High Resolution, High-Precision Peptide Mapping of a Monoclonal Antibody Digest

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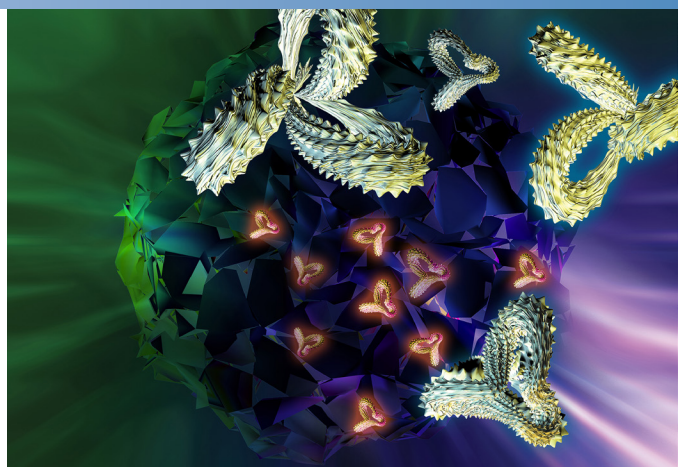
Key Words
Biotherapeutics, Column Thermostatting, Peak Capacity, Protein Characterization

Goal

Described a high resolution, high retention time precision method for reversed phase UHPLC-UV separation of a monoclonal antibody digest



Introduction

Reversed phase liquid chromatography (RPLC) of peptides is routinely used in the biopharmaceutical industry to provide information on the nature and quality of protein therapeutics. RPLC in combination with UV detection is common in stability studies, process control, quality control, and other cases where all the important attributes of the peptide sequence are extrapolated directly from the chromatogram. In this case the assignment of a peptide to a peak is based on retention time comparison between the investigated sample and the reference one, provided that the identity of the peaks in the reference chromatogram has been elucidated by mass spectrometry beforehand. UHPLC is the preferred approach, as highly efficient columns will provide sufficient resolution to resolve closely eluting peptides. Since peak assignment is solely based on retention time, high run-to-run retention time precision is required in order to avoid incorrect peptide identification.

Here we demonstrate that the Thermo Scientific™ Dionex™ UltiMate™ 3000 BioRS system is capable of meeting the requirements of biopharmaceutical analysis for the UHPLC-UV analysis of mAb digests.

Experimental

Equipment

- UltiMate 3000 Biocompatible Rapid Separation (BioRS) UHPLC system equipped with:
  - DGP-3600RS pump (P/N 5040.0066)
  - VWD-3400RS detector (P/N 5074.0010)
  - Semi-micro Flow Cell for VWD-3000 Series, PEEK, 2.5 µL volume, 7 mm pathlength (P/N 6074.0300)
- WPS-3000 TBRS well plate autosampler (P/N 5841.0020)
- TCC-3000RS thermostatted column compartment (P/N 5730.0000)
- Pre-column heater, 2 µL bio-compatible (P/N 6723.0232)
- Thermo Scientific™ Dionex™ Chromeleon™ 7.2 Chromatography Data System (P/N CHROMELEON7)

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Chromatographic Conditions		
Column:	Thermo Scientific™ Acclaim™ RSLC 120, C18, 2.2 µm Analytical (2.1 × 250 mm) (P/N 074812)	
Mobile Phase A:	0.05% TFA in water	
Mobile Phase B:	0.04% TFA in 8/2 acetonitrile/water (v/v)	
Gradient:	Time (minutes)	%B
	0	4
	30	55
	31	90
	35	90
	36	4
	45	4
Flow Rate:	0.4 mL/min	
Column Compartment Temperature:	80 °C	
Injection Volume:	0.2–1 µL UV 214 nm. Data collection rate: 10 Hz. Time constant: 0.12 sec	

Results and Discussion

A high resolution UHPLC separation of a mAb tryptic digest is depicted Figure 1. Average peak width at half height was 2.7 seconds, resulting in peak capacity on the order of 400. The peak distribution was highly homogeneous across the whole chromatogram. The gradient time was 30 minutes, and the total run time was 45 minutes, including column wash with 100% strong eluent and re-equilibration. The peak capacity achieved with this method was remarkable, considered the relatively short analysis time. The method considering required column thermostatting at 80 °C. Due to the high temperature in the column, pre-heating of the mobile phase was essential to avoid thermal mismatch. Temperature differences between column and mobile phase can cause additional band dispersion, hence loss of resolving power. In this work, to overcome this problem the mobile phase was heated using a bio-compatible pre-column heater in the UltiMate 3000 BioRS system. The design of the heater allowed proper mobile phase thermostatting without adding any substantial extra-column dispersion.

Retention time precision was evaluated for 10 randomly selected peaks from 5 consecutive injections. The relative standard deviation was between 0.076% and 0.022% (Table 1).

Table 1. Retention time relative standard deviation (RSD) calculated for a selection of peaks. Sample mAb tryptic digest 10 mg/mL. Injection volume 0.2 µL.

Retention Time (min)	RSD% (n = 5)
7.369	0.076
9.473	0.074
13.838	0.036
15.895	0.056
18.370	0.042
20.000	0.057
22.964	0.028
25.150	0.034
28.459	0.049
30.112	0.022

Conclusion

The method here described, provides sufficient peak capacity to address the challenges of mAb digest analysis by RPLC-UV. The excellent precision of the method is compatible with workflows that base peak assignment on retention time.

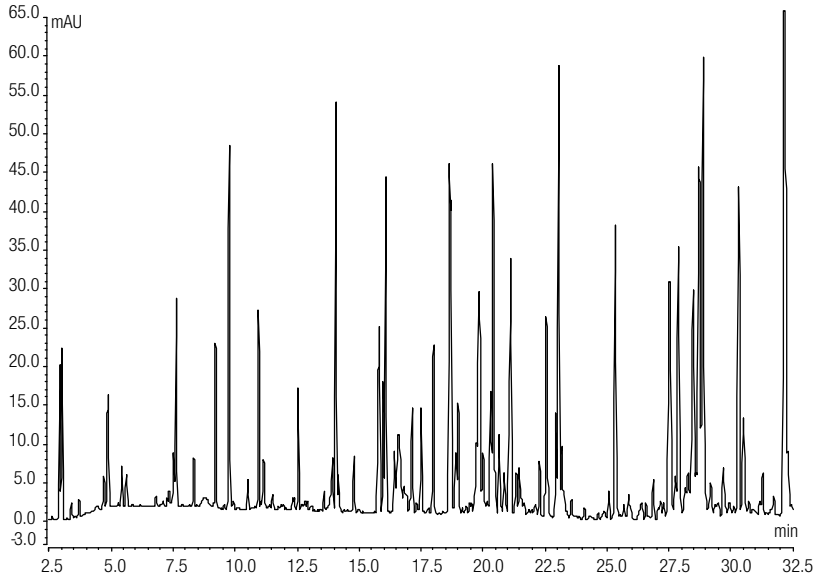

Figure 1. Separation of mAb 10 mg/mL tryptic digest. Injection volume 1 µL.

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