A New Chemically-Resistant and Heat-Stable Reversed-Phase Column for Protein and Peptide Separations

Introduction

Reversed-phase high-performance liquid chromatography is the most common choice for the separation of polypeptides. Silica-based reversed-phase columns provide high efficiency, but lack stability in extremely harsh conditions. Chemical stability is becoming increasingly important because of the need for chemical sterilization of the adsorbent prior to purification and cleaning and/or regeneration after its use.

With this demand in mind, we have developed a new polymer-based reversed-phase adsorbent (259VHP) specifically for separations of polypeptides. This new adsorbent is based on porous (300Å pore diameter), highly crosslinked polystyrene-divinylbenzene spheres. Because of the high crosslinkage, this new adsorbent gives high mechanical stability with a minimum of shrinking in aqueous and swelling in organic solvents. Its chemical stability was examined by testing chromatographic performance before and after washing with both strong base and acid. No noticeable changes were observed. Its separation performance for proteins and peptides is demonstrated by comparing it with high quality silica-based reversed-phase columns. The results show that performance equivalent to silica-based reversed-phase columns can be obtained with this new polymer-based column.

Chemical Stability Test

Figure 1 shows the effect of extensive washing with strong acid and strong base on peptide retention and resolution on a Vydac 259VHP5415 column. These results indicate

- no noticeable change in separation after a 400 column-volume wash with strong base.
- the strong acid wash did not alter chromatographic performance.

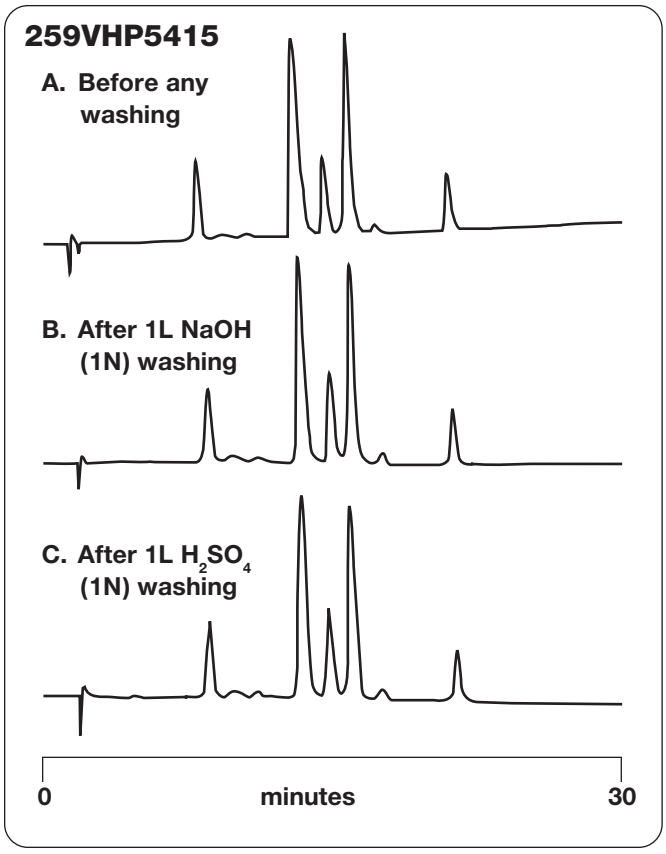

Figure 1. Effect of washing with strong base and strong acid on 259VHP5 column performance. Column: 259VHP5415 5µm 300Å polymer reversed-phase, 4.6 mm ID x 150 mm L. Conditions: 220 nm. 1.0 mL/min. A = 0.1% TFA (v/v):15% ACN in water. B = 0.1% TFA (v/v):30% ACN in water. Gradient from 0 to 100% B in 30 minutes. Sample: 3 µL standard peptide mixture. Oxytocin, bradykinin, angiotensin II, eledoisin, neurotensin (in order of elution).

Comparison of Peptide Separation on Polymer- and Silica-based Columns

Figure 2 compares a standard peptide separation on the 259VHP5415 polymer column to the same separation performed on Vydac's 218TP5415 silica-based reversed-phase column. The two columns provide

- comparable high efficiency
- different selectivity

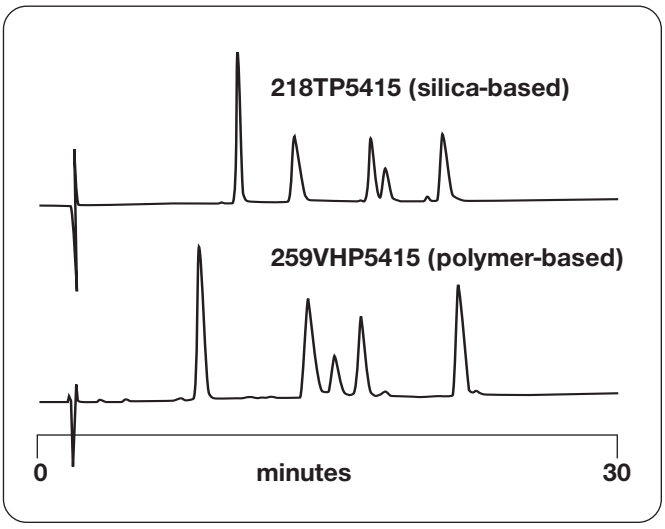

Figure 2. Comparison of Vydac polymer- and silica-based reversed-phase columns. Columns: Both 5 µm, 300Å, 4.6 mm ID x 150 mm L. Conditions: 220nm. 1.0 mL/min. A = 0.1% TFA (v/v):15% ACN in water. B = 0.1% TFA (v/v):30% ACN in water. Gradient from 0 to 100% B over 30 minutes. Sample: Standard peptide mixture. Oxytocin, bradykinin, angiotensin II, eledoisin, neurotensin.

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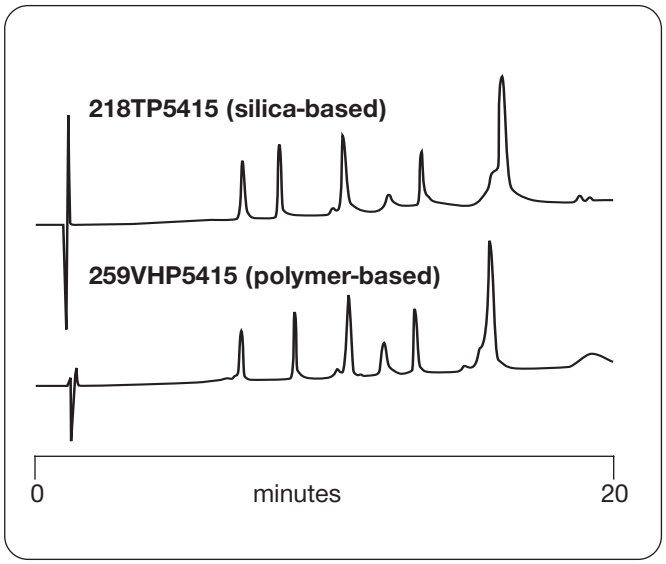

Figure 3. Comparison of Vydac polymer- and silica-based reversed-phase columns. Columns: Both 5 µm, 300Å, 4.6 mm ID x 150 mm L. Conditions: 220nm. 1.5 mL/min. 26° C. A = 0.1% TFA (v/v) in water. B = 0.1% TFA (v/v):95% ACN in water. Gradient from 20 to 75% B over 20 minutes. Sample: Standard protein mixture. RNase, insulin, lysozyme, BSA, myoglobin, ovalbumin.

Comparison of Protein Separation on Polymer- and Silica-based Columns

Figure 3 compares a standard protein separation on the 259VHP5415 polymer column to the same separation performed on Vydac's 218TP5415 silica-based reversed-phase column. The two columns provide

- comparable high efficiency
- slightly different selectivity

Heat and Pressure Stability

259VHP columns are not only acid and alkali stable from pH 0 to 14. They are also heat stable up to 80°C, and can be operated at pressures up to 3000 psi. These characteristics make them ideal for applications requiring extreme conditions, for example, separation of difficult proteins including

- membrane proteins
  - structural proteins
    - viral coat proteins
      - crude inclusion bodies

all of which nature has designed to be insoluble. Such proteins can be eluted using harsh conditions, for example gradients from 10% isopropanol (IPA) to 50% IPA with 3M guanidine HCl at 60°C. (Elevated temperature reduces viscosity, allowing reasonable flow rates.) The chromatogram in Figure 4 shows a separation of serum proteins at 65°C as an example.

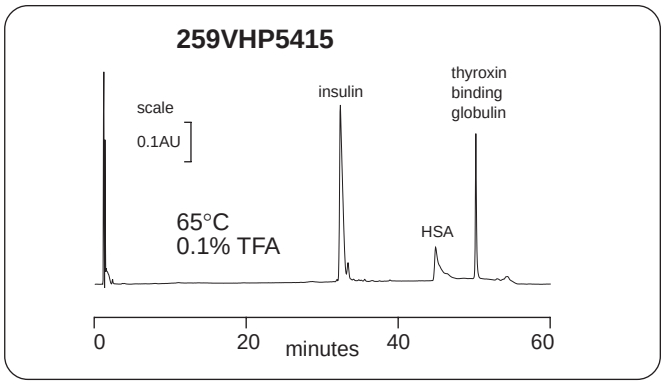

Figure 4. Reverse-phase separation of a mixture of human serum proteins. The extremely sharp peaks indicate that high performance separations can be obtained with the 259VHP column. Elevated temperature improves chromatography of larger, more complex peptides and proteins. Column: 259VHP5415, 4.6mm ID x 150mm L. Conditions: 1.5 mL/min, absorbance at 220nm, 65°C, linear gradient over 45 minutes from 10% to 45% ACN in water with 0.1% TFA (w/v).

Protein Loading Capacity

Protein	M.W.	Capacity (mg/g)
Lysozyme	13930	73
Ovalbumin	43500	56
BSA	67000	28

Frontal loading, 10 mg/mL protein solutions. Column: 259VHP5405, 4.6 mm ID x 50 mm L. Conditions: Loading mobile phase = 80:20 H2O:acetonitrile with 0.1% (v/v) TFA. Flow rate = 1 mL/min.

- Loading capacity is a function of protein molecular weight.

Conclusions

- The new 259VHP5 polymer-based reversed-phase material is chemically stable in both strong base and acid.
- The 259VHP5 material is also solvent resistant, heat stable to 80°C, and pressure stable to 3000 psi.
- The separation performance of this new column is comparable to silica-based reversed-phase columns.
- The polymer-based reversed-phase column provides alternative selectivity to silica-based reversed-phase columns.

ORDERING INFORMATION:

Cat. No.	Description
259VHP5415	Column, Polymer Reverse-Phase, 5µm, 300Å, 4.6mm ID x 150mm L

Other analytical and preparative column dimensions available upon request.

To place an order, contact The Nest Group toll-free at 800-347-6378.