ADVANCING ANALYTICAL APPROACHES FOR ROBUST EVALUATION OF LARGE MOLECULE BIOLOGICS USING LIQUID CHROMATOGRAPHY

Corey E. Reed, Andrew Steere, Paula Hong
Waters Corporation, Milford, MA 01757 (USA)

INTRODUCTION

- Size-Exclusion Chromatography (SEC) and Ion-Exchange Chromatography (IEX) are prominent analytical techniques for the analysis of biomolecules
- This study uses these two methods for the analysis of the monoclonal antibody (mAb) Infliximab
- The SEC method is used to compare results on a legacy high-performance liquid chromatography (HPLC) system, the Alliance™ iS Bio HPLC System, and a Competitive Bio HPLC System looking at relative areas for aggregation, fragmentation, and the monomer peak
- The IEX method is used to assess the robustness of the Alliance iS Bio HPLC System with a 30 day study using a high-salt gradient method and evaluating retention time and relative area stability.

SEC METHOD

- Systems:
  1. Alliance iS Bio HPLC System
  2. Alliance e2695 HPLC System
  3. Competitive Bio HPLC
- Mobile Phase: 60.3 mM K_2HPO_4, 140.3 mM KH_2PO_4, 249.5 mM KCl @ pH 6.2
- Column: XBridge™ Premier Protein SEC Column, 4.6 X 300 mm, 250 Å, 2.5 µm (p/n 186009960)
- Flow Rate: 0.3 mL/min (Isocratic)
- Column Temperature: ambient
- Sample Temperature: 6°C
- Injection Volume: 10 µL

IEX METHOD

- System: Alliance iS Bio HPLC System
- Column: BioResolve™ SCX mAb Column, 4.6 X 100 mm, 3 µm (p/n 186009060)
- Mobile Phase A: 100 mM MES Monohydrate
- Mobile Phase B: 100 mM MES Sodium Salt
- Mobile Phase C: 1 M NaCl
- Mobile Phase D: Water
- Flow Rate: 0.5 mL/min
- Sample Temp.: 6°C
- Injection V.: 10 µL
- Column Temp.: 30°C
- Gradient:

Time (min)	% A	% B	% C	% D	Curve
0.00	4.7	15.3	2.5	77.5	Initial
1.00	4.7	15.3	2.5	77.5	6
31.00	4.5	15.5	7.5	72.5	6
32.00	3.8	16.2	50.0	30.0	6
35.00	3.8	16.2	50.0	30.0	6
35.01	4.7	15.3	2.5	77.5	6
50.00	4.7	15.3	2.5	77.5	6

RESULTS & DISCUSSION

Size-Exclusion Comparison

SEC is a widely used analytical technique for monitoring aggregation and fragmentation of biologics as the separation mechanism (under ideal conditions) should rely purely on the hydrodynamic radii of the analytes in solution. Additionally, because there is no re-focusing of analyte at the head of the column in SEC, system dispersion can have a major effect on the quality of results in the form of peak shape. Figure 1 displays the same SEC separation on 3 competitive HPLC systems. The Alliance iS Bio HPLC System demonstrates the lowest in class system dispersion, best peak shape, and greatest resolution between the monomer and high-molecular weight species (HMWS) out of the systems tested. This translates into more easily integrated results and a more accurate quantitative measurement on the amounts of aggregation (HMWS) and fragmentation (LMWS) in the sample. Results for the relative areas of each component in the sample are given in Table I.

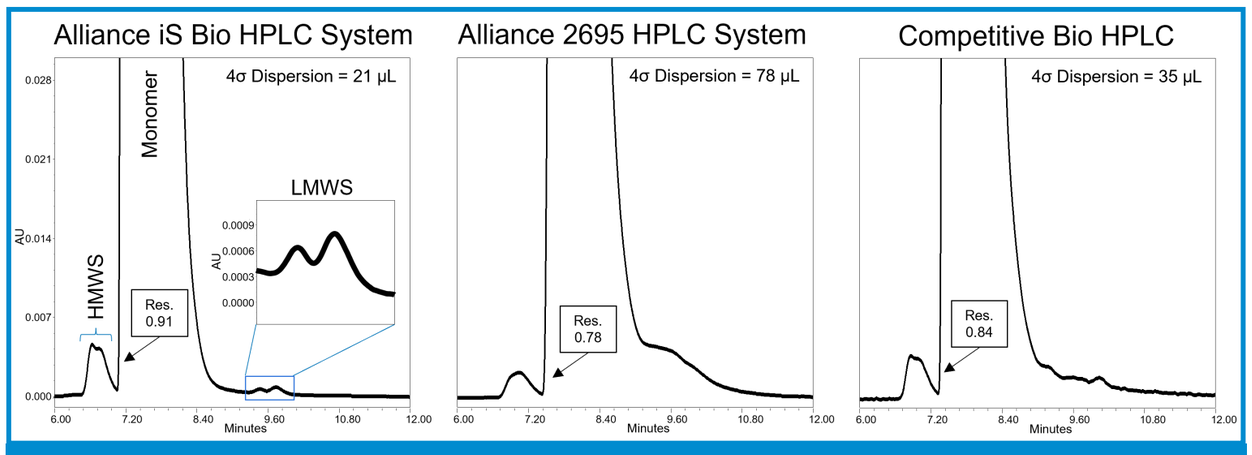

Figure 1: SEC separations of Remicade™ (Infliximab) on 3 HPLC systems. HMWS = high-molecular weight species. LMWS = low-molecular weight species. Resolution (Res.) = USP resolution. The drug product shown is analyzed past expiry. Chromatograms are a representative single injection on each system.

Table I:			
Measure	Alliance iS Bio System	Alliance e2695 System	Competitive Bio
HMWS % Area	0.47	0.54	0.47
Monomer % Area	99.44	99.46	99.50
LMWS % Area	0.09	0.00	0.04

Ion-Exchange Salt Gradient Robustness Testing

IEX is a widely used method for the analysis of lysine charge variants of mAbs. In order to analyze the robustness of the Alliance iS Bio HPLC System for use in a high salt application a salt gradient IEX separation was performed over the course of 30 days. (Figure 2) The Alliance iS Bio HPLC System demonstrated excellent repeatability over the course of this study, achieving %RSDs for relative area of the 3 charge variant peaks well below 1%. (Table II) Additionally, acidic and basic variants of the lysine charge variants remain consistent over the course of the study. In total 1,290 injections were performed on the Alliance iS Bio HPLC System over 30 days (including blanks and standards) and retention times for the lysine charge variant peaks remained consistent, demonstrating %RSDs of less than 1.5%. (Figure 3)

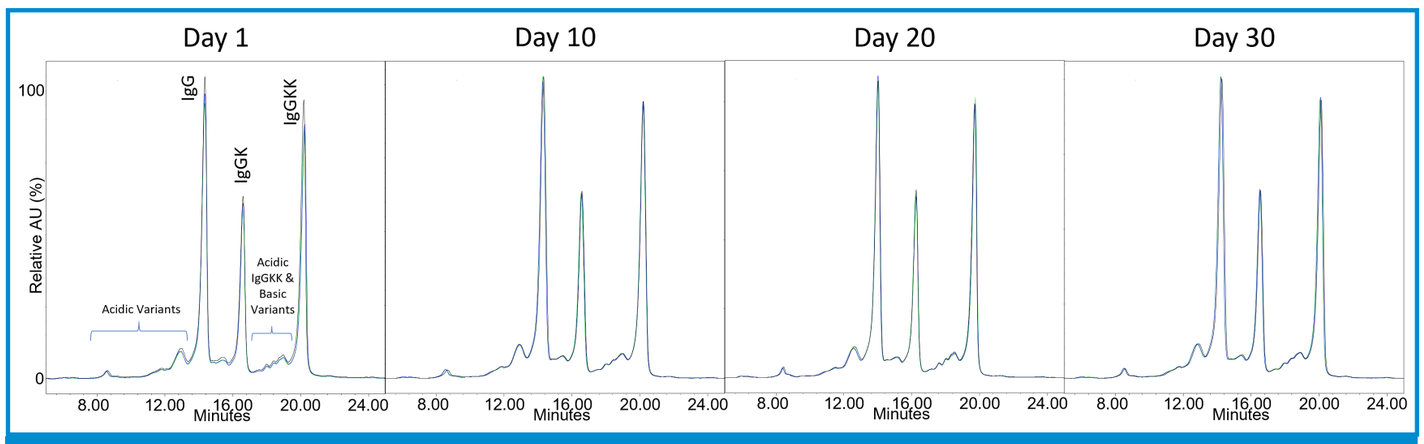

Figure 2: IEX salt gradient separation of Infliximab over 30 days on an Alliance iS Bio HPLC System. Responses are shown as a percent in relative AU. N=3 in all cases. IgG = Immunoglobulin G. IgGK = Immunoglobulin G with a single additional lysine. IgGKK = Immunoglobulin G with 2 additional lysine. The drug product shown is analyzed past expiry. RSDs are calculated from the average of each day, minus day 18 where an empty vial resulted in null-injections (N=29).

Table II:	
Component	Relative Area % RSD
IgG % Area	0.39%
IgGK % Area	0.22%
IgGKK % Area	0.34%

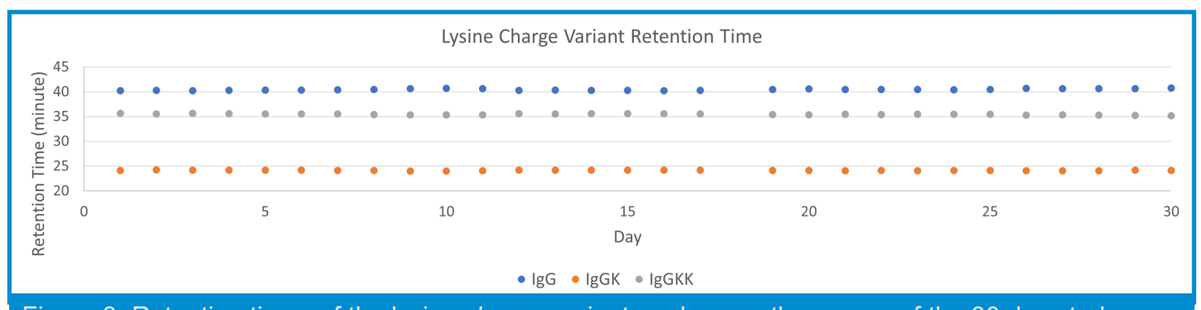

Figure 3: Retention times of the lysine charge variant peaks over the course of the 30 day study. Series in blue are IgG, orange are IgGK, and grey are IgGKK. Day 18 is excluded due to an empty vial resulting in null-injections. Each data point is an average of triplicate injections.

CONCLUSION

- **Lower dispersion of the Alliance iS Bio HPLC System results in more reliable SEC results for aggregate and fragment monitoring of mAbs**
- **Robustness of the Alliance iS Bio HPLC System results in relative area %RSDs of less than 1% and retention time % RSDs of less than 1.5% for lysine charge variant detection of a mAb using a salt gradient IEX method over the course of a 30-day study**
- **The Alliance iS Bio HPLC System is suitable for use in the analysis of biomolecules, particularly for high-salt applications such as SEC and IEX**

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