# Seamless Method Transfer from an Agilent 1260 Infinity Bio-inert LC to an Agilent 1260 Infinity II Bio-inert LC

## Charge Variant Analysis of Rituximab Innovator and Biosimilar

## Application Note

Biologics & Biosimilars


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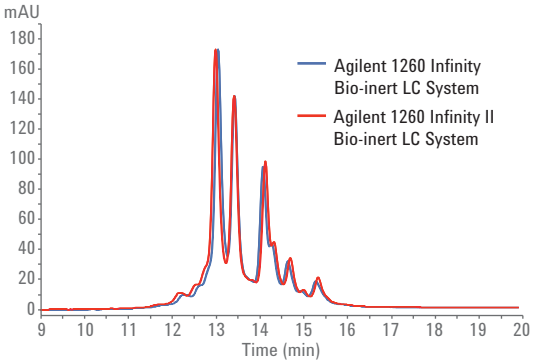
### Abstract

Monitoring the charge heterogeneity of biopharmaceuticals is critical to the production of effective and safe drugs because changes in the charge pattern could lead to adverse immunological reactions. This Application Note shows the analysis of charge variants for the innovator as well as for the biosimilar of rituximab. The charge variants showed similarity in peak pattern, but clear differences in the intensities of the single peaks from innovator to biosimilar. Method transfer between the Agilent 1260 Infinity Bio-inert LC and the Agilent 1260 Infinity II Bio-inert LC produced equivalent results. Excellent agreement of retention times was found with a maximum deviation of less than 0.5 %. Both systems showed highly precise results regarding retention time and area.



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## Introduction

Instrument-to-instrument method transfer is an important topic for laboratories throughout different industries[1]. For validated methods in the pharma- and biopharmaceutical industry, equivalent method transfer from one instrument to another is mandatory, for example, due to lab modernization actions.

Developments in the biopharmaceutical industry with many emerging monoclonal antibodies (mAbs) as expensive drugs, led to the continuous development of less costly biosimilars. Comparison of innovator and biosimilar drugs is crucial to evaluate the safety and efficacy of the biosimilar mAbs. These extensive studies include important quality attributes such as titer analysis, aggregation studies, charge variant and glycan profiling, peptide mapping, and many others. Two of the most important quality attributes are aggregation studies as well as the analysis of the charge variant pattern[2]. The mAbs used in this Application Note are innovator and biosimilar of rituximab, which is applied in the treatment of diffuse large B-cell lymphoma along with multiagent chemotherapy[3].

This Application Note analyzes the charge variant patterns of rituximab innovator and biosimilar using weak cation exchange chromatography (WCX). Two different bio-inert systems, the Agilent 1260 Infinity Bio-inert LC and the Agilent 1260 Infinity II Bio-inert LC system, were compared to prove equivalency of both tested systems.

## Experimental

### Instrumentation

The experiments were carried out on an Agilent 1260 Infinity Bio-inert LC and on an Agilent 1260 Infinity II Bio-inert LC system. The systems were composed of the following modules:

Agilent 1260 Infinity Bio-inert Quaternary LC System

- Agilent 1260 Infinity Bio-inert Quaternary Pump (G5611A)
- Agilent 1260 Infinity High-Performance Bio-inert Autosampler (G5667A)
- Agilent 1290 Infinity Thermostat (G1330B) for sample cooling
- Agilent 1290 Infinity Thermostatted Column Compartment (G1316C) with bio-inert solvent heat exchangers
- Agilent 1260 Infinity Diode Array Detector (G1315C) with a 10 mm bio-inert standard flow cell

Agilent 1260 Infinity II Bio-inert Quaternary LC System

- Agilent 1260 Infinity II Bio-inert Pump (G5654A)
- Agilent 1260 Infinity II Bio-inert Multisampler (G5668A) with sample cooler (Option #100)
- Agilent 1260 Infinity II Multicolumn Thermostat (G7116A) with bio-inert heat exchanger (Option #019)
- Agilent 1260 Infinity II Diode Array Detector WR (G7115A) with bio-inert flow cell (Option #028)

### Column

Agilent Bio mAb, nonporous, 2.1 × 250 mm, 5 µm HPLC column, PEEK (p/n 5190-2411)

### Software

- Agilent OpenLAB CDS Version 2.1
- Agilent Buffer Advisor A.01.01 [009]

LC instrument control as well as LC data analysis was carried out using Agilent OpenLAB CDS Version 2.1. OpenLAB CDS Version 2.1 offers a single software system for liquid chromatography, gas chromatography, and single-quadrupole mass spectrometry. It provides a flat user interface and customized and interactive reporting with drag-and-drop template creation.

### Samples

Rituximab innovator and biosimilar samples were purchased in local pharmacies in India.

### Chemicals

All solvents used were LC grade. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22 µm membrane point-of-use cartridge (Millipak). Sodium phosphate monobasic, sodium phosphate dibasic, and sodium chloride were purchased from Sigma-Aldrich, St. Louis, USA.

### Chromatographic conditions

| Parameter | Value | | | | |
|---|---|---|---|---|---|
| Salt gradient 0–200 mM NaCl, 30 mM sodium phosphate buffer, pH 6.8 (gradient calculated from Buffer Advisor) | Time (min) | A) Water | B) NaCl (1,000 mM) | C) $NaH_2PO_4$ (55 mM) | D) $NaH_2PO_4$ (50 mM) |
| | 0.00 | 43.1 | 0.0 | 31.0 | 25.9 |
| | 30.00 | 22.3 | 20.0 | 22.7 | 35.0 |
| | 35.00 | 22.3 | 20.0 | 22.7 | 35.0 |
| Stop time | 35 minutes | | | | |
| Post time | 30 minutes | | | | |
| Flow rate | 0.25 mL/min | | | | |
| Injection volume | 2 µL | | | | |
| Temperature sampler | 10 °C | | | | |
| Temperature column | 25 °C | | | | |
| DAD | 280 nm/4 nm, Ref.: OFF | | | | |
| Peak width | > 0.025 minutes (0.5 seconds response time) (10 Hz) | | | | |

## Results and Discussion

Method development in ion exchange chromatography usually begins with pH scouting to find the optimal chromatographic conditions to separate the charge variants. For the separation of the rituximab charge variants, a phosphate buffered gradient was chosen using sodium chloride for a linear elevation of the ionic strength. The pH range for optimal buffering capacity of phosphate buffer lies between 6 to 7.5 pH. Therefore, pH scouting was carried out starting at pH 6.2, and was gradually increased in 0.2 steps to pH 7.2. Figure 1 shows the pH scouting chromatograms using the rituximab biosimilar. Clear differences were observed in the retention time as well as the resolution depending on the adjusted pH. The quaternary gradients were calculated with Agilent Buffer Advisor software using the same four stock solutions for all adjusted pH values. Working with dynamically mixed four-component gradients, calculated by Buffer Advisor software, shortened and simplified the workflow for pH scouting[4]. Especially, when compared to the manual preparation of buffers for premixed two-component gradients, the use of dynamically mixed gradients calculated with Buffer Advisor software resulted in a significant decrease in buffer preparation time. In addition, it eliminated the need to interrupt the analysis sequence to introduce and flush the new buffer composition. pH 6.8 was chosen as the one with the highest resolution, and was used for all further analyses.

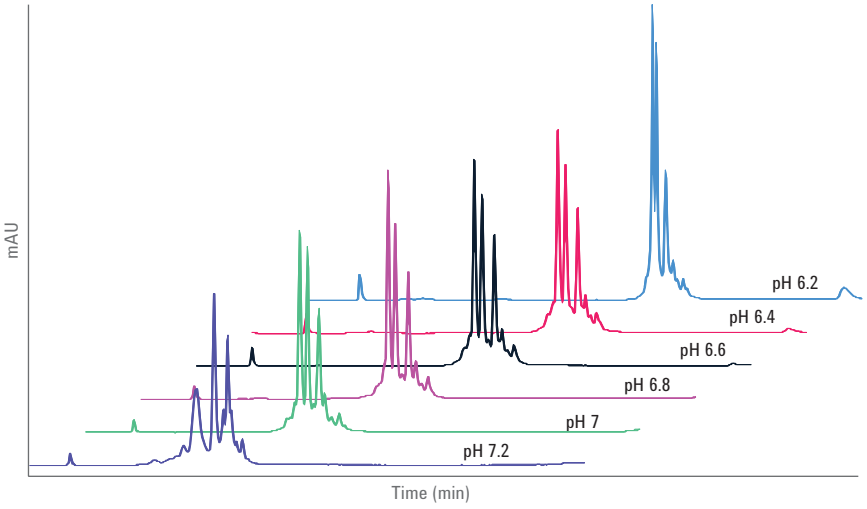


Figure 1. pH scouting using rituximab biosimilar from pH 6.2 to 7.2 on an Agilent 1260 Infinity II Bio-inert LC.

Figure 2 shows the comparison of rituximab innovator and biosimilar at pH 6.8. A clear difference between the intensities of the single peaks can be seen. An overlay of both chromatograms reveals that the pattern looks similar in terms of peak quality, whereas the quantity, especially of the basic variants, differs to a great extent (Figure 3). The two basic variants with the highest intensities of the biosimilar were identified as C-terminal lysine variants in previous Application Notes[5].

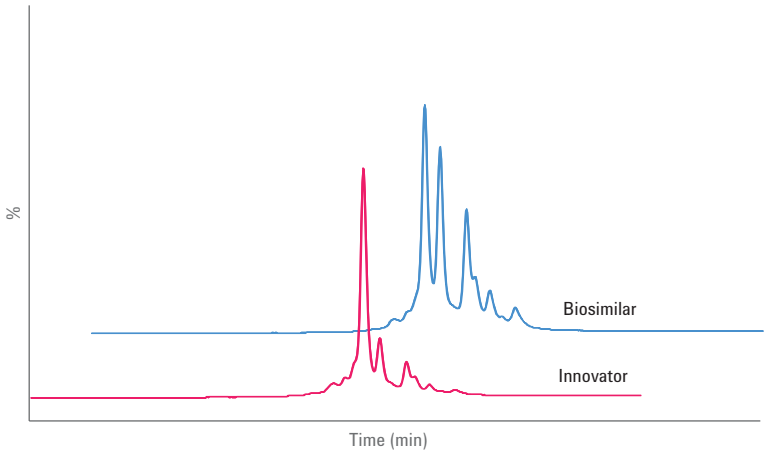


Figure 2. Comparison of the charge variant profiles at pH 6.8 of rituximab innovator and biosimilar on an Agilent 1260 Infinity II Bio-inert LC.

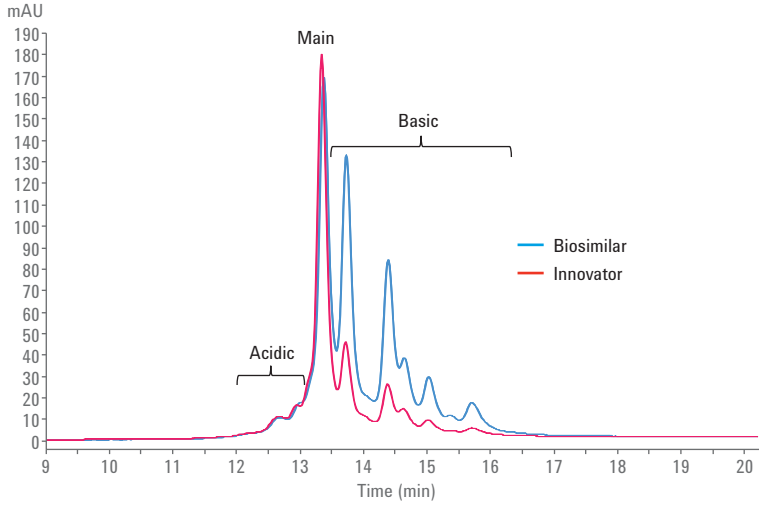


Figure 3. Direct overlay of the charge variant profiles of rituximab innovator and biosimilar on an Agilent 1260 Infinity II Bio-inert LC.

To prove equivalency, the method for the charge variants analysis was applied without any changes on the 1260 Infinity Bio-inert LC and the 1260 Infinity II Bio-inert LC. Figure 4 shows an overlay of the WCX chromatograms on both systems: the 1260 Infinity Bio-inert LC (blue trace) and the 1260 Infinity II Bio-inert LC (red trace). Table 2 shows the compared retention times. With a maximum deviation of less than 0.5 %, excellent agreement of retention times between both bio-inert systems was observed.

The precision of retention times and area was evaluated on the 1260 Infinity Bio-inert LC and the 1260 Infinity II Bio-inert LC using five subsequent injections of rituximab biosimilar. The resulting separation, the corresponding retention time, and area precisions are presented in Figure 5 for the 1260 Infinity Bio-inert LC, and in Figure 6 for the 1260 Infinity II Bio-inert LC. Both systems revealed highly precise chromatographic results in terms of retention times and areas.

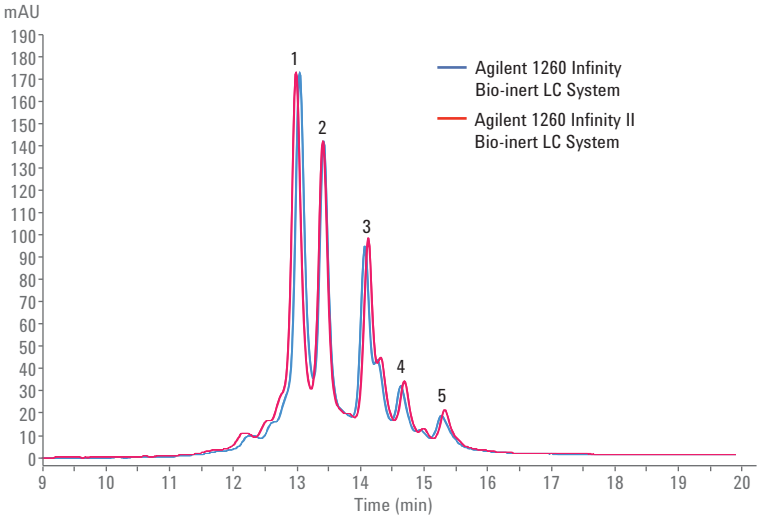


Figure 4. Overlay of the biosimilar WCX separation at pH 6.8 on an Agilent 1260 Infinity Bio-inert LC (blue trace) and an Agilent 1260 Infinity II Bio-inert LC (red trace).

Table 2. Comparison of retention times of the biosimilar WCX separation at pH 6.8 on an Agilent 1260 Infinity Bio-inert LC and an Agilent 1260 Infinity II Bio-inert LC.

| | Agilent 1260 Infinity Bio-inert LC System (min) | Agilent 1260 Infinity II Bio-inert LC System (min) | Deviation in % |
|---|---|---|---|
| Peak 1 | 13.37 | 13.31 | 0.47 |
| Peak 2 | 13.75 | 13.73 | 0.17 |
| Peak 3 | 14.40 | 14.44 | -0.28 |
| Peak 4 | 14.95 | 15.01 | -0.39 |
| Peak 5 | 15.59 | 15.65 | -0.41 |

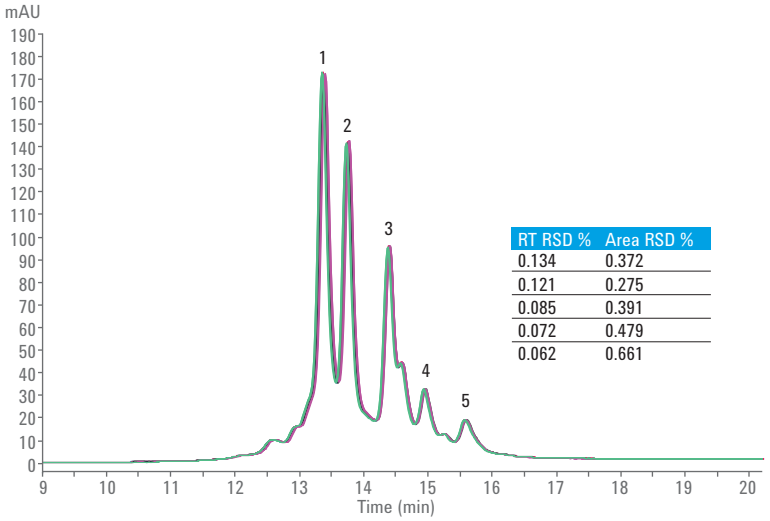


Figure 5. Overlay of five subsequent injections of the charge variants of the rituximab biosimilar on an Agilent 1260 Infinity Bio-inert LC.

## Conclusion

The charge variant analysis of rituximab innovator and biosimilar was analyzed with weak cation exchange chromatography. After method development/pH scouting with Agilent Buffer Advisor software calculated gradients from pH 6.2 to 7.2, pH 6.8 was chosen as the pH where the best resolution was achieved for these samples in a phosphate buffered system. After comparing the charge variant pattern of the innovator with the biosimilar, similarity in the peak pattern was found, however, with clear differences in the intensities of the single peaks. The separation at pH 6.8 was run on the Agilent 1260 Infinity Bio-inert LC as well as the Agilent 1260 Infinity II Bio-inert LC. Excellent agreement of retention times was observed with a maximum deviation of less than 0.5 %, proving equivalency of both bio-inert systems. In addition, highly precise results were found regarding retention time and area with both systems.

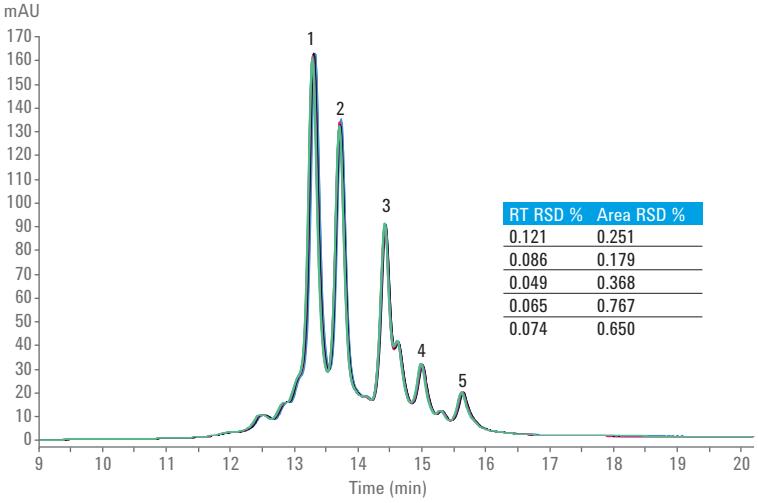


Figure 6. Overlay of five subsequent injections of the charge variants of the rituximab biosimilar on an Agilent 1260 Infinity II Bio-inert LC.

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