Fast Protein and Peptide Analyses with Vydac 3µm 300Å Reversed-Phase Columns

Decreasing the particle size of an HPLC packing facilitates rapid chromatography by speeding diffusion between the mobile phase and adsorptive surfaces of the stationary phase. For small molecule separations this leads to

- **sharper peaks**
- **resolution on shorter columns**
- **reduced analysis time**
- **reduced solvent consumption.**

In fact, much of the performance gain of modern HPLC over earlier chromatographic techniques is the result of improvements in the manufacture and use of small-particle adsorbents.

Unlike small molecules, peptides and proteins typically do not partition by repeatedly adsorbing and desorbing from the stationary-phase as they move through a chromatography column. Instead, separation is based on mobile-phase gradients. Strong adsorption is followed by selective release when the concentration of organic component reaches a specific value for each analyte. Retention of proteins and peptides is more an all-or-nothing phenomenon.

The main effect of reducing adsorbent particle size for this type of separation is to allow mobile phase molecules to move rapidly in and out of the packing. This permits a quick and uniform release of each adsorbed analyte as the critical mobile-phase composition is attained. Performance advantages are somewhat different from those for small molecules. Much protein and peptide chromatography is already run on short columns with 5µm to 10µm packings. The 3µm particle size speeds exchange between the mobile phase and packing, and allows you to

- **increase the mobile phase flow rate**
- **run a shallower gradient on a per-volume basis during the same run time.**

This results in the fast, high resolution separations shown here with Vydac's 3µm 300Å reversed phases.

Figure 1 shows a comparison of separations of five standard proteins run on a 100-mm-long 3µm reversed-phase column (Vydac 238TP3410) with a flow rate of 2.5 mL/minute, and using the same gradient timing on a 50-mm-long column with the same packing (Vydac 238TP3405) at a higher flow rate of 4.0 mL/minute. The higher flow rate is made possible by the lower back pressure of the shorter column. Note the faster analysis time together with improved resolution on the shorter column due to the shallower elution gradient.

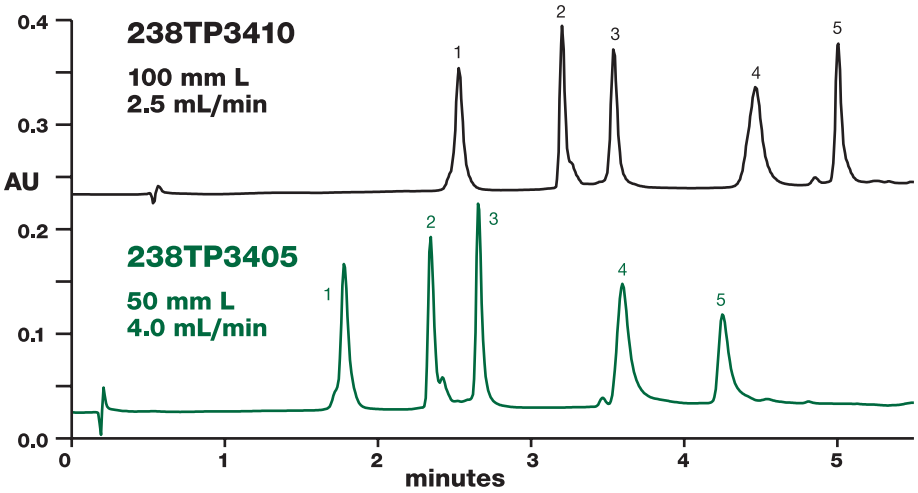

Figure 1. Comparison of protein separations on 3µm 300Å reversed-phase columns of 100 mm and 50 mm lengths. Note the higher flow rate on the 50 mm column which produces a shallower mobile-phase composition gradient on a per-volume basis. Columns: Vydac 238TP3410 "monomeric" C18 reversed-phase, 3µm particle size, 300Å pore size, 4.6mmID x 100mmL, and Vydac 238TP3405, identical packing in 4.6mmID x 50mmL column. Flow rates: 2.5 mL/min and 4.0 mL/min, respectively.
Mobile phase: A = 20% acetonitrile (v/v) in water with 0.1% TFA (w/v). B = 45% acetonitrile (v/v) in water with 0.1% TFA (w/v). Gradient: 0% to 100% B in 4 minutes for both columns. Detection: 215nm.
Sample: Standard protein mixture. Peaks: (1) ribonuclease, (2) insulin, (3) cytochrome C, (4) BSA, and (5) myoglobin.

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3µm 300Å Reversed-Phase Columns for Proteins and Peptides

The same five proteins (Figure 2) and also a mixture of six standard peptides (Figure 3) were run under the fast separation conditions on 3µm 50-mm-long columns with three different reversed-phase chemistries for a comparison of selectivities. The 218TP and 238TP packings are both octadecyl (C18) phases on the same 300Å silica base, but with polymeric and monomeric bonding chemistries, respectively. The 214TP packing is a polymerically bonded butyl (C4) phase.

Good separations of the five proteins were obtained on all three columns, with the C4 column providing the best peak shape and resolution.

In the case of the peptide mixture, the C4 column (separation not shown) failed to resolve peaks 2 and 3. Both C18 columns were effective in resolving all components, with the monomeric bonding chemistry (238TP) providing the best resolution and peak shapes.

These results suggest that Vydac's 238TP monomeric C18 may be the best all-around choice for both peptide and protein analyses. Still, for mixtures of uncertain composition the ability to screen on a variety of reversed-phase chemistries with varying selectivity provides added assurance that all components will be seen.

Vydac's 3µm columns make rapid analysis possible and are ideal for:

- **in-process testing**
- **combinatorial library screening**
- **screening of recombinant clones**
- **studies of reaction kinetics**

Ordering Information Call 800.347.6378

Cat. No.	Description
	50 mm long columns:
238TP3405	Column, Octadecyl (C18), Monomeric, 3µm, 300Å, 4.6mm ID x 50mm L
218TP3405	Column, Octadecyl (C18), Polymeric, 3µm, 300Å, 4.6mm ID x 50mm L
214TP3405	Column, Butyl (C4), Polymeric, 3µm, 300Å, 4.6mm ID x 50mm L
	100 mm long columns:
238TP3410	Column, Octadecyl (C18), Monomeric, 3µm, 300Å, 4.6mm ID x 100mm L
218TP3410	Column, Octadecyl (C18), Polymeric, 3µm, 300Å, 4.6mm ID x 100mm L
214TP3410	Column, Butyl (C4), Polymeric, 3µm, 300Å, 4.6mm ID x 100mm L

Proteins

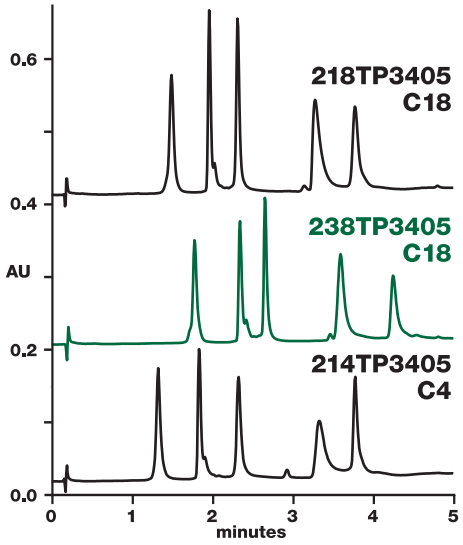

Figure 2. Comparison of protein separation on three 3µm 300Å reversed-phase columns.
Flow rate: 4.0 mL/min. Columns: Vydac 218TP3405 "polymeric" C18, 238TP3405 "monomeric" C18, and 214TP3405 C4, all 4.6mmID x 50mmL. Conditions: As described for Figure 1. Sample: Standard protein mixture. Peak order: Same as Figure 1.

Peptides

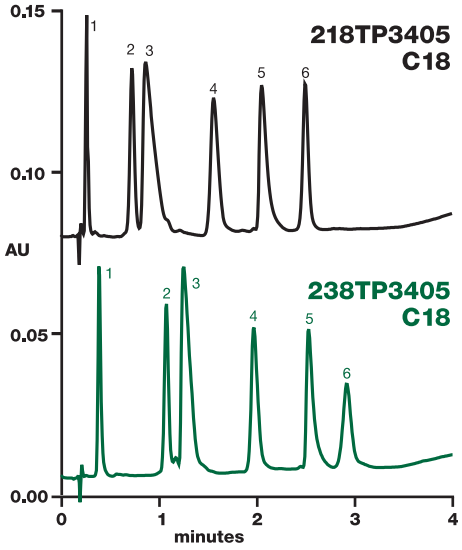

Figure 3. Comparison of peptide separation on two 3µm 300Å reversed-phase columns.
Flow rate: 4.0 mL/min. Columns: Same as Figure 2. Mobile phase: A = 15% acetonitrile (v/v) in water with 0.1% TFA (w/v). B = 25% acetonitrile (v/v) in water with 0.1% TFA (w/v). Gradient: 0% to 100% B in 3 minutes. Detection: 215nm. Sample: Peptide mixture. Peaks: (1) neurotensin fragment 1-8, (2) oxytocin, (3) neurotensin fragment 8-13, (4) angiotensin II, (5) neurotensin, and (6) angiotensin I.