**INTRODUCTION**

Peptide mapping with reversed phase HPLC is an established technology for the analysis of protein structure. Coupling the chromatographic separation to mass spectral detection significantly increases the information available. In characterizing variant structures in a protein sample, complications can arise. Low abundance peptides, representing trace modifications, may be over-shadowed or lost in the more intense signal representing the native structure. We have optimized peptide separations to ensure the highest quality spectral data for characterizing trace modifications of a protein. An essential element of the optimization process for higher resolution than is possible with HPLC.

**COMPARING HPLC AND UPLC**

- **UPLC** offers improved resolution and peak shape.
- **HPLC** provides better sensitivity, broader dynamic range, and lower solvent consumption.

**DEVELOPING FAST, FOCUSED METHODS**

Cascading gradient optimization is necessary to achieve separation with acceptable baseline characteristics and high resolution. The UPLC gradient runs were designed to be as similar as possible to the corresponding HPLC gradient.

**RESULTS**

- **Figure 3.** HPLC and UPLC Separation at Constant Gradient (90-100% B). The UPLC run is 40% faster than the HPLC run.
- **Figure 4.** HPLC and UPLC Gradient Separation (from 10 to 90% B). The UPLC run is 30% faster than the HPLC run.

**EFFECT OF TEMPERATURE**

- **Figure 9.** 12.5 to 34°C in 34 minutes at various temperatures. Small changes in temperature produce large changes in selectivity. The data is gathered with extracted mass chromatograms.

**CONCLUSION**

- **UPLC** improves resolution in peptide mapping.
- **Improved resolution** can be achieved with a more complex separation in the same time as HPLC.
- **Improved resolution** can be achieved at a reduced run time.

With optimization, faster methods can improve selectivity and sensitivity.

Small changes in gradient slope and temperature give improved results.

Improved chromatographic resolution can improve the signal-to-noise ratio in these separations and can simplify interpretation of those spectra.