Guide to Column Care and Use: Vydac® Product Focus

The purpose of this guide is to provide information that will help you successfully use VYDAC® HPLC columns. General instructions and specific column recommendations are described in the following sections:

Section 1 General Instructions
Section 2 Protein/Peptide Reversed-Phase Columns
Section 3 201TP Specialty Reversed-Phase Columns
Section 4 302IC Ion Chromatography Columns

SECTION 1

Starting to use your VYDAC HPLC column

Remove the plastic nuts at each end. PEEK Finger-Tight™ compression fittings are recommended to secure 1/16" stainless steel or PEEK tubing to the column inlet and outlet. These PEEK fittings are available from Grace Davison Discovery Sciences (e.g., Alltech part # 32233), or from your local distributor. For 2.1 and 4.6 mm hardware, stainless steel compression fittings (10-32 UNF, CPI Standard) also may be used.

Column protection

Column lifetime can be extended by filtering all solvents and samples prior to use. We recommend using a low-dead-volume in-line filter (available from Grace Vydac: Cat. No. CPF10, pkg of 10) between the injector and column to trap particles from solvents, pumps, the mixing chamber, and the injector. We also recommend using a guard column if samples contain insoluble or strongly adsorbed materials that may clog the column. Cartridge-type guard columns are available for most VYDAC HPLC columns.

Pressure and temperature limits

VYDAC silica-based HPLC columns are stable from 0-60°C and at pressures up to 5000 psi (335 bar).

Typical backpressure of VYDAC silica-based reversed-phase columns

Particle Size (µm)	Column Size (mm)	Flow Rate (mL/min.)	Typical Backpressure (with 50:50 Acetonitrile:Water)
5	2.1 x 250	0.2	1000 – 1800 psi
5	4.6 x 250	1.0	1000 – 1800 psi
5	4.6 x 150	1.0	600 – 1200 psi
5	10 x 250	5.0	1000 – 1800 psi
10	4.6 x 250	1.0	500 – 1000 psi
10	10 x 250	5.0	500 – 1000 psi
10	22 x 250	25	500 – 1000 psi

If you have a problem with a VYDAC column

HPLC columns may become contaminated by strongly adsorbed sample constituents, causing an increase in column backpressure or a loss of resolution. If a column appears to be contaminated:

- If the backpressure is high, disconnect the column from the injector and run the pumps to ensure that the back-pressure is due to the column and not the HPLC system.
- If the column backpressure is high, the column may be reversed and rinsed to try to flush contaminants from the inlet frit. Begin the reverse rinse at a low flow rate (0.5 mL/min for a 4.6 mm ID column) for 10-15 minutes and then increase the flow to 1.5-2.0 mL/min (for a 4.6 mm ID column).
- Wash the column either with 10-20 column volumes of a strong eluent (high organic solvent for reverse phase columns; high salt for ion exchange columns) or run 2-3 “blank” (that is without sample injection) gradients as normally run to remove less strongly adsorbed contaminants.

SECTION 2

VYDAC Protein/Peptide Reversed-Phase Columns:
208, 214, 218, 219, and 238 TP and MS; 238EV

Except for 238TP, 238MS, and 238EV, VYDAC silica-based reversed-phase HPLC columns consist of hydrocarbon compounds chemically bonded to “TP” 300-Angstrom pore-size silica through the use of multifunctional chlorosilanes. The resulting"polymeric" reversed phases are very resistant to hydrolysis. VYDAC 238MS, 238TP, and 238EV adsorbents are bonded using mono-silane reagents, leading to a “monomeric” C18 phase. This produces subtle differences in selectivity that can be exploited to optimize peptide and protein separations. The 238TP, 238MS, and 238EV adsorbents are exhaustively end-capped.

Performance testing

Each lot of reversed-phase material is tested for selectivity with a set of peptides and a set of proteins. (219MS and 219TP are tested only with proteins, and 238MS and 238TP with peptides). Each column is individually tested for packing efficiency using either biphenyl (for analytical columns) or benzoate esters (for preparative scale columns). Test conditions and results are included with the column documentation. If you would like to test or verify column performance,

we recommend repeating the selectivity and/or efficiency test.

Shipping
VYDAC Protein/Peptide Reversed-Phase columns are shipped in ACN:water (50:50 for C4 and diphenyl, 60:40 for C8 and C18 adsorbents). Preparative columns are shipped in methanol:water.

Column Conditioning
Because of the nature of the reversed-phase surface, column performance (resolution, retention) may change slightly during the first few injections of polypeptide. "Column conditioning" occurs for most polypeptides larger than 10,000 MW. A column can be conditioned by repeated injections of a polypeptide until the column characteristics remain constant (typically requires injection of about 100 µg of polypeptide on a 4.6 mm ID x 250 mm column) or by injection of 100 µg of a commonly available protein such as ribonuclease, followed by elution of the column with a typical acetonitrile gradient with 0.1% TFA.

Column storage
VYDAC Protein/Peptide Reversed-Phase columns may be stored in any combination of organic solvent and water. For long term storage ion-pair reagent should be flushed from the column and the organic content should be at least 50%. The column should be sealed with the plastic plugs originally supplied.

Chemical Stability
VYDAC Reversed-Phase Protein/Peptide columns are stable in most common organic solvents including acetonitrile, methanol, ethanol, isopropanol, dichloromethane and chloroform. When switching solvents it is important to verify that subsequent solvents are miscible with the previous solvent used. Protein/Peptide columns are very resistant to hydrolysis, can be used with eluents as low as pH 2 (such as 0.1% trifluoroacetic acid) for long periods of time, and are stable to occasional use at lower pH if columns are stored at pH higher than 2. **Silica-based Protein/Peptide Reversed-Phase columns should not be used above pH 7. USE ABOVE pH 7 IS LIKELY TO REDUCE THE COLUMN LIFETIME!** Common protein detergents such as sodium dodecylsulfate (SDS) can be used without harm to columns and may be removed by rinsing the column with acetonitrile or isopropanol. However, detergents are likely to affect the resolution of the column during the run in which they are present. Oxidative eluents or sample additives should be avoided.

Recommended elution conditions
VYDAC Protein/Peptide Reversed-Phase columns are typically eluted with an increasing gradient of organic solvent in the presence of an ion-pairing agent. The most common organic solvent used is acetonitrile due to its low viscosity, good UV transparency and high volatility. Ethanol or isopropanol are also occasionally used. Trifluoroacetic acid (TFA) is the most commonly used ion-pair reagent and is usually present at concentrations of 0.05 - 0.2% (w/v). However, VYDAC LC/MS grade columns (208MS, 214MS, 218MS, 219MS, 238MS, 238EV) produce excellent separations with TFA concentrations as low as 0.01% (w/v) or no TFA and one of the following ion-pairing reagents: acetic acid, formic acid, heptafluorobutyric acid (for basic polypeptides), triethylamine phosphate (TEAP), phosphoric acid.

Typical flow rates and loading capacities for VYDAC Protein/Peptide Reversed-Phase columns

Column Diameter (mm)	Typical Flow Rate (1)	Sample Capacity (2)	Maximum Practical Sample Load (3)
0.075	0.25 µL/min.	0.05 µg	
0.15	1 µL/min.	0.2 µg	
0.30	5 µL/min.	1 µg	
0.50	10 µL/min.	2 µg	
1.0	25 – 50 µL/min.	0.05 – 10 µg	
2.1	100 – 300 µL/min.	0.2 – 50 µg	
4.6	0.5 – 1.5 mL/min.	1 – 200 µg	10 mg
10	2.5 – 7.5 mL/min.	1000 µg	50 mg
22	10 – 30 mL/min.	5 mg	200 mg
50	50 – 100 mL/min.	25 mg	1000 mg
100	150 – 300 mL/min.	125 mg	5000 mg

1. Actual flow rates can be a factor of two higher or lower depending on the method.
2. Sample capacity is the quantity of polypeptide that can be loaded onto the column without reducing resolution.
3. Maximum practical sample load is approximately the maximum quantity of sample that can be purified with reasonable yield and purity on the column.

Column cleaning and regeneration
VYDAC Protein/Peptide Reversed-Phase HPLC columns may become contaminated by strongly adsorbed sample constituents causing a loss in column performance. If the recommendations given on page 1 fail to correct the problem:

- Inject up to 12% of the column volume with 1% SDS solution (CV) (500 µL for 4.6 mm x 250 mm column). Then run a 20-minute gradient from 5% to 95% ACN with 0.1% (v/v) TFA.
- If lipids or very hydrophobic small molecules are causing the change in column performance, we recommend rinsing the column with several column volumes (CV) of solvent in the following order: Acetonitrile (10 CV), Dichloromethane (10 CV), Hexane (10 CV), Dichloromethane (10 CV), Acetonitrile (10 CV)
- If the loss in column performance appears to be due to adsorbed protein we recommend rinsing any of the polymeric-bonded columns with a mixture of one part 0.1 N nitric acid and four parts isopropanol. Rinsing at a low flow rate (20% of normal) overnight is most effective. **NOTE: WASHING WITH NITRIC ACID IS NOT RECOMMENDED FOR 238TP, 238MS, and 238EV "MONOMERIC" REVERSED-PHASE COLUMNS.**
- Use 6M Guanidine HCl in water. Filter the Guanidine prior to mixing 1:1 with isopropanol. Inject 5% of the CV (for an analytical column 4.6x250 mm, inject 200 µL) while running 50% IPA in water. This achieves a "plug flow" of Guanidine, which will break loose protein contaminants from the column.
- Synthetic Peptides: The cleavage of synthetic peptides off solid phase resins generates very reactive carbonium ions that are "scavenged" by anisole and thioanisole. These scavenger-carbonium ion reactions yield large, organic soluble, aromatic molecules that can be found in the cleavage solutions. Washing the column with 100% acetonitrile (ACN) or 100% methanol may not elute these molecules. Or, at times, washing the column with these organic solvents may remove some contaminants giving a waxy precipitate that are at times mistakenly assumed to be the C18 phase coming off the column. *To clean*: Wash with isopropanol, 0-100% over 10 column volumes, hold at 100% isopropanol or until baseline. Monitor at 260 nm. Rinse with 100% dichloromethane until baseline. Remove dichloromethane with isopropanol before use. This method is very effective at removing contaminants introduced by the synthesis process.

Example of how to calculate the column volume CV for a column of the dimensions 4.6 mm ID x 250 mm L:
$CV = \pi r^2 L = (3.14)(2.3\ mm)^2(250\ mm) =$
$4153\ mm^3 \times (1\ cm^3/1000\ mm^3) = 4.15\ cm^3 = 4.15\ mL$

$1\ cm^3 = 1\ mL$
$10\ mm = 1\ cm$
$(10\ mm)^3 = (1\ cm)^3$
$1000\ mm^3 = 1\ cm^3$

SECTION 3

VYDAC 201TP Specialty Reversed-Phase Columns
VYDAC 201TP reversed-phase HPLC columns consist of C18 hydrocarbon chains bonded to TP (300 angstrom) silica using multifunctional silanes that result in a "polymeric" cross-linked reverse-phase. 201TP adsorbents are not end-capped.

Performance testing
Every lot of 201TP reversed-phase material is tested for selectivity using the sixteen EPA priority-pollutant polyaromatic hydrocarbons.

Shipping
VYDAC 201TP reversed-phase columns are shipped in 60:40 acetonitrile:water.

Recommended elution conditions
Elution conditions for 201TP reversed-phase columns are as specified by EPA when used for analysis of priority pollutant PAHs.

Common recommendations
Aside from items noted above, information and recommendations are as offered in Section 2 for VYDAC Protein/Peptide Reversed-Phase columns.

SECTION 4

VYDAC 302IC Ion Columns
VYDAC 302IC ion-chromatography columns contain a low-capacity anion-exchange (quaternary amine) material based on high-purity large-pore silica. They can be used with ordinary HPLC systems for analysis of common anions and organic acids.

Performance testing
Every lot and each column of 302IC ion-chromatography material is tested for selectivity and efficiency. Results from the

selectivity/efficiency test are enclosed with the column documentation. If you would like to test or verify column performance we recommend repeating the selectivity/efficiency test.

Shipping
Vydac 302IC columns are shipped in 50:50 methanol:water.

Column storage
VYDAC 302IC columns may be stored in any combination of organic solvent and water after first rinsing the column free of salts, buffers or acids. For long-term storage the organic content should be at least 50%.

Chemical Stability
VYDAC 302IC columns are stable in common organic solvents such as acetonitrile, methanol, isopropanol and dichloromethane. When switching solvents it is important to verify that subsequent solvents are miscible with the previous solvent used. The recommended pH range for 302IC columns is pH 2 - 6.5. **THE USE OF 302IC COLUMNS ABOVE pH 6.5 WILL REDUCE THE COLUMN LIFETIME.**

Recommended elution conditions
VYDAC 302IC columns are eluted with buffered aqueous eluents. The conditions shown on the selectivity test chromatogram are the best starting point for developing a new separation. The recommended flow rate is that used in the original column test, although somewhat lower flow rates may be effectively used.

Column cleaning and regeneration
VYDAC 302IC columns may become contaminated by strongly adsorbed sample constituents, causing a loss of column performance. If the recommendations on page 1 do not restore performance, 302IC columns can often be regenerated by rinsing the column with strong buffer (2-3 X the strength of the buffer normally used). Columns can also be rinsed with 0.05M EDTA to remove adsorbed anions or with 0.05M nitric acid to remove adsorbed cations. To remove adsorbed hydrophobic molecules, rinse the column with several column volumes of dichloromethane or chloroform. When changing from water to chloroform or dichloromethane or back again it is important to rinse the column with a mutually miscible intermediate solvent such as isopropanol or acetone between the two less miscible solvents.