

Small Molecule Separations and Fast LC on 90 Å and 300 Å Reversed-Phase Columns

Although it is generally accepted that small-molecule analyses by reversed-phase HPLC are performed on small-pore (60-100 Å) adsorbents, this is in some respects a historical accident. During the development of HPLC technology, wide-pore 300 Å silica-based adsorbents were the last to become available.

When VYDAC® 300 Å pore-size synthetic silica was developed in the early 1980s, it was introduced as an alternative HPLC silica with advantages over existing small-pore packings. The new 300 Å material rapidly caught the attention of protein and nucleic acid chemists. However,

because small-molecule HPLC applications were already established on 60-100 Å pore-size silicas, the new 300 Å silica received less attention from chemists doing small-molecule analyses. Nonetheless, 300 Å silica-based reversed-phase columns can be used, often with advantage, for small molecule separations. For example, Figure 1 shows how a small-molecule separation on a 300 Å C18 column succeeds in resolving two sample components that coelute on a 90 Å C18 adsorbent. In addition, the 300 Å adsorbent produces the separation in the same overall run time with less organic solvent in the mobile phase.

Antihistamines on Wide-Pore and Small-Pore Reversed Phase Columns

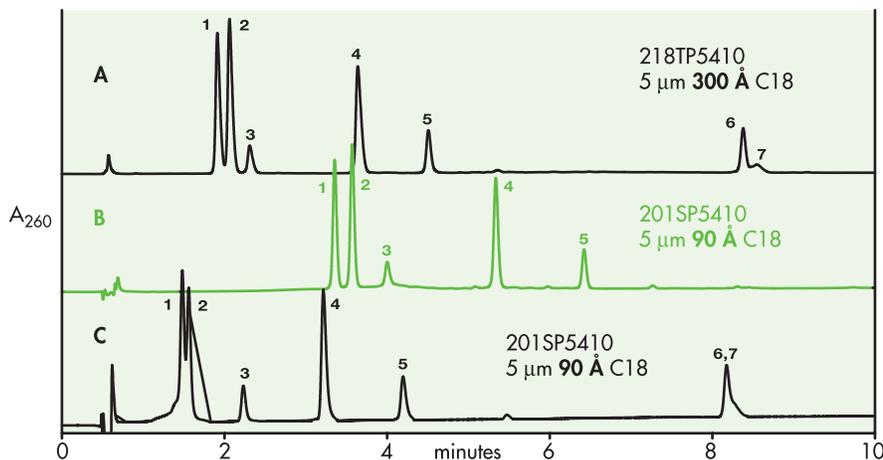


Figure 1. Separation of antihistamines on Vydac large-pore and small-pore reversed-phase columns. Column dimensions: All 4.6 mm ID x 100 mm. Flow: 2.5 mL/min. Mobile phase: A = 0.1% (v/v) TFA in H₂O. B = 0.1% (v/v) TFA in ACN. Gradient, A & B: 5% to 35% B in 10 minutes. Gradient, C: 10% to 40% B in 10 minutes. Peaks: 1) pheniramine; 2) doxylamine; 3) methapyraline; 4) chlorpheniramine; 5) orphenadrine; 6) diphenylpyraline; 7) promethazine. The 300 Å polymeric C18 column provides better resolution of peaks 6 and 7 compared to the 90 Å reversed-phase material. Eluting all peaks in similar time on the 90 Å column (chromatogram C) requires 25% more organic solvent, a significant cost in routine analytical HPLC.

Reversed-Phase Bonding Types

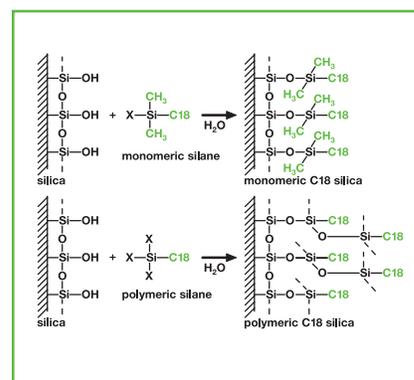


Figure 2. Monomeric vs. polymeric C18 bonding. Use of trifunctional bonding reagent results in a more complex, multilayered C18 bonded phase.

Wide-Pore Advantages

Wide-pore reversed-phase adsorbents complement small-pore adsorbents by providing alternative media that can more successfully accomplish certain separations and analytical objectives. Their potential advantages fall into three basic areas:

Advantage #1

The larger silica pore size allows added variety in bonding chemistries. Polyfunctional silanes can be used to produce polymeric reversed-phase layers (Fig. 2). With small-pore silicas monomeric bonded phases are generally used because the more complex polymeric phases tend to block the pores and limit chromatographic performance. On 300 Å silica, polymeric phases provide practical adsorbents with subtly different selectivities for small molecules as well as proteins and peptides. Phases with bulky rigid substituents such as diphenyl are

also possible, providing another variant in selectivity. Figure 3 shows the same antihistamine separation as in Figure 1, this time run on two different 300 Å reversed-phase columns – one with a polymeric C18

bonded phase and one with a monomeric C18 phase. This demonstrates that the greater selectivity for diphenylpyraline and promethazine is really due to the polymeric bonded phase, and not pore size.

Antihistamines on 300 Å Polymeric C18 and Monomeric C18 Reversed Phases

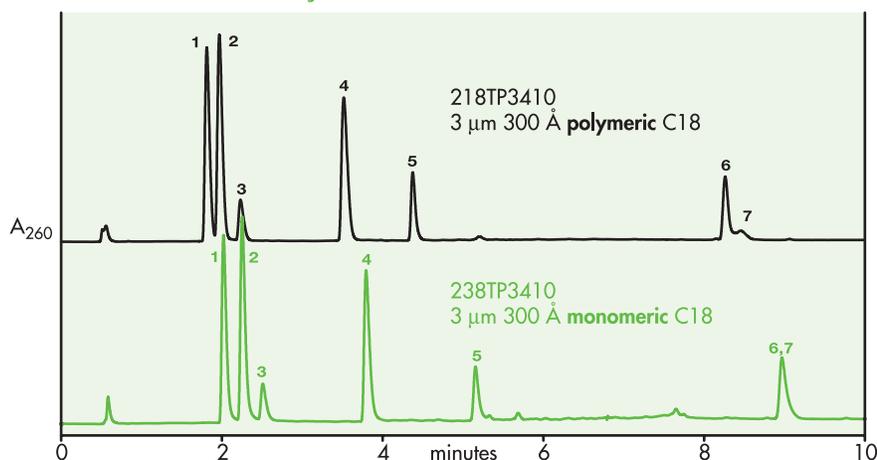


Figure 3. Comparison of antihistamine separations on monomeric and polymeric C18 phases on 300 Å silica-based adsorbents. Column dimensions: Both 4.6 mm ID x 100 mm. Flow: 2.5 mL/min. Mobile phase: A = 0.1% (v/v) TFA in H₂O. B = 0.1% (v/v) TFA in ACN. Gradients: 5% to 35% B in 10 minutes. Peaks: Same as Figure 1. Resolution of peaks 6 and 7 is better on the polymeric bonded phase than on the monomeric phase under the same conditions.

Fast Screening of Cat's Claw Extract

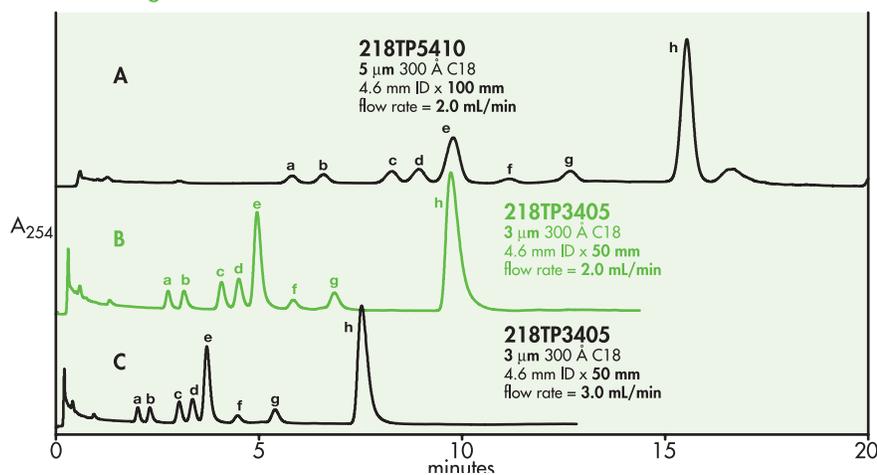


Figure 4. Fast screening of extract of Cats Claw. Mobile phase: A = 10 mM KH₂PO₄, pH 6.6, 20% ACN, 10% MeOH. B = 100% ACN. Gradient: 0% to 5% B in 5 minutes, then ramp to 13% B in 5 minutes, and to 100% B in 3 minutes. An excellent example of the benefits of short, fast columns: Elution of the eight alkaloids in chromatogram A takes about 15 minutes. In chromatogram B, on a shorter column, the separation is accomplished in 10 minutes with a time and solvent savings of 33%. Increasing flow rate in chromatogram C saves another 25% in time while increasing solvent consumption by only 12.5% compared to B.

Advantage #2

It should also be remembered that not all small molecules, in the sense of non-polymers, are really “that small.” Many non-polymeric molecules of interest, particularly in biochemistry and pharmaceutical sciences, are actually quite large – steroids, certain antibiotics, retinoids, taxanes and other natural products to name a few. These “large” small molecules often show better retention and improved selectivity on 300 Å reversed-phase adsorbents because they are able to more freely enter the pores for access to the entire adsorbent surface.

Advantage #3

The lower total surface area of a 300 Å silica – roughly 70 m²/g vs. 250 m²/g for 90 Å silica – produces lower retentivity on similar surface chemistry while maintaining selectivity. This means that less organic solvent is needed in the mobile phase for elution. Methods on 300 Å adsorbents reduce solvent usage, and with that comes reduced solvent cost.

Fast LC

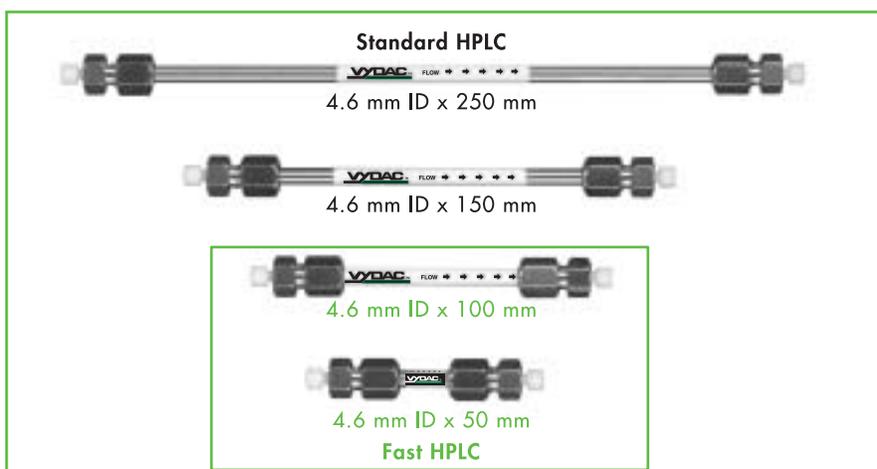
Extending the solvent-reduction advantages of wide-pore adsorbents, Vydac 300 Å reversed-phase materials are now available in 3 µm particle diameters. The smaller 3 µm particles improve mass transfer between the mobile phase and stationary phase in the column by decreasing the distances that analyte molecules must diffuse within the pores to interact with adsorptive surfaces. Improved mass transfer permits separations to be performed more rapidly using a combination of shorter columns, higher mobile-phase flow rates and faster gradients without sacrificing peak sharpness or resolution. With modern high-efficiency chromatography systems, the use of 3 µm adsorbents in short 100 mm or 50 mm columns for “Fast LC” provides not only

faster analyses, but also additional reductions in solvent use and mobile-phase disposal cost.

The advantages of fast LC on short columns packed with 3 μm 300 \AA reversed-phase adsorbents are clearly demonstrated

in the separations shown in Figures 4 through 7. For complex separations such as the screening of Cats Claw extracts for alkaloids (Fig. 4) and analysis of taxanes (Fig. 5), time savings of 50% or more and solvent savings of 20% or more are clearly

possible. For simple routine analyses such as the prednisolone QC separation of Figure 6, very high analytical throughputs can be achieved.



Fast Screening of Taxanes

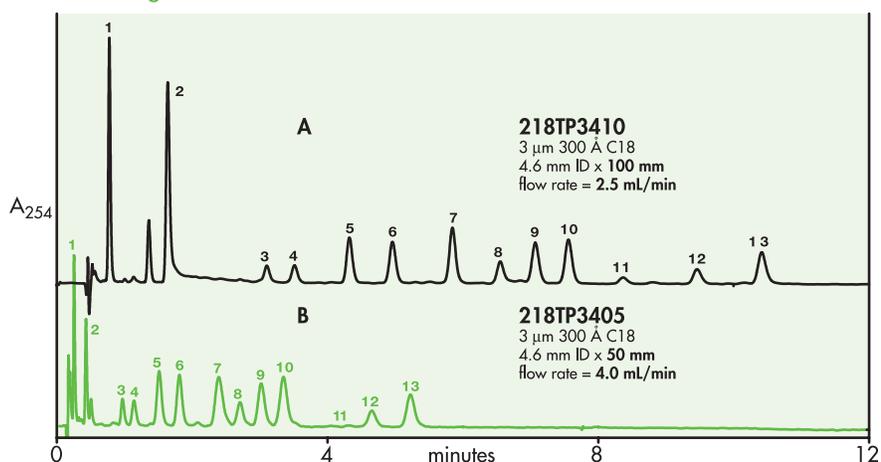


Figure 5. Fast screening of taxanes. Mobile phase: A = 50 mM NaOAc, pH 6.7. B = ACN. Gradient: 30% to 40% B in 10 minutes, then hold for 2 minutes. Peaks: 1) 10-deacetylbaccatin III; 2) baccatin III; 3) 10-deacetyl-7-xylosyltaxol B; 4) taxinine M; 5) 10-deacetyl-7-xylosyltaxol; 6) 10-deacetyl-7-xylosyltaxol C; 7) 7-xylosyltaxol; 8) cephalomannine; 9) 10-deacetyl-7-epitaxol; 10) paclitaxel; 11) 10-deacetyltaxol; 12) taxol C; 13) 7-epitaxol. In chromatogram B, a complex separation of thirteen compounds is achieved in less than six minutes. By increasing the flow rate to 4.0 mL/min and reducing the column length to 50 mm, the time savings is approximately 50%. Not so obvious is the solvent savings, which is about 5.5 mL per run, or 20%.

Fast Quality Control

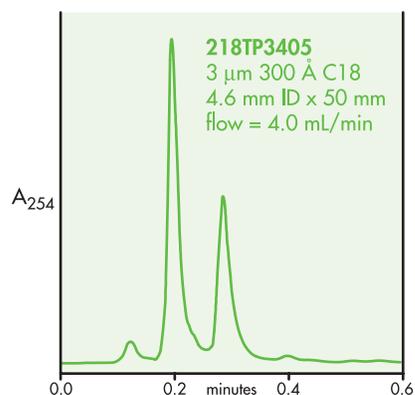


Figure 6. Fast QC of prednisolone. Mobile phase: Isocratic. 40% ACN in water. Peaks: 1) prednisolone acetate; 2) prednisolone. In this day of high-throughput screening, short fast columns are the way to go. This separation was done at a flow of 4.0 mL/min in less than half a minute using isocratic elution. Using an automated system, at 0.5 minutes per analysis, this would result in 960 analyses in an eight hour working day. (Over lunch, add another 120.)

Fast HPLC of Priority Pollutants

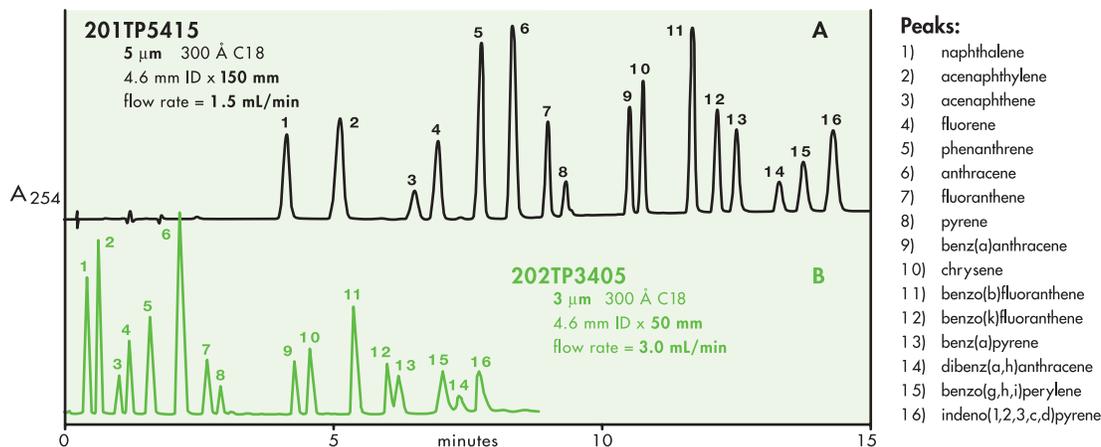


Figure 7. Fast LC of priority pollutant polyaromatic hydrocarbons (PAHs). Reference: EPA Methods 550, 550.1, 610, and 8310. Columns VYDAC C18 specialty reversed phases as indicated. Flow, A: 1.5 mL/min. Flow, B: 3.0 mL/min. Mobile phase: A = water. B = acetonitrile. Gradient, A: Hold 50% B for 3 minutes, then 50% to 100% B in 7 minutes. Gradient, B: Ramp 40% to 95% B in 8 minutes.

Figure 7 shows separations of sixteen priority pollutant polyaromatic hydrocarbons (PAHs) on VYDAC 300 Å C18 columns and illustrates the time savings that can be achieved using a shorter 50 mm column containing 3 µm particles for fast LC.

Benefits of VYDAC 300 Å columns for small molecule HPLC:

- Better selectivity
- Savings on solvent use and disposal
- Faster separations

Ordering Information: Columns for Fast LC

Cat. No. Description

50 mm columns:

202TP3405	Column, Octadecyl (C18), Specialty, 3µm, 300Å, 4.6mm ID x 50mm
238TP3405	Column, Octadecyl (C18), Monomeric, 3µm, 300Å, 4.6mm ID x 50mm
218TP3405	Column, Octadecyl (C18), Polymeric, 3µm, 300Å, 4.6mm ID x 50mm
214TP3405	Column, Butyl (C4), Polymeric, 3µm, 300Å, 4.6mm ID x 50mm

100 mm columns:

238TP3410	Column, Octadecyl (C18), Monomeric, 3µm, 300Å, 4.6mm ID x 100mm
218TP3410	Column, Octadecyl (C18), Polymeric, 3µm, 300Å, 4.6mm ID x 100mm
214TP3410	Column, Butyl (C4), Polymeric, 3µm, 300Å, 4.6mm ID x 100mm

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