Guide to Column Care and Use

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 259VHP Polymer Reversed-Phase Columns
Section 4 201TP Specialty Reversed-Phase Columns and 302IC Ion Columns

SECTION 1

Starting to use your VYDAC HPLC column

Remove the plastic nuts at each end and connect the column to your HPLC system using low-dead-volume Valco-compatible male connectors. Two Valco nuts and ferrules are included with each column.

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. (See the enclosed High-Performance Guard Column product sheet).

Pressure and temperature limits

VYDAC silica-based HPLC columns are stable from 0-60°C and at pressures up to 5000 psi (335 bar). The upper pressure limit of the HPLC system used should be set to a maximum of 5000 psi (335 bar).

VYDAC 259VHP polymer reversed-phase columns are stable from 0-80°C, but pressure-limited to 3000 psi (200 bar). See specific information for 259VHP columns in Section 3.

The back-pressure of VYDAC silica-based reversed-phase columns under standard test conditions (50-70% acetonitrile in water) is initially in the range given below:

Column Size	Particle Diameter	Flow Rate	Back-Pressure Range
2.1 x 250 mm	5 µm	0.20 mL/min	1000 - 2000 psi
4.6 x 250 mm	5 µm	1.0 mL/min	1000 - 2000 psi
4.6 x 150 mm	5 µm	1.0 mL/min	600 - 1400 psi
10.0 x 250 mm	5 µm	5.0 mL/min	1000 - 2000 psi
4.6 x 250 mm	10 µm	1.0 mL/min	500 - 1200 psi
10.0 x 250 mm	10 µm	5.0 mL/min	500 - 1200 psi
22.0 x 250 mm	10 µm	25.0 mL/min	500 - 1200 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 back-pressure or loss of resolution. If a column appears to be contaminated:

- If the back-pressure 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 back-pressure 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; 1.0-1.5 mL/min for polymer columns).
- 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.

(continued on page 2)

- If these steps do not restore column performance, follow the recommendations for column regeneration found in the section dealing with your specific column.
- If column performance is not restored by any of these steps, please contact Grace Vydac in USA at (760) 244-6107 for technical assistance with the following information:
  - ✔ The type and lot number of the column. The lot number is the series of numbers found at the top center of the column label (e.g. E980204-3-2) after the catalog number.
  - ✔ Describe the problem. If possible, send a FAX to USA (760) 244-1984 with a chromatogram illustrating the problem. This will help in rapidly assessing and correcting the problem.

SECTION 2

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

Except for 238MS and 238TP, 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 and 238TP adsorbents are bonded using mono-silane reagents, leading to a "monomeric" C18 phase. This produces subtle differences in selectivity which can be exploited to optimize peptide and protein separations. The 238MS and 238TP 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!**

We suggest using a polymer reversed-phase column (VYDAC 259VHP, Section 3) under basic elution conditions.

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) 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 for VYDAC Protein/Peptide Reversed-Phase columns are:

Capillary	75 µm diameter	0.25 µL/min
	100 µm diameter	1 µL/min
	300 µm diameter	5 µL/min
	500 µm diameter	10 µL/min
Microbore	1.0 mm diameter	25-50 µL/min
Narrow Bore	2.1 mm diameter	100-300 µL/min
Analytical	4.6 mm diameter	0.5-1.5 mL/min
Semipreparative	10.0 mm diameter	2.5-7.5 mL/min
Preparative	22 mm diameter	10-30 mL/min
Process	50 mm diameter	50-100 mL/min
	100 mm diameter	150-300 mL/min

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 500 µL of 1% SDS solution at 1 mL/min (for 4.6 mm ID column). Then run a 10-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 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.
- 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 238MS AND 238TP "MONOMERIC" REVERSED-PHASE COLUMNS.**

SECTION 3

VYDAC 259VHP Polymer Reverse-Phase Columns

VYDAC 259VHP reversed-phase columns contain highly cross-linked polystyrene-divinylbenzene copolymer beads with 300 Å pores. A proprietary surface modification enhances separation performance. 259VHP columns are designed for separation of proteins and peptides, but can also be used for separating small molecules. They are stable from 1 N acid to 1 N base at ambient temperature. This provides a broad choice of mobile phases, including conditions of pH where silica-based reversed-phase columns can not be used. 259VHP columns are compatible with most common organic solvents.

Performance testing

Every lot of 259VHP material is tested for selectivity using protein and peptide standards.

Shipping and first use

259VHP columns are shipped in 50:50 acetonitrile:water. Prior to use, we recommend rinsing with 10 column volumes (25 mL for a 259VHP5415 column) of the weak mobile phase (solvent A) followed by a blank gradient from 0 to 100% B.

Pressure limit

259VHP columns are stable to 3000 psi (200 bar). The upper pressure limit of your HPLC system should be set at less than 3000 psi (200 bar). For best column lifetime, a flow rate of 1.0 mL/min or lower is recommended for 259VHP5415 (4.6 mm ID X 15 cm). For columns with other dimensions, the flow rate can be adjusted accordingly.

Chemical and temperature stability

VYDAC 259VHP columns are stable from pH 1 to pH 14 and temperatures from 0 to 80°C. However, **strong-acid and strong-base cleaning should NOT be done at elevated temperatures**.

Column cleaning and regeneration

Strongly adsorbed proteins and other contaminants that are not removed by normal gradients can lead to loss of column performance. Should this occur, 259VHP columns can often be cleaned by spot injections of common denaturants.

All denaturant solutions should be membrane filtered immediately before use to remove particles greater than 0.5 µm. Syringe filters are convenient for small volumes. For strong base (1 N NaOH), be sure the polyimide injector rotor is replaced with a base-resistant material and use a PTFE membrane filter to remove particulates.

All procedures should be performed at room temperature unless otherwise noted.

Connect the column in reverse-flow direction. Program the HPLC system to run 10 minute gradients from 0 to 100% B at flow rate 1.0 mL/min (for 4.6 mm ID column) with A = 0.1% TFA (v/v) in 10:90 ACN/water, and B = 0.1% TFA (v/v) in 90:10 ACN/water. Monitor absorbance continuously at 280 nm. For each procedure, inject the denaturant in solvent A then run the gradient. Repeat the procedure several times until the baseline profile remains constant from run to run, indicating absence of further desorption.

Depending on the contaminants, one or more denaturants may be effective. Amounts per injection are for 4.6 mm ID column.

1) 1% or 2% SDS in 10:90 ACN/water. Inject 500 µL.
2) 50:50 IPA:6 M guanidine HCl. Inject 200 µL at 0.2 mL/min. (This solution is very viscous, necessitating the low flow rate. Also, it is very important to filter the guanidine HCl solution two or three times, before and after adding IPA, in order to avoid introducing particles that can clog the column.)
3) Any of the following at low flow (0.2 mL/min):
   - ✔ 100% TFA. Inject 200 µL.
   - ✔ Glacial acetic acid. Inject 1 mL.
   - ✔ Formic acid. Inject 1 mL.
   - ✔ 1M phosphoric acid. Inject 1 mL.

   Repeat step 2. Steps 1 through 3 are strongly solubilizing for proteins and lipids.
4) 1 N NaOH. Inject 2-3 mL at 0.2 mL/min. (Base hydrolysis of proteins and peptides breaks them into smaller units with greater solubility.)

Test column performance under normal gradient conditions when the 280 nm trace indicates that the column is clean.

Other procedures to try:

- Using your normal mobile phase buffer, heat the column to 60°C and run a 0 to 100% ACN gradient several times.
- Repeat steps 1 through 4 using IPA (a stronger solvent) in place of ACN in the mobile phase. Use a lower flow rate (0.5 mL/min suggested) to allow for the higher viscosity of this mobile phase.
- If these steps do not appear to remove all lipids or other non-polar contaminants, the column can be cleaned by rinsing with chloroform or dichloromethane, as described in Section 3 for silica-based protein-peptide reversed phase columns, being sure to proceed from water through intermediate rinses with mutually miscible solvents such as isopropanol or acetone.

SECTION 4

VYDAC 201TP Specialty Reversed-Phase Columns

VYDAC 201TP reversed-phase HPLC columns consist of C_{18} hydrocarbon chains bonded to TP (300 angstrom) silica using multifunctional silanes which 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.

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.

If you need additional information or have questions not answered in this guide, please contact Grace Vydac via phone, FAX, or email as indicated below and ask for technical assistance.

Or visit Grace Vydac's online FAQ page at
www.gracevydac.com

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