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APPLICATION NOTE 72598

High-throughput protein aggregate analysis of monoclonal antibodies using a novel dual-channel UHPLC instrument

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Keywords

NIBRT, biopharmaceutical, biotherapeutic, quality control, monoclonal antibody, mAb, IgG, bevacizumab, Vanquish Flex Duo UHPLC system, Vanquish Flex Quaternary, size exclusion chromatography, MAbPac SEC-1

Application benefits

- Dual LC workflow demonstrates the ability to employ two chromatography channels (columns, pumps, and detectors) simultaneously with no loss in data quality.
- Higher number of sample injections can be performed during analysis time resulting in efficient sample analysis and overall lower costs.
- Separate software modules for each LC system result in easy data processing.

Goal

To demonstrate the high-throughput capabilities of the newly developed Thermo Scientific™ Vanquish™ Flex Duo UHPLC system. This study focused on data reproducibility and the quality of chromatography, while comparing technology performance to the standard Thermo Scientific™ Vanquish™ Flex Quaternary UHPLC system.

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Introduction

In the current scientific environment, there is an increased need for the rapid and robust high-throughput analysis of biotherapeutics, in particular monoclonal antibodies (mAbs). This is due to the rise in the use of mAbs for the treatment of diseases.[1] As a result, standardized chromatographic methods and excellent reproducibility are essential for sample analysis in quality control (QC) laboratories. Although standard UHPLC systems can analyze samples simply and rapidly, there is typically only the option to use one stationary phase at any given time. As a result, analysts are limited regarding the number of injections that a chromatography system can perform, leading to less efficient sample analysis and additional costs. In the biomanufacturing pipeline, biopharmaceutical handling and storage can cause a number of unintentional size variants of the original product that are potentially harmful for human health. Size-exclusion chromatography (SEC) is considered a gold standard for monitoring the formation and level of mAb aggregates and fragments[2] and is probably one of the most frequently performed analyses in QC laboratories.

In this study, the Vanquish Flex Duo UHPLC system was used for the high-throughput analysis of a mAb. One hundred injections of bevacizumab were performed on two identical Thermo Scientific™ MAbPac™ SEC-1 size exclusion columns. SEC is commonly used for the high-resolution separation of mAb aggregates and fragments.

A number of chromatographic parameters were determined—including analyte retention time, peak width, area, symmetry, and efficiency—highlighting the similar results between each of the channels of the Vanquish Flex Duo UHPLC system and the low % RSD values obtained. These results were also obtained on a standard Vanquish Flex Quaternary UHPLC system for direct comparison.

Experimental

Recommended consumables

- Deionized water, 18.2 MΩ·cm resistivity
- Thermo Scientific MAbPac SEC-1 column, 5 µm, 300 Å, 4.0 x 300 mm (P/N 074693)
- Fisher Scientific™ sodium phosphate dibasic anhydrous (P/N 10440481)
- Fisher Scientific™ sodium phosphate monobasic anhydrous (P/N 10751135)
- Fisher Scientific™ sodium chloride (P/N 119641051)

Sample preparation

Bevacizumab (25 mg/mL) was diluted 1:10 in 100 mM sodium phosphate, pH 6.8, 300 mM NaCl. Diluted samples were aliquoted and stored at -20 °C.

Separation conditions

Instrumentation

Thermo Scientific Vanquish Flex Duo UHPLC system (Figure 1), consisting of the following:

- Dual Pump F (P/N VF-P32-A-01)
- Dual Split Sampler FT (P/N VF-A40-A-02)
- (2) Column Compartment H (P/N VH-C10-A-02)
- (2) Diode Array Detector HL (P/N VH-D10-A)
- (2) Thermo Scientific™ LightPipe™ 10 mm Standard Flow Cell (P/N 6083.0100)
- System Base Vanquish Dual (P/N VF-S02-A-02)

Thermo Scientific Vanquish Flex Quaternary UHPLC system, consisting of the following:

- Quaternary Pump F (P/N VF-P20-A)
- Split Sampler FT (P/N VF-A10-A-02)
- Column Compartment H (P/N VH-C10-A-02)
- Diode Array Detector HL (P/N VH-D10-A)
- LightPipe 10 mm Standard Flow Cell (P/N 6083.0100)
- System Base Vanquish Duo UHPLC system (P/N VF-S01-A-02)

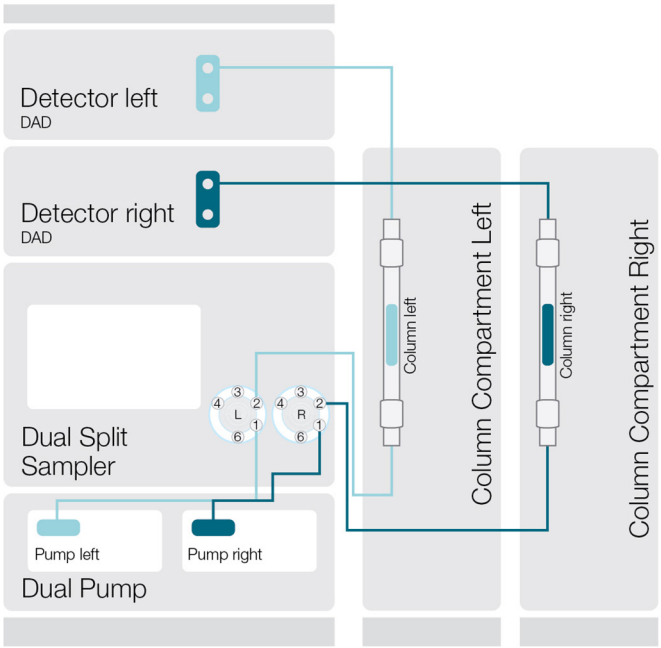

Figure 1. Vanquish Flex Duo UHPLC system

LC conditions	
Mobile phase:	100 mM sodium phosphate, pH 6.8 in 300 mM NaCl, isocratic
Flow rate:	0.25 mL/min
Run time:	16 min
Column temperature:	30 °C (Still air mode)
Autosampler temperature:	5 °C
UV wavelength:	Data collected at 214 nm and 280 nm
Injection volume:	2 µL of 25 mg/mL bevacizumab
Injection wash solvent:	MeOH/H_2O (20:80 v/v)
Needle wash:	Enabled pre-injection

Data processing and software

Thermo Scientific™ Chromeleon™ Chromatography Data System, version 7.2.8, was used for data acquisition and analysis.

Results and discussion

To evaluate the high-throughput capabilities of the Vanquish Flex Duo UHPLC system, 100 injections of bevacizumab (Avastin®) were performed simultaneously on both channels and two size exclusion columns.

For a direct comparison with the Dual LC workflow, 100 injections of bevacizumab were performed on a Vanquish Flex Quaternary UHPLC system. A third MAbPac SEC-1 analytical column was used for this analysis.

Peak information following 100 injections of bevacizumab on the Vanquish Flex Duo UHPLC system (left and right channel) and Vanquish Flex Quaternary system were evaluated using the Chromeleon Chromatography Data System (Table 1 and Figures 2, 3).

Table 1. SEC-UV analysis of bevacizumab. Comparison of performances of left and right channels of Vanquish Flex Duo UHPLC system and Vanquish Flex Quaternary system.

LC System	Ret. Time (min)	Ret. Time %RSD	Relative Peak Area (%)	Relative Peak Area %RSD	Peak Width at 50% Height	Peak Width at 50% %RSD	Asymm. (EP)	Theor. Plates (EP)
Vanquish Flex Duo system Left Channel	9.587	0.012	96.91	0.11	0.232	0.20	0.99	9484
Vanquish Flex Duo system Right Channel	9.596	0.021	96.89	0.14	0.234	0.22	1.02	9291
Vanquish Flex Quaternary system	9.439	0.011	97.88	0.53	0.238	0.51	1.00	8751

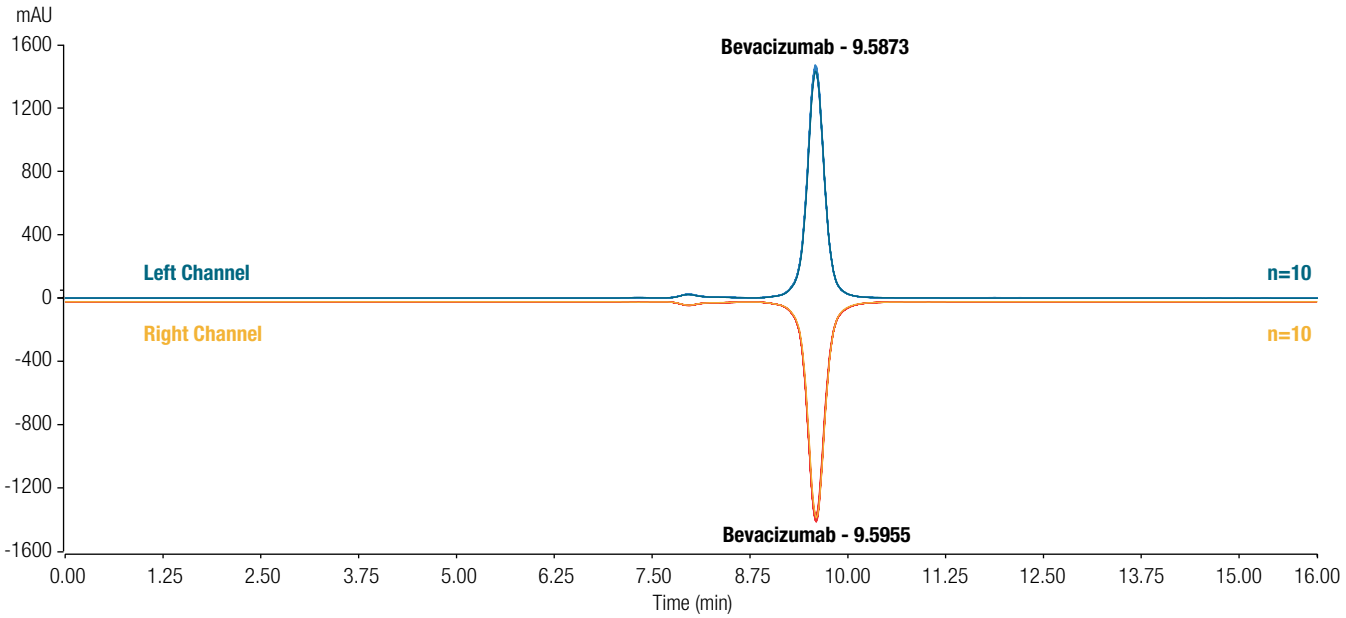

Figure 2. SEC-UV chromatograms mirror plot. Comparison of Vanquish Flex Duo UHPLC system left and right channels.

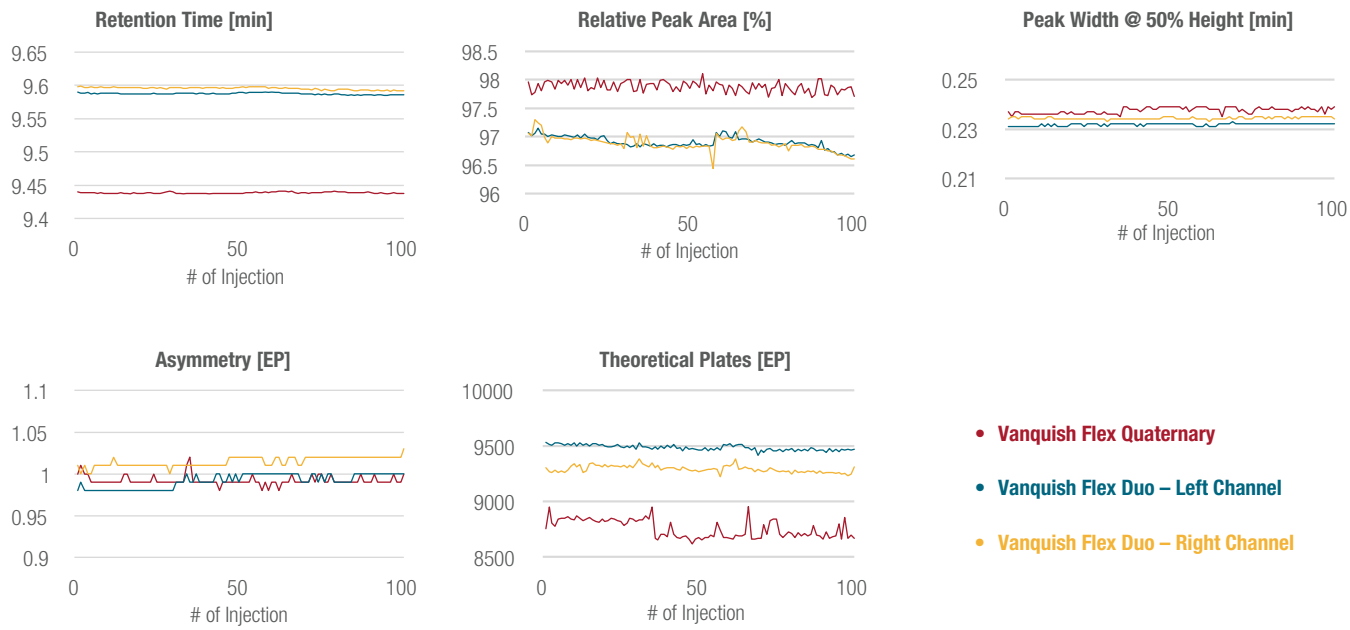

Figure 3. Chromatographic results of SEC-UV analysis of bevacizumab. Data for 100 repetitive injections are shown for the left and right channel for Vanquish Flex Duo UHPLC system and the Vanquish Flex Quaternary system.

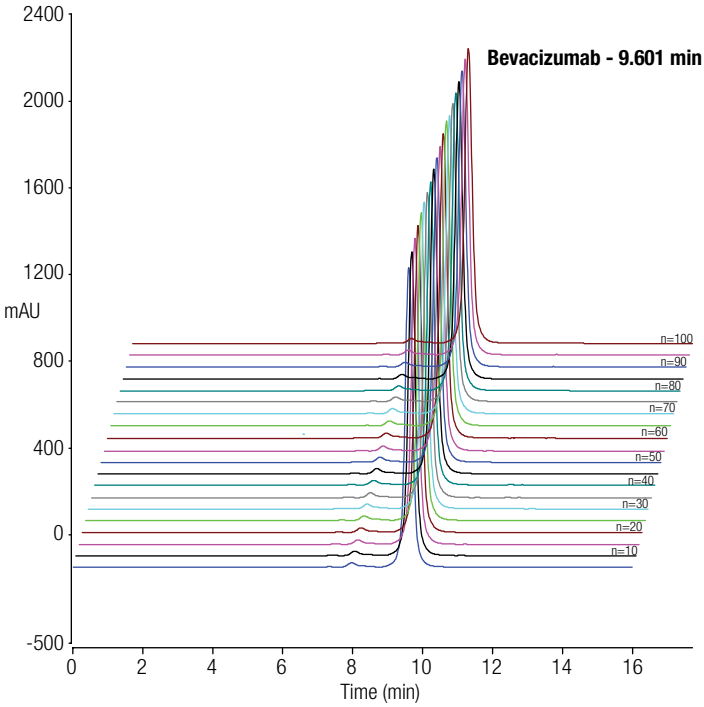

Figure 4. SEC-UV chromatograms of bevacizumab. Direct comparison of traces obtained every 5 injections for 100 repetitive injections on one channel of the Vanquish Flex Duo UHPLC system.

The Vanquish Flex Duo UHPLC system has shown to be an easy-to-use chromatography system for the analysis of biotherapeutics. This Dual LC workflow provides the possibility of high-throughput analysis of biotherapeutic samples with no compromise on the quality of analysis, which is highly advantageous in a QC environment.

Conclusions

- The Vanquish Flex Duo UHPLC system provides simple and rapid high-throughput analysis of biotherapeutics.
- Analysis of mAbs gives excellent quality data on the Vanquish Flex Quaternary system and the Vanquish Flex Duo system for Dual LC, with high confidence in results. Excellent reproducibility with RSD values below 1% for a number of analytical parameters was obtained for both LC systems.
- Data was comparable to that obtained on a standard Vanquish Flex Quaternary system.

References

1. Ecker, D.M.; Jones, S.D.; Levine, H.L. The therapeutic monoclonal antibody market, *mAbs,* **2015,** *7*(1), 9–14.
2. Tassi, M.; De Vos. J.; Chatterjee, S.; Sobott, F. Bones, J.; Eeltink, S. Advances in native high-performance liquid chromatography and intact mass spectrometry for the characterization of biopharmaceutical products, *J. Sep. Sci.,* **2017,** DOI: 10.1002/jssc.201700988.

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