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Application Note

USP Monograph mAb SEC Method Robustness on the XBridge Premier Protein SEC Column

Hua Yang, Stephan M. Koza, Ying Qing Yu

Waters Corporation

Abstract

Size-exclusion chromatography (SEC) has been widely used for size-based impurity analysis. We show here that the USP monograph method for SEC-based mAb analyses is robust using Waters XBridge Premier Protein SEC 250 Å, 2.5 mm Columns for measuring HMWS, monomers, and LMWS of monoclonal antibodies via the coupling of SEC performance benefits using Waters MaxPeak Premier High Performance Surfaces (HPS) and BEH polyethylene SEC Particle technologies. The USP method run on XBridge Premier Protein SEC Columns can serve as an effective starting point in method development for measuring size-based impurities in other monoclonal antibodies.

Results obtained using the XBridge Premier Protein SEC 250 Å, 2.5 µm Columns were consistent with those using a 7.8 x 300 mm column packed with 5 um particles and a 250 Å pore size L59 SEC Column and mobile phase as specified in the USP method. Additionally, the XBridge Column was shown to be robust with respect to SEC mobile phase variations in pH and ionic strength, and column-to-column variation.

Benefits

- Robust USP SEC method for mAb size variant analysis obtained using Waters XBridge Premier Protein SEC

250 Å, 2.5 µm Column

- Waters XBridge Premier Protein SEC 250 Å, 2.5 µm Column is a USP listed L59 column that provides comparable quantitative results to the results obtained from the L59 column specified in the USP monograph for mAb analysis. Additionally, the XBridge Column also provides significantly greater resolution for both HMWS and LMWS impurities
- Demonstrated column-to-column reproducibility for the USP mAb SEC analysis

Introduction

United States Pharmacopoeia (USP) General Chapter <129> (Analytical Procedures for Recombinant Therapeutic Monoclonal Antibodies) provides the procedure for determining impurities in recombinant therapeutic monoclonal antibodies (mAbs) by size exclusion chromatography (SEC).[1] The method uses a 7.8 x 300 mm SEC Column containing 5 um particles with a 250 Å pore size to monitor high molecular weight species (HMWS), mAb monomers, as well as low molecular weight species (LMWS). The LMWS is presumed to be the one-third of the mAb (Fab or Fc domain).[2]

The coupling of Waters advancement using MaxPeak Premier High Performance Surfaces (HPS) with novel BEH-based, hydroxy-terminated polyethylene SEC particle bonding technologies synergistically combine to minimize non-desired secondary ionic interactions and hydrophobic interactions to help obtain reliable SEC analysis of mAb size variants.

In this application note, we show that the USP method is robust for the USP Monoclonal IgG standard when using a Waters XBridge Premier Protein SEC 250 Å, 7.8 x 300 mm Column. In addition, consistent results were obtained using the XBridge Column compared to use of a 7.8 x 300 mm SEC Column packed with 5 µm particles as specified for the USP method in measuring HMWS and LMWS of the four biosimilar monoclonal antibodies (mAbs) currently marketed in the United States of America.

Experimental

Sample Description

Monoclonal IgG System Suitability standard was purchased from USP. Biosimilar mAbs were bevacizumab (Mvasi), infliximab (Avsola), and rituximab (Ruxience) and trastuzumab was the originator (Herceptin).

LC Conditions

LC system:	ACQUITY UPLC H-Class Bio
Detection:	ACQUITY UPLC TUV Detector with 5 mm titanium flow cell, wavelength: 280 nm
Vials:	Polypropylene 12 x 32 mm Screw Neck Vial, with Cap and Pre-slit PTFE/Silicone Septum, 300 µL Volume, 100/pk (p/n: 186002639)
Column(s):	XBridge Premier Protein SEC 250 Å, 2.5 µm, 7.8 x 300 with mAb Size Variant Standard (p/n: 176005070) BioSuite Diol (OH) Column, 250 Å, 5 µm, 7.8 x 300 mm (p/n: 186002165)
Column temperature:	Ambient
Sample temperature:	10 °C
Injection volume:	5–20 uL

Flow rate:	0.5–1 mL/min
Mobile phase A:	400 mM potassium phosphate, monobasic
Mobile phase B:	400 mM potassium phosphate, dibasic
Mobile phase C:	1 M potassium chloride
Mobile phase D:	water
Buffer concentrated to deliver:	200 mM

Gradient Table (an Auto·Blend Method, an empirical table was used)

Time (min)	Flow (mL/min)	pH	Salt (mM)	Salt curve
0.0	0.5	6.2	250	
35.0	0.05	6.2	250	11

An equivalent gradient table for a generic quaternary LC system is shown above.

Time (min)	%A	%B	%C	%D
0.0	36.7	13.3	25.0	25.0
35.0	36.7	13.3	25.0	25.0

Results and Discussion

A. Robustness of the USP Method on XBridge Premier Protein SEC Column

As shown in Figure 1, USP Monoclonal IgG was analyzed on an XBridge Premier Protein SEC 250 Å, 2.5 µm Column (7.8 x 300 mm). The mobile phase used in the USP method is 200 mM potassium phosphate and 250 mM potassium chloride, pH 6.2. Four additional mobile phase conditions were also run to investigate the robustness of the method by varying the pH (6.0 and 6.4) and the ionic strength (200 mM and 300 mM potassium chloride).

Similar separations were obtained using various mobile phase conditions (Figure 1b), indicating that the USP method is robust within the pH and ionic strength range tested using the same XBridge Premier Protein SEC 250 Å, 2.5 µm, 7.8 x 300 Column. The quantitative criteria set in the USP General Chapter <129> is that the percent peak area of the HMWS must be 0.4%–0.67% and that the percent peak area of the LMWS must be no more than (NMT) 0.2%. As shown in Figure 1b, the HMWS eluted between 10.7–12.3 minutes and the LMWS eluted between 15.5–16.3 minutes. The average percent peak area of both HMWS and LMWS meets the criteria under all the mobile phase conditions, as indicated in the chromatograms (n=2).

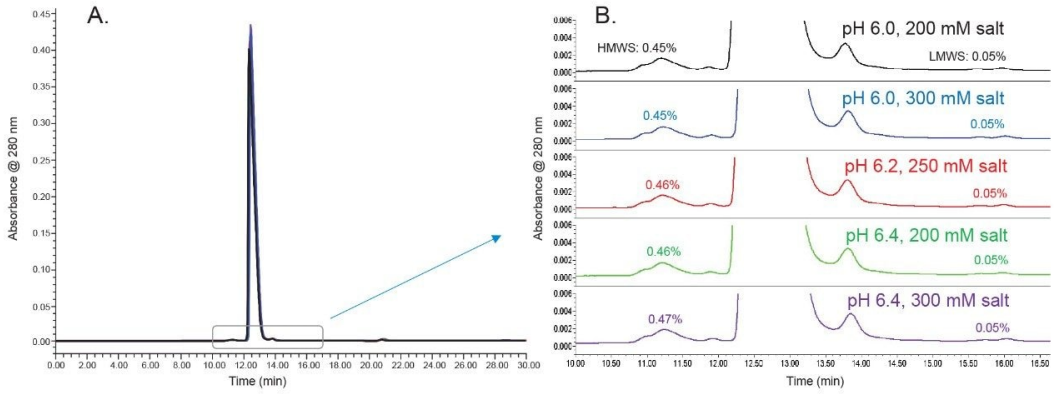

Figure 1. USP Monograph Method for Monoclonal IgG size variant determinations was analyzed on an XBridge Premier Protein SEC 250 Å, 2.5 µm SEC Column under five mobile phase conditions. A. Overlay of the chromatograms in full scale. B. Zoomed-in view of HMWS and LMWS, the average percent peak area of which shown on each chromatogram (n=2).

Figure 2 shows the analysis on a L59 SEC Column containing 5 um particles (*i.e.* USP Method column for Monoclonal IgG size variant determinations) using the USP specified mobile phase. The average percent peak areas of HMWS and LMWS are shown in the chromatogram and meet the quantitative criteria (n=2).

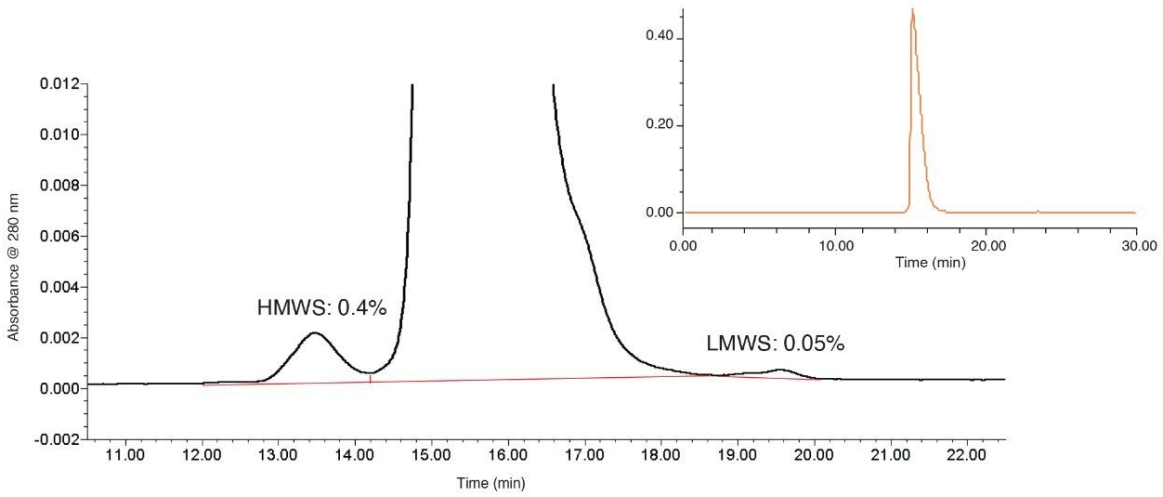

Figure 2. USP Monograph Method for Monoclonal IgG size variant determinations was analyzed on an USP SEC Method Column containing 5 µm particles using the mobile phase conditions specified in General Chapter <129> (200 mM potassium phosphate and 250 mM potassium chloride, pH 6.2). The average percent peak area of HMWS and LMWS is shown on the chromatogram (n=2). The insert shows a full-scale view of the chromatogram.

B. Impurity Analysis of Biosimilar mAbs Using the USP Method

The USP Monoclonal IgG and samples of the four mAbs (bevacizumab, infliximab, rituximab, trastuzumab) currently marketed in the USA were analyzed using the USP method on three XBridge Premier Protein SEC 250 Å, 2.5 µm columns from different batches and one SEC Column containing 5 µm particles (Figures 3–7). The flow rate is 0.5 mL/min for all the columns except that for the Premier Column #3, the flow rate is 1 mL/min when analyzing the biosimilar and originator mAbs. Duplicate injections were made on most the columns (n=2). The error bar indicates the range of results.

Consistent results were obtained for both the HMWS and the LMWS for all five mAbs using the XBridge Premier Protein SEC 250 Å, 2.5 µm particles from three batches as well as the USP Method recommended column detailed above. For LMWS1, Premier Column #3 resulted in lower percentage, likely due to loss of resolution between the monomer peak and LMWS1 at the higher flow rate. In most cases, however, the percent peak area of HMWS and LMWS

is very similar among all three XBridge Premier Protein SEC 250 Å, 2.5 µm Columns regardless of the flow rate, suggesting that the XBridge Premier Protein SEC 250 Å, 2.5 µm Column can be used for higher sample throughput. For all the mAbs tested, LMWS1 was not adequately separated on the USP Method column for reliable quantitation. Therefore, no data was included in the plot. In some cases, the measured percent peak area of HMWS1 is lower on the USP Method column than on the XBridge Premier Protein SEC 250 Å, 2.5 µm Columns, likely because the resolution between the HMWS1 and the monomer peak is lower on the USP Method column containing 5 µm particles.

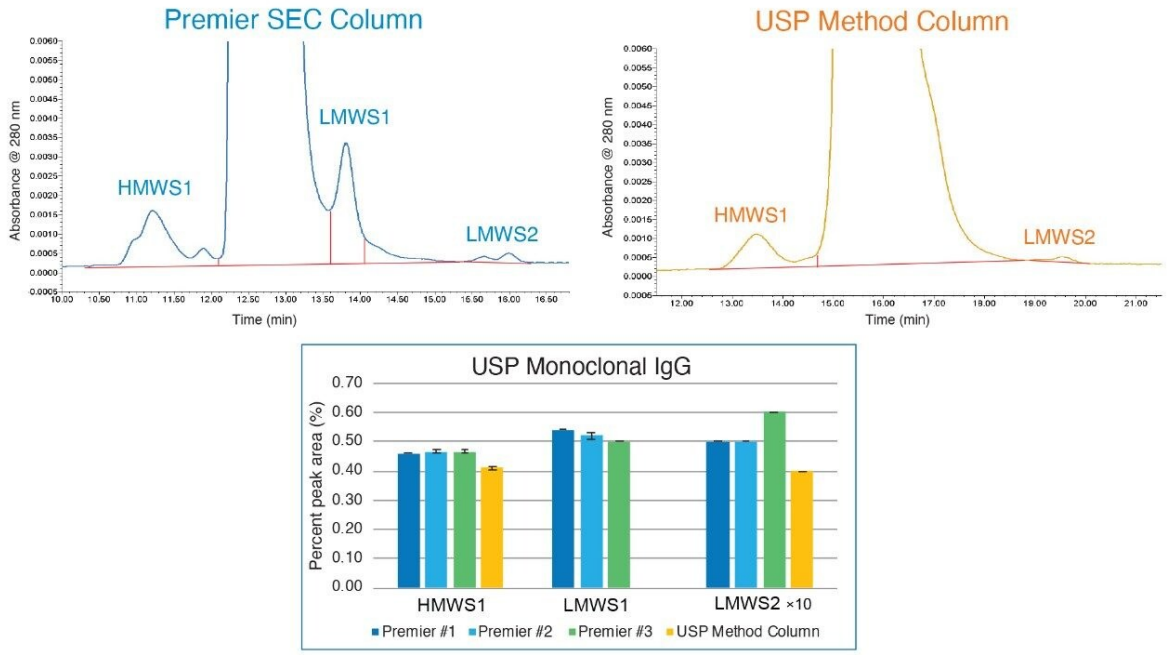

Figure 3. Comparison of the results obtained from USP Monoclonal IgG size variant determinations on XBridge Premier Protein SEC 250 Å, 2.5 µm SEC Columns from three different batches and one USP SEC Method Column containing 5 µm particles using the mobile phase conditions specified in General Chapter <129>. For better observation, the percent peak area for LMWS2 is plotted 10x higher than the actual values.

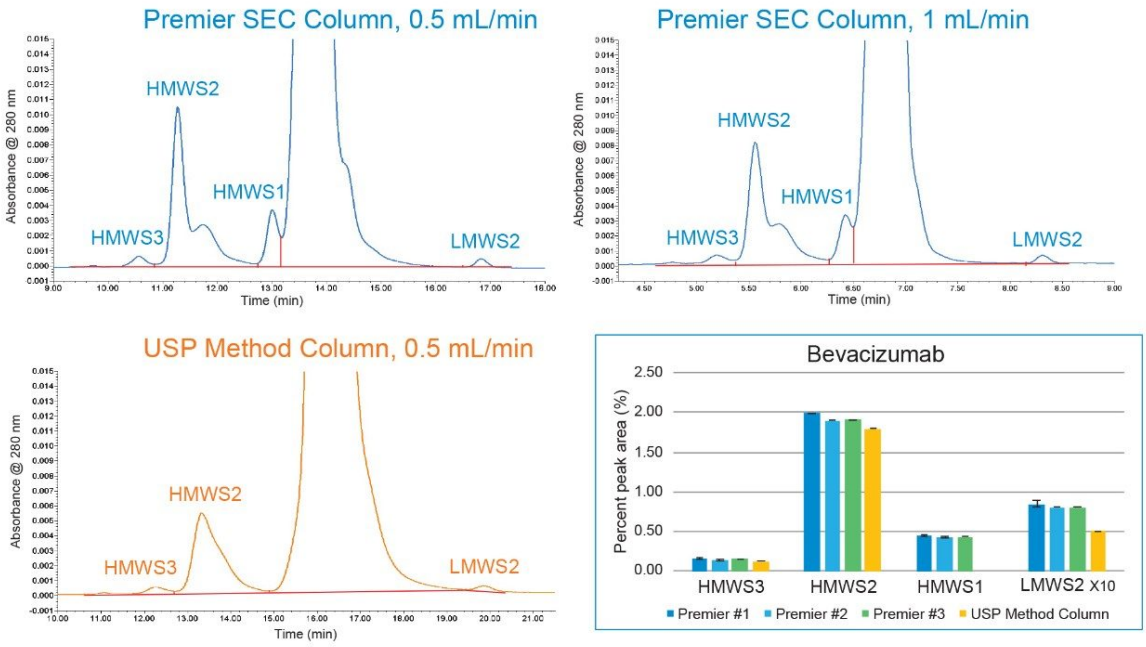

Figure 4. Comparison of the results obtained from analysis of bevacizumab biosimilar on XBridge Premier Protein SEC 250 Å, 2.5 µm SEC Columns from three different batches and one USP SEC Method Column containing 5 µm particles using the mobile phase conditions specified in General Chapter <129>. The flow rate is 0.5 mL/min, except that for Premier Column #3, the flow rate is 1 mL/min. For better observation, the percent peak area for LMWS2 is plotted 10x higher than the actual values.

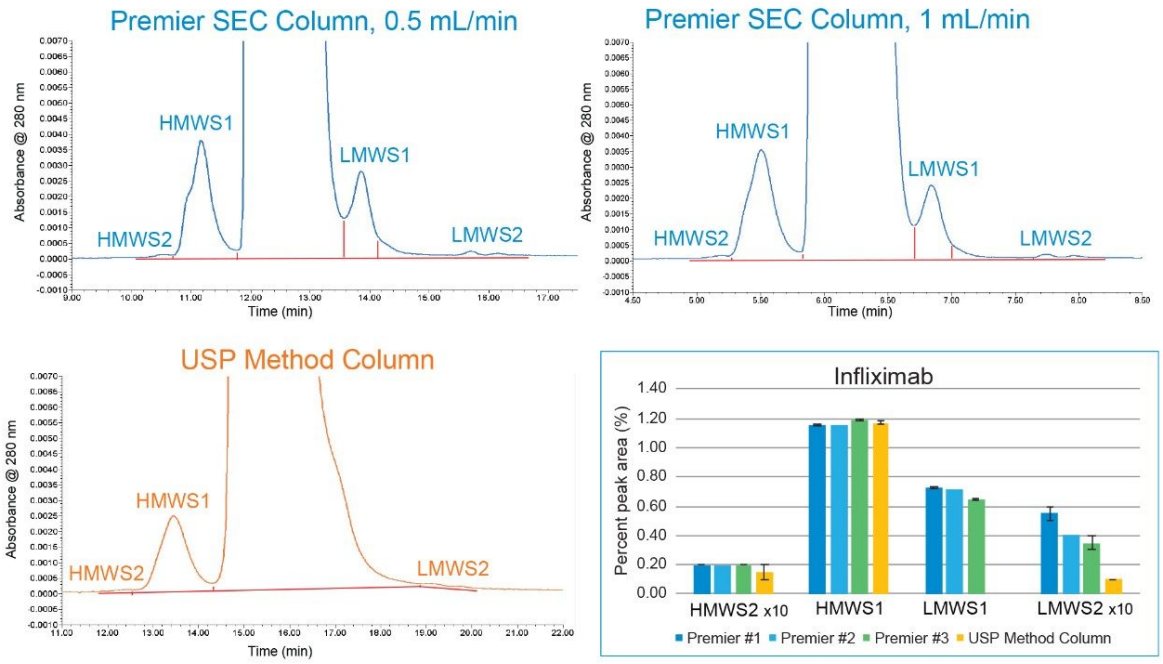

Figure 5. Comparison of the results obtained from analysis of infliximab biosimilar on XBridge Premier Protein SEC 250 Å, 2.5 µm SEC Columns from three different batches and one USP SEC Method Column containing 5 µm particles using the mobile phase conditions specified in General Chapter <129>. The flow rate is 0.5 mL/min, except that for Premier Column #3, the flow rate is 1 mL/min. For better observation, the percent peak area for HMWS2 and LMWS2 is plotted 10x higher than the actual values.

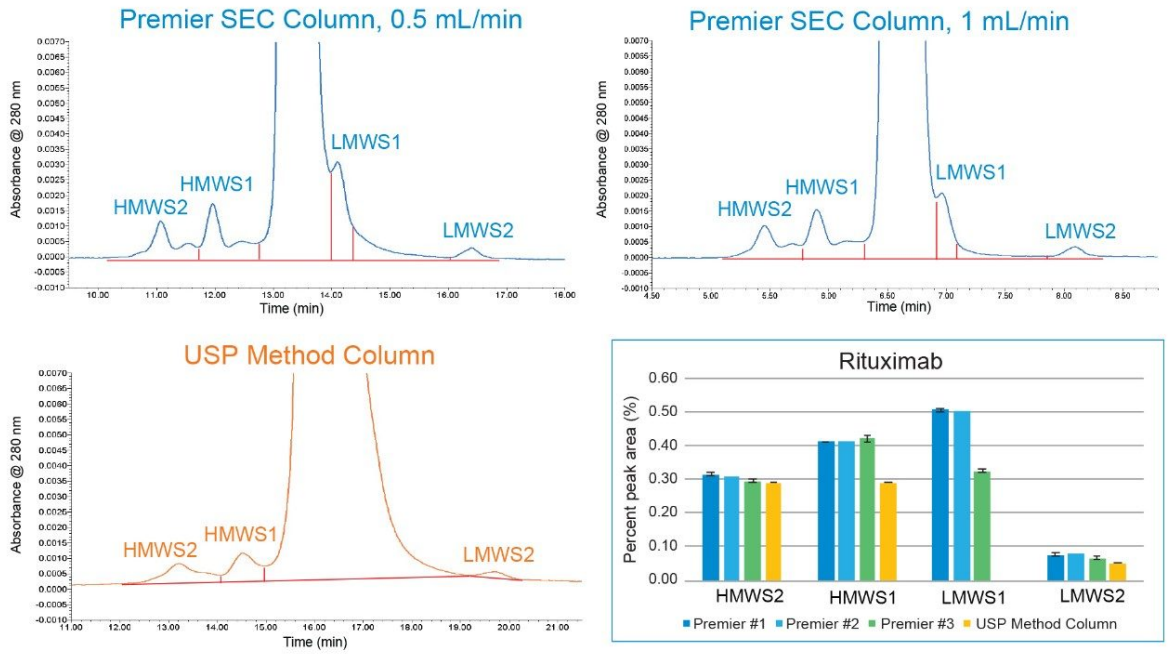

Figure 6. Comparison of the results obtained from analysis of rituximab biosimilar on XBridge Premier Protein SEC 250 Å, 2.5 µm SEC Columns from three different batches and one USP SEC Method Column containing 5 µm particles using the mobile phase conditions specified in General Chapter <129>. The flow rate is 0.5 mL/min, except that for Premier Column #3, the flow rate is 1 mL/min.

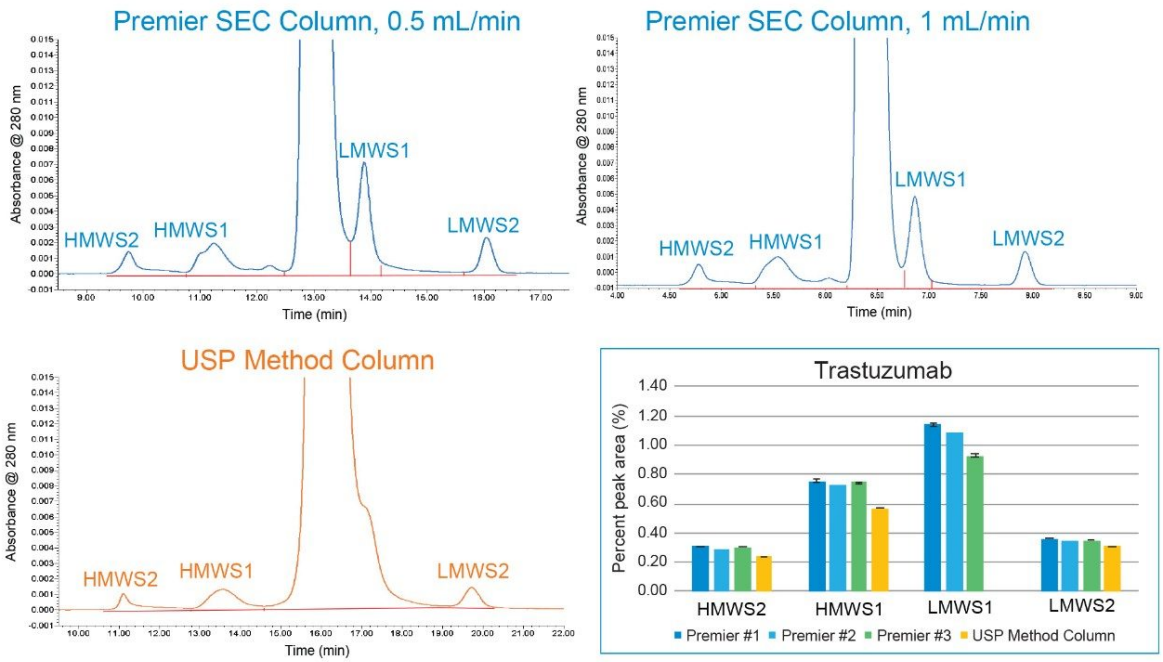

Figure 7. Comparison of the results obtained from analysis of trastuzumab on XBridge Premier Protein SEC 250 Å, 2.5 µm SEC Columns from three different batches and one USP SEC Method Column containing 5 µm particles using the mobile phase conditions specified in General Chapter <129>. The flow rate is 0.5 mL/min, except that for Premier Column #3, the flow rate is 1 mL/min.

Conclusion

The USP method for measuring HMWS and LMWS of mAbs is robust using Waters XBridge Premier Protein SEC 250 Å, 2.5 µm Columns. Consistent results were obtained using the XBridge Premier Protein SEC 250 Å, 2.5 µm Columns and comparable to results obtained on the USP Method column containing 5 µm particles.

Therefore, the USP monograph mAb SEC method run on the XBridge Column can serve as a starting point in method development for measuring size-based impurities in monoclonal antibodies. In addition, the XBridge Column also permits a 2-fold higher sample throughput in comparison to use of the specified USP SEC Method Column packed with 5 µm particles.

References

1. Analytical Procedures for Recombinant Therapeutic Monoclonal Antibodies. USP General Chapter <129>. 2017.
2. Hong P.; Koza S. M.; Fountain K. J. Analysis of Proteins by Size-Exclusion Chromatography Coupled With Mass Spectrometry Under Non-denaturing Conditions. Waters Application Note. 720004254EN. 2012.

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