Optimizing Peptide Purifications

Introduction

Diversity is an innate characteristic of peptide structures. Even closely related peptides, for example the target product and contaminating side products from a synthetic process, typically have chemical differences at multiple sites consisting of various hydrophobic, polar, and charged acidic or basic constituents.

This remarkable diversity is what lets peptides exhibit such a wide range of biological activities. It also provides chromatographers with an ability to change selectivity and control the resolution in reversed-phase separations of peptides to a much greater extent than is normally possible when separating smaller, simpler molecules.

Summary

Selectivity trials for a specific partially purified cyclic peptide using a range of columns and mobile phases suggest the following conclusions.

- Wide-pore (300Å) packings provide the best reversed phase separations.
- Separation at near neutral pH produces better resolution than the typical 0.1% TFA acidic mobile phase.
- Orthogonal separations using different stationary phase chemistries or mobile phases can be useful for identifying and removing problem contaminants.

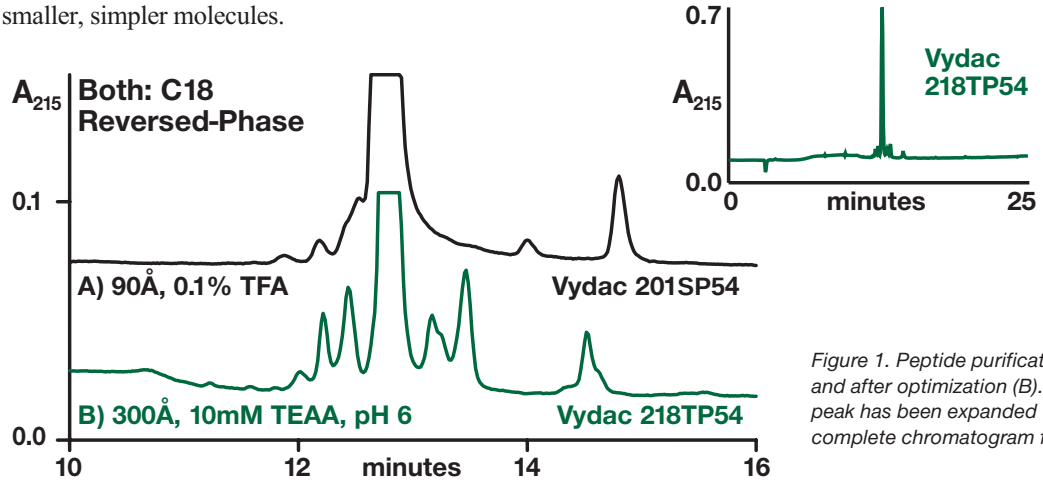

Figure 1. Peptide purification under typical conditions (A) and after optimization (B). The region around the main peak has been expanded to show detail. (Inset shows complete chromatogram for 218TP54.)

A Range of Reversed-Phase Performance

The chromatograms of Figure 1 show separations of the same synthetic peptide mixture on two different reversed-phase columns under identical conditions of flow, temperature, and organic solvent gradient. The separations differ in the pore-size of the packing and the pH and buffer ions in the aqueous mobile-phase component. They represent the extremes of a series of resolution trials that were aimed at finding the best conditions for purification of the major component, a partially purified cyclic peptide with a C-terminal acyl hydrazide. Each complete chromatogram (inset) reveals one large peak of target product surrounded by several much smaller contaminating peaks. In the figures, the vertical axis in the region containing the major peak has been expanded to show resolution in detail.

Chromatogram A was the first attempt at separation using a 90Å pore-size C18 reversed-phase column (Vydac 201SP54) and the mobile phase most commonly used for peptide separations, TFA/ACN. Chromatogram B, a separation at pH 6.0 in triethylamine acetate (TEAA) buffer on a 300Å pore-size C18 reversed-phase column (Vydac 218TP54), represents perhaps the best option resulting from the series of trials shown in Figure 2 (next page).

ORDERING INFORMATION FOR COLUMNS USED IN THIS WORK:

Cat. No.	Description
201SP54	Column, Octadecyl (C18), Monomeric, 5µm, 90Å, 4.6mm ID x 250mm L
218TP54	Column, Octadecyl (C18), Polymeric, 5µm, 300Å, 4.6mm ID x 250mm L
238TP54	Column, Octadecyl (C18), Monomeric, 5µm, 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.

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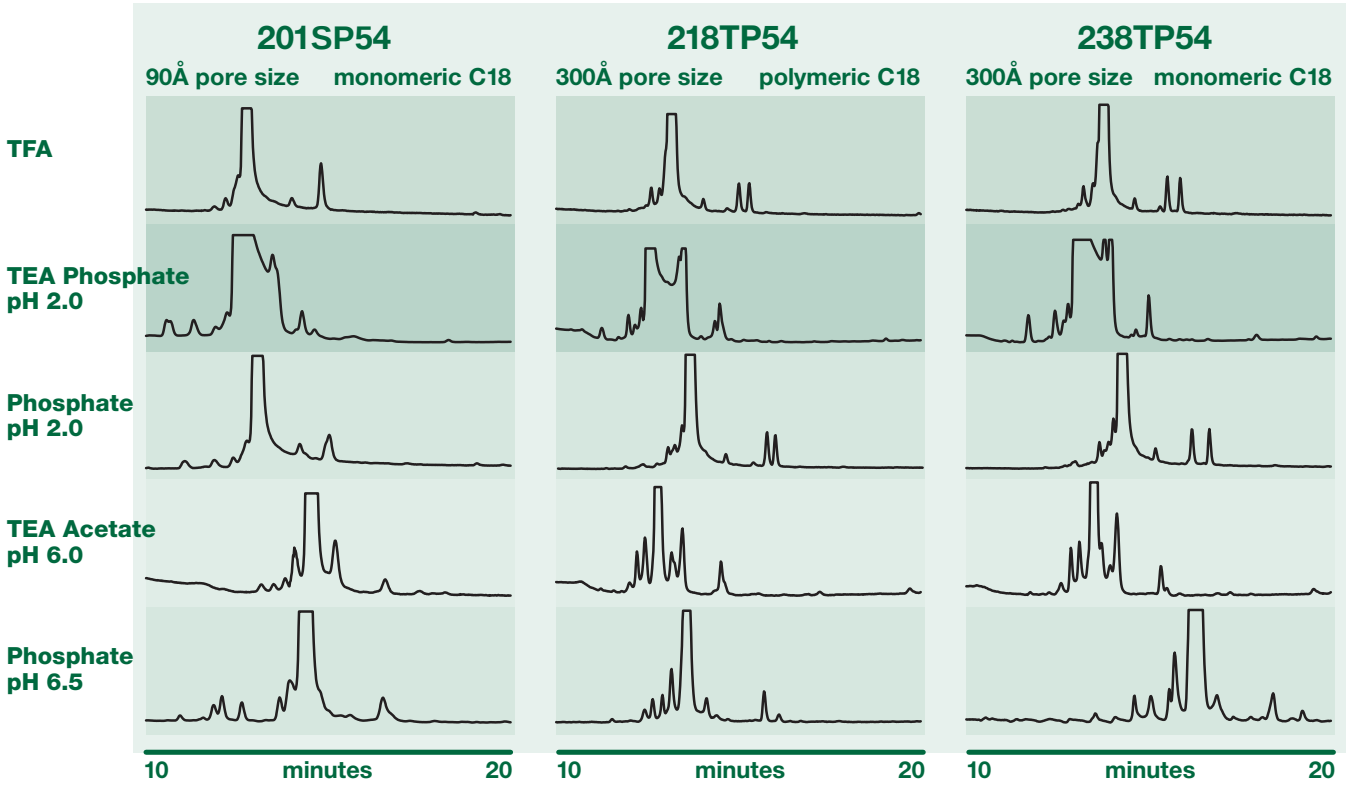

Figure 2. Comparison of synthetic peptide separation on three columns under five different buffer conditions. All columns: 5µm, 4.6mmID x 250mmL. Temperature: Ambient. Flow rate: 1.0 mL/min. Buffers: 0.1% TFA (v/v). 10mM TEA Phosphate, pH 2.0. 10mM NaH2PO4, pH 2.0. 10mM TEA acetate, pH 6.0. 10mM NaH2PO4, pH 6.5. Mobile phase A: Buffer as indicated. Mobile phase B: Buffer as indicated (except 0.09% for TFA), 90% ACN (except 80% ACN for phosphate buffers). Gradient: Initial 5% B to 2 minutes. Then 20 minute linear to 45% B. Then 2 minutes to 100% B. Hold 2 minutes. Return to 5% B in 1 minute. Detection: 215 nm. Sample: Partially purified cyclic peptide with C-terminal acyl hydrazide.

The Complete Trial-Separation Set

Several conclusions can be drawn from inspection of the complete series. First, it is clear from comparisons under any of the mobile phase conditions tried that the 300Å wide-pore reversed phases produce narrower peaks and improved resolution when compared to the 90Å adsorbent. Peptides of significant size, or rigidity as in the case of a cyclic peptide, will be at least partially excluded from the pores of the 90Å material. In addition, narrow pores restrict diffusion. Both effects contribute to peak broadening. Thus, for peptide separations in general, 300Å wide-pore silicas should be the adsorbents of choice.

Effects of buffers and pH are dramatic. In general, separation at mildly acidic pH (6.0 or 6.5) appears to produce better peak shape and resolution, or at least a greater number of resolved components, than separation at highly acidic pH. This observation is significant in view of the fact that highly acid conditions such as 0.1% TFA tend to be the norm for reversed-phase peptide chromatography. The results shown here suggest that pH 6.0 or 6.5 separations are more likely to separate difficult to resolve components. However, it should be noted that these results are specific to the particular peptide sample used. The conclusions may or may not apply to other samples.

Significant selectivity differences are seen between the two chemically distinct 300Å reversed phases. Vydac 218TP is a polymeric C18 produced with multifunctional silanes, while 238TP is a monomeric C18 produced with monofunctional silanes. In specific situations, one or the other of these adsorbents may produce better resolution between critical components. In some instances, it may be desirable to run sequential separations on different adsorbents or with different buffers to eliminate specific problem contaminants.

Conclusion

As shown here, a series of trial separations can be a great help in choosing optimal conditions for a specific peptide purification. For process development, where extensive method workup can be justified, use of a resolution mixture will also yield additional information about the sample, assist in optimizing conditions, and provide a means for process validation.

Data for this note courtesy of

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