Molecular Characterization of Biotherapeutics

The Agilent 1260 Infinity Multi-Detector Bio-SEC Solution with Advanced Light Scattering Detection

Application Note

Biologics and Biosimilars

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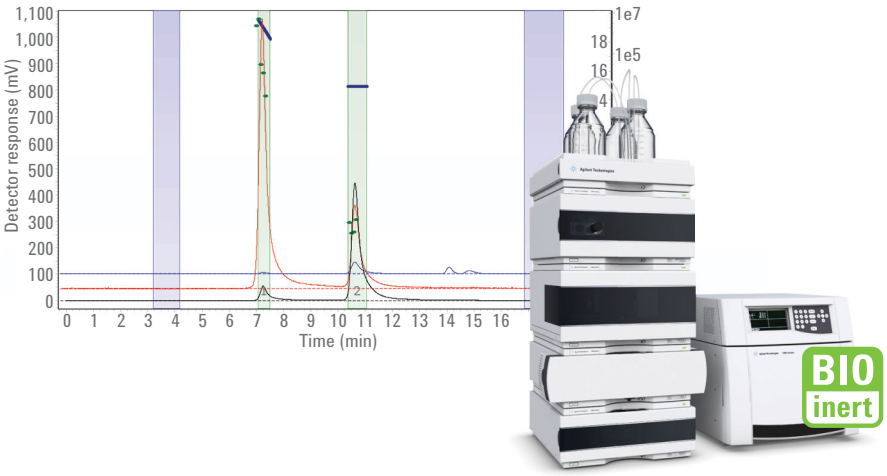
Abstract

This Application Note describes the molecular characterization of two monoclonal antibodies (rituximab) used as biotherapeutics to compare innovator versus biosimilar using the Agilent 1260 Infinity Multi-Detector Bio-SEC Solution. The molecular weight and size of innovator and biosimilar were found to be comparable. In addition, aggregate formation was monitored after 1 hour at 60 °C and analyzed using area percent. No major differences were detected between innovator and biosimilar. In particular, light scattering detection provided significantly higher sensitivity for mAb aggregates at 90° compared to UV detection.



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Introduction

Biopharmaceuticals such as recombinant monoclonal antibodies (mAbs) are a class of advanced, but expensive, drug products. This leads to a demand for generic versions to lower the costs of these important medicines. The development of mAbs is more complicated compared to the production of generic small molecule pharmaceutical, which are exact copies of the innovator product. However, producing an exact copy of a biopharmaceutical innovator is technically impossible[1]. Biopharmaceuticals are usually produced in genetically modified cell lines and extracted with complex and cumbersome purification procedures. Hence, the manufacturing process will always be different as this is proprietary knowledge[2]. The produced biologics are unique molecules rather than identical generic copies. The term biosimilar was introduced to describe a product that is not identical, but similar to the innovator product[3].

Due to the complex production procedures, biopharmaceuticals are susceptible to a variety of modifications, including aggregations and charge variants, among others. This makes the characterization of the molecules extensive and difficult[4]. The efficacy of protein-based therapeutic agents like monoclonal antibodies is highly dependent on the correct primary, secondary, ternary, and quaternary structure. This includes correct amino acid composition, post-translational modifications, conformation, and aggregation status.

Size exclusion chromatography (SEC) has been widely used as the standard method to characterize protein aggregates in immunoglobulins. The Agilent 1260 Infinity Multi-Detector Bio-SEC Solution with advanced light scattering detection comprises the Agilent 1260 Infinity Multi-Detector Suite with static and dynamic light scattering in combination with the Agilent 1260 Infinity Bio-inert Quaternary LC with the Agilent 1260 Infinity Diode Array Detector (DAD) with bio-inert flow cell. Static and dynamic light scattering detection enables the measurement of both molecular weight (MW) and hydrodynamic radius (Rh)[5,6].

In this Application Note, innovator and biosimilar of rituximab were chosen for analysis. Rituximab is a monoclonal antibody against the protein CD 20, a protein that is mainly found on the surface of B cells. As rituximab is directed against B cells, it is used as a treatment for diseases that overexpress B cells, for example, cancer types of leukemia or many lymphomas, but also transplant rejection or autoimmune disorders.

Innovator and biosimilar are compared with respect to aggregation status after non-ideal storage conditions that can cause aggregation, such as storage at room temperature or heat exposure.

Experimental

The Agilent 1260 Infinity Multi-Detector Bio-SEC Solution consisted of the following modules:

- 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 exchanger
- Agilent 1260 Infinity DAD (G1315C) with bio-inert standard flow cell, 10 mm
- Agilent 1260 Infinity Multi-Detector Suite (G7805A) featuring 1260 Infinity Bio-inert Dual angle and Dynamic Light Scattering Detector (G7809A)

Instrumental conditions

Parameter	Value
Mobile phase	PBS, pH 7.4
Flow rate	0.75 mL/min
Run time	25 minutes
Injection volume	5 µL
Thermostat autosampler	5 °C
Column temperature	30 °C
DAD detection	Peak width 280 nm/4 nm Ref OFF, > 0.05 minutes (1.0 seconds response time) (5 Hz)
Light scattering detection	30 °C, 5 Hz
DLS parameters	
Correlator run time	5 s
Correlator function clip time	10 µs
R^2	0.80
Eluent viscosity	0.0079 p (viscosity of water at 30 °C)
Eluent refractive index	1.333 (refractive Index of water)

Column

The column was an Agilent Bio SEC-3, 300Å, 7.8 × 300 mm, 3 µm (p/n 5190-2511). An inline filter was used in front of the light scattering detector (p/n G7808-64001) with 0.2-µm filter membranes (Supor-200, 13 mm (p/n 60298), Pall Life Sciences, Port Washington, NY, USA).

Software

Agilent Bio-SEC Software Version A.01.01 Build 4.30989

Solvents and samples

All chemicals and solvents were HPLC grade and highly purified water from a Milli Q water purification system (Millipore Elix 10 model, USA) was used. Sodium phosphate dibasic, sodium phosphate monobasic, sodium chloride and gel filtration markers were purchased from Sigma-Aldrich Corp., St. Louis, USA. The phosphate buffered saline (PBS) was prepared by using 0.0779 % monosodium phosphate, monohydrate, 0.5187 % disodium phosphate, heptahydrate, and 0.9 % sodium chloride in ultra-pure water.

The PBS was triple filtered using a 0.2-µm membrane filter. In addition, the samples were filtered using an Agilent Captiva Premium Syringe Filter, with a regenerated cellulose membrane, 4-mm diameter, and 0.2-µm pore size (p/n 5190-5106).

Results and Discussion

Two versions of the biopharmaceutical mAb rituximab (innovator and biosimilar) were analyzed with the Multi-Detector Bio-SEC Solution with advanced light scattering detection. Both mAbs were analyzed after 24 hours storage at room temperature and after 1 hour at 60 °C. After 24 hours storage at room temperature, no aggregation formation was observed. Therefore, these data are not discussed. Figure 1 shows UV signals of rituximab from fresh (A) innovator and after 1 hour at 60 °C (B). Both Figure 1A and 1B show an overlay of seven subsequent sample runs.

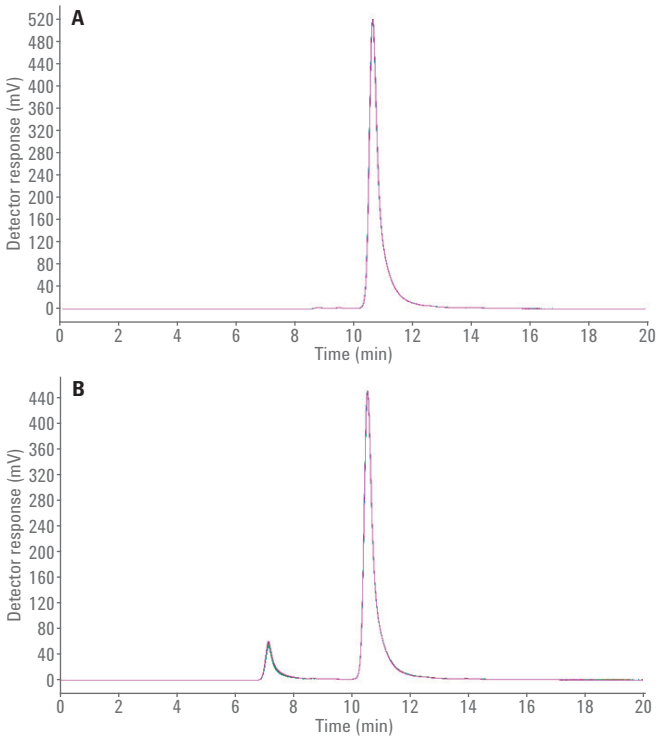

Figure 1. Comparison of UV signals of rituximab from A) fresh innovator and B) after one hour at 60 °C analyzed with the Agilent 1260 Multi-Detector Bio-SEC Solution. After one hour at 60 °C, a clear aggregation peak was visible.

Table 1 shows the retention time, area, molecular weight, and size determination together with precision values for seven subsequent runs for fresh rituximab innovator and biosimilar. Both rituximab samples were comparable. In addition, excellent precision was found for retention time, area, and molecular weight determination.

Table 1. Retention time, area, molecular weight, and hydrodynamic radius together with precision of the fresh rituximab innovator and biosimilar.

	RT (mean)	RSD RT (%)	Area (mean)	RSD area (%)	Bulk MW monomer (mean)	RSD bulk MW (%)	Rh (mean)	RSD Rh (%)
Innovator	10.62	0.025	10,631	0.137	155,786	0.059	4.8	6.4
Biosimilar	10.67	0.368	10,545	1.164	156,407	0.154	5.5	9.3

Figure 2 shows all signals in the analysis of the heat-stressed rituximab innovator, including the DAD signal at 280 nm, the LS at 90° and the DLS signal. The blue lines represent the calculated MW (LS at 90°) and the green dots the hydrodynamic radius measured with DLS. The blue areas represent baseline determination and the green areas define the peaks. The light scattering signal of the aggregate peak (at ~7 minutes) was significantly higher compared to the UV signal at 280 nm, making light scattering a considerably more sensitive method for aggregate determination.

A comparison of the two heat-stressed rituximab samples from innovator and biosimilar showed similar results (Figure 3 and Table 2). Similar results were found for retention time (RT), area, area %, MW, and Rh. Area % is one of the most interesting parameters, describing the percentage of aggregate present in the mAb sample. In addition, the percentage of aggregate found after 1 hour at 60 °C was nearly the same in both rituximab samples from innovator (9.72 %) and biosimilar (9.94 %). Moreover, excellent precision was achieved for RT, area, and MW.

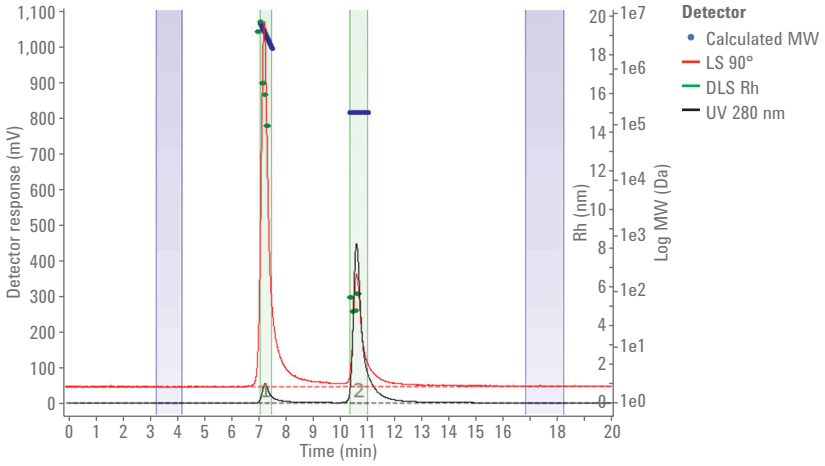

Figure 2. Determination of MW and Rh of heated rituximab innovator using the Agilent 1260 Bio-SEC Solution, including DAD and LS/DLS signals.

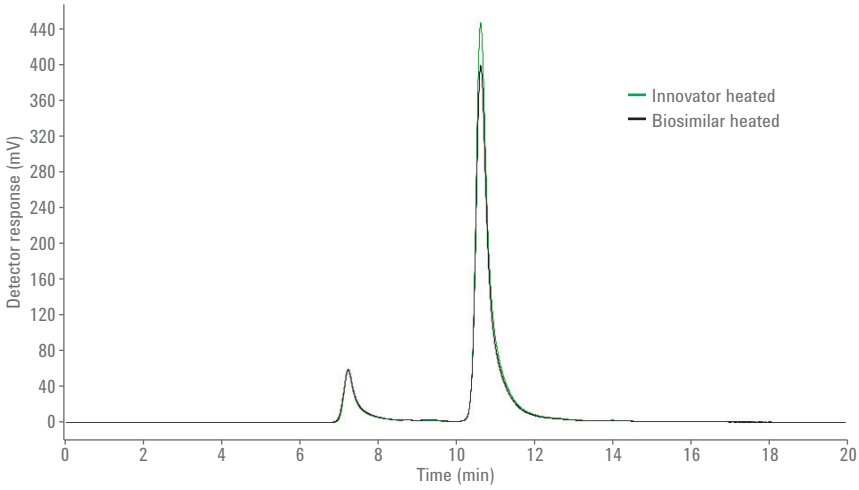

Figure 3. Overlay of heat stressed rituximab samples (innovator and biosimilar).

Table 2. Comparison of heated rituximab samples with respect to retention time, area, molecular weight, and hydrodynamic radius together with precision.

	RT (mean)	RSD RT (%)	Area (mean)	RSD area (%)	Area (%)	Bulk MW (mean)	RSD bulk MW (%)	Rh (mean)	RSD Rh (%)
Innovator aggregate	7.25	0.024	938	4.184	9.72	4,114,774	0.666	16.4	5.0
Innovator monomer	10.62	0.013	8,862	0.329	90.28	161,120	0.130	5.2	7.2
Biosimilar aggregate	7.37	0.68	882	2.541	9.94	4,500,390	0.591	16.9	5.8
Biosimilar monomer	10.74	0.030	8,590	0.281	90.06	164,849	0.149	5.3	11.1

Conclusions

Two rituximab monoclonal antibodies from an innovator and biosimilar were characterized using the Agilent 1260 Infinity Bio-SEC Solution. No major differences with respect to MW and size were found between innovator and biosimilar. After 1 hour at 60 °C, the amount of aggregate formation was compared using area percent, and similar results were found for the innovator and biosimilar. In addition, light scattering detection provided significantly higher sensitivity for mAb aggregates at 90° compared to UV detection.

References

1. Schellekens, H. Follow-on biologics: challenges of the 'next generation'. *Nephrol. Dial. Transplant*. **2005**, *20*, iv31–iv36.

2. Mellstedt, H.; Niederwieser, D.; Ludwig, H. The challenge of biosimilars. *Ann. Oncol.* **2008**, *19*, pp 411-4119.

3. Niederwieser, D.; Schmitz, S. Biosimilar agents in oncology/ haematology: from approval to practice. *Eur. J. Haematol.* **2011**, *86*, pp 277-288.

4. Kuhlmann, M.; Covic, A. The protein science of biosimilars. *Nephrol. Dial. Transplant*. **2006**, *21*, v4-v8.

5. Schneider, S. Detailed Aggregation Characterization of Monoclonal Antibodies Using the Agilent 1260 Infinity Multi-Detector Bio-SEC Solution with Advanced Light Scattering Detection, *Agilent Technologies Application Note*, publication number 5991-3954EN, **2014**.

6. Schneider, S. Determination of Protein Molecular Weight and Size Using the Agilent 1260 Infinity Multi-Detector Bio-SEC Solution with Advanced Light Scattering Detection, *Agilent Technologies Application Note*, publication number 5991-3955EN, **2014**.

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