TOSOH

APPLICATION NOTE

UHPLC ANALYSIS OF BIOSIMILARS USING SIZE EXCLUSION CHROMATOGRAPHY

INTRODUCTION

A biosimilar is a biological medicine highly similar to an approved biological medicine (originator) in terms of structure, biological activity and efficacy, safety and immunogenicity profile. In order to demonstrate biosimilarity, the biosimilar manufacturer must generate an array of data comparing the proposed product to the approved reference product; a detailed analytical characterization and comparison of the products marks the first step in this process.

A series of analytical chromatographic techniques are used to characterize similarity. When it comes to the monitoring of aggregation and fragmentation of a therapeutic protein, SEC is the standard technology for the characterization of a biosimilar molecule versus the reference product. In this application, a TSKgel® UP-SW3000 UHP-SEC column was used to elucidate the molecular similarity between Ipilimumab and Adalimumab biosimilar and the reference product Yervoy® and Humira®.

UHPLC ANALYSIS OF YERVOY AND BIOSIMILAR

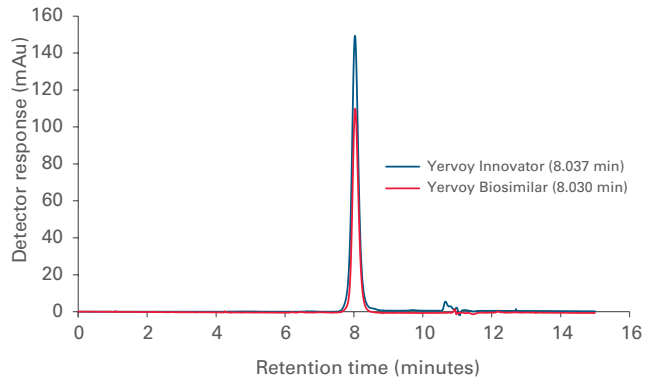

Figure 1

UHPLC ANALYSIS OF YERVOY AND BIOSIMILAR - ZOOMED-IN PROFILE

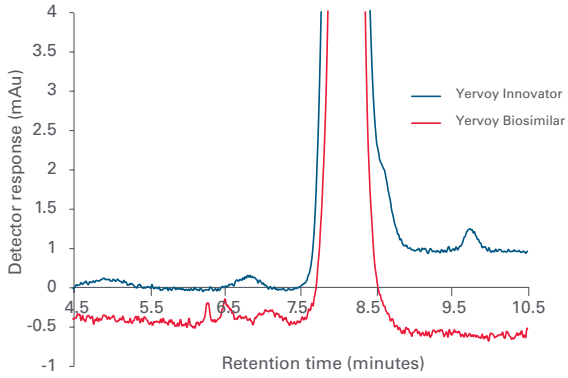

Figure 2

EXPERIMENTAL HPLC CONDITIONS

Column:	TSKgel UP-SW3000, 2 µm, 4.6 mm ID × 30 cm L
Mobile phase:	100 mmol/L KH_2PO_4/Na_2HPO_4, pH 6.7, 100 mmol/L, Na_2SO_4, 0.05% NaN_3
Gradient:	isocratic
Flow rate:	0.35 mL/min
Detection:	UV @ 280 nm
Temperature:	25 °C
Injection vol.:	5 µL
Samples:	Humira Innovator Reference Product (5 mg/mL) Humira Biosimilar (4 mg/mL) Yervoy Innovator Reference Product (5 mg/mL) Yervoy Biosimilar (3.7 mg/mL)

Humira is a registered trademark of AbbVie Inc.

Yervoy is a registered trademark of Bristol-Myers Squibb Company.

RESULTS AND DISCUSSION

Yervoy and its biosimilar were subsequently injected onto a TSKgel UP-SW3000, 2 µm SEC column in order to analytically assess the similarities between the two products. Figure 1 shows that Yervoy and its biosimilar display similar retention times at.

In Figure 2, a zoomed-in profile provides a closer look at the baseline and impurities present in each sample. Table 1 shows the numerical values that are depicted in Figure 2. The relative percent peak area calculation clearly shows minimal impurities in each sample; both monomers are >99% pure.

In order to assess the reproducibility of results, retention time, asymmetry and theoretical plates, were plotted over

COMPARISON OF RETENTION TIME AND PERCENT PEAK AREA FOR YERVOY AND BIOSIMILAR

	Retention time (minutes)			
	HMW	Dimer	Monomer	Fragment
Yervoy Innovator	4.837	6.780	8.030	9.707
Yervoy Biosimilar	6.19, 6.223, 6.447	6.987	8.037	-
	% Peak area (mAU* min)			
	HMW	Dimer	Monomer	Fragment
Yervoy Innovator	0.18	0.20	99.47	0.15
Yervoy Biosimilar	0.26	0.23	99.56	0

Table 1

six consecutive injections for both Yervoy as well as its biosimilar. Table 2 shows that the relative standard deviation (RSD) was low across all of the peak parameters that were analyzed.

Humira and its biosimilar were then analyzed using a TSKgel UP-SW3000, 2 µm SEC column in order to elucidate and compare the SEC profiles of the two molecules (Figure 3).

REPRODUCIBILITY OF PEAK PARAMETERS FOR YERVOY AND BIOSIMILAR ACROSS MULTIPLE INJECTIONS

	Yervoy Innovator			Yervoy Biosimilar		
	RT (min)	As	N	RT (min)	As	N
	8.04	1.10	10617	8.03	1.05	8279
	8.04	1.08	10618	8.03	1.10	8279
	8.04	1.06	10614	8.02	1.11	8276
	8.04	1.07	10628	8.02	1.09	8276
	8.04	1.07	10550	8.01	1.10	8255
	8.04	1.08	10455	8.02	1.09	8572
Avg.	8.04	1.08	10580.33	8.02	1.09	8322.83
Std. dev.	0.00	0.01	67.52	0.01	0.02	122.40
% RSD	0.02	1.27	0.64	0.07	1.92	1.47

Table 2

UHPLC ANALYSIS OF HUMIRA AND BIOSIMILAR

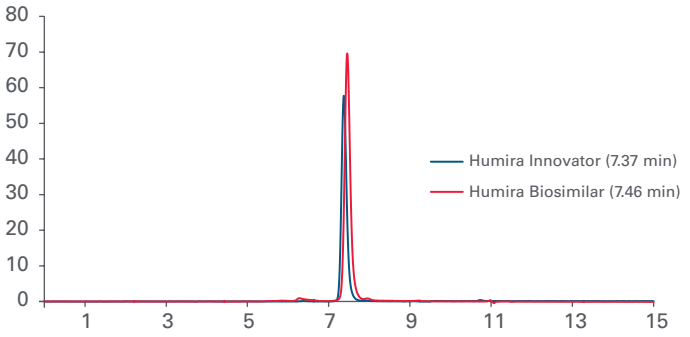

Figure 3

UHPLC ANALYSIS OF HUMIRA AND BIOSIMILAR WITH TSKgel UP-SW3000: ZOOMED-IN PROFILE

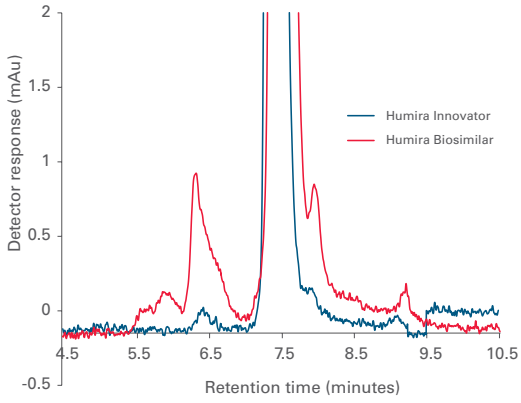

Figure 4

The zoomed-in profile depicted in Figure 4 provides a better look at the aggregates and fragments present in each sample. Table 3 outlines the retention time and relative percent peak area values that are shown in the chromatogram. The relative peak area calculation suggests a larger percentage of impurities, predominantly aggregate, are present in the biosimilar compared to the innovator drug.

Reproducibility was then analyzed by plotting retention time, asymmetry and theoretical plates over six consecutive injections for Humira and its biosimilar. Table 4 shows that results were reproducible from injection-to-injection.

COMPARISON OF RETENTION TIME AND PERCENT PEAK AREA FOR HUMIRA AND BIOSIMILAR

	Retention time (minutes)			
	HMW	Dimer	Monomer	Fragment
Humira Innovator	0	6.387	7.37	7.883, 9.097
Humira Biosimilar	5.843	6.283	7.46	7.960, 9.217
	% Peak area (mAU* min)			
	HMW	Dimer	Monomer	Fragment
Humira Innovator	0	0.27	99.59	0.14
Humira Biosimilar	0.15	1.73	97.66	0.46

Table 3

REPRODUCIBILITY OF PEAK PARAMETERS FOR HUMIRA AND BIOSIMILAR ACROSS MULTIPLE INJECTIONS

	Yervoy Innovator			Yervoy Biosimilar		
	RT (min)	As	N	RT (min)	As	N
	7.38	1.20	15082	7.46	1.20	13781
	7.38	1.20	14876	7.46	1.20	13781
	7.37	1.20	15082	7.46	1.20	13704
	7.37	1.20	15254	7.46	1.20	13607
	7.37	1.20	15247	7.46	1.20	13506
	7.37	1.20	15282	7.46	1.20	13487
Avg.	7.37	1.20	15137.17	7.46	1.20	13644.33
Std. dev.	0.00	0.00	155.55	0.00	0.00	131.30
% RSD	0.03	0.00	1.03	0.00	0.00	0.96

Table 4

CONCLUSIONS

UHP-SEC using a TSKgel UP-SW3000 column is a powerful tool to show the similarity between the innovator drug and the biosimilar with regard to the HMW and LMW impurities, as a function of hydrodynamic radii of the molecules. Reproducibility of consecutive injections yielded very low percent RSD when analyzing peak parameters such as retention time, peak area, peak asymmetry, and number of theoretical plates. Robustness and reproducibility are indispensable prerequisites for using SEC columns in quantitative analysis.