



Simultaneous analysis of monoclonal antibodies using a novel dual-channel UHPLC instrument and orthogonal chromatography

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Keywords

NIBRT, biopharmaceutical,
biotherapeutic, quality control,
monoclonal antibody, mAb, IgG,
bevacizumab, infliximab, Vanquish
Flex Duo UHPLC system for Dual
LC, size exclusion chromatography,
strong cation exchange
chromatography, reversed phase
chromatography

Application benefits

- The Dual LC workflow demonstrates the ability to employ two chromatography channels with different separation mechanisms at one time.
- 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 results in easy data processing.

Goal

To demonstrate the flexibility of the newly developed Thermo Scientific™ Vanquish™ Flex Duo UHPLC system for Dual LC. This study focused on the ability of the Dual LC workflow to employ two different chromatographic mechanisms at one time, without compromising data reproducibility and the quality of chromatography.

Introduction

Although standard UHPLC systems can analyze samples simply and rapidly, typically only one stationary phase can be used at any given time, although there are multiple features to monitor and each requires its own method.¹ 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. The Vanquish Flex Duo system for Dual LC can address these problems using two simultaneous LC channels for the same sample.

In this study, we focused on the flexibility of the Vanquish Dual LC workflow. Orthogonal analyses were performed on the same sample at the same time. The two analyses use different elution solvents and different instrument methods, varying in data collection wavelengths, gradient, or isocratic conditions and different run times.

A number of chromatographic parameters were determined—including analyte retention time, peak width, area, symmetry, and efficiency—highlighting the similar results between each LC channel of the Vanquish Flex Duo system for Dual LC and the low % RSD values obtained.

Experimental

Recommended consumables

- Deionized water, 18.2 M Ω ·cm resistivity
- Thermo Scientific™ MAbPac™ SEC-1 column, 5 μ m, 300 Å, 4.0 × 300 mm (P/N 074693)
- Thermo Scientific™ MAbPac™ RP column, 4 μ m, 2.1 × 100 mm (P/N 088647)
- Thermo Scientific™ MAbPac™ SCX-10 RS column, 5 μ m, 2.1 × 50 mm (P/N 082675)
- Fisher Scientific™ sodium phosphate dibasic anhydrous (P/N 10440481)
- Fisher Scientific™ sodium phosphate monobasic anhydrous (P/N 10751135)
- Fisher Scientific™ sodium chloride (P/N 11964051)

Sample preparation

Bevacizumab (25 mg/mL) was diluted 1:10 in water. Infliximab was prepared at a concentration of 10 mg/mL in water. Diluted mAb samples were aliquoted and stored at -20 °C.

Separation conditions

Instrumentation

Vanquish Flex Duo system for Dual LC (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 Duo for Dual LC (P/N VF-S02-A-02)

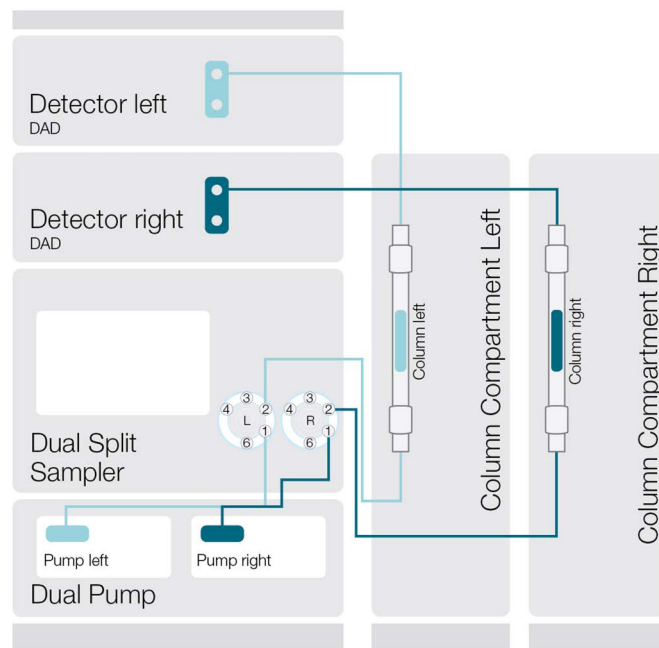


Figure 1. Vanquish Flex Duo system for Dual LC.

LC conditions

Size exclusion chromatography

Mobile phase
(Isocratic): 100 mM sodium phosphate, pH 6.8 in 300 mM NaCl, filtered through 0.2 µm filter membrane before use

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/H₂O (20:80 v/v)

Needle wash: Enabled pre-injection

Strong cation exchange chromatography

Eluent A: 10-fold dilution of CX-1 buffer A (pH 5.6) with DI water

Eluent B: 10-fold dilution of CX-1 buffer B (pH 10.2) with DI water (stored in amber borosilicate glass bottle to protect from light). Mobile phases filtered through 0.2 µm filter membrane before use.

Column temperature: 30 °C (Still air mode)

Autosampler temperature: 5 °C

UV wavelength: Data collected at 280 nm

Injection volume: 1 µL of 10 mg/mL infliximab
2 µL of 25 mg/mL bevacizumab

Injection wash solvent: MeOH/H₂O (20:80 v/v)

Needle wash: Enabled pre-injection

Charge variant gradient conditions: Tables 1 and 2

Table 1. Charge variant gradient conditions for infliximab.

Time (min)	Flow rate (mL/min)	% A	% B
0.0	0.2	85	15
25.0	0.2	60	40
25.1	0.2	85	15
30.0	0.2	85	15

Table 2. Charge variant gradient conditions for bevacizumab.

Time (min)	Flow rate (mL/min)	% A	% B
0.0	0.3	85	15
25.0	0.3	60	40
25.1	0.3	85	15
30.0	0.3	85	15

Reversed phase chromatography

Eluent A: H₂O/TFA (99.9:0.1 v/v)

Eluent B: MeCN/H₂O/TFA (90:9.9:0.1 v/v)
Mobile phases filtered through 0.2 µm filter membrane before use.

Flow rate: 0.6 mL/min

Pre-column heater temperature: 80 °C

Column temperature: 80 °C (Still air mode)

Post-column cooling temperature: 40 °C

Autosampler temperature: 5 °C

UV wavelength: Data collected at 214 nm and 280 nm

Injection volume: 0.5 µL of 10 mg/mL infliximab

Injection wash solvent: MeOH/H₂O (20:80 v/v)

Needle wash: Enabled pre-injection

Gradient conditions: Table 3

Table 3. Reversed phase conditions for infliximab.

Time (min)	Flow rate (mL/min)	% A	% B
0	0.6	80	20
5.5	0.6	50	50
6.0	0.6	50	50
6.5	0.6	80	20
10.0	0.6	80	20

Data processing and software

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

Results and discussion

To evaluate the flexibility of the Vanquish Flex Duo system for Dual LC, two separate studies were conducted, each employing two different stationary phases.

Flexibility study 1

The Vanquish Flex Duo system for Dual LC was evaluated using bevacizumab (Avastin®). Two different analyses were carried on each channel: in one channel, aggregate analysis was performed on a Thermo Scientific MAbPac SEC-1, 5 µm, 300 Å, 4.0 × 300 mm column while charge variant analysis was performed in the second channel on a Thermo Scientific SCX-10 RS, 5 µm, 2.1 × 50 mm column. Ten technical replicates were performed. Results are shown in Figure 1 and Tables 4 and 5; the main peak characteristics have been evaluated.

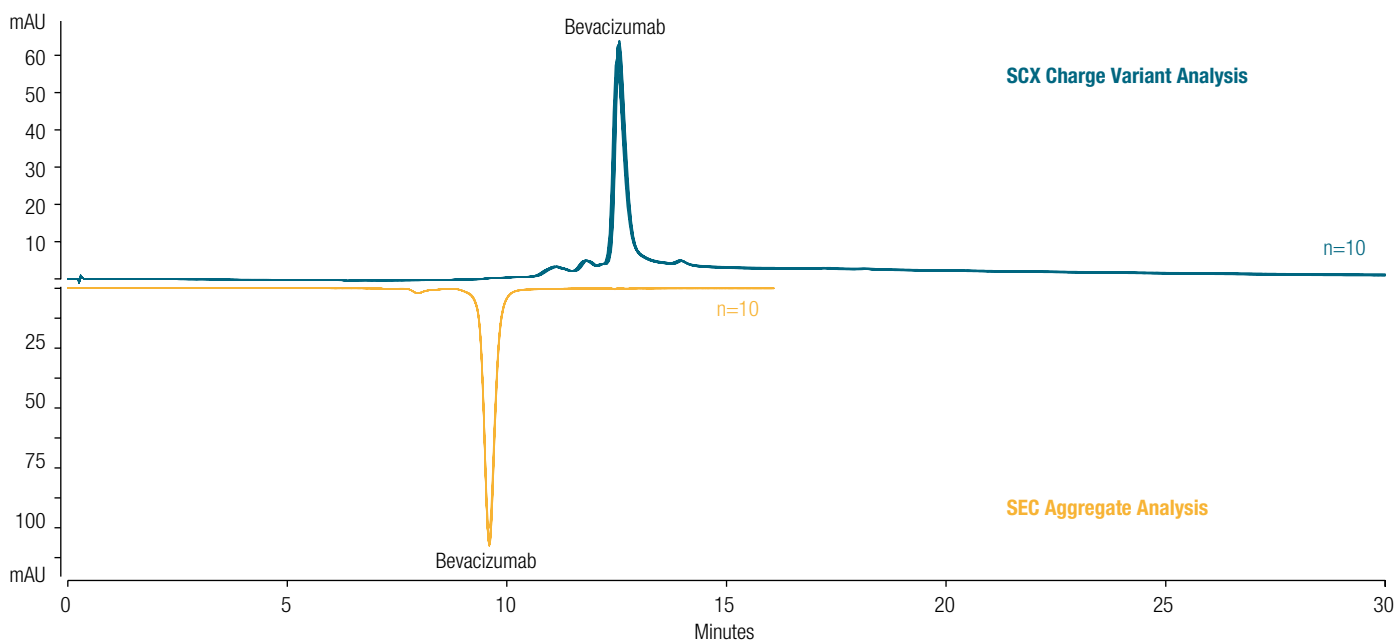


Figure 2. LC-UV chromatograms for the right (blue) and left (orange) LC channel. On the right channel, charge variant analysis of bevacizumab was performed, while on the left channel, size exclusion intact analysis profile was obtained.

Table 4. Performance on charge variant analysis of bevacizumab.

SCX Analysis of Bevacizumab (n=10)				
	Retention Time (min)	Relative Peak Area (%)	Peak Width at 50% Height (min)	Asymmetry (EP)
Average	12.515	85.01	0.302	1.81
% RSD	0.16	0.30	5.00	1.48

Table 5. Performance on aggregate analysis of bevacizumab.

SEC Analysis of Bevacizumab (n=10)				
	Retention Time (min)	Relative Peak Area (%)	Peak Width at 50% Height	Asymmetry (EP)
Average	9.554	96.57	0.259	1.08
% RSD	0.03	0.29	0.25	1.00

Flexibility study 2

The Vanquish Flex Duo system for Dual LC workflow was evaluated using infliximab (Remicade®). Two different analyses were carried on the two channels: charge variant analysis was performed in the first channel on a Thermo Scientific SCX-10 RS, 5 µm, 2.1 × 50 mm column, while intact protein analysis was performed on a Thermo Scientific MAbPac RP, 4 µm, 2.1 × 100 mm column in the second channel (Figure 3). Ten technical

replicates were performed. Charge variant analysis for infliximab was re-run on a Thermo Scientific Vanquish Flex Quaternary UHPLC system to compare the performances.

Results are shown in Tables 6 and 7; the main peak characteristics have been evaluated using the Chromeleon CDS.

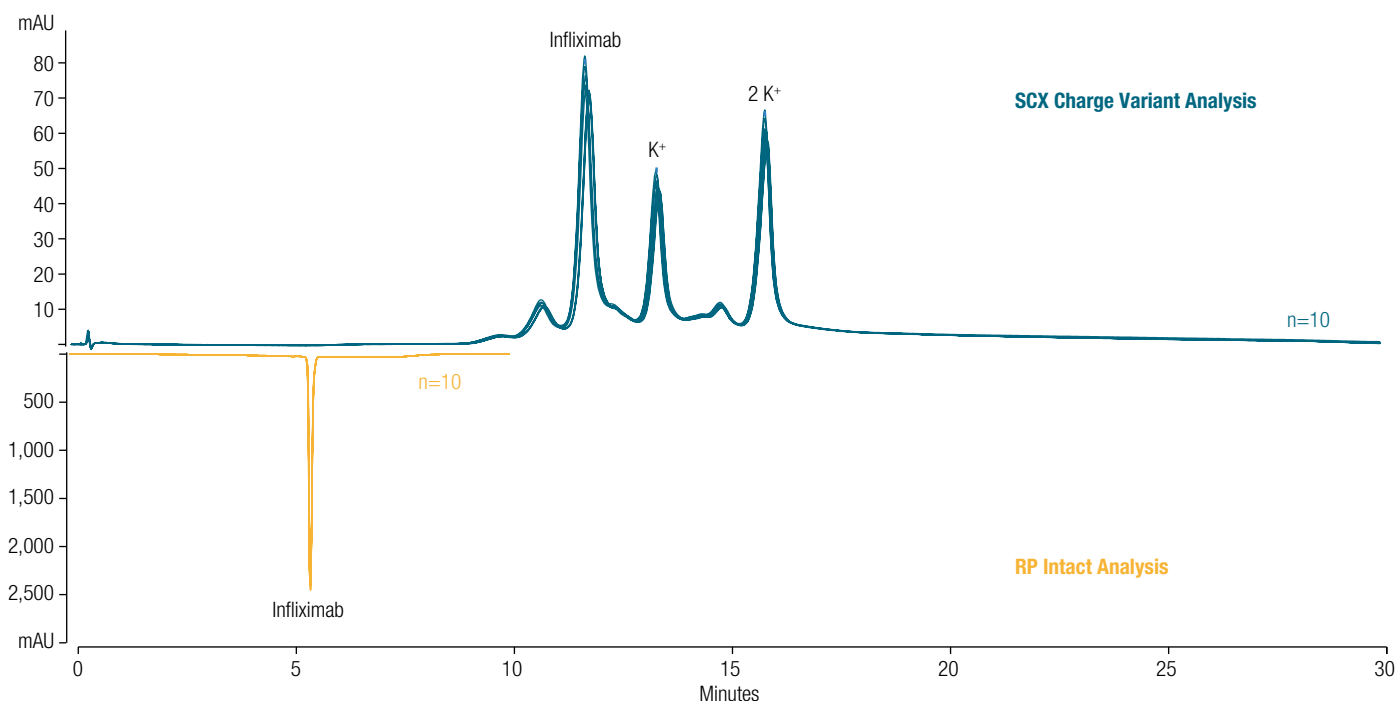


Figure 3. LC-UV chromatograms for the right (blue) and left (orange) LC channel. On the right system charge variant analysis of infliximab was performed, while on the left system reversed phase intact analysis profile was obtained.

Table 6. Performance on charge variant analysis of infliximab.

SCX Analysis of Infliximab (n=10)				
	Retention Time (min)	Relative Peak Area (%)	Peak Width at 50% Height (min)	Asymmetry (EP)
Average	11.856	54.08	0.293	1.03
% RSD	0.15	0.57	1.92	1.66

Table 7. Performance on reversed phase intact analysis of infliximab.

RP Analysis of Infliximab (n=10)				
	Retention Time (min)	Relative Peak Area (%)	Peak Width at 50% Height (min)	Asymmetry (EP)
Average	5.477	100.00	0.0746	1.34
% RSD	0.072	0.00	1.92	1.57

These data show excellent reproducibility of the instrument and outstanding performances on both channels for the two simultaneous analyses, allowing time savings and cost reduction. The Vanquish Flex Duo system for Dual LC has shown to be an easy-to-use chromatography system for the analysis of biotherapeutics. This Dual LC workflow provides the possibility to analyze biotherapeutic samples using different chromatographic techniques at one time with no compromise on the quality of analysis, which is highly advantageous in a QC environment.

Conclusion

- The Vanquish Flex Duo system for Dual LC provides simple and rapid high-throughput analysis of biotherapeutics.
- Analysis of monoclonal antibodies gives excellent quality data on both chromatographic channels with high confidence in results. Excellent reproducibility with low % RSD values for a number of analytical parameters was obtained for both chromatographic channels.

Reference

1. Beck, A.; Wagner-Rousset, E., Ayoub, D.; Van Dorsselaer, A.; Sanglier-Cianferani, S. Characterization of Therapeutic Antibodies and Related Products, *Anal. Chem.* **2013**, *85*, 715–736.

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