Separation of Biosimilar Using a 2 µm Silica Based Size Exclusion Chromatography Column

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Introduction

- A biosimilar is a “biological product that is highly similar to and has no clinically meaningful differences from an existing FDA-approved reference product” with regards to safety and effectiveness.[1]
- A reference product is “the single biological product, already approved by FDA, against which a proposed biosimilar product is compared”.[1]
- It is expected that the biologics market will increase at a rate of more than 20% per year, and that by 2025 more than 70% of New Drug Approvals will be biological products.[2]
- Analytical characterization of several batches of the reference product must be compared to the analysis of several batches of the biosimilar drug in order to demonstrate molecular similarity.
- A series of other analytical chromatographic techniques are used to characterize the similarity such as size exclusion chromatography (SEC), reverse 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 presentation, a silica-based SEC column containing 2 µm particles with 250Å pores was used to determine the molecular similarity between Humira and Yervoy biosimilars and the corresponding innovator reference products using UV detection.

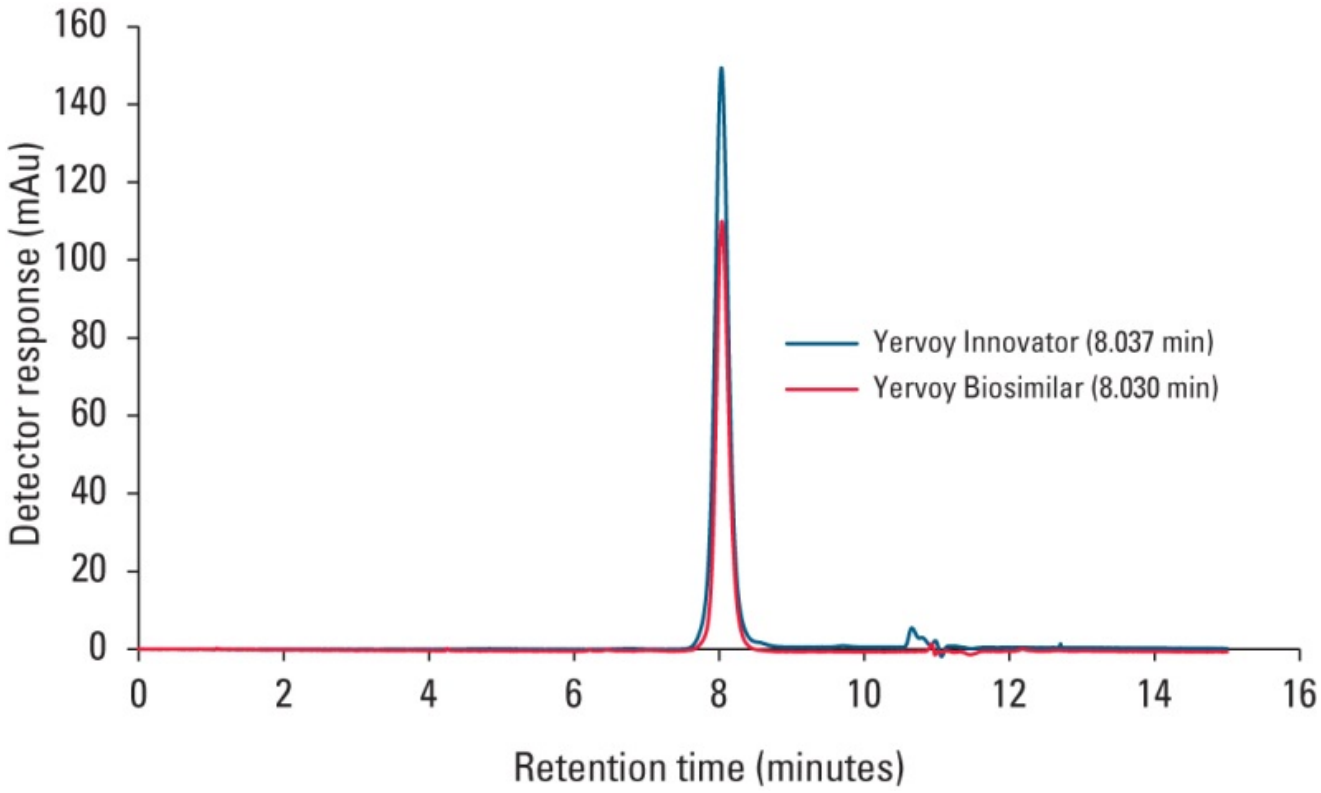
Column Attributes

TSKgel® Column	TSKgel UP-SW3000
Column size	4.6 mm ID × 30 cm
Base material	Silica
Stationary phase	Diol
Particle size	2 µm
Pore size	25 nm
Exclusion limit (Proteins)	800 kDa
Separation range (Proteins)	10 - 500 kDa

Materials and Methods

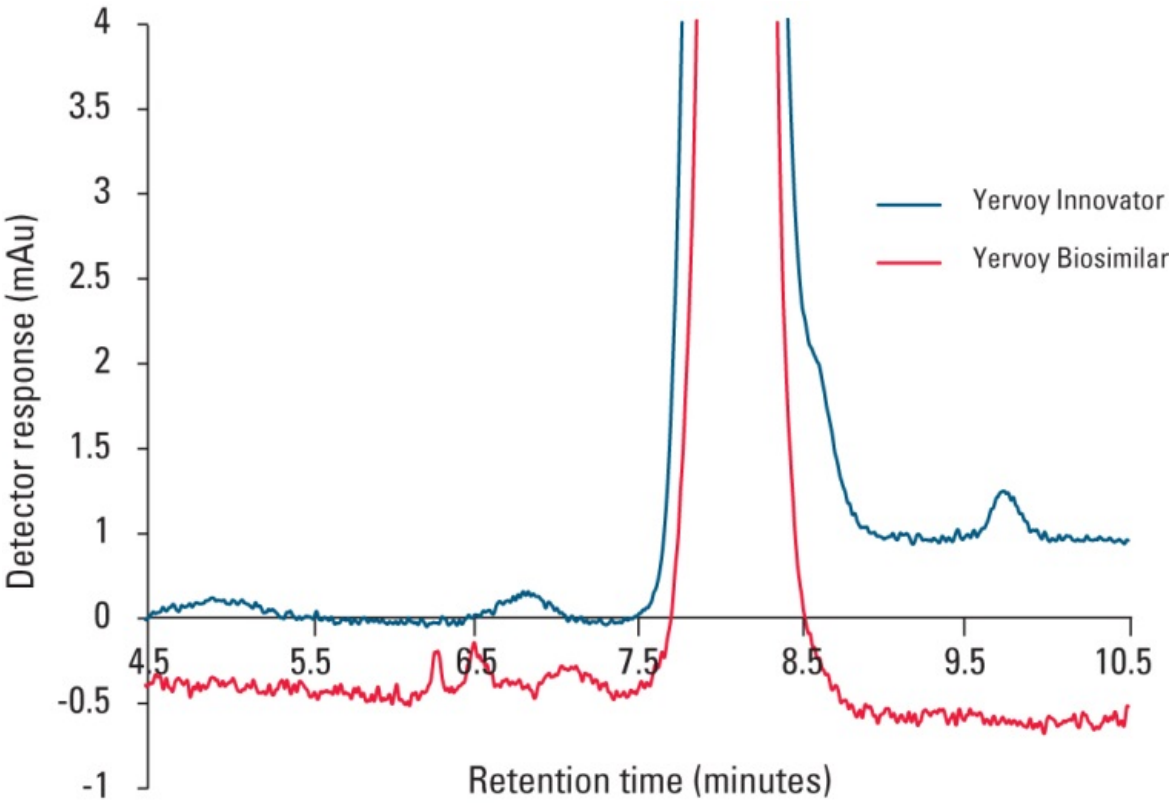
Column:	TSKgel UP-SW3000, 2 µm, 4.6 mm ID x 30 cm
Instrument:	Thermo Fisher Dionex Ultimate® 3000 with Chromeleon® v. 6.8
Mobile phase:	100 mmol/L KH_2PO_4/Na_2HPO_4, pH 6.7, 100 mmol/L Na_2SO_4, 0.05% NaN_3
Flow rate:	0.35 mL/min
Pressure:	28.5 MPa
Detection:	UV @ 280 nm
Temperature:	25˚ C
Injection vol.:	5 µL unless stated
Sample:	Humira® Innovator (5 mg/mL)
	Humira Biosimilar (4 mg/mL)
	Yervoy® Innovator (5 mg/mL)
	Yervoy Biosmilar (3.7 mg/mL)

Analysis of Yervoy Innovator and its Biosimilar



The Yervoy innovator and biosimilar exhibit similar retention times.

Analysis of Yervoy Innovator and its Biosimilar



The zoomed-in profile provides a closer look at the baseline and impurities present in each sample. Relative % peak area of the monomer and impurities are shown below.

Analysis of Yervoy Innovator and its Biosimilar

Retention time (minutes)				
	HMW	Dimer	Monomer	Fragment
Yervoy Innovator	4.837	6.780	8.030	9.707
Yervoy Biosimilar	6.19, 6.447, 6.223	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

- **The relative % peak area calculation clearly shows minimal impurities in each sample. Both monomers are >99% pure.**
- **This study shows that this 2 µm SEC column can be used to compare the consistency of the Yervoy innovator and its biosimilar.**

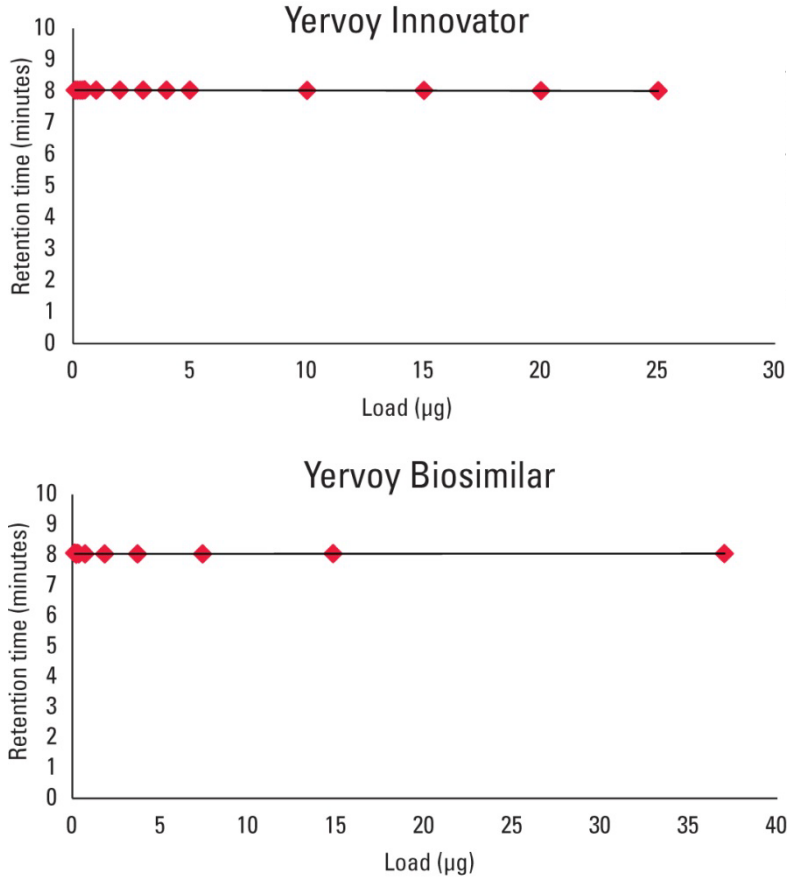
Analysis of Yervoy Innovator and its Biosimilar

Yervoy Innovator			
	RT (min)	As	N
	8.04	1.10	10617
	8.04	1.08	10618
	8.04	1.06	10614
	8.04	1.07	10628
	8.04	1.07	10550
	8.04	1.08	10455
Avg.	8.04	1.08	10580.33
Std. dev.	0.00	0.01	67.52
% RSD	0.02	1.27	0.64

Yervoy Biosimilar			
	RT (min)	As	N
	8.03	1.05	8279
	8.03	1.10	8279
	8.02	1.11	8276
	8.02	1.09	8276
	8.01	1.10	8255
	8.02	1.09	8572
Avg.	8.02	1.09	8322.83
Std. dev.	0.01	0.02	122.40
% RSD	0.07	1.92	1.47

6 consecutive injections yielded low % RSD for the peak parameters such as retention time, peak asymmetry and theoretical plates.

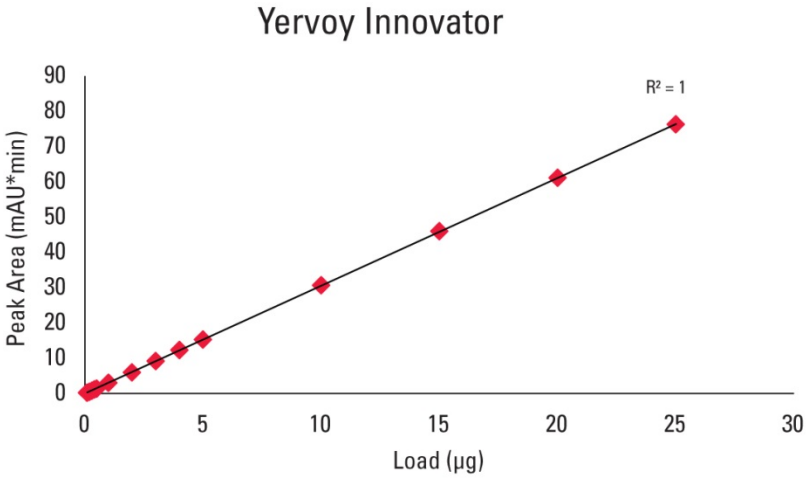
Loading Study – Yervoy Innovator and its Biosimilar

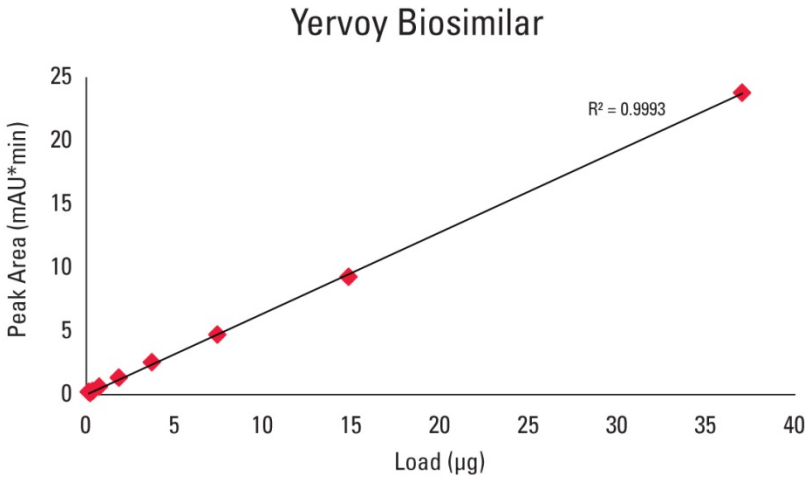


	Yervoy Innovator	Yervoy Biosimilar
Load (ug)	RT (min)	RT (min)
0.2	8.03	8.07
0.3	8.03	8.03
0.4	8.04	8.06
0.5	8.04	8.04
1	8.04	8.04
2.5	8.04	8.04
5	8.03	8.04
10	8.03	8.04
20	8.03	8.04
25	8.02	8.04
Avg.	8.03	8.04
Std. dev.	0.01	0.01
% RSD	0.08	0.15

Retention time was reproducible within the experimental sample load range with a low % RSD.

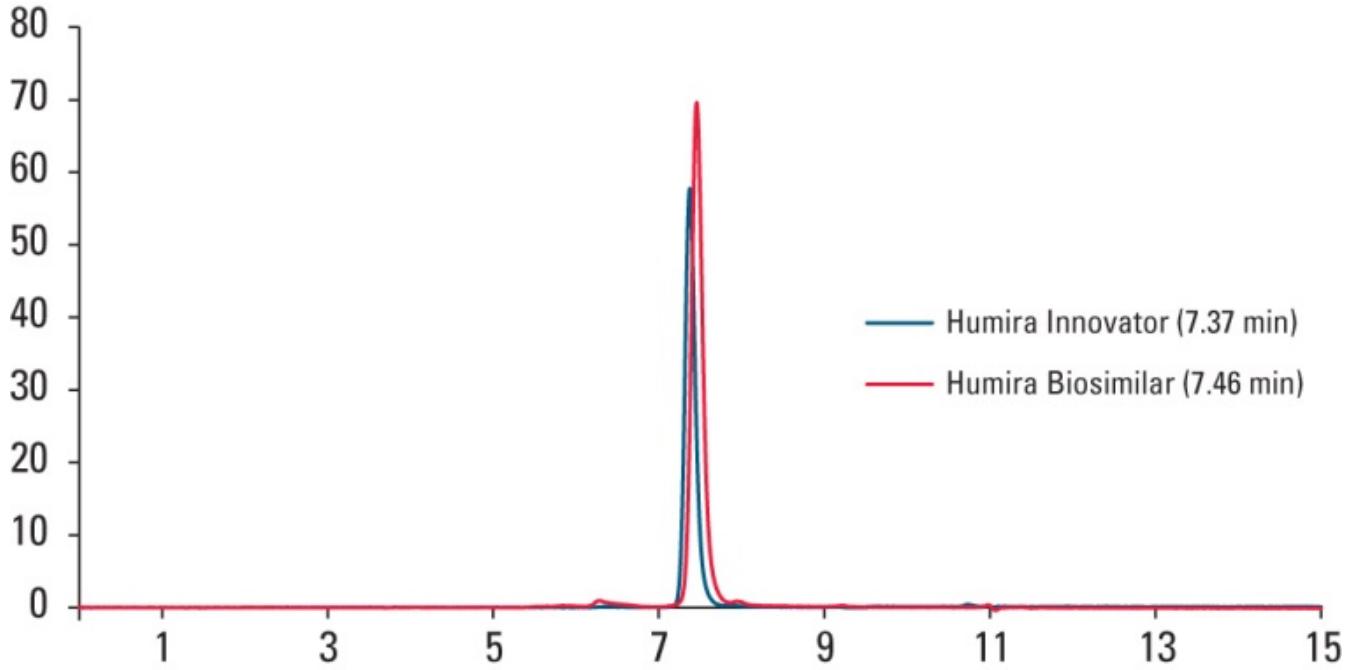
Linearity Analysis – Yervoy Innovator and its Biosimilar





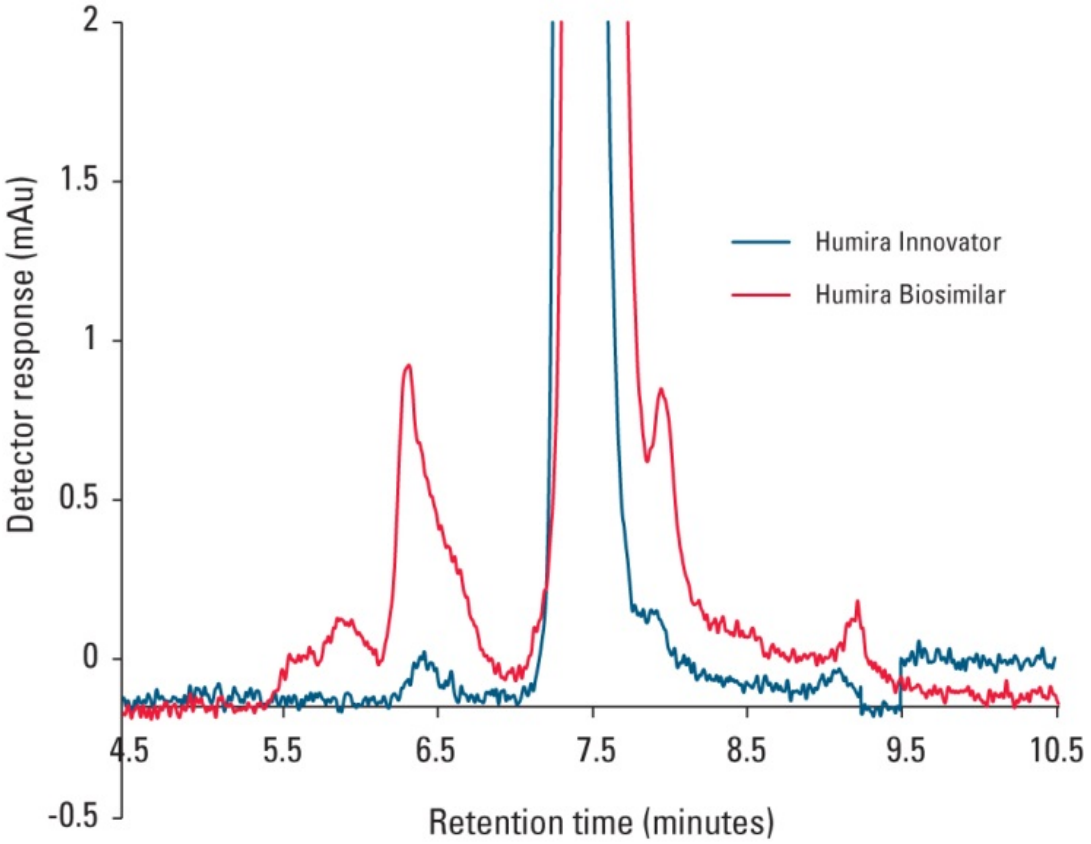
Linear regression shows favorable R^2 values for both Yervoy and the biosimilar, indicating that the TSKgel UP-SW3000 column is suitable for the analysis of Yervoy and its biosimilar across the specified loading range.

Analysis of Humira Innovator and its Biosimilar



The Humira innovator and biosimilar exhibit similar retention times.

Analysis of Humira Innovator and its Biosimilar



The zoomed-in profile provides a closer look at the baseline and impurities present in each sample. Relative % peak area calculation shown below.

Analysis of Humira Innovator and its 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

- **The relative % Peak area calculation suggests a larger percentage of impurities, predominantly aggregate, are present in the biosimilar compared to the innovator drug.**
- **This study shows that this 2 µm SEC column can be used to compare the consistency of the Humira innovator and its biosimilar.**

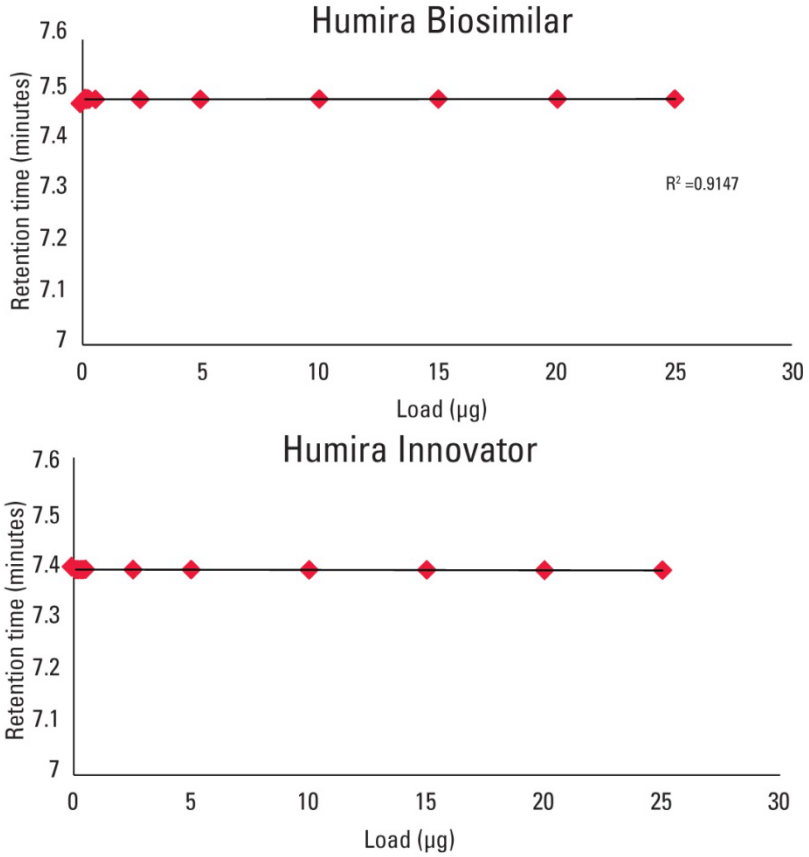
Analysis of Humira Innovator and its Biosimilar

Humira Innovator			
	RT (min)	As	N
	7.38	1.20	15082
	7.38	1.20	14876
	7.37	1.20	15082
	7.37	1.20	15254
	7.37	1.20	15247
	7.37	1.20	15282
Avg.	7.37	1.20	15137.17
Std. dev.	0.00	0.00	155.55
% RSD	0.03	0.00	1.03

Humira Biosimilar			
	RT (min)	As	N
	7.46	1.20	13781
	7.46	1.20	13781
	7.46	1.20	13704
	7.46	1.20	13607
	7.46	1.20	13506
	7.46	1.20	13487
Avg.	7.46	1.20	13644.33
Std. dev.	0.00	0.00	131.30
% RSD	0.00	0.00	0.96

6 consecutive injections yielded low % RSD for the peak parameters such as retention time, peak asymmetry and theoretical plates.

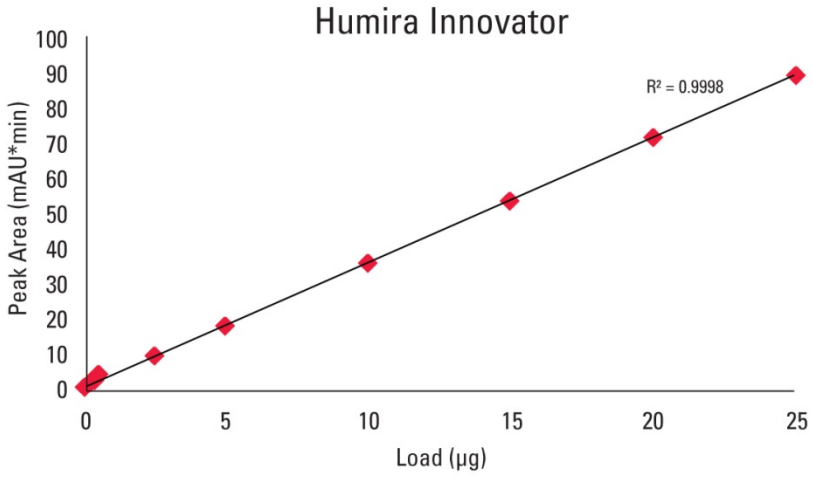
Loading Study – Humira Innovator and its Biosimilar

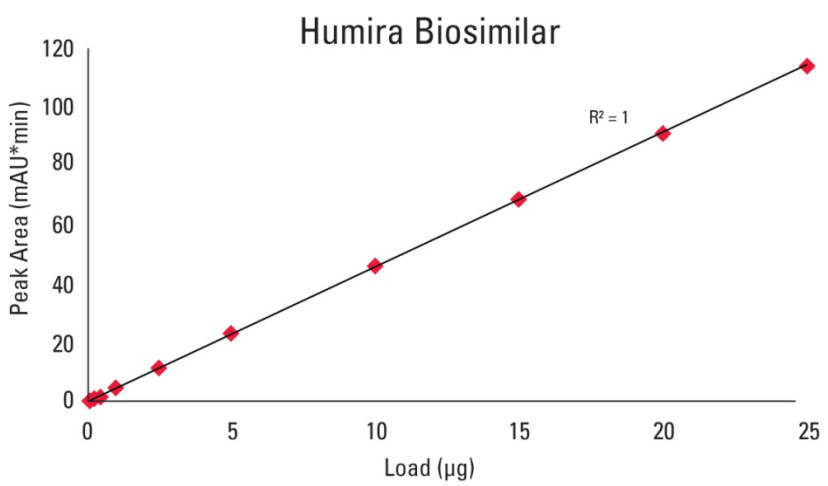


	Humira Innovator	Humira Biosimilar
Load (ug)	RT (min)	RT (min)
0.2	7.37	7.45
0.3	7.37	7.45
0.4	7.37	7.45
0.5	7.37	7.46
1	7.37	7.46
2.5	7.37	7.46
5	7.37	7.46
10	7.37	7.46
15	7.38	7.46
20	7.39	7.47
25	7.38	7.47
Avg.	7.37	7.46
Std. dev.	0.01	0.01
% RSD	0.09	0.09

Retention time was reproducible within the experimental sample load range with a low % RSD, as was the case with Yervoy.

Linearity Analysis – Humira Innovator and its Biosimilar





Linear regression shows favorable R^2 values for both Humira and the biosimilar, indicating that the TSKgel UP-SW3000 column is suitable for the analysis of Humira and its biosimilar across the specified loading range.

Reproducibility Analysis – Yervoy Innovator and its Biosimilar

Innovator

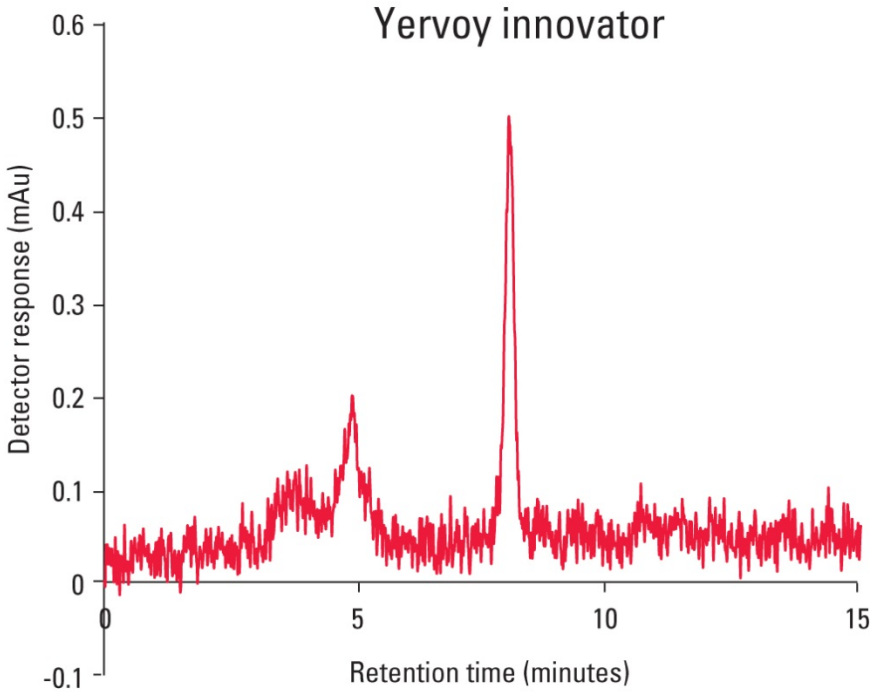
Day	RT (min)	Relative % peak area	As	N
1	8.03	99.47	1.05	8576
2	8.02	99.61	1.09	8512
3	8.01	99.49	0.98	8566

Biosimilar

Day	RT (min)	Relative % peak area	As	N
1	8.04	99.56	1.10	9810
2	8.05	99.63	1.10	9312
3	8.04	99.50	0.97	9315

- **The TSKgel UP-SW3000, 2 µm SEC column yielded excellent reproducibility in the analysis of both Yervoy and its biosimilar.**
- **This column yielded similar day to day reproducibility with Humira and its biosimilar as well (data not shown here).**

Limit of Detection (LOD) and Limit of Quantitation (LOQ)



- **A representative chromatogram of LOD of Yervoy innovator is shown above. The TSKgel UP-SW3000 column yielded similar results with Yervoy biosimilar.**
- **LOD for Yervoy innovator and its biosimilar is 50 ng; LOQ is 0.5 ug. This column reliably detected a load of 50 ng of Yervoy.**
- **Signal to noise ratio at this concentration is 4:1 for the monomer and 2:1 for the dimer.**
- **This study shows that the TSKgel UP-SW3000 column can be used for LOD/LOQ studies of similar innovator drugs and the corresponding biosimilars.**

Conclusions

- This study shows 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 % RSD in analyzing peak parameters such as retention time, peak area, peak asymmetry, and number of theoretical plates and showed that this column could be successfully used for the quantitative analysis.
- Overall this study indicates utility of the TSKgel UP-SW3000 column for analyzing the molecular similarity between innovator and its biosimilar molecule.
- This study shows that the 2 um SEC column can be used for the LOD/LOQ studies of similar innovator drug and the corresponding biosimilar.
- Future studies include further characterization of the molecular similarity using other modes of chromatography (abstract submitted for ASMS 2018).

References

[1]FDA. Biosimilar Product Regulatory Review and Approval. https://www.fda.gov/downloads/Drugs/DevelopmentApprovalProcess/HowDrugsareDevelopedandApproved/ApprovalApplications/TherapeuticBiologicApplications/Biosimilars/UCM581309.pdf

[2]Erickson BE. Untangling Biosimilars. Chem Eng News. 2010;88:25-27

[3]First Supplement to USP 40–NF 35 General Information / á1225ñ Validation of Compendial Procedures.