GRACE VYDAC **APPLICATION NOTE**

#0103

Base-Stable Reversed-Phase Columns for Preparative LC of Proteins and Peptides

VYDAC® 259VHP polymer-based reversed-phase adsorbent consists of porous divinylbenzene with a stable polar surface modification. As shown in Figure 1, VYDAC 259VHP is very stable in the presence of both strong base and strong acid. It is a unique and versatile tool for protein and peptide chromatography. Its exceptional chemical resistance makes it ideal for separations of difficult proteins or peptides that require harsh conditions to maintain solubility, and also for more routine applications where it is desired to clean and sanitize columns between runs, for example by washing with strong alkali.

- acid and alkali stable, pH 0 to 14
- heat stable to 80°C
- pressure stable to 3000 psi
- 300 Å pore size for proteins and peptides

Chemical Stability

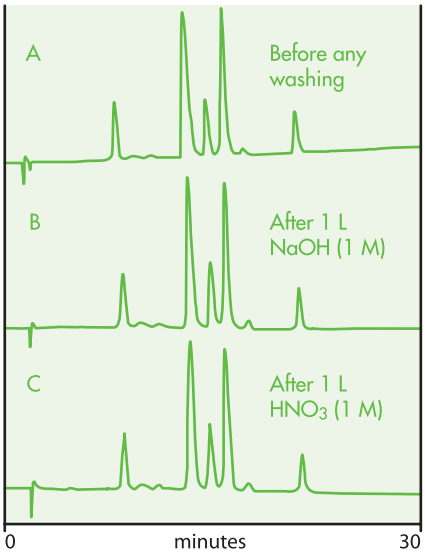

Figure 1. *Chemical stability test on VYDAC 259VHP polymer-based reversed-phase adsorbent. There is no noticeable change in the peptide separation after a 400-column-volume wash with strong base or strong acid.*

Peptides on VYDAC 259VHP

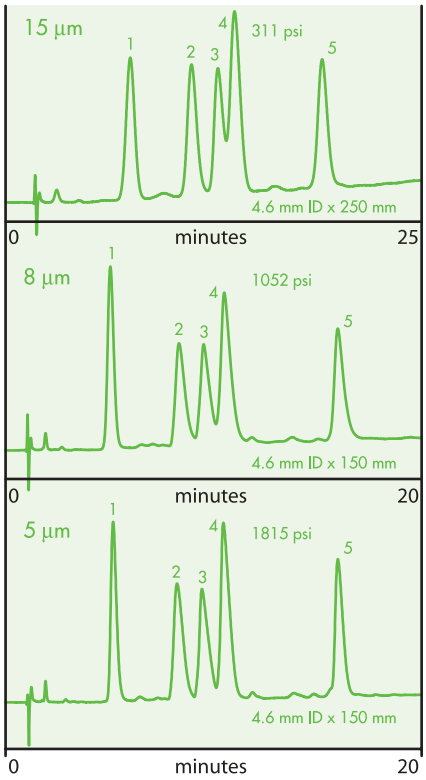

Figure 2. *Peptides on VYDAC 259VHP. A reversed-phase separation of five peptides was compared on 259VHP adsorbents of three different particle sizes. Conditions identical for all three columns. Column sizes and backpressures as shown. Detection: 220 nm. Flow: 1.5 mL/min. Mobile phase: A = 0.1% TFA (w/v) in water. B = 0.1% TFA (w/v) in 25:75 water:ACN. Gradient: Linear, 20% to 40% B over 30 minutes. Peaks: 1. oxytocin, 2. bradykinin, 3. eledoisin-related peptide, 4. angiotensin II, 5. neurotensin.*

The VYDAC 259VHP polymer has a 300 Å pore size to provide access to all adsorbent surfaces for large peptides and proteins. It is mechanically stable under normal HPLC operating pressures and produces resolution comparable to 300 Å silica-based reversed-phase adsorbents.

VYDAC 259VHP material has been available for several years in the form of 5 µm diameter particles for analytical and semipreparative HPLC. Now two larger particle sizes – 8 µm and 15 µm – are also available for preparative scaleup.

The chemistry of the larger particles is identical to that of the 5 µm particles, allowing easy scaleup with minimal modifications to conditions developed using analytical columns. Comparisons of peptide and protein separations run under identical conditions on different 259VHP particle sizes are shown in Figures 2 and 3.

Figures 4 shows a preparative separation of a crude recombinant human insulin preparation. Analysis of selected fractions, shown in Figure 5, demonstrates the resolving power of the VYDAC 259VHP 15µm pore-size adsorbent under preparative load conditions.

Grace Vydac can supply 259VHP in columns or as preparative separation media. Please contact us for pricing on media.

Proteins on VYDAC 259VHP

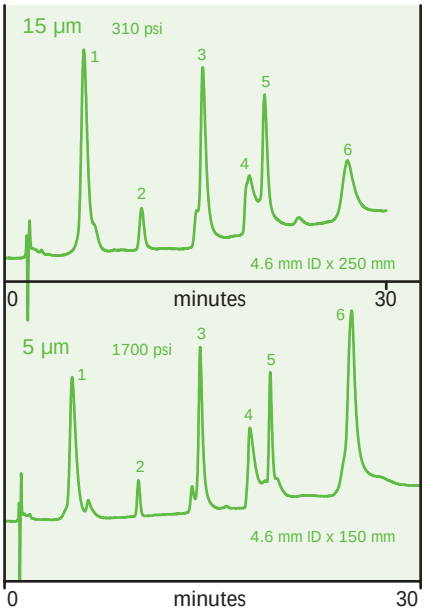

Figure 3. *Proteins on 259VHP. A reversed-phase separation of six proteins was compared on VYDAC 259VHP adsorbents of two different particle sizes. Conditions identical for both columns. Column sizes and backpressures as shown. Detection: 220 nm. Flow: 1.5 mL/min. Mobile phase: A = 0.1% TFA (w/v) in water. B = 0.1% TFA (w/v) in 25:75 water:ACN. Gradient: Linear, 33% to 80% B over 30 minutes. Peaks: 1. ribonuclease A, 2. insulin, 3. lysozyme, 4. BSA, 5. myoglobin, 6. ovalbumin.*

Preparative Separation of Insulin

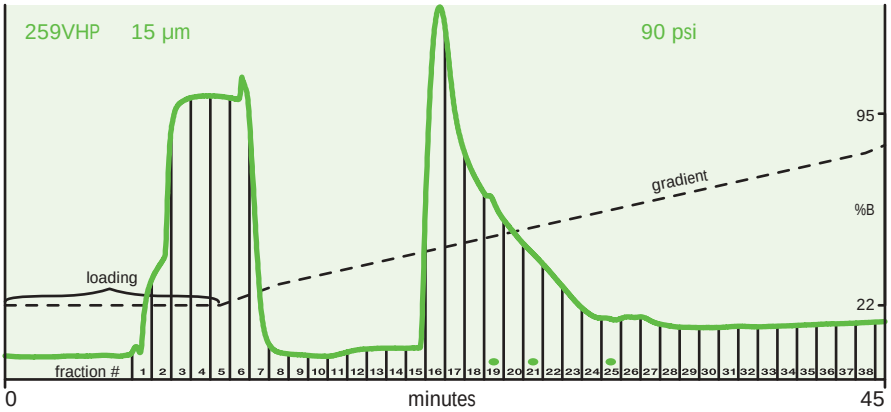

Figure 4. *Preparative separation of insulin. Sample: 45 µg of crude recombinant human insulin in 7.5 mL of 0.25 M HOAc/20% ACN. Column: VYDAC 259VHP, 15 µm particle diameter, 10 mm ID x 150 mm. Detection: 220 nm. 0.7 ODFS. Mobile phase: A = 0.25 M HOAc in H_2O. B = 0.25 M HOAc in 70:30 ACN:H_2O. Program: Equilibrate column at 22% B. Load sample in 10 mL loop and inject at 1 mL/min for 11 minutes. Elute at flow of 3.8 mL/min with gradient ramp from 22% to 30% B in 3 minutes, then from 30% to 80% B in 30 minutes. Collect one-minute fractions with Gilson collector beginning at 6.5 minutes.*

Ordering Information

Cat. No.	Description
259VHP5415	Analytical Column, Polymer-Based Reversed Phase, 300 Å, 5 µm, 4.6 mm ID x 150 mm
259VHP810	Semiprep Column, Polymer-Based Reversed Phase, 300 Å, 8 µm, 10 mm ID x 250 mm
259VHP822	Prep Column, Polymer-Based Reversed Phase, 300 Å, 8 µm, 22 mm ID x 250 mm
259VHP1522	Prep Column, Polymer-Based Reversed Phase, 300 Å, 15 µm, 22 mm ID x 250 mm

Other column sizes and media are available for both analytical and preparative applications.

Analyses

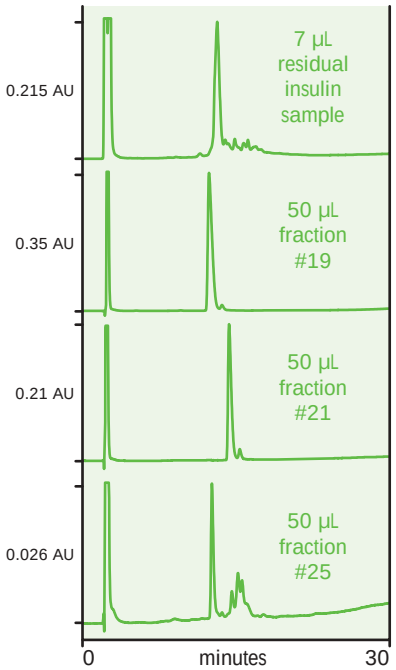

Figure 5. *Analyses of sample and selected fractions from preparative insulin separation of Figure 4. Column: VYDAC 259VHP5415, 5 µm, 4.6 mm ID x 150 mm. Detection: 220 nm. Absorbance normalized to insulin peak. Flow: 0.8 mL/min. Mobile phase: A = 0.1% TFA in H_2O. B = 0.1% TFA in 70:30 ACN:H_2O. Gradient: 37% to 68% B in 30 minutes.*

To place an order, call (800) 347-6378, fax (508) 485-5736 The Nest Group your Vydac distributor.
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