditioning of the HPLC column can lead to a significant rise in the separating capacity.
- In case of NH2-phases, a wash-round from the RP mode to the NP mode should be avoided. If it is necessary, the mid-cycle rinsing should be performed using THF.

Maximum working pressure:

Standard HPLC columns	up to 400 bar
UHPLC columns	up to 900 bar

The following cases represent deviations:

Filling material with a pore size of 300 A	up to 300 bar
Filling material with a pore size of 1000 A	up to 200 bar

Column parameters

Column size	ID (mm)	Flow rate (ml/min)	Volume* (ml)	Bulk quantity* (g)
Preparative	62	182	754,7	454
Preparative	50	118	490,9	295
Preparative	40	75,6	314,2	189
Preparative	32	48,4	201,1	121
Preparative	25	29,5	122,7	73,8
Semi-preparative	20	18,9	78,5	47,3
Semi-preparative	16	12,1	50,3	30,2
Semi-preparative	10	4,7	19,6	11,8
Semi-preparative	8	3,0	12,6	7,6
Analytical	4,6	1,0	4,15	2,5
Analytical	4	0,76	3,14	1,89
Analytical	3	0,43	1,77	1,06
Micro	2	0,19	0,785	0,473
Micro	1	0,047	0,196	0,118
Nano	0,5	0,012	0,049	0,0295

*Based on a length of 250 mm and 2.5 g of bulk material for the column (250 x 4.6 mm)

2.3 Storage of the HPLC columns

In principle, it is recommended that all HPLC columns be stored in the original eluent (delivery of the column). The storage temperature should be 15-30°C. Do not use any eluents containing buffer solutions for storage. On account of potential contamination by metal ions [e.g. iron (III)], methanol is not suitable for long-term storage (> 3 days). The columns should be firmly sealed with the seal plugs in order to prevent drainage.

The following regulations are also applicable:

NP phases	Can also be stored in n-hexane or toluol. Do not use any polar solvents (e.g. eluents with H2O), or any solvents with a low boiling point (e.g. THF or dichloromethane).	
HILIC phases	In addition to the original eluents, acetonitrile:water (80:20) can also be used.	
NH2 phases	A wash-round from the RP mode to the NP mode is not recommended for storage.	
PEI phase	**Short time (overnight)**	**Long time (several days)**
	Methanol	Acetonitrile
	2M Na-acetate solution (pH 6)	0.1% Na-acid in 1M KH2PO4solution
	2M (NH4)2SO4solution (pH 6)	0.1% Na-acid in 2M Na-acetate solution (pH 6)
	2M KH2PO4 solution (pH 6)	

2.4 Regeneration of an HPLC column

If an analytical HPLC column becomes dry during storage, rinse it with approximately 10 column volumes of the storage eluent, with a flow rate of 0.1-0.2 ml/min.
If you want to regenerate a column, it is important to localise the cause of the problem in advance. It often helps to use a fresh eluent, which is filtered through a 0.2 or 0.45-µm membrane filter.

Normal phases (NP phases)
→ Rinse with ca. 5 column volumes of n-hexane/iso-propanol (80/20)
→ Rinse with ca. 10 column volumes of tetrahydrofuran to remove non-polar and medium-polar organic compounds
→ If necessary, rinse in the opposite flow direction, at 25% of the original flow rate
→ Use n-heptane to condition the HPLC column along the original flow direction

Reversed phases (RP phases)
→ Rinse with ca. 10 column volumes of acetonitrile/water (10/90) to remove buffers
→ Rinse with methanol to remove polar organic contaminants
→ Rinse with acetonitrile to remove medium-polar organic contaminants
→ Rinse with tetrahydrofuran to remove non-polar organic contaminants
→ If necessary, rinse in the opposite flow direction, at 25% of the original flow rate In case of persistent contamination
→ Rinse with water (2-3 drops of phosphoric acid/l) until the minimum pressure is reached, in order to remove acidic dispersible substances
→ To remove alkaline dispersible substances, inject ca. 50 µl of triethylamine and rinse with water until the minimum pressure is reached
→ Condition the HPLC column along the original flow direction, with the help of the used eluent
(Be mindful of the miscibility of the solvents!)

Ion exchanger phases

NH2 phase
→ Depending on the application, regenerate as described above in the NP or RP mode

PEI phase
→ Rinse with ca. 10 column volumes - 2M sodium acetate solution (pH 7-8)
→ Rinse with ca. 5 column volumes of distilled water
→ Rinse with ca. 10 column volumes of 10% acetic acid
→ Rinse with ca. 5 column volumes of distilled water
→ Rinse with ca. 5 column volumes of DMSO/distilled water (50/50)
→ Condition vis-à-vis the working buffer
(Be mindful of the miscibility of the solvents!)

HILIC phases
→ Rinse with ca. 10 column volumes of the most polar component used in the working eluent
→ Rinse with ca. 20 column volumes of methanol/distilled water (50/50)
Alternative
→ Rinse with ca. 10 column volumes of dichloromethane/methanol (95/5)

3. Preparative HPLC columns

Special features of preparative columns
→ Before it is used, the column should be conditioned with at least one column volume of the mobile phase.
→ The columns should not be operated against the specified flow direction, but an exception can be made for situations in which deposits on the inlet frit are being removed.
→ On account of the large volume of the mobile phase during the usage of the preparative column, attention should be paid to the toxicity and flammability.

→ Maximum working pressure	16 to 20 mm ID:	350 bar
	> 25 mm ID	200 bar

A higher working pressure can destroy the package or lead to leakages.

4. Polymer columns

4.1 Polymer phases

Cation exchanger	**pH range**	**Max. temp.**	**Max. pressure**	**Flow rate**
CarbEx II H-form 9 µm	1-3	90°C	100 bar	1.0 ml/min
CarbEx II Ca-form 9 µm	5-9	90°C	100 bar	1.0 ml/min
CarbEx II Pb-form 9 µm	5-9	90°C	100 bar	1.0 ml/min
Optigel CH-org. acids 8 µm		90°C	100 bar	1.0 ml/min
Optigel CH-wine 8 µm		90°C	100 bar	1.0 ml/min
Anion exchanger				
Optigel ZAO-wine 7 µm	2-8	70°C	100 bar	1.0 ml/min

4.2 Usage and handling of polymer columns

The new column should be conditioned with at least one column volume of the mobile phase, and a flow of 0.2-0.3 ml/min. Always let the pressure rise or fall in a gradual manner (more than ca. 2 min). Above all, a rapid rise in pressure should be avoided. Such a rise could compromise the package and lead to peak-tailing and a loss of separating performance.

When the column is to be tempered, raise the temperature gradually.

When organic modifiers are used in the eluent, the column should be rinsed at the rate of 0.1 ml/min. with no more than 5% of the modifier in the eluent. This should be done until the base line stabilises. The desired modifier concentration can then be used. This prevents rapid swelling of the phase, which in turn rules out the resultant high pressure or destruction of the polymer particles.

Eluents used:

	Eluent	Organ. Modifier
CarbEx II H-Form 9 µm	5mM H_2SO_4 sol. H_3PO_4 sol.	max. 10% acetonitrile; ethanol; isopropanol
CarbEx II Ca-form 9 µm	double-distilled H_2O	as above
CarbEx II Pb-form 9 µm	double-distilled H_2O	as above
Optigel CH-org. acids 8 µm	double-distilled H_2O	as above
Optigel CH-wine 8 µm	5mM H_2SO_4 sol.	as above
Optigel ZAO-wine 7 µm	5mM H_2SO_4 sol.	as above

Avoid the following:

CarbEx II H-form 9 µm	Methanol, salts, bases, metal ions, amines and other organic solvents pH >3 in H_2O
CarbEx II Ca-form 9 µm	Methanol, acids, bases, other salts
CarbEx II Pb-form 9 µm	Methanol, acids, bases, other salts
Optigel CH-org. acids 8 µm	Methanol, acids, bases, other salts
Optigel CH-wine 8 µm	Methanol, salts, bases, metal ions, amines and other organic solvents pH >3 in H_2O
Optigel ZAO-wine 7 µm	Methanol, acids, bases, other salts

In order to avoid solubility problems, dissolve the samples in the mobile phase and filter them using a 0.45-µm membrane filter.

4.3 Storage of the polymer columns

Storage in the original eluent.

4.4 Cleaning of the phases

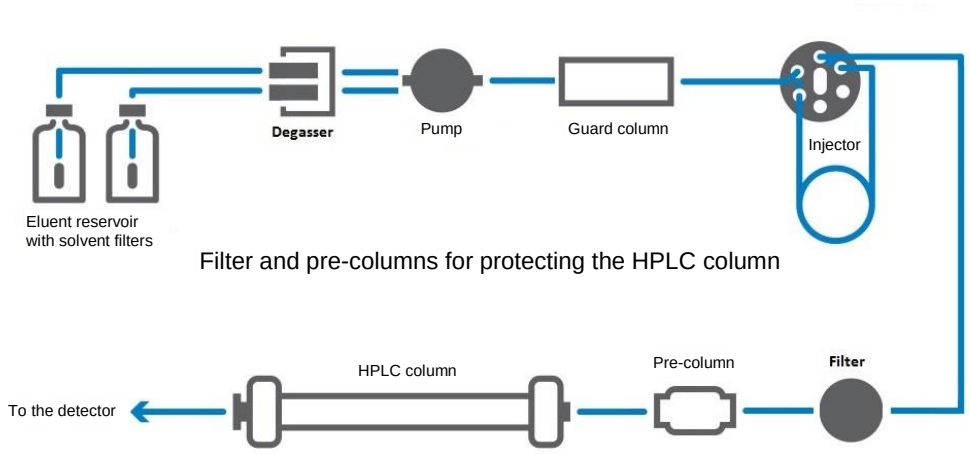
CarbEx II H-form 9 µm	1) Rinse with 5% acetonitrile in 5mM H_2SO_4 sol. 2) Rinse with 30% acetonitrile in 5mM H_2SO_4 sol.
CarbEx II Ca-form 9 µm	Rinse with acetonitrile/double-distilled water (30/70)
CarbEx II Pb-form 9 µm	Rinse with acetonitrile/double-distilled water (30/70)
Optigel CH-org. acids 8 µm	Rinse with acetonitrile/double-distilled water (30/70)
Optigel CH-wine 8 µm	1) Rinse with 5% acetonitrile in 5mM H_2SO_4 sol. 2) Rinse with 30% acetonitrile in 5mM H_2SO_4 sol.
Optigel ZAO-wine 7 µm	Rinse with acetonitrile/double-distilled water (30/70)

4.5 Regeneration of the phases

CarbEx II H-form 9 µm	Rinse with 25mM H_2SO_4 sol.
CarbEx II Ca-form 9 µm	Rinse with 0.1M $Ca(NO_3)_2$ sol.
CarbEx II Pb-form 9 µm	Rinse with 30% acetonitrile in 0.1M $Pb(NO_3)_2$ sol. (pH 4.0)
Optigel CH-org. acids 8 µm	Rinse with 0.1M $Ca(NO_3)_2$ sol.
Optigel CH-wine 8 µm	Rinse with 25mM H_2SO_4 sol.
Optigel ZAO-wine 7 µm	1) Rinse for 60 min. with distilled water, at 0.3 ml/min. 2) Rinse for 120 min. with 0.1M NaOH at 0.3 ml/min. (Change of the counter-ion into OH-) 3) Rinse for 60 min. with distilled water at 0.3 ml/min. 4) Rinse for 60 min. with 0.1M HCl at 0.3 ml/min. (Change of the counter-ion into Cl-)

5. Protective measures for columns

a) Syringe filter	for sample filtration
Membrane filter	for the pre-filtration of the eluent
Mobil phase filter	for eluent filtration
In-line filter	for the filtration between injector and column
b) Pre-columns and pre-column cartridges	to protect the main column
c) Column-test standards	to check the column performance
d) Eluent-degasser	for de-gassing of the eluent



Filter and pre-columns for protecting the HPLC column

Product recommendations:

Syringe filter:
VDS optilab offers a large selection of 17-mm and 30-mm HPLC syringe filters for sample filtration. Please ask for our catalogue of vials.

Degasser:
Upon request, we would be happy to provide you with the optimal degasser for your HPLC system.

Mobile phase filter:
E.g. PP mobile phase filter for 1/8" pipes, pack containing 50 pieces, **item no.: 3210.1200**

Guard filter between pump and injector:
E.g. guard filter kit: Filter holder and 5 filter cartridges - 10 x 4.0 mm, **item no.: 1546.4100K1-FK**

In-line solvent filter between injector and HPLC column:
A very large selection of in-line solvent filters and solvent suction filters can be found in the VDS optilab catalogue of accessories.

Pre-column cartridge head for direct connection onto the column:
- For 5-mm long cartridges with ID of 2.0, 3.0, 3.9, 4.0 and 4.6 mm, **item no.: 15020508K05**
- For 10- and 20-mm long cartridges with ID of 2.0, 3.0, 3.9, 4.0 and 4.6 mm, **item no.: 15020508**

Pre-column cartridge holder (stand-alone):
- For 10-mm long cartridges with ID of 2.0, 3.0, 3.9, 4.0 and 4.6 mm, **item no.: 1546.4100K1**
- For 20-mm long cartridges with ID of 2.0, 3.0, 3.9, 4.0 and 4.6 mm, **item no.: 1546.4100K2**

The respective pre-columns can be found in the VDS optilab price list for HPLC columns.

6. Manual - Troubleshooting

a) Base line (unsteady or drift)	
→ Equilibrium with the eluent not yet established	Increase the run-in time of the column
→ Eluent contaminated	Use fresh eluent
→ Temperature problems	Temper column
b) Wide peaks	
→ Mixture and/or diffusion before/after the column	Use capillaries to minimise the dead volume
→ Large sample volume	Reduce injection quantity
c) Double peaks	
→ Large dead volume; sagging package	Partially blocked column inlet sieve or frit / clean or replace sieve or frit; If necessary, use new column
→ Silica gel is dissolved due to excessively high pH value of the eluent	Be mindful of the pH-stability of the phase / use new column
→ Faulty fittings/connections	Replace fittings and/or ferrules
d) Peak-tailing	
→ Column overloaded	Reduce injection quantity
e) Insufficient separation	
→ Elution capacity of the eluent too high	Change eluent composition
→ Phase not suitable	Use other phase/modification
→ Due to column temperature or flow rate	Change parameters
→ Phase surface contains, for example, greases, oils, lipids from the sample	Remove contaminants associated with sample preparation Clean the column in accordance with the manual (see above).
→ Protonation of the NH2-groups due to acidic buffer	Rinse with weakly basic solutions in the eluent
f) High back pressure	
→ Particles on stainless steel sieve or frit	Replace eluent / Back-flush column / Replace sieve or frit / Use in-line filter
→ Separation phase surface occupied	Rinse column (refer to 2d), inject DMF if necessary
→ Buffer salts falling out	Be mindful of the solubility of the buffer salts / Rinse column (refer to 2d)
→ Blocked capillaries	Replace tubing
g) Drifting of retention times	
→ In case of pure silica phases	Stabilise the water content of the eluent by adding, for example, specific hydrate salts or dioxane
→ In case of modified phases	Use eluents that do not remove the bonded groups, or use a new column

Notes:

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