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Advantages of Isopropanol for the Reverse Phase Analysis of Peptide Pharmaceuticals

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[Background]

Why would using Isopropanol be beneficial?

- There is currently a worldwide acetonitrile shortage; as a result of the shortage, acetonitrile costs have risen dramatically.

What makes Isopropanol a suitable replacement for acetonitrile?

- The major reason isopropanol is avoided is its higher viscosity. As we show, with the right columns, this is not an issue.
- Acetonitrile can produce unwanted artifacts during gradient elution. While isopropanol has its own unwanted gradient behaviors, they often do not overlap with acetonitrile, making isopropanol the preferred solvent in the examples presented here.

[Summary]

For a recent product, extant analytical methods were all release methods of marginal use for in-process analysis. The peptide contains cysteines, and is synthesized in a totally reduced form. The active peptide is folded into a compact form via the formation of –S-S- bridges, thus the "linear" reduced peptide must be oxidized to produce the correct product. The first two columns employed were not able to resolve the reduced from the oxidized form. A release method column could resolve these species, but the gradient was not optimal, and we altered mobile phase composition and the gradient to maximize resolution, and to mitigate a very noisy baseline. With particles smaller than 5 microns (3.5 or 3 µ), the baseline noise with acetonitrile was particularly bad where the product eluted.

To address this, we developed a method employing isopropanol/water gradients instead of the acetonitrile/water. We employed Cadenza CD-C18 reverse-phase columns; 3 µ columns notable for high resolution (200,000 plates/m) and low backpressure. The low backpressure, combined with the greater eluent strength of isopropanol, compensated for its increased viscosity. With a 4.6 x 150 mm column we were able to run at 1 ml/min and stay within the recommended pressure limits of the column. More importantly, we obtained excellent separation of reduced and oxidized peptide. In view of the current supply issues and higher expense of acetonitrile, we think this approach deserves consideration.

[Materials & Methods]

We employed either a Cadenza CD-C18 column or an Intrada WP-RP. The Cadenza is 4.6 x 150 mm with 3 µm particles containing 12 nm pores; the Intrada is 4.6 x 250 mm with 3 µm particles containing 30 nm pores. The mobile phases were the same for both columns:

Mobile Phase A : 0.1% TFA in 8.6% IPA/water
Mobile Phase B : 0.1% TFA in 96% IPA/water

For the Cadenza:

Flow Rate: 1.0 mL/min
Temperature : 40 °C
Wavelength: 220 nm
Injection Volume: 10 µL
Gradient %B (interval in min): 0-2 (5), 2-8(15), 8-12(25), 12-100(30),100(33), 0(50)

For the Intrada:

Flow rate mL/min (interval in min): 0.6-1.0 (30), 1.0 (42), 0.6 (48)
Temperature : 55 °C
Wavelength : 214 nm
Injection Volume : 10 µL
Gradient %B (interval in min) : 9-15 (30), 15-100 (35), 100 (38), 9 (48)

The Cadenza was employed for the chromatographs presented in figures 1-4; the Intrada was employed for the chromatographs presented in figure 5. Figures 1, 3 and 4 are analyses of the cysteine peptide mentioned in the introduction. Figure 2 is an analysis of a synthetic step for a different peptide, and figure 5 shows the development of an analytical method for a third peptide.

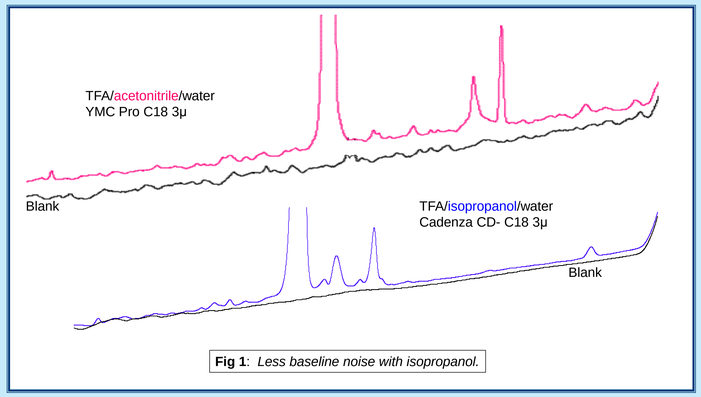

Fig 1: *Less baseline noise with isopropanol.*

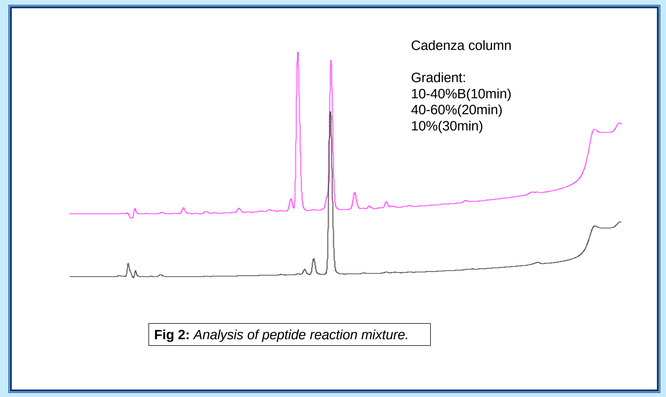

Fig 2: *Analysis of peptide reaction mixture.*

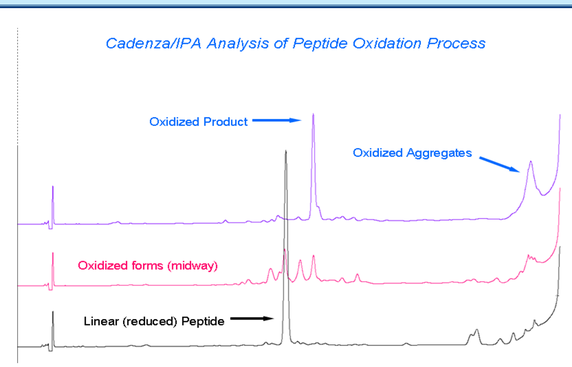

Figure 3: *Separation of reduced from oxidized peptide during disulfide bridge formation.*

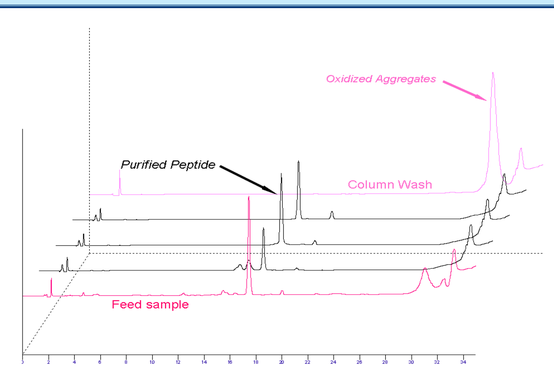

Figure 4: *Purification of correctly folded product.*

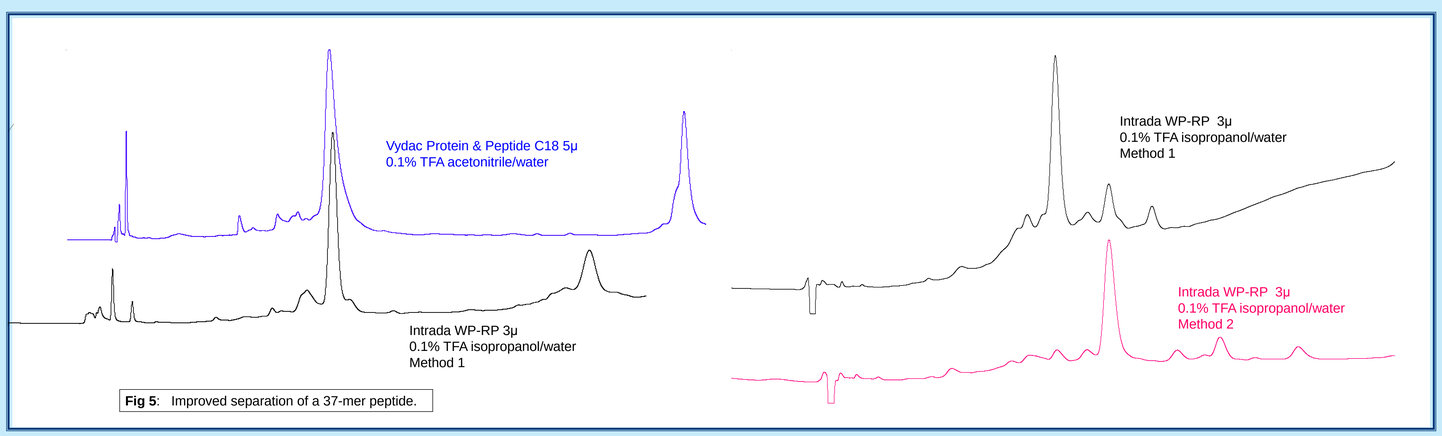

Fig 5: Improved separation of a 37-mer peptide.