Reversed-Phase Separation of Intact Therapeutic Antibodies Using the Vanquish Flex UHPLC System

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Key Words

Biotherapeutics, Biosimilars, Intact Proteins, Stability, mAbs

Goal

Provide examples of reversed-phase chromatography of monoclonal antibodies with the Thermo Scientific™ Vanquish™ Flex UHPLC System and Thermo Scientific™ MAbPac™ RP column.

Introduction

In biopharmaceutical early analytical development, characterization of monoclonal antibodies is required to support process development. Separation techniques such as hydrophobic interaction chromatography, size-exclusion chromatography, ion-exchange chromatography, and reversed-phase chromatography are routinely applied to profile the therapeutic protein during this stage of development. Reversed-phase chromatography can be run with mass spectrometry (MS)-compatible mobile phase, hence the method can be easily transferred to MS characterization laboratories when required.

Besides providing separation of impurities based on hydrophobicity, reversed-phase chromatography is an excellent tool for protein quantitation of main compound and minor variants. Reversed-phase separation of intact proteins is typically run at high temperatures to improve peak shape and recovery of proteins. Thus, high-resolution columns, packed with temperature-stable material are required. In addition, the method should be sufficiently fast, in order to allow the processing of a large number of samples in a reasonable time. An initial stability evaluation of the new biological entities has to be provided by early development laboratories. The analytical methods for the early stability assessment need to be able to indicate, and approximately quantify, sample degradation.
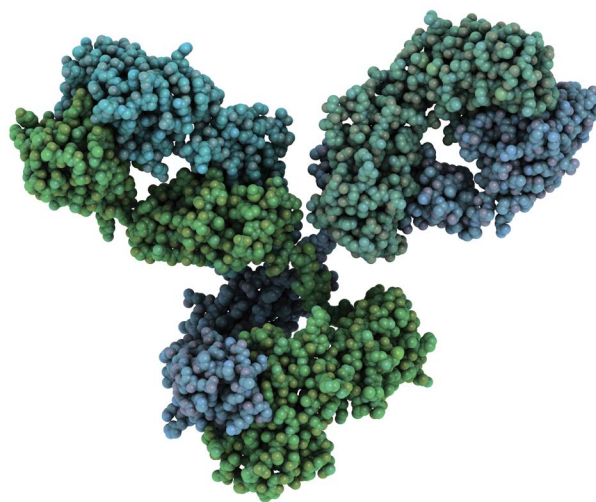
The MAbPac RP column is dedicated to separations of intact proteins. It is based on supermacroporous 4 µm polymer particles with exceptional thermal stability. The Vanquish Flex UHPLC system offers column thermostatting up to 120 °C and features a low-dispersion active pre-column eluent heater. This device actively regulates the thermal balance between the mobile phase and the stationary phase. The accurate temperature control allows avoiding loss of efficiency due to temperature mismatch between the column and the incoming solvent.

In this work, the MAbPac-RP column was operated with the Vanquish Flex UHPLC system for the reversed-phase chromatography of several intact therapeutic antibodies. To assess the suitability for stability studies, the chromatograms of a reference and a stressed mAb were compared.

Experimental

Instrumentation

Vanquish Flex UHPLC system, equipped with:

- System Base (P/N VH-S01-A)

- Quarternary Pump F (P/N VF-P20-A)

- Split Sampler FT (P/N VF-A10-A)

- Column Compartment H (P/N VH-C10-A) with Active Pre-heater VH-C1 (P/N 6732.0110) and Post-column Cooler 1 µL VH-C1 (P/N 6732.0510)

- Diode Array Detector HL (P/N VH-D10-A) equipped with LightPipe™ Standard Flowcell (P/N 6083.0100)

Chromatographic Conditions

Column:	MAbPac RP (2.1 x 50 mm) (P/N 088648)
Mobile phase A:	0.1:100 TFA/water (v/v)
Mobile phase B:	0.1:90:10 TFA/acetonitrile/water (v/v/v)
Flow rate:	300 µL/min
Column compartment temperature settings:	Column compartment: 80 °C Forced air mode Active pre-heater: 80 °C Post column cooler: 50 °C
Detector settings:	Detection wavelength: 280 nm Data acquisition rate: 10 Hz Response time: 0.4 s

Data Processing

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

Results and Discussion

Four commercial antibodies were eluted with a 10 minute linear gradient. The linear increase of acetonitrile in the mobile phase was (9%)/min, in the case of trastuzumab (Figure 1), and (3.6%)/min for cetuximab (Figure 4). In all cases, the elution of the intact antibodies resulted in very sharp peaks. The peak width at half height spanned from 1.6 seconds to 3.1 seconds for the steepest and the shallowest gradient, respectively. Peak symmetry was excellent for all mAbs, as it can be observed in Figures 1–4.
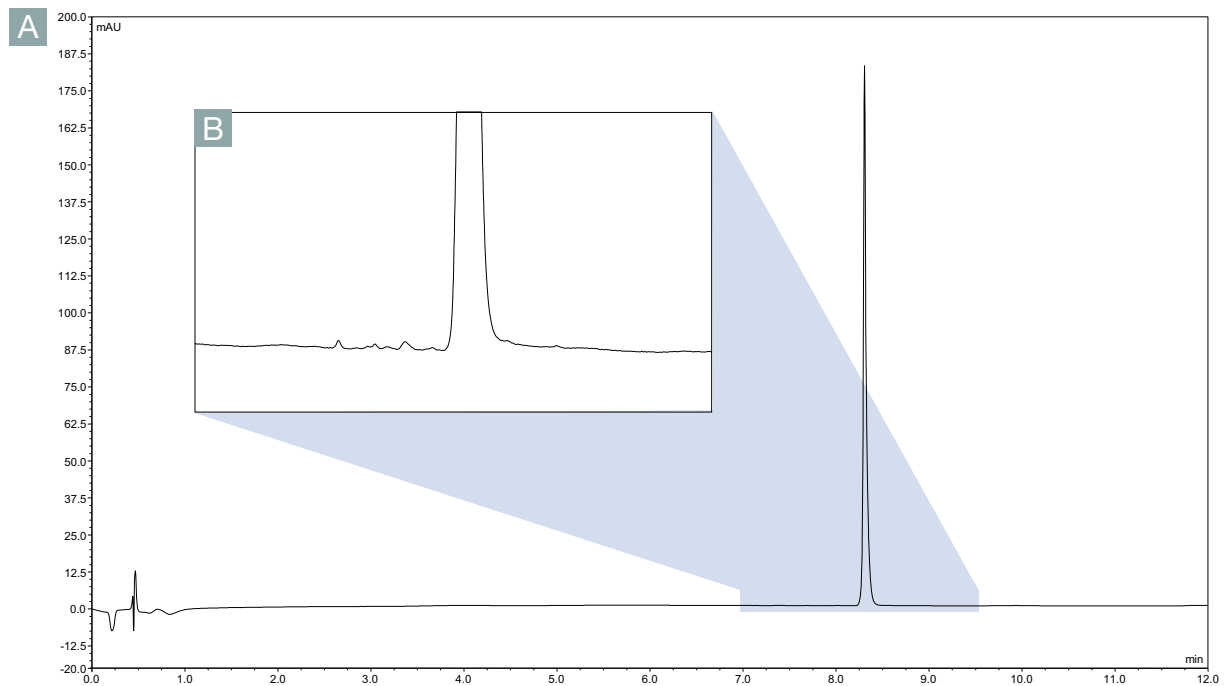

Figure 1. Injection of 4.2 µg of trastuzumab. Gradient 0–100% B in 10 minutes. Full view (a) and enlarged view (b).
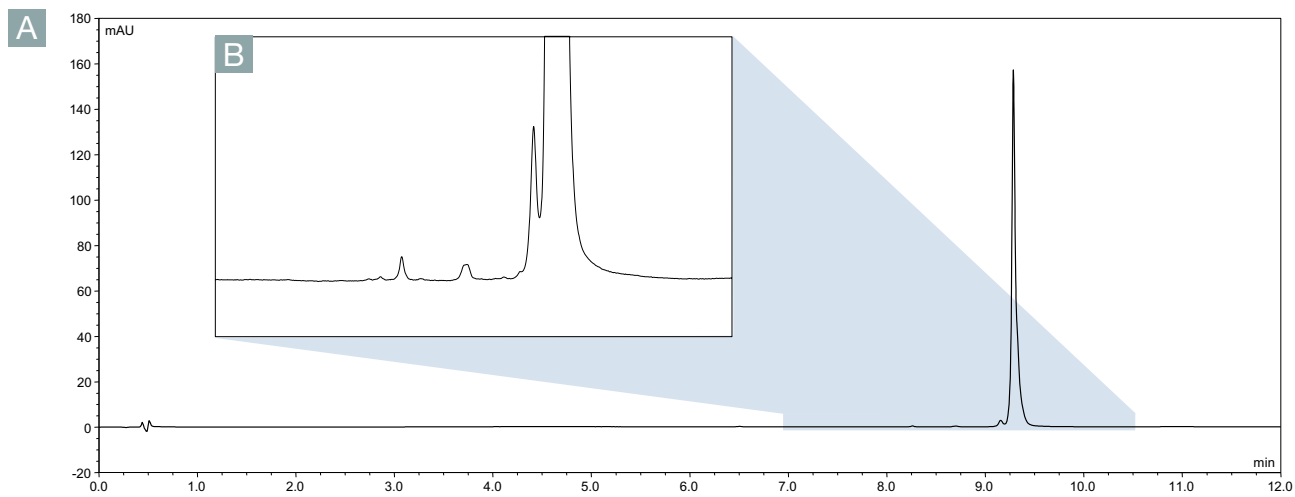
Figure 2. Injection of 25 µg of bevacizumab. Gradient 10−60% B in 10 minutes. Full view (a) and enlarged view (b).
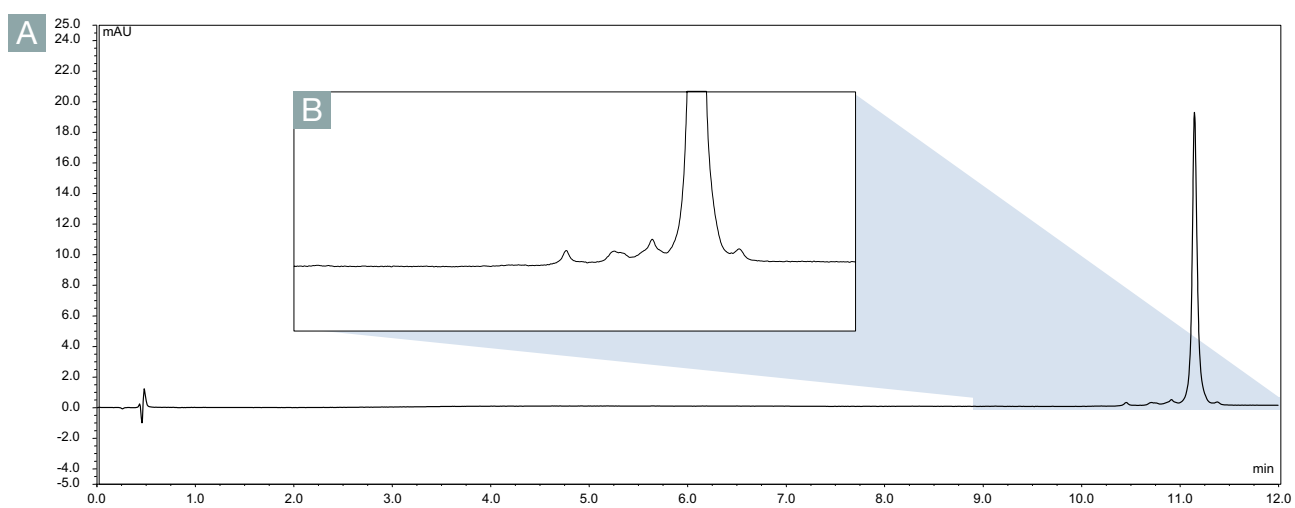

Figure 3. Injection of 1.25 µg of cetuximab. Gradient 20−45% B in 10 minutes. Full view (a) and enlarged view (b).
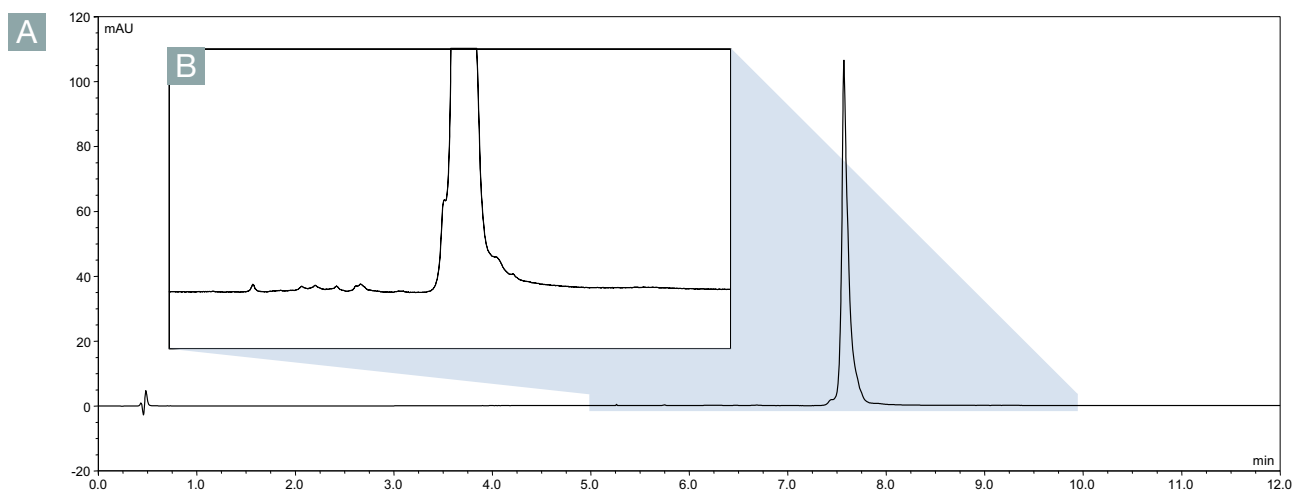

Figure 4. Injection of 1 µg of rituximab. Gradient 22−60% B in 10 minutes. Full view (a) and enlarged view (b).

Detailed views of the intact antibodies chromatograms revealed that the MAbPac RP column provides selectivity to separate minor variants, even with relatively simple and fast gradient programs. This feature can be exploited in cases such as preliminary stability studies. In Figure 5, the chromatogram of a reference antibody is compared to a stressed one. The sample was donated by a customer and the stressing conditions were not disclosed. Here, the effects of stress-related degradation of an antibody are observed by running a simple 10 minute gradient from 0 to 100% B. The increased relative area of the impurities eluting before the main peak of the stressed mAb, confirmed the degradation of the sample. Additionally the degradation/denaturation of the sample can be estimated by the increased width of the main peak. The width of the main peak at half height was 2.0 seconds for the reference, and 3.3 seconds for the stressed sample. This effect is likely caused by close-eluting species present in the stressed antibody but not in the reference one.

Conclusion

Reversed-phase chromatography is a powerful and convenient tool for the characterization of intact antibodies. With the extended thermostatting temperature range of the Vanquish Flex system and the new MAbPac RP column, very fast and efficient separations are achieved. Chromatograms with sharp symmetrical peaks are obtained that can be used to assess antibody purity in a very straightforward way.
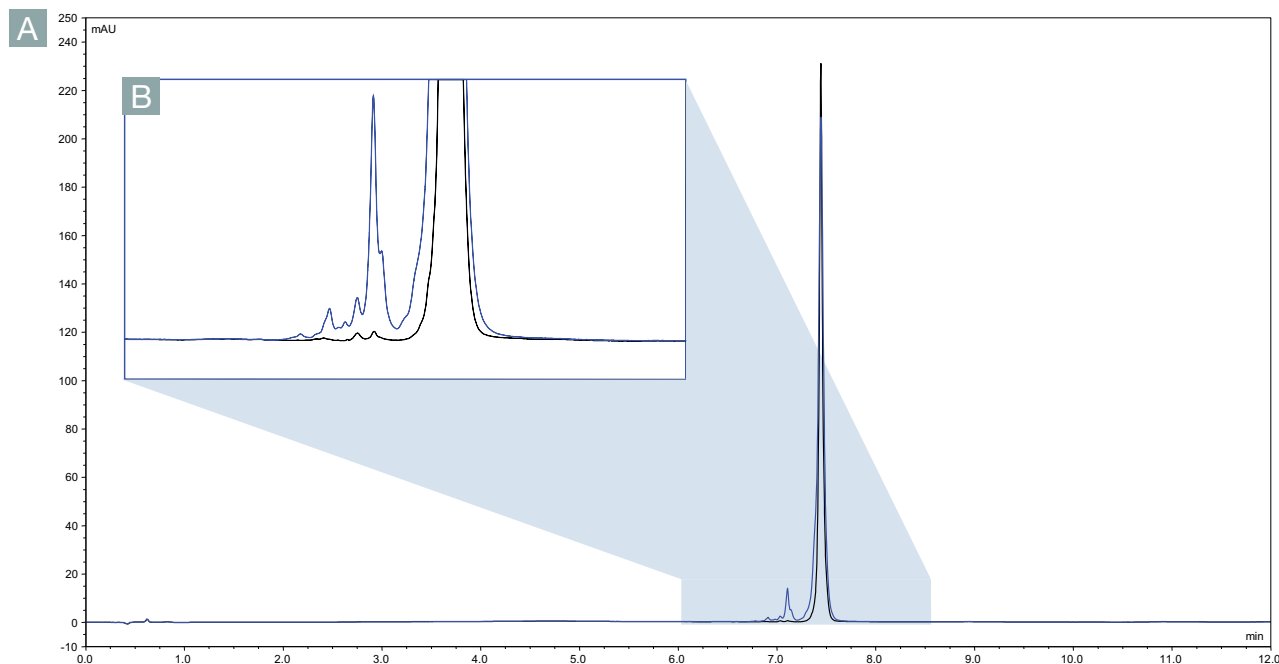

Figure 5. Comparison between a reference (black trace) and a stressed antibody (blue trace). Gradient: 0–100% B in 10 minutes. Full view (a) and enlarged view (b).

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