# Structure, Stability, Similarity, and Stress Characterization of Trastuzumab and Two Biosimilar Drugs Using Microfluidic Modulation Spectroscopy (MMS)

Valerie I. Collins,[1] Richard Huang,[1] Maria Ma,[1]* James Mosedale,[2] David J. Sloan[1]
[1]RedShift BioAnalytics, Inc., 80 Central Street, Boxborough, MA 01719, USA
[2]Ichorbio, Boston House, Grove Business Par, Downsview Rd, Wantage OX12 9FF, UK
*Presenting Author

## Abstract

Biologic drugs are complex and difficult to replicate, however, a biosimilar can be approved if it is shown to be highly similar to the originator. In this study, MMS was used to: 1) show the structural similarity between Trastuzumab and two research-grade biosimilar drugs, 2) compare their structure in different formulations, and 3) test their stability under different stress conditions. None of the three drugs showed structural differences in different buffers, but heat stress had a greater structural effect on Trastuzumab in PBS than in formulation buffer. Future work will replicate the heat stress study with the biosimilars to understand their thermal stability.

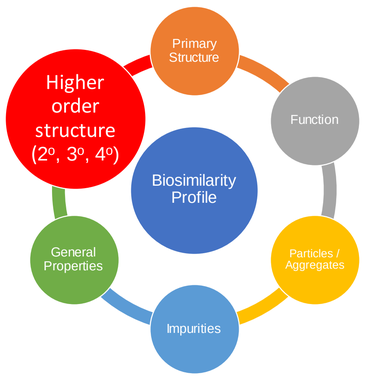



**Figure 1. FDA guidance on a biosimilarity profile including attribute comparisons.**

## Introduction

Trastuzumab (or Herceptin®) is a biologic used for HER2 positive cancers.[1] Significant time and money are invested in producing biosimilars as they must pass rigorous characterizations to show identical properties to the originator.[2] Here, we tested the structure of the originator and two research-grade biosimilars using MMS under normal and stressed conditions. MMS is a disruptive technique invented by RedShiftBio. Its quantum cascade laser interrogates the amide I band of the IR spectrum to probe protein structures, while its microfluidic flow cell modulates between the sample and reference buffer for real-time background subtraction. It's fully automated from measurement to analysis with powerful and user-friendly software. **Overall, MMS:**

- **Measures samples in the formulation condition of interest:**
  - ✓ maximized buffer compatibility
  - ✓ a concentration range from 0.1 to 200 mg/mL
- **Is ultra-sensitive and reproducible**
  - ✓ Up to 30x more sensitive than traditional technologies
  - ✓ >98% similarity between replicates is typical
- **Applications:**
  - ✓ Lot-to-lot similarity; stability; structural vs. colloidal aggregation; structural drug candidate screening; formulations; process conditions; testing of computational models on structure and stability
  - ✓ Non-destructive, fully recoverable samples

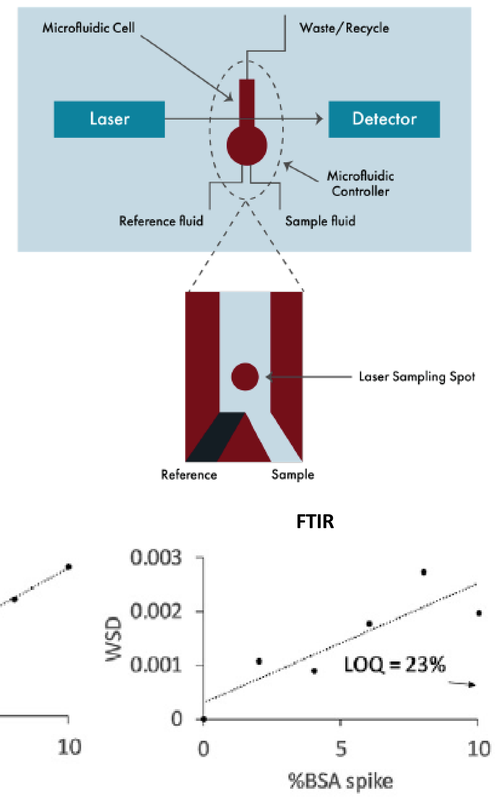
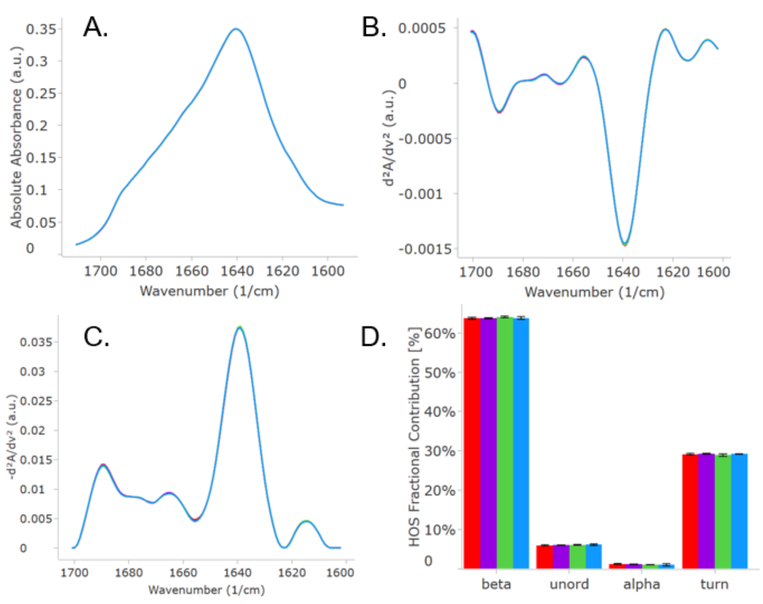



**Figure 2.** Schematic representation of the flow cell. MMS data compared to FTIR data from a benchmarking spiked study showing MMS has a limit of quantitation (LOQ) of just 0.76%, whereas FTIR has an LOQ of 23%.

## Result I: Comparing originator to biosimilars:

All samples in formulation conditions are equivalent in secondary structure.

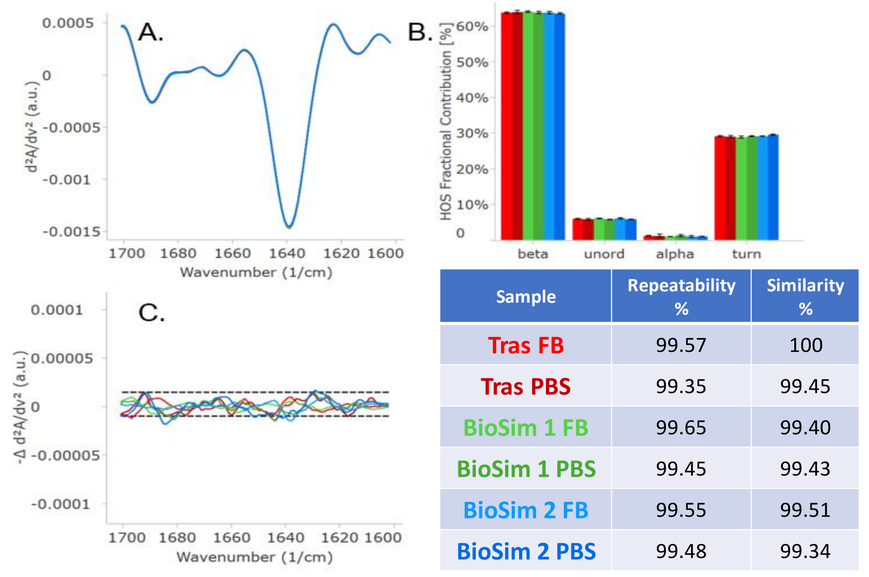



| Sample Name | Conc (mg/mL) | Repeatability (%) | Similarity (%) |
|---|---|---|---|
| Trastuzumab | 5 | 99.57 | 100 |
| Trastuzumab | 21 | 99.73 | 99.73 |
| Biosimilar 1 | 5 | 99.65 | 99.40 |
| Biosimilar 2 | 5 | 99.55 | 99.51 |

**Figure 3.** Trastuzumab at 5 and 21 mg/mL and both biosimilars are all perfectly overlaid, showing no spectral differences in (A) the absolute AU plot that normalizes for concentration, (B) second derivative plot, and (C) similarity plot which is the second derivative spectra inverted and baseline corrected. (D) HOS bar graph demonstrates the identical structures.
**Table 1.** Samples, concentrations and similarities show no change in structure.

## Result II: Formulation buffer vs PBS:

None of the three drugs showed structural differences in buffers tested.



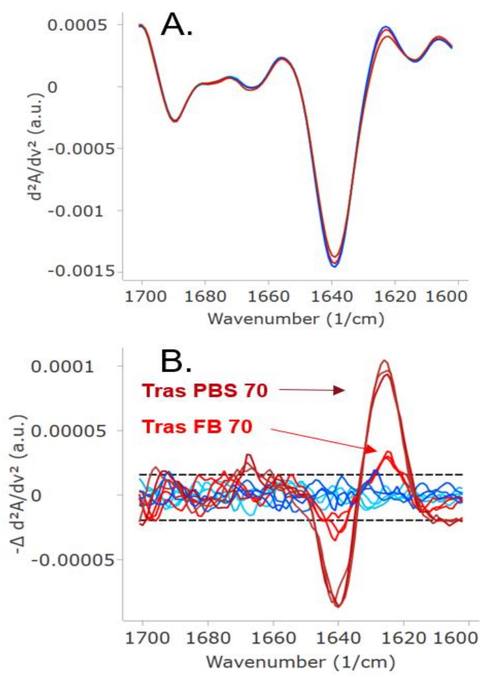

| Sample | Repeatability % | Similarity % |
|---|---|---|
| Tras FB | 99.57 | 100 |
| Tras PBS | 99.35 | 99.45 |
| BioSim 1 FB | 99.65 | 99.40 |
| BioSim 1 PBS | 99.45 | 99.43 |
| BioSim 2 FB | 99.55 | 99.51 |
| BioSim 2 PBS | 99.48 | 99.34 |

**Figure 4.** All spectra are perfectly overlaid in the second derivative plot (A). HOS bar graph (B) shows the similarity in structures along with the delta plot (C).
**Table 2.** Repeatability and similarity for each sample quantitatively shows the lack of change due to buffer.

## Result III: Heat stress

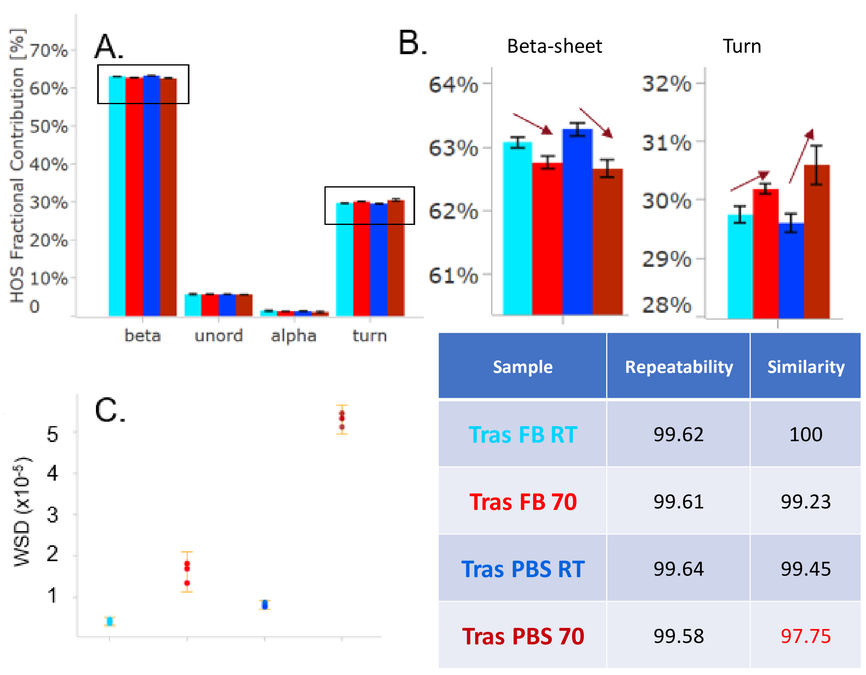
Trastuzumab in its original formulation buffer (FB) and in PBS were exposed to 70 °C for 30 mins. Both heated samples show an increase in the spectra around 1624 $cm^{-1}$ (intermolecular beta-sheet) and a decrease in the region at 1640 $cm^{-1}$ (native beta-sheet). Heat stress had a greater structural effect on Trastuzumab in PBS than in formulation buffer.

**Figure 5.** (A) Second derivative (B) and delta plot of Trastuzumab RT (blue) and 70C (red) in FB (lighter shades) and PBS (darker shades). The dashed lines represent the signal variance of the replicates of the control. Therefore, any traces that fall above or below those dashed lines reveal significant spectral change.




Quantitatively, Figure 6 (B) shows that heat stress caused a decrease in beta-sheet content and an increase in turn structure. Both the WSD in Figure 6 (C) and the percent similarities in Table 3, show that the FB heated sample undergoes subtle structural change, and the heated PBS sample undergoes more significant structural change.

**Figure 6.** HOS bar graph (A) and a focused view of beta-sheet and turn structures (B). Weighted spectral difference plot (C).
**Table 3.** Repeatability and similarity percentages for each RT and heated sample in FB and PBS.




| Sample | Repeatability | Similarity |
|---|---|---|
| Tras FB RT | 99.62 | 100 |
| Tras FB 70 | 99.61 | 99.23 |
| Tras PBS RT | 99.64 | 99.45 |
| Tras PBS 70 | 99.58 | 97.75 |

## Conclusions

- Trastuzumab and both research-grade biosimilars have comparable secondary structures as they maintain >99% similarity when compared to the control (Trastuzumab).
- Formulation buffer and PBS do not affect the structure of the antibodies as determined using the delta plot and area of overlap percent similarities.
- Heat stress of 70°C for 30 mins does affect the structure of Trastuzumab and the drug in PBS is more affected than the drug in formulation buffer.
- Heat stress causes a loss in beta-sheet content and a gain in turn structure, possibly indicating unwinding of the native beta-sheet structure.
- MMS is a robust and automated tool that can be used to quickly and easily analyze protein structure and determine change qualitatively, using the delta plots, or quantitatively using the percent similarity, HOS percentages, or the weighted spectral difference, simplifying the decision-making process when analyzing samples for comparability.

**Abbreviations:** HOS: higher order structure; MMS: Microfluidic Modulation Spectroscopy; AU: absorption unit; FB: formulation buffer; WSD: weighted spectral difference; LOQ: limit of quantitation

## References

1. Spector, Neil L., and Kimberly L. Blackwell. "Understanding the mechanisms behind trastuzumab therapy for human epidermal growth factor receptor 2–positive breast cancer." *Journal of Clinical Oncology* 27.34 (2009): 5838-5847.
2. Vulto, Arnold G., and Orlando A. Jaquez. "The process defines the product: what really matters in biosimilar design and production?." *Rheumatology* 56.suppl_4 (2017): 14-29.
3. Kendrick, Brent S., et al. "Determining spectroscopic quantitation limits for misfolded structures." *Journal of pharmaceutical sciences* 109.1 (2020): 933-936.