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Biopharma / Nexera Bio UHPLC

Analysis of mAb Aggregates by Nexera™ Bio UHPLC with a Shim-pack™ Bio Diol (Size Exclusion) Column

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❑ Introduction

Monoclonal antibodies (mAb) are important biologics for the treatment of cancers. The production of mAb biosimilar is challenging, because various variations may occur in upstream and downstream processing (DSP). Protein aggregation is one of such variations – a biological phenomenon in which mAb accumulate and clump together. Aggregation is a critical quality attributes (CQAs), as the aggregates not only reduce the efficacy of mAb biosimilar drug, but also can stimulate immunogenic responses, leading to various adverse events in treatment. Thus, the accurate analysis of monomer, dimer, and higher aggregates of mAb is required for developing higher quality therapeutics. We present here a robust, well-established size exclusion chromatography (SEC) approach for mAb aggregate analysis.

❑ Experimental

A bevacizumab biosimilar sample of 1 mg/mL was prepared in 50 mmol/L Tris-HCl (pH 8.0) buffer. The Tris-HCl buffer was prepared by dissolving 6.06 g of trizma base with 1 L of water, and adjust to pH 8.0 with 1 mol/L HCl. The sample was directly injected and analyzed by a Shimadzu Nexera Bio UHPLC with a UV detector. Analytical conditions are shown in Table 1.

Table 1: Aanlytical conditions of SEC for characterization of mAb aggregation

LC system	: Nexera Bio UHPLC
Column	: Shim-pack Bio Diol-300 (150 mm x 4.6 mm; 2 µm)
Column temperature	: Ambient
Mobile phase	: 150 mmol/L Sodium Phosphate buffer, pH 7.0, by mixing 150 mmol/L NaH_2PO_4 and 150 mmol/L Na_2HPO_4 stock solutions to the desired pH
Flow rate	: 0.2 mL/min
Elution mode	: Isocratic flow
Elution time	: 15 min
Injection volume	: 5 µL
Detector	: UV, 220 nm

❑ Results and Discussion

Figure 1 exhibits the relative abundance of monomer and dimer of bevacizumab biosimilar, in which the peaks were eluted quickly within 10 minutes. The result indicated that the bevacizumab biosimilar consists of about 1.9% dimer and 98.1% monomer. Injection-to-injection variability of UHPLC-UV system was evaluated as shown in Figure 2.

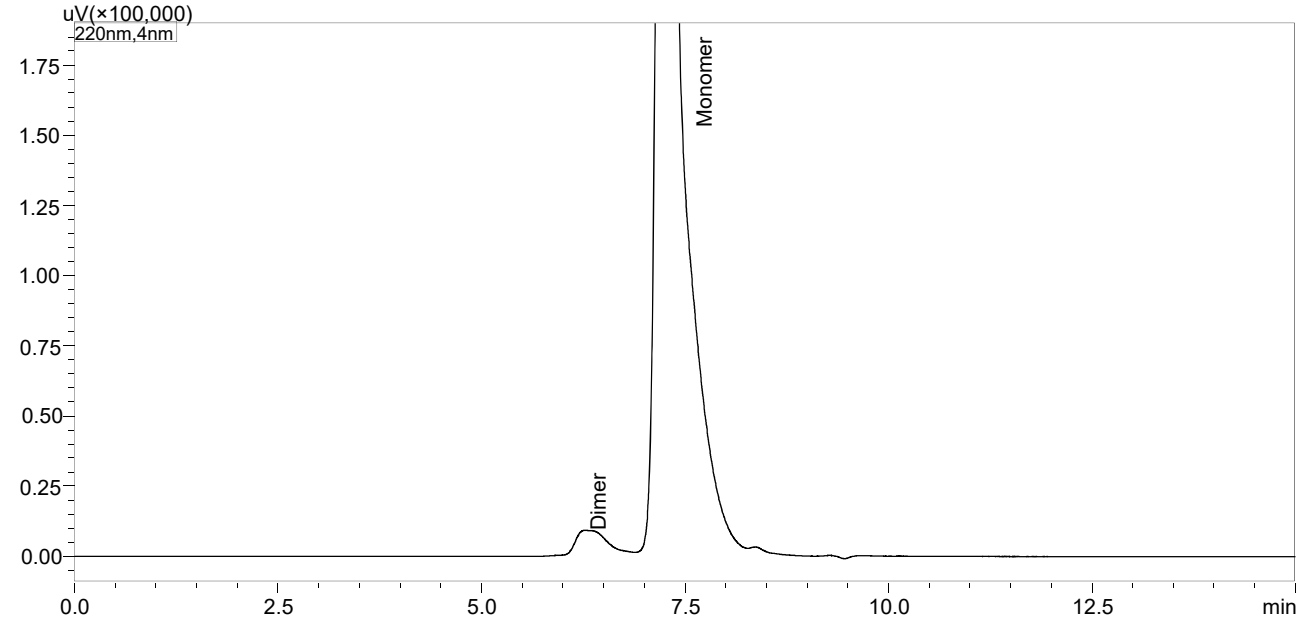

Figure 1: UHPLC-UV (220 nm) chromatogram of bevacizumab biosimilar

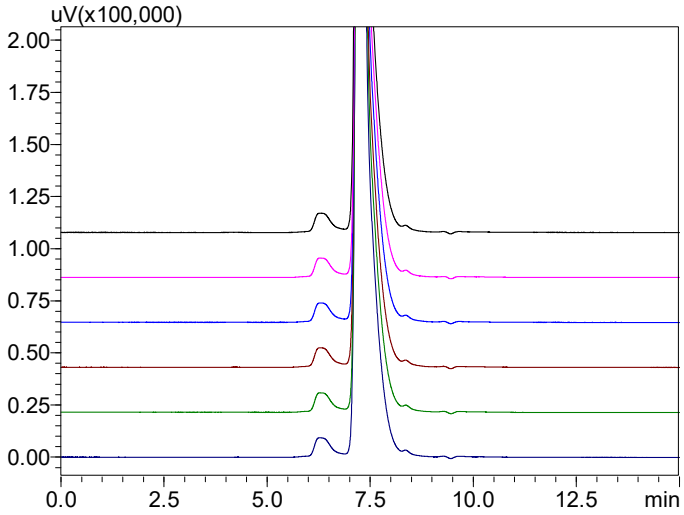

Figure 2 UHPLC-UV (220 nm) chromatograms of six injections of bevacizumab biosimilar sample.

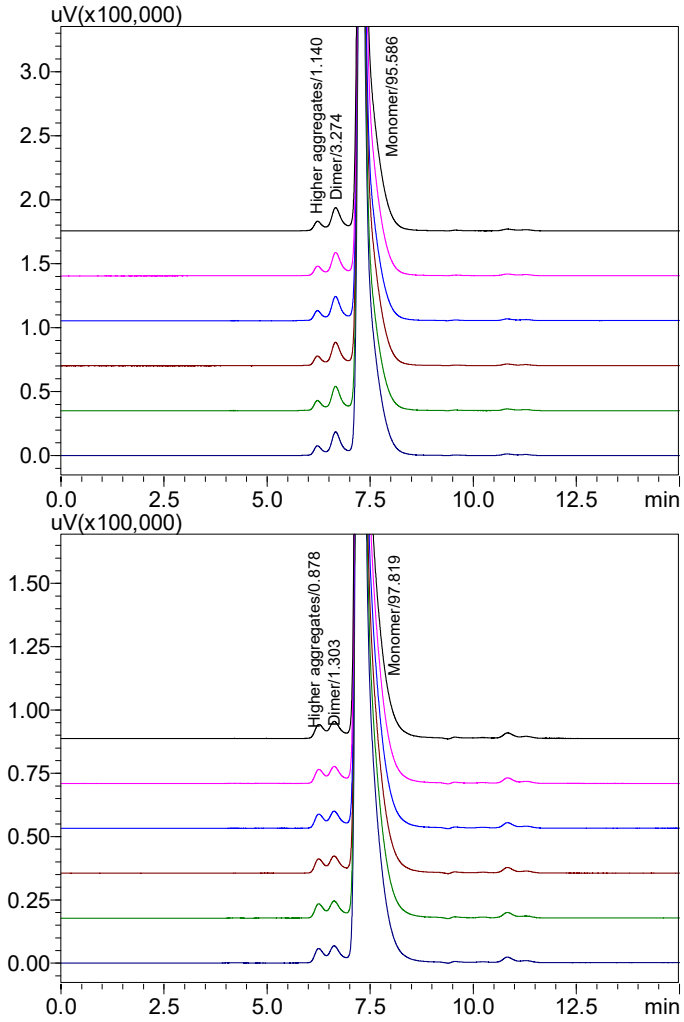

Figure 3 UHPLC-UV (220 nm) chromatograms of six injections of two unknown mAb (top and bottom). It shows perfect repeatability of chromatograms.

Table 2: Injection-to-injection repeatability of retention time and peak area (*n* = 6) of dimer and monomer of bevacizumab biosimilar

	Dimer		Momomer	
	RT	Area	RT	Area
Injection _1	6.276	213,014	7.245	11,104,320
Injection _2	6.287	213,566	7.252	11,101,718
Injection _3	6.289	213,197	7.246	11,100,827
Injection _4	6.291	212,678	7.249	11,092,490
Injection _5	6.292	212,686	7.251	11,066,707
Injection _6	6.291	212,615	7.244	11,035,553
Average	6.288	212,959	7.248	11,083,553
%RSD	0.094	0.176	0.046	0.246

The variations in retention time and peak area of six injections were less than 1% RSD for both monomer and dimer (Table 2). Moreover, the method and the UPHLC-UV system were used to analyze two other mAb samples (Figure 3). The results indicate that both mAb samples have a purity of more than 95% monomeric.

❑ Conclusions

A fast SEC method on the Nexera Bio UHPLC system is established and used for quantification of mAb aggregates. The method was evaluated for stability and repeatability in retention time and peak area (RSD < 1%).

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