TOSOH

Application Note

UHPLC Analysis of Biosimilars Using Size Exclusion Chromatography

Introduction

In order to provide more treatment possibilities, increase accessibility to lifesaving medicines and possibly decrease health care costs through competition, Congress implemented a shortened licensure path for biological products that are proven to be biosimilar to an FDA-approved biological product. In order to demonstrate biosimilarity, the manufacturer of the proposed biosimilar must generate an array of data comparing the proposed product to the FDA-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 the similarity such as size exclusion chromatography (SEC), reversed phase chromatography (RPC), hydrophilic interaction chromatography (HILIC), and ion exchange chromatography (IEX). SEC is an important chromatographic technique for the analytical characterization of a biosimilar molecule versus the reference product due to its utility in monitoring the aggregation and fragmentation of the molecule. In this application note, a TSKgel® UP-SW3000 SEC column was used to elucidate the molecular similarity between Humira® and Yervoy® biosimilars and the corresponding innovator reference products using UV detection.

Experimental HPLC Conditions

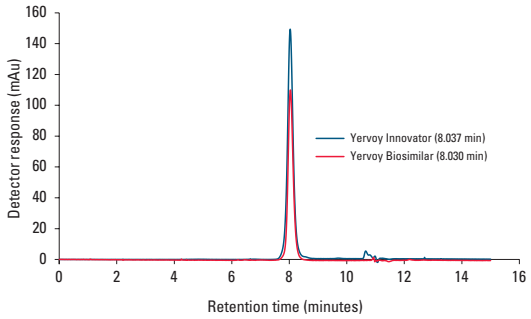
Quality Control Conditions

Column:	TSKgel UP-SW3000, 2 µm, 4.6 mm ID × 30 cm
Instrument:	Thermo Fisher Dionex Ultimate® 3000 with Chromeleon® v. 6.8 UHPLC
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)

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 8.037 minutes and 8.030 minutes respectively.

Figure 1. UHPLC Analysis of Yervoy and Biosimilar with TSKgel UP-SW3000: Retention Time Comparison



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.

Figure 2. UHPLC Analysis of Yervoy and Biosimilar with TSKgel UP-SW3000: Zoomed-in Profile

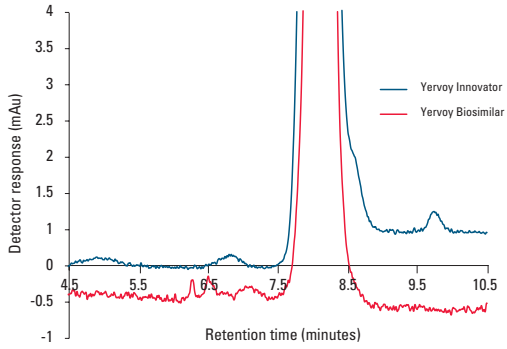


Table 1. Comparison of Retention Time and Percent Peak Area for Yervoy and Biosimilar

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

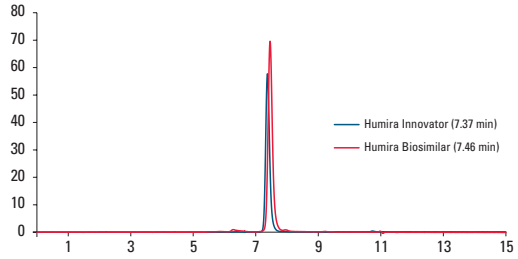
In order to assess the reproducibility of results, retention time, asymmetry and theoretical plates, were plotted over six consecutive injections for both Yervoy as well as its biosimilar. *Table 2* shows that the relative standard deviation (RSD) was low across all of the peak parameters that were analyzed.

Table 2. 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	Avg.	8.02	1.09	8322.83
Std. dev.	0.00	0.01	67.52	Std. dev.	0.01	0.02	122.40
% RSD	0.02	1.27	0.64	% RSD	0.07	1.92	1.47

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* shows that Humira and its biosimilar show slight variability in retention times at 7.37 minutes and 7.46 minutes respectively.

Figure 3. UHPLC Analysis of Humira and Biosimilar with TSKgel UP-SW3000: Retention Time Comparison



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.

Figure 4. UHPLC Analysis of Humira and Biosimilar with TSKgel UP-SW3000: Zoomed-in Profile

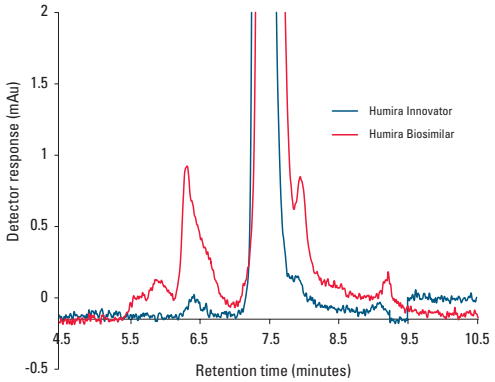


Table 3. Comparison of Retention Time and Percent Peak Area for Humira and Biosimilar

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

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.

Table 4. Reproducibility of Peak Parameters for Humira and Biosimilar across Multiple Injections

Humira Innovator				Humira 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	Avg.	7.46	1.20	13644.33
Std. dev.	0.00	0.00	155.55	Std. dev.	0.00	0.00	131.30
% RSD	0.03	0.00	1.03	% RSD	0.00	0.00	0.96

Conclusions

This application note confirms that a TSKgel UP-SW3000, 2 µm SEC column can be successfully used to show the similarity between the innovator drug and the biosimilar, as well as the 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 indicating that this column can be utilized for quantitative analysis.