

# Increased Long-term Stability of Peptide Mapping Using the Vanquish UHPLC System

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## Key Words

Acclaim C18 RSLC, Biocompatible UHPLC, Biopharma, Biotherapeutics Characterization, Monoclonal Antibodies, Protein Digest, Vanquish UHPLC System

## Goal

Evaluate the long-term stability of peptide mapping on a Vanquish UHPLC system

## Introduction

Peptide mapping is frequently used to study the primary structure of proteins, to characterize post-translational modifications or to confirm genetic stability, especially when interfaced with mass spectrometry. UV detection is still the preferred technique for peptide mapping especially in stability studies and quality control environments due to its inherent robustness and ease of use. The reversed-phase based separations combined with UV are especially challenging since a high number of peptides need to be baseline resolved. Chromatographic runs consist typically of long shallow gradients with run times in the range of 30 to 120 minutes. With such applications, the identification of peptides in the sample of interest is based on a comparison of retention times to a reference sample.

As peak assignment is based on retention times, highly reproducible chromatographic runs over an extended time frame are essential for confident data interpretation. When evaluating these aspects together, it highlights the importance of highly reliable and reproducible peptide mapping over extended time periods. This challenges the column robustness and the UHPLC instrumentation, including the consistency of flow delivery and column thermostating.



The Thermo Scientific™ Vanquish™ UHPLC system is uniquely positioned to meet these analytical requirements due to advancements in system design such as the new parallel pump design which delivers a highly stable flow with extremely low pulsation. Additionally, the Vanquish autosampler compresses the sample before injecting it. This feature contributes to improve the flow consistency of the Vanquish pump resulting in ultra high retention time precision. Since the pre-compression protects the analytical column from any pressure drop during the injection, the column lifetime is extended.

The injection-to-injection reproducibility was evaluated previously.<sup>1</sup> This study investigates the reproducibility of peptide mapping over a period of 4 days. The retention time and peak area precision are reported as reproducibility indicators. In addition, the peak capacity is given to evaluate the separation quality.

## Equipment

Vanquish UHPLC system consisting of:

- System Base (P/N VH-S01-A)
- Binary Pump H (P/N VH-P10-A)
- Mixer Kit, 200  $\mu$ L, VH-P1 (6268.5120)
- Split Sampler HT (P/N VH-A10-A)
- Column Compartment H (P/N VH-C10-A)
- Active pre-heater (6732.0110)
- Diode Array Detector HL (P/N VH-D10-A)
- LightPipe flow cell, standard (10 mm; P/N 6083.0100)

Thermo Scientific™ Dionex™ Chromeleon™  
Chromatography Data System (CDS) software 7.2

## Experimental

### Conditions

Column:	Thermo Scientific™ Acclaim™ RSLC 120, C18, 2.2 $\mu$ m Analytical (2.1 $\times$ 250 mm), P/N 074812
Mobile Phase:	A: 0.05% TFA in water, P/N TFA 85183 B: 0.04% TFA in 8/2 acetonitrile/water (v/v), P/N acetonitrile TS-51101
Gradient:	0–30 min: 4–55% B, 30–31 min: 55–100% B, 31–35 min: 100% B, 35–36 min: 100–4% B, 36–56 min: 4% B
Flow Rate:	0.3 mL/min
Temperature:	50 °C; Still Air Mode
Injection Volume:	2 $\mu$ L
Detection:	214 nm Data Collection Rate: 10 Hz Response Time 0.4 s
Flow Cell:	10 mm LightPipe™

## Results and Discussion

For this study we repeatedly injected a tryptic digest of bovine serum albumin (BSA) or a solvent blank over a time period of more than 80 hours. The separation was achieved using a 250 mm long Acclaim RSLC C18 column. The optional 200  $\mu$ L mixer setup was installed on the Vanquish pump.<sup>2</sup> In Figure 1, ten randomly chosen samples, representing the entire time frame of the experiment, are shown. Visual inspection of these chromatograms clearly demonstrates that the separation is highly reproducible during the entire sample sequence of 86 injections. Figure 2 gives the retention time trend plot for six peptides eluting over the entire gradient. No shift in retention time can be observed for any of the peptides. Table 1 gives the quantitative data on retention time and peak area as relative standard deviation. The relative standard deviation of the retention time was in the range of 0.16% (standard deviation of 0.005 min) for the least retained peptide and 0.06% (standard deviation of 0.016 min) for substance F representing a late eluting peptide.

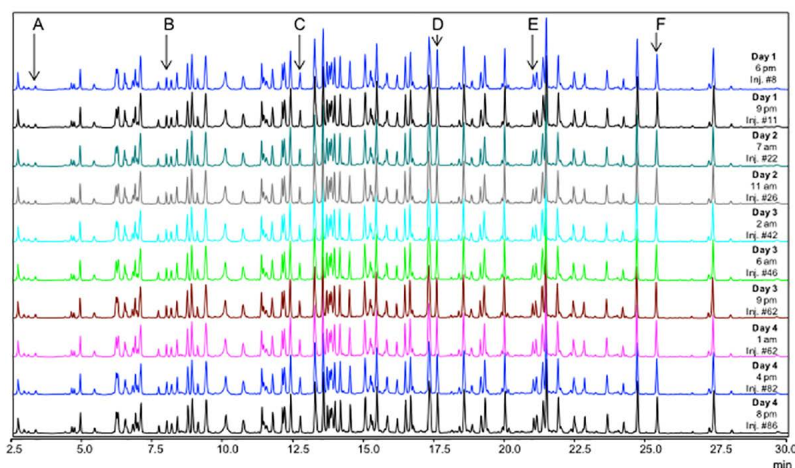


Figure 1. Ten randomly chosen chromatograms for the peptide mapping of a tryptic BSA digest. In total 86 injections were performed covering a time frame of 80 hours without any user interference.

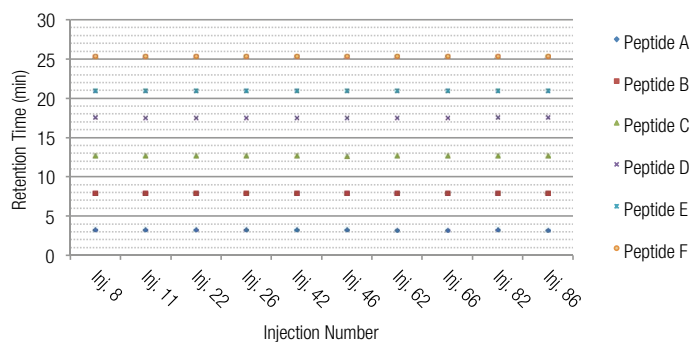


Figure 2. Trend plot of retention times of peptides A-F in 10 randomly chosen chromatograms covering a time period of more than 80 hours.

Table 1. Retention time and peak area precision of six selected peaks eluting over the entire gradient and spanning a wide concentration range. Chromatograms used for the calculation span a time period of more than 80 hours.

Peak ID	Retention Time (min)	Retention Time SD (min)	Retention Time RSD (%)	Average Area (mAU*min)	Peak Area SD (mAU * min)	RSD Area (%)
A	3.289	0.005	0.16	0.147	0.003	2.34
B	7.937	0.008	0.10	0.375	0.004	1.13
C	12.673	0.010	0.08	0.532	0.003	0.54
D	17.535	0.014	0.08	1.490	0.005	0.34
E	20.945	0.015	0.07	0.442	0.004	0.87
F	25.328	0.016	0.06	1.412	0.007	0.48

To evaluate the separation quality, the peak capacity was calculated for all chromatograms recorded during the experiment. The peak capacity was calculated according to:

$$n_c = \frac{t_w}{1.7 \cdot w_{1/2}} + 1$$

with  $t_w$  being the gradient time (30 min) and  $w_{1/2}$  representing the peak width at half height. The calculation was based on the peak width of six well resolved peptides. The peak capacities for the selected chromatograms are shown in Figure 3.

The peak capacity in the beginning of the sample list was at a remarkable value of 392 highlighting the separation power of the Vanquish system in combination with the Acclaim RSLC column. During the course of that 80 hours experiment the peak capacity did not significantly decrease. For injection number 86 the peak capacity was still at 390.

## Conclusion

Stability of retention times and peak areas are crucial for the evaluation of peptide mapping experiments especially in LC-UV type experiments e.g. in process control or stability studies. In this study we demonstrated that the Vanquish UHPLC system provided extraordinary stability of retention time, peak area and peak capacity of a very large sample set of 86 injections over more than 80 hours. This stability will enable the analysts to confidently attribute a change in retention time or peak area to a real peptide modification and avoids any misinterpretation of the data.

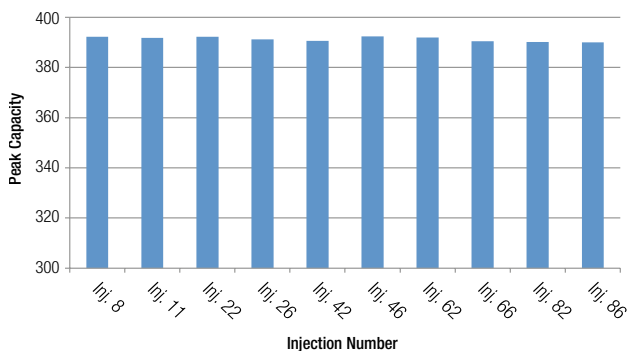


Figure 3. Peak capacity of 10 randomly chosen chromatograms during the peptide mapping of a tryptic BSA digest.

## References

1. Thermo Scientific Application Note 1124: Providing the Highest Retention Time and Peak Area Reproducibility for Maximal Confidence in Peptide Mapping Experiments, 2015.
2. Thermo Scientific Technical Note 108: Reliable Solvent Mixing in UHPLC, 2011..

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