

## Assessing Polypeptide Integrity: Reversed-Phase HPLC vs. PAGE

Sensitive, reproducible analytical methods are essential to development of well-characterized pharmaceuticals. For polypeptides, polyacrylamide gel electrophoresis (PAGE) and reversed-phase HPLC are commonly used. Here we offer a comparison of both methods by analyzing a common polypeptide, chymotrypsin, after exposure to conditions expected to cause degradation.

Chymotrypsin, purchased commercially, was dissolved at 1 mg/mL in water, PBS, and 0.1% TFA/5% ACN. Portions of each solution were incubated as indicated, then analyzed by reversed-phase chromatography (Figure 1) and PAGE (Figure 2). Sample 1 was the original aqueous solution held at 4°C for comparison. Lane 0 of the gel is a standard polypeptide mixture.

### Results

Both methods clearly show decomposition in the incubated samples and formation of aggregates. However, some decomposition products visible in chromatograms 6 and 7 were not seen with PAGE and probably ran off the gel. On the other hand, the large contaminant seen clearly in lane 4 with PAGE appears as a very broad peak and is not as apparent with reversed-phase HPLC. Reversed-phase HPLC and PAGE are complementary methods. Used together they provide a more definitive indication of purity.

**Data for this note courtesy of**

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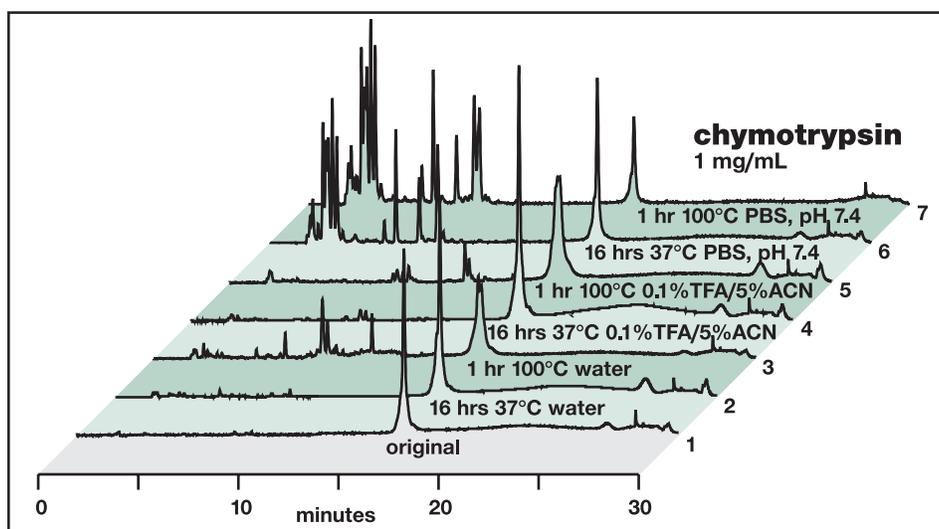
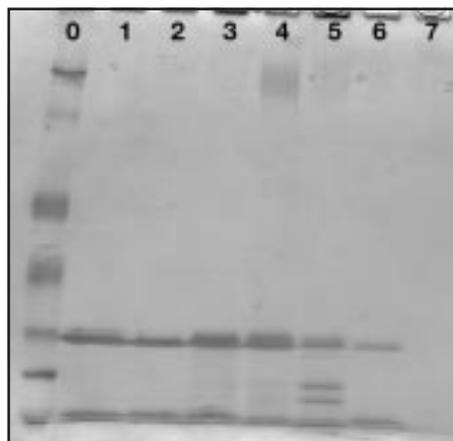


Figure 1. Reversed-phase HPLC of chymotrypsin samples. Column: Vydac 214TP54 C4 5 $\mu$ m 4.6mmID x 250mmL. Conditions: 1 mL/min. 280nm. Solvent A = 0.1% TFA (w/v) in water. Solvent B = 0.09% (w/v) TFA in ACN. Gradient: 20% to 60% B in 40 minutes.

Figure 2. PAGE analysis of chymotrypsin samples. A Novex 12% tris glycine gel, 1.5 mm thick, was run under reducing conditions and silver stained. Numbered lanes correspond to chromatograms of Figure 1.



## Reversed-Phase Column Cleaning: SDS is OK!

Vydac's *Handbook of Analysis and Purification of Peptides and Proteins by Reversed-Phase HPLC* cautions (page 40) about the adverse effects of residual surfactant in samples. Even trace amounts of SDS can noticeably degrade a reversed-phase polypeptide chromatogram.

Also noted, however, is that surfactants appear to be removed by the gradient and do not permanently harm a column. This leads to a question: "Can SDS be used for cleaning contaminated reversed-phase columns?" The answer, as shown by the chromatograms of Figure 3, is "Yes!" Separation of six polypeptides on a C18 column (A) became degraded (B) after contamination of the column by protecting group and scavenger products from a peptide synthesis. We cleaned the column by injecting 500  $\mu$ L of 1% SDS solution at 1 mL/min, followed by a 10-minute gradient from 5% to 95% acetonitrile with 0.1% (v/v) TFA. After equilibrating to starting conditions, the peptide separation was restored (C).

This demonstrates that even large amounts of SDS are effectively removed by a solvent gradient. While there's no guarantee it will restore all columns, SDS is a reasonable agent to try for cleaning. As with any cleaning procedure, periodic routine application is preferable to catastrophic intervention after heavy contamination has accumulated.

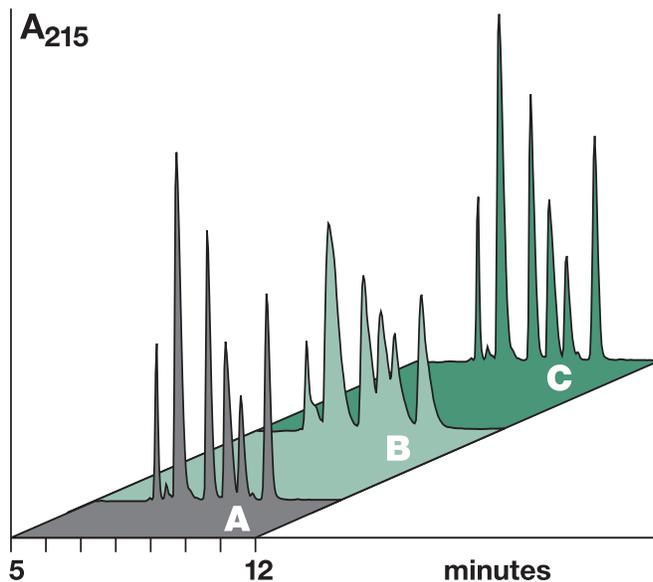


Figure 3. Polypeptide chromatograms. A. Original. B. Contaminated column. C. After SDS cleaning. Column: Vydac 218TP54, C18, 4.6 x 250 mm. Mobile phase: A = 15% ACN in water. B = 27% ACN in water. Both with 0.1% (v/v) TFA. Gradient: 0 to 100% B in 15 minutes.

### Vydac Reversed-Phase Columns:

Cat. No.	Description
208TP54	Column, Octyl (C8), Polymeric, 5 $\mu$ m, 300Å, 4.6mm ID x 250mm L
214TP54	Column, Butyl (C4), Polymeric, 5 $\mu$ m, 300Å, 4.6mm ID x 250mm L
218TP54	Column, Octadecyl (C18), Polymeric, 5 $\mu$ m, 300Å, 4.6mm ID x 250mm L
219TP54	Column, Diphenyl, Polymeric, 5 $\mu$ m, 300Å, 4.6mm ID x 250mm L
238TP54	Column, Octadecyl (C18), Monomeric, 5 $\mu$ m, 300Å, 4.6mm ID x 250mm L
259VHP54	Column, Polymer Reversed Phase, 5 $\mu$ m, 300Å, 4.6mm ID x 250mm L

Other analytical and preparative column dimensions available upon request.

**To place an order, call The Nest Group, 800.347.6378 your local Vydac distributor.**