Application Note 1132

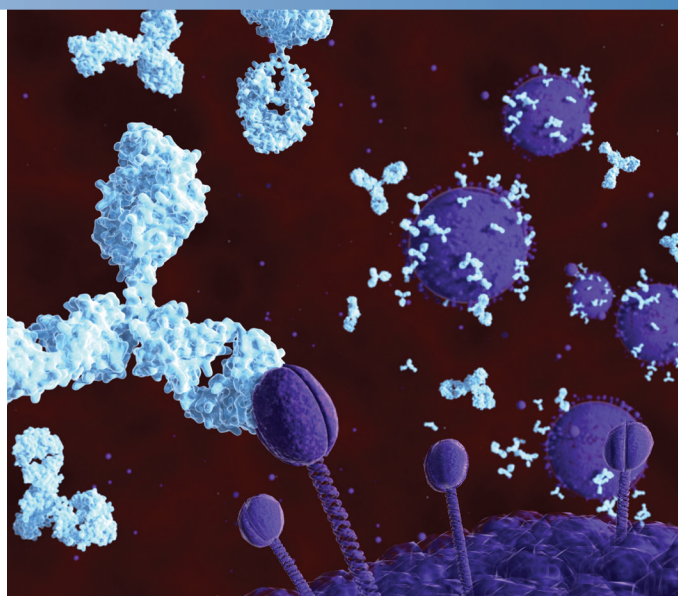
Reliable Results in Peptide Mapping Using the Vanquish Flex UHPLC System

Carsten Paul, Mauro De Pra, and Evert-Jan Sneekes
Thermo Fisher Scientific, Germering, Germany

Key Words
Monoclonal Antibodies, Acclaim C18 RSLC Column, Biocompatible UHPLC, Protein Digest, Biotherapeutics Characterization, Biopharma

Goal
Prove the suitability of the Thermo Scientific™ Vanquish™ Flex UHPLC system for efficient and reliable peptide mapping experiments.

Introduction
Peptide mapping is one of the routine methods for biotherapeutics characterization. This technique, combined with mass spectrometry, is utilized in research environments for the determination of the primary sequence of an antibody or the identification of post-translational modifications. UV is often used as a unique detection tool, for instance in stability studies. The data interpretation of these experiments is based on retention times as qualitative and peak area as quantitative information. The high complexity of the sample emphasizes the need for a highly efficient separation that can be achieved with the Thermo Scientific™ Acclaim™ column technolology.[1] In addition, for a reliable comparison of different samples, for instance of a reference standard to a new batch of antibody, high retention time reproducibility is mandatory for reliable peptide identification and correct data interpretation.



The new Vanquish Flex UHPLC system features a quaternary pump[2] for highest application flexibility. In addition, identically to the Vanquish UHPLC system[3], the sample is pressurized prior to the injection into the high-pressure flow path. This results in a highly stable flow delivery and thus significantly improves the retention time precision. Thereby the confidence in peak assignment is increased.

In this work, the separation of peptides obtained from a bovine serum albumin (BSA) digest is demonstrated. The retention time and peak area precisions are evaluated for repeated injections.

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Experimental

Equipment

Vanquish Flex UHPLC system consisting of:

- System Base (P/N VF-S01-A)
- Quaternary Pump Flex (P/N VF-P20-A)
- Split Sampler FT (P/N VF-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)

Experimental Conditions

Column:	Acclaim RSLC 120, C18, 2.2 µm Analytical (2.1 x 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
Injection Volume:	2 µL
Detection:	214 nm Data collection rate: 10 Hz Response time: 0.4 s
Flow Cell:	10 mm LightPipe

Data Processing

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

Results and Discussion

The separation of a tryptic digest of BSA was obtained with a 30 minute gradient and a total analysis time of 56 minutes, including column wash with high organic eluent and re-equilibration at initial conditions. Figure 1 shows excellent precision for the overlay of five consecutive BSA digest injections.

The reproducibility was assessed using the retention time standard deviation (SD) and relative standard deviation (RSD) of all peptides automatically detected by Chromeleon CDS. The relative standard deviation was below 0.05% for 80% of all peptides (Figure 2). Even retention RSDs below 0.02% were achieved in 27% of all peptides. Only a minor portion of the peptides had retention time RSDs above 0.1%. These peptides were early eluting substances (< 6 min) which have naturally higher RSD values due to the mathematical procedure of the RSD calculation. All retention time SDs were in the range of maximal 0.008 min and minimal 0.0008 min (Figure 3). This data shows excellent flow delivery precision across the entire gradient.

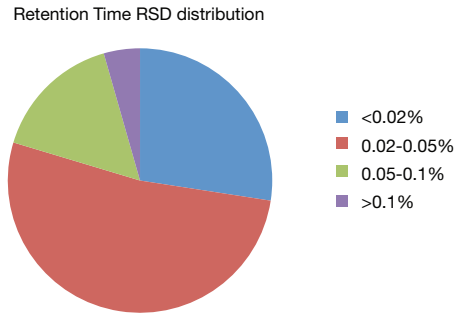

Figure 2. Distribution of retention time RSDs of a Vanquish Flex system for the analysis of a BSA digest.

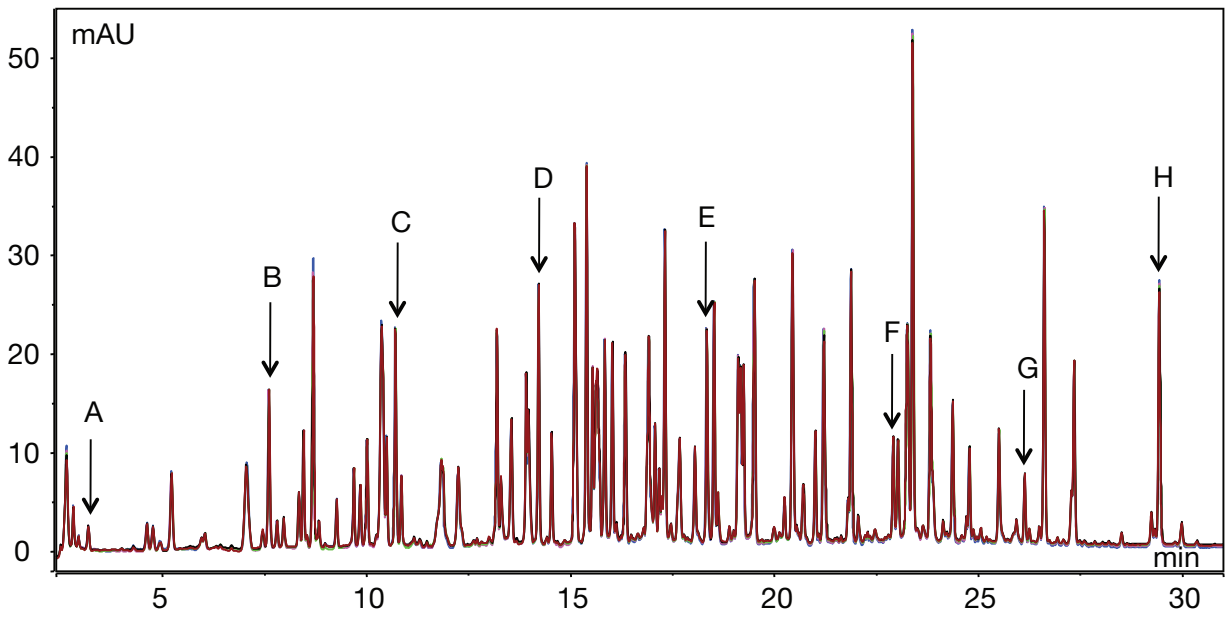

Figure 1. Overlaid chromatograms of five subsequent injections of BSA tryptic digest.

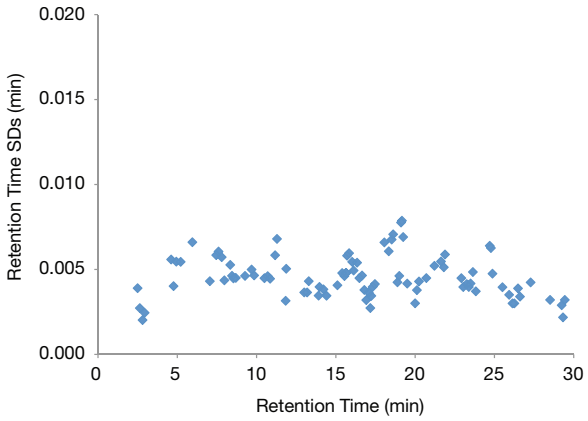

Figure 3. Retention time standard deviation measured for five repeated injections of a BSA tryptic digest using a Vanquish Flex system.

The peak area precision was calculated for nine peptides homogeneously distributed throughout the retention window (Table 1). The peak area RSD of eight of the evaluated peptides was below 0.8% with a minimal value of 0.2%. The higher area RSD for peptide H is only observed for this peptide and therefore this variation is attributed to the sample and not the system.

Table 1. Retention time and peak area RSD of nine selected peaks eluting over the entire gradient.

Peak ID	Retention Time (min)	RSD Area (%)
A	3.171	0.61
B	7.601	0.31
C	10.702	0.22
D	14.217	0.24
E	18.345	0.77
F	22.912	0.79
G	26.137	0.20
H	29.438	2.10

Conclusion

The reproducibility of retention times for qualitative and peak areas for quantitative information is critical for the correct data evaluation in UV detection-based peptide mapping. The Vanquish Flex system delivers outstanding retention time and peak area precision; that precision will help chromatographers to compare their results acurately and interpret their data reliably.

References

1. Dionex (now part of Thermo Fisher Scientific) Application Update 183: Separation of Peptides from Enzymatic Digestion on Different Acclaim Columns: A Comparative Study. Sunnyvale, CA, 2011.
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3. Thermo Scientific Application Note 1123: Increased Long-term Stability of Peptide Mapping using the Vanquish UHPLC System. Germering, Germany, 2015.
4. Thermo Scientific Application Note 1124: Providing the Highest Retention Time and Peak Area Reproducibility for Maximal Confidence in Peptide Mapping Experiments. Germering, Germany, 2015.

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