

High throughput, flexible chromatographic analysis of monoclonal antibodies

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ABSTRACT

To demonstrate the high-throughput capabilities of the newly developed Thermo Scientific™ Vanquish™ Flex Duo UHPLC system for Dual LC workflows. This study focused on data reproducibility and the quality of chromatography, while comparing technology performance to the standardly used Thermo Scientific™ Vanquish™ Flex Quaternary UHPLC system. The second part 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

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. 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, biopharmaceuticals 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 is considered a gold standard for monitoring the formation and level of mAb aggregates and fragments and is probably one of the most frequently performed analyses QC laboratories.

In this study, the Vanquish Flex Duo UHPLC system for Dual LC 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 mAbs. 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 system and the low % RSD values obtained. These results were also obtained on a standardly used Vanquish Flex Quaternary UHPLC system for direct comparison.

The second part of the study 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 and the low % RSD values obtained.

MATERIALS AND METHODS

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.

Test Methods

Instrumentation

Thermo Scientific Vanquish Flex Duo UHPLC 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 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 Flex (P/N VF-S01-A-02)

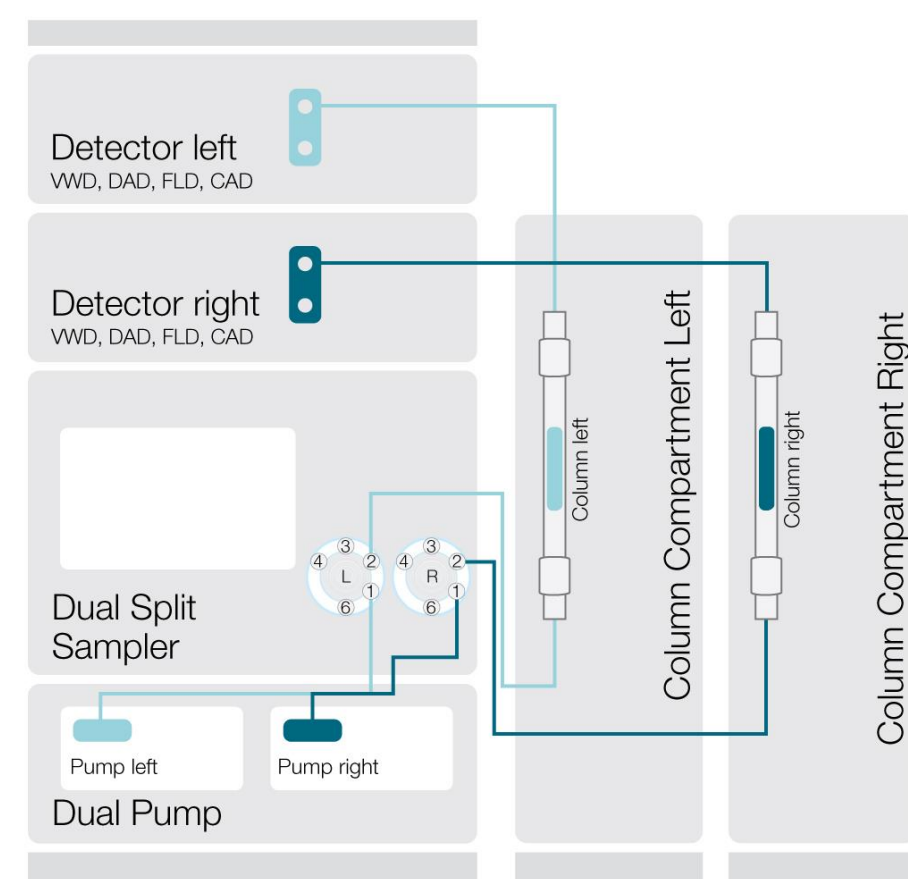


Figure 1. Vanquish Flex Duo UHPLC system for Dual LC workflows

Table 1. LC method parameters for the separations of intact mAbs.

| Parameter | Size exclusion chromatography | Strong cation exchange chromatography | Reversed phase chromatography |
|-------------------------|--|--|--|
| Mobile phase A | 100 mM sodium phosphate, pH 6.8 in 300 mM NaCl, filtered through 0.2 µm filter membrane before use | 10-fold dilution of CX-1 buffer A (pH 5.6) with DI water | H ₂ O/TFA (99.9:0.1 v/v) |
| Mobile phase 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. | MeCN/H ₂ O/TFA (90:9:0.1 v/v) Mobile phases filtered through 0.2 µm filter membrane before use. |
| Flow rate | 0.25 mL/min | See table 2 | 0.50 mL/min |
| Column temperature | 30 ° C (Still air mode) | 30 ° C (Still air mode) | 80 ° C (Still air mode) |
| Autosampler temperature | 5 ° C | | |
| UV wavelength | 214 nm, 280 nm | 280 nm | 214 nm, 280 nm |
| Injection volume | 2 µL of 25 mg/mL bevacizumab | 1 µL of 10 mg/mL infliximab 2 µL of 25 mg/mL bevacizumab | 0.5 µL of 10 mg/mL infliximab |
| Injection wash solvent | MeOH/H ₂ O (20:80 v/v) | | |
| Run time | 16 min | 30 min | 10 min |
| Needle wash | Enabled pre-injection | | |
| Gradient | isocratic | See table 2 | See table 2 |

Three MAbPac SEC-1 columns, 5 µm, 300 Å, 4.0 × 300 mm (P/N 074693) were used to compare the throughput capabilities of the Vanquish Flex Duo system to the Vanquish Flex Quaternary system.

For the second part of the study the Thermo Scientific MAbPac™ RP column, 4 µm, 2.1 × 100 mm (P/N 088647) and the Thermo Scientific™ SCX-10 RS column, 5 µm, 2.1 × 50 mm (P/N 082675) were used to demonstrate the flexibility of the system.

Table 2. LC gradient conditions for the separations of intact mAbs.

| Strong cation exchange chromatography | | | | | | | | |
|---------------------------------------|--------|--------|--------------------|---|--------|--------|--------------------|--|
| Infliximab | | | Bevacizumab | | | | | |
| Time [min] | A1 [%] | B1 [%] | Flow Rate [mL/min] | Time [min] | A1 [%] | B1 [%] | Flow Rate [mL/min] | |
| 0 | 85 | 15 | 0.2 | 0 | 85 | 15 | 0.3 | |
| 25.0 | 60 | 40 | 0.2 | 25.0 | 60 | 40 | 0.3 | |
| 25.1 | 85 | 15 | 0.2 | 25.1 | 85 | 15 | 0.3 | |
| 30.0 | 85 | 15 | 0.2 | 30.0 | 85 | 15 | 0.3 | |
| Reversed phase chromatography | | | | | | | | |
| Time [min] | A1 [%] | B1 [%] | Flow Rate [mL/min] | Data Analysis | | | | |
| 0.0 | 80 | 20 | 0.6 | The data were acquired and analyzed exclusively with the Thermo Scientific™ Chromleon™ Chromatography Data System (CDS), version 7.2.8. | | | | |
| 5.5 | 50 | 50 | 0.6 | | | | | |
| 6.0 | 50 | 50 | 0.6 | | | | | |
| 6.5 | 80 | 20 | 0.6 | | | | | |
| 10.0 | 80 | 20 | 0.6 | | | | | |

RESULTS

Part 1: High-throughput capabilities of Vanquish Duo system

To evaluate the high-throughput capabilities of the Vanquish Flex Duo UHPLC system for Dual LC, 100 injections of bevacizumab (Avastin®) were performed simultaneously on both channels and two identical 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 for Dual LC (left and right channel) and Vanquish Flex Quaternary UHPLC system were evaluated using the Chromleon CDS (Figures 2, 3 and Table 3).

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

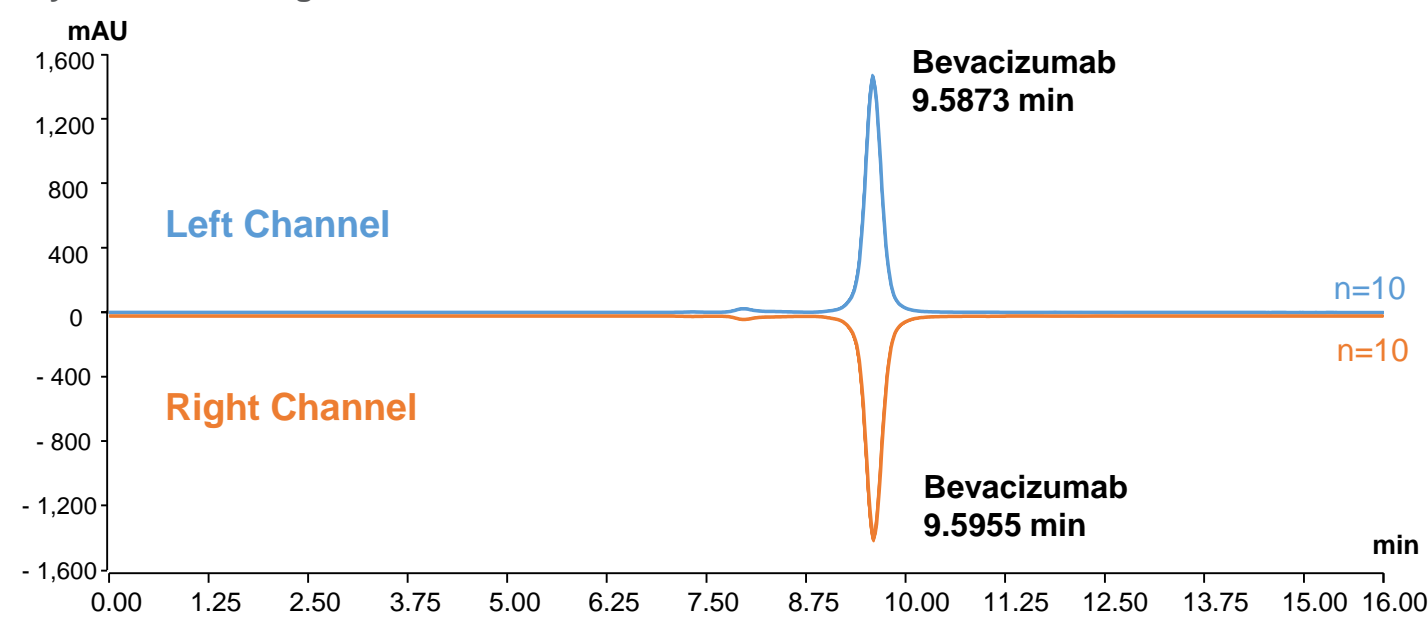
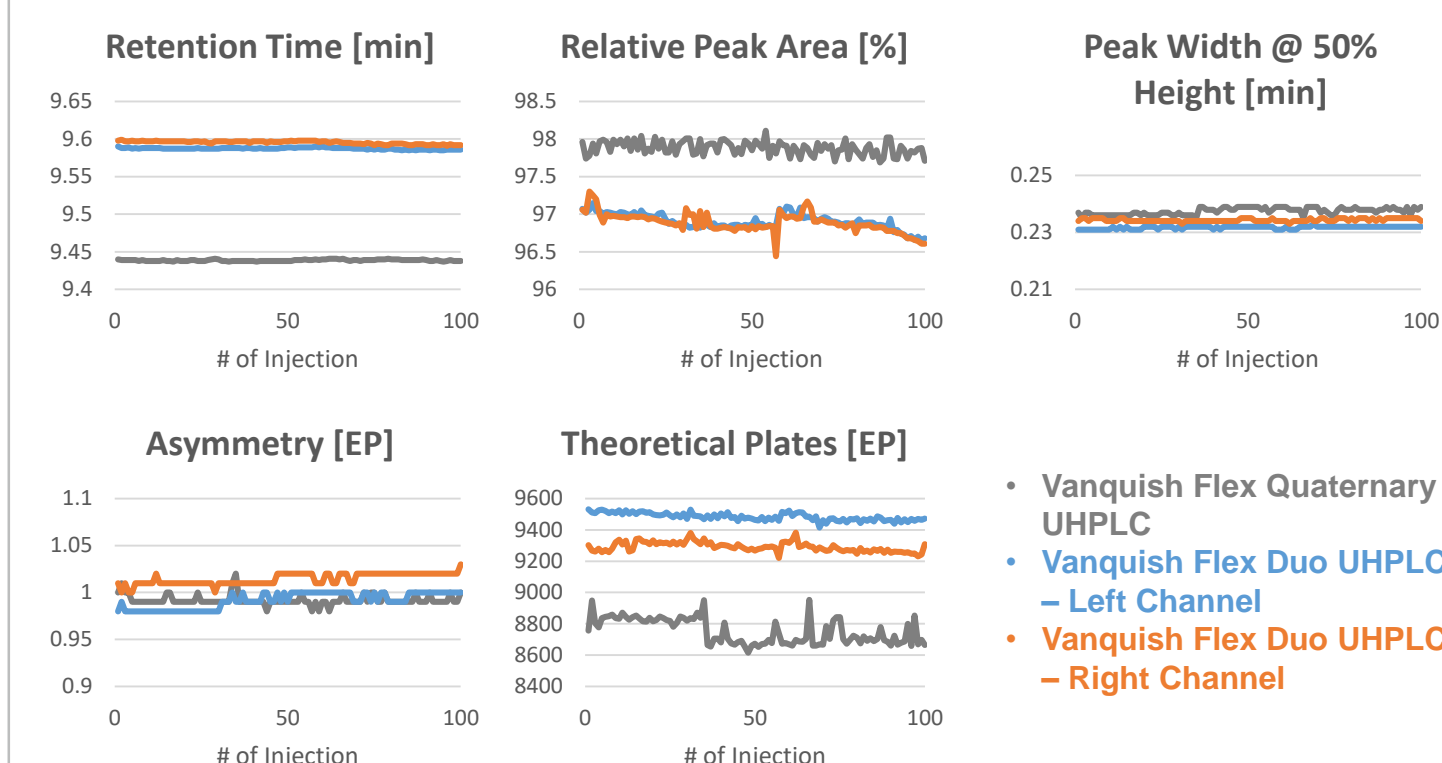


Table 3. SEC-UV analysis of bevacizumab. Comparison of performances of left and right channels of Vanquish Flex Duo for Dual LC system and Vanquish Flex Quaternary UHPLC system. Data for 100 repetitive injections.

| LC System | RT (min) | RSD RT (%) | Relative Peak Area (%) | Relative Peak Area %RSD | Peak Width at 50% Height | Peak Width at 50% %RSD | Asymm. (EP) | Theor. Plates (EP) |
|----------------------------|----------|------------|------------------------|-------------------------|--------------------------|------------------------|-------------|--------------------|
| Vanquish Duo Left Channel | 9.587 | 0.012 | 96.91 | 0.11 | 0.232 | 0.20 | 0.99 | 9484 |
| Vanquish Duo Right Channel | 9.596 | 0.021 | 96.89 | 0.14 | 0.234 | 0.22 | 1.02 | 9291 |
| Vanquish Flex Quaternary | 9.437 | 0.011 | 96.88 | 0.53 | 0.238 | 0.51 | 1.00 | 8751 |

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 UHPLC system.



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 of high-throughput analysis of biotherapeutic samples with no compromise on the quality of analysis, which is highly advantageous in a QC environment.

Part 2: Flexibility capabilities

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

Flexibility study 1

The Vanquish Flex Duo UHPLC 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 4 and Table 4; the main peak characteristics have been evaluated.

Figure 4. LC-UV chromatograms for the left (blue) and right (orange) LC channel. On the left channel, charge variant analysis of bevacizumab was performed, while on the right channel, size exclusion intact analysis profile was obtained.

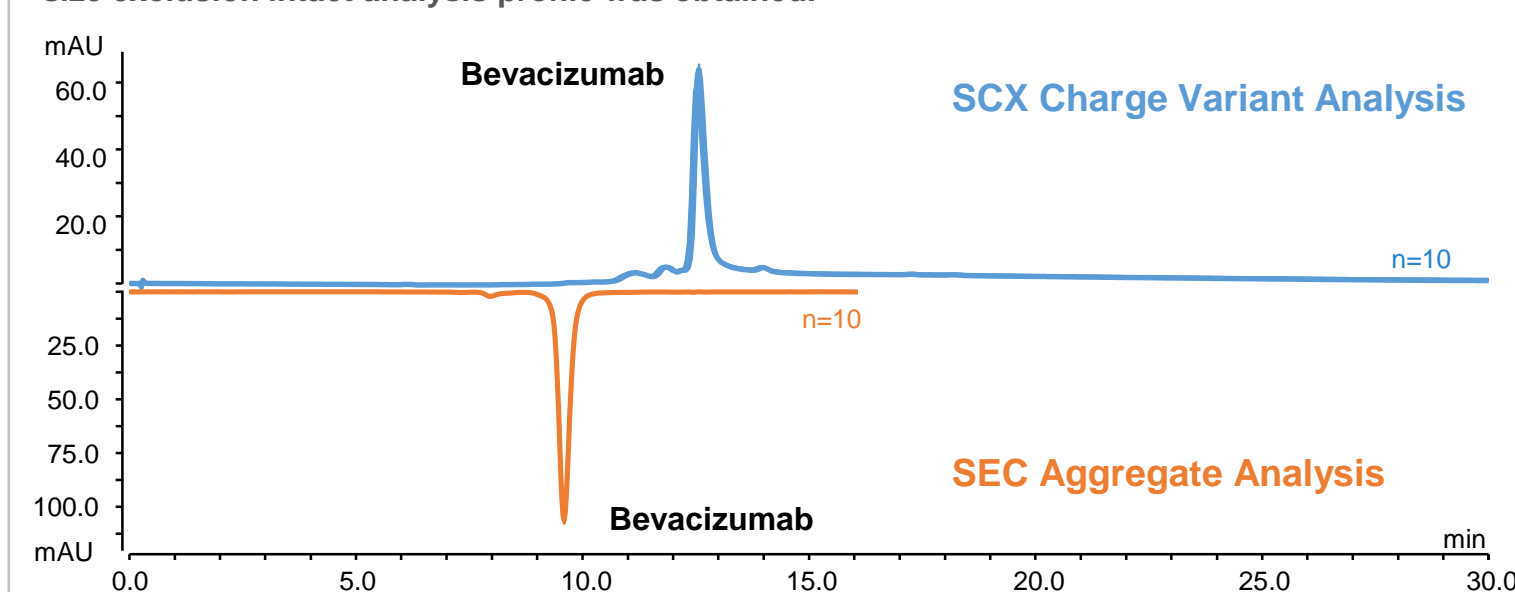


Table 4. SCX and SEC Analysis of bevacizumab – Vanquish Flex Duo

| | RT (min) | Relative Peak Area (%) | Peak Width at 50% Height (min) | Asymm. (EP) |
|--------------------|----------|------------------------|--------------------------------|-------------|
| Average (n=10) SCX | 12.515 | 85.01 | 0.302 | 1.81 |
| % RSD (n=10) SCX | 0.16 | 0.30 | 5.00 | 1.48 |
| Average (n=10) SEC | 9.554 | 96.57 | 0.259 | 1.08 |
| % RSD (n=10) SEC | 0.03 | 0.29 | 0.25 | 1.00 |

Flexibility study 2

The Vanquish Flex Duo 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 5). Ten technical replicates were performed. Charge variant analysis for infliximab was rerun on a Thermo Scientific Vanquish Flex UHPLC system to compare the performances. Results are shown in Figure 5 and Table 5; the main peak characteristics have been evaluated using the Chromleon Chromatography Data System.

Figure 5. LC-UV chromatograms for the left (blue) and right (orange) LC channel. On the left system charge variant analysis of infliximab was performed, while on the right system reversed phase intact analysis profile was obtained.

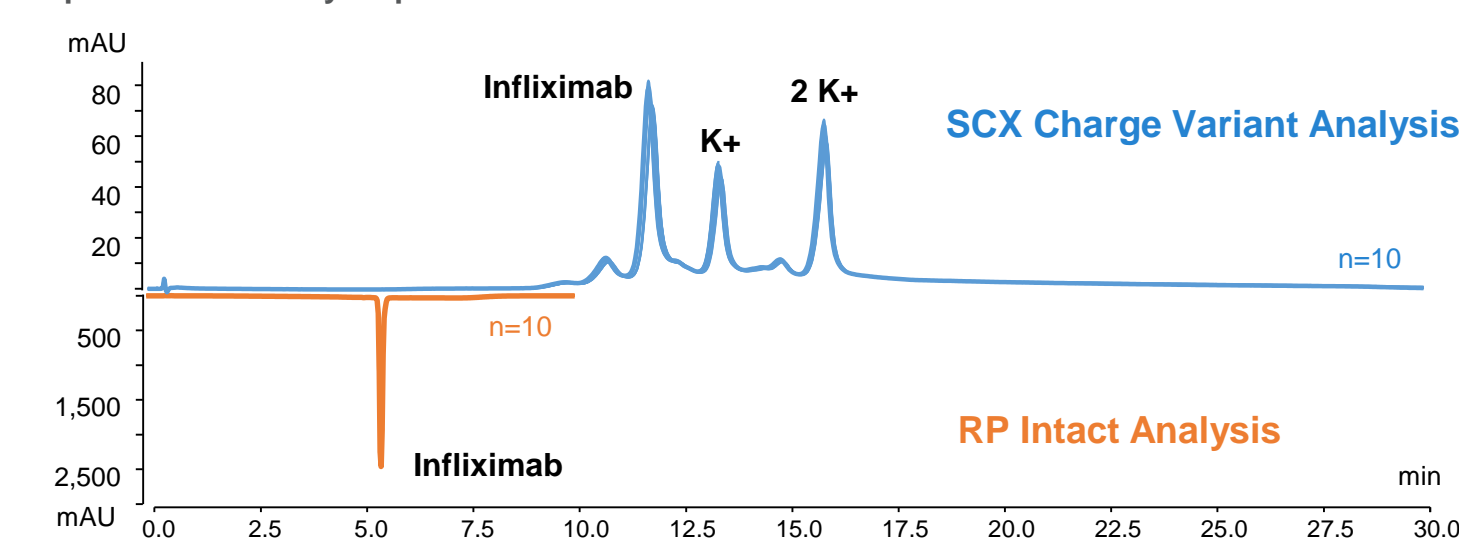


Table 5. SCX and RP Analysis of infliximab – Vanquish Flex Duo

| | RT (min) | Relative Peak Area (%) | Peak Width at 50% Height (min) | Asymm. (EP) |
|--------------------|----------|------------------------|--------------------------------|-------------|
| Average (n=10) SCX | 11.856 | 54.08 | 0.293 | 1.03 |
| % RSD (n=10) SCX | 0.15 | 0.57 | 1.92 | 1.66 |
| Average (n=10) RP | 5.477 | 100.00 | 0.075 | 1.34 |
| % RSD (n=10) RP | 0.07 | 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 UHPLC 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 technique at one time with no compromise on the quality of analysis, which is highly advantageous in a QC environment.

CONCLUSIONS

- The Vanquish Flex Duo UHPLC system for Dual LC workflows provides simple and rapid high-throughput analysis of biotherapeutics.
- Analysis of mAbs 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.
- Data was comparable to that obtained on a standard Vanquish Flex Quaternary UHPLC system.

TRADEMARKS/LICENSING

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