Biosimilar Characterization for Regulatory Submission and Process Development

Overview of Company and Capabilities

Shantani™

Advancing Technologies and Applications of Proteome Analysis

Premises, Problem Statement and Solution

mAb / Vaccine / biotherapeutics development program underway but

- Some or all components of physico-chemical characterization missing
- Product characterization needed for the purpose of regulatory filing and process development

Consider Shantani's more than a decade old Expertise in Protein Analysis

Why Shantani

- International Scientific Team
- Cutting-Edge Technologies
- High-end Instrumentation

Global Quality Standards

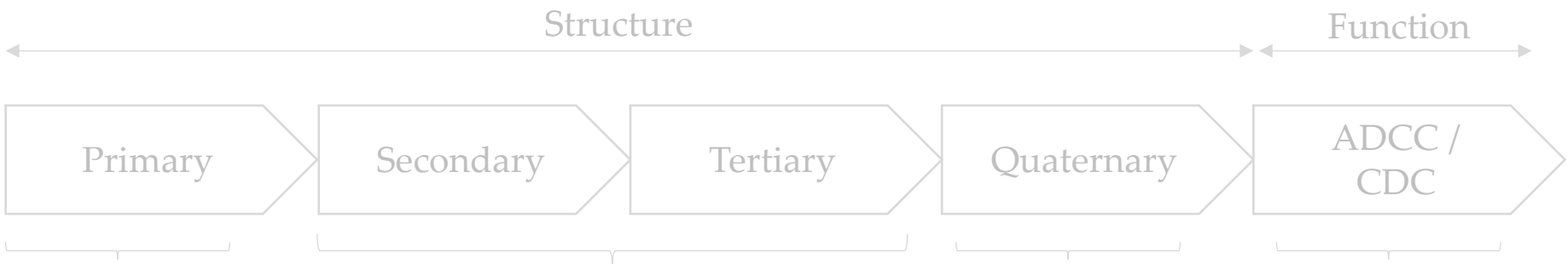
- Located in Pune, India
- Consultative Approach
- 'Fit-for-Purpose' Analysis

Faster Turn Around Time

- Completed > 20 Biologics Characterization
- Supported > 12 Regulatory Submissions
- Worked with > 6 Biosimilar Developers

Successful Track Record of Delivery

Drug Substance and Drug Product Characterization Workflow



- Intact Mass
- Peptide Mapping
- Glycan Profiling
- Charge Variant Analysis

- Circular Dichroism
- Fluorescence Spectroscopy
- Disulfide Bond Analysis
- FTIR

- AFM
- SEM / TEM

- Cytotoxicity Assays

- Host-Cell Protein / Host-Cell DNA / Aggregate Analysis

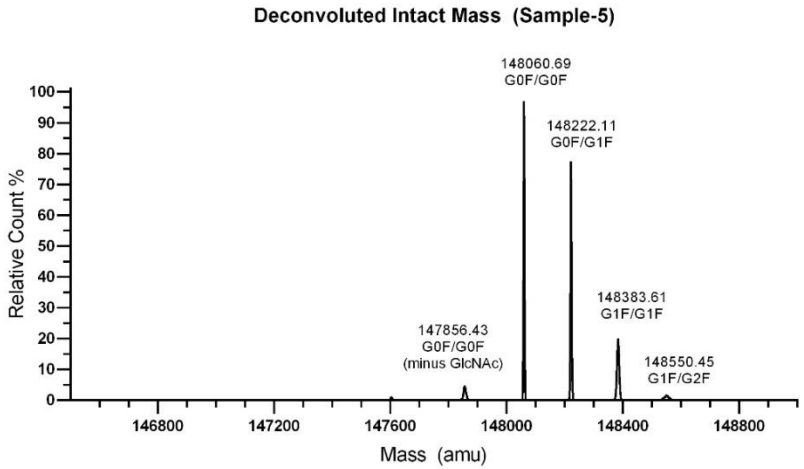
Biosimilar Characterization and Comparability Analysis

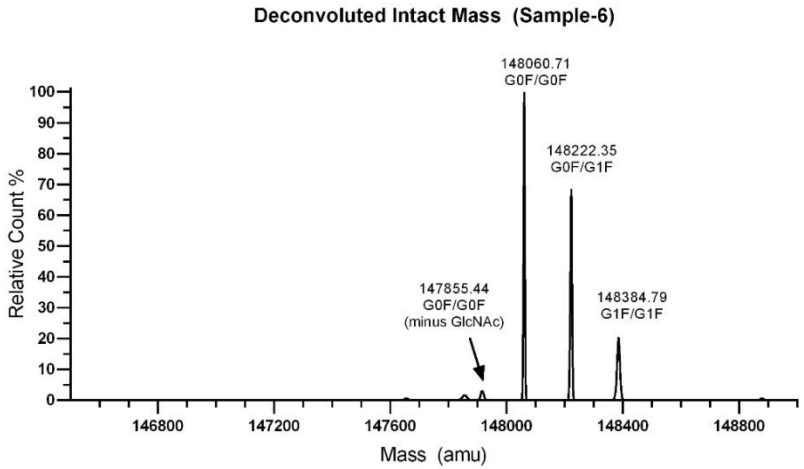
Intact Mass Analysis

- Typically carried out using ESI-Q-TOF (Agilent 6540 UHDA)
- Standard and well-established protocols for Biotherapeutics
  - Underivatized / Derivatized
  - Glycosylated / De-glycosylated
  - Reduced / Non-reduced
- Successful looking at protein up to 240 kDa size

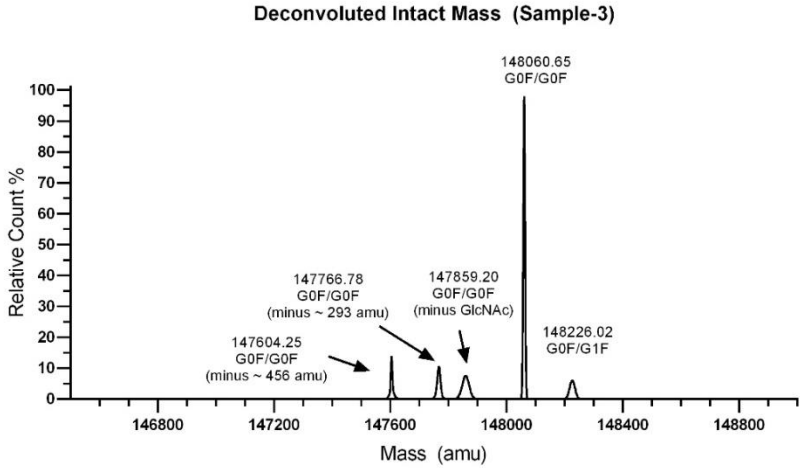
Determination of Intact Mass of mAbs using ESI-MS

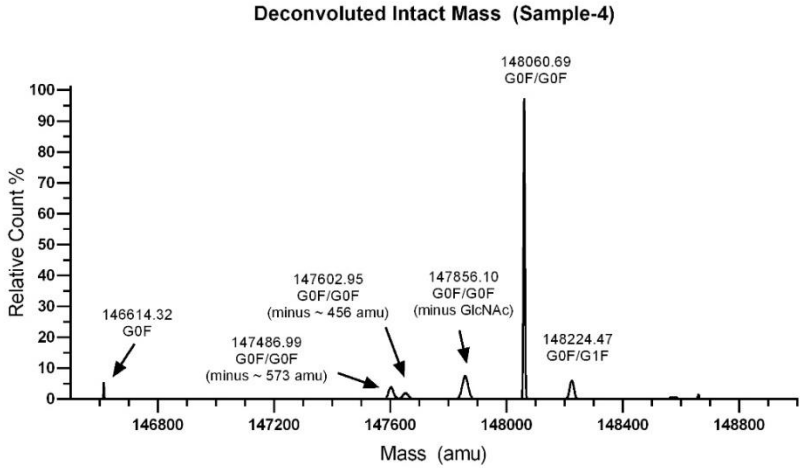
Innovator's Samples →





Biosimilars →



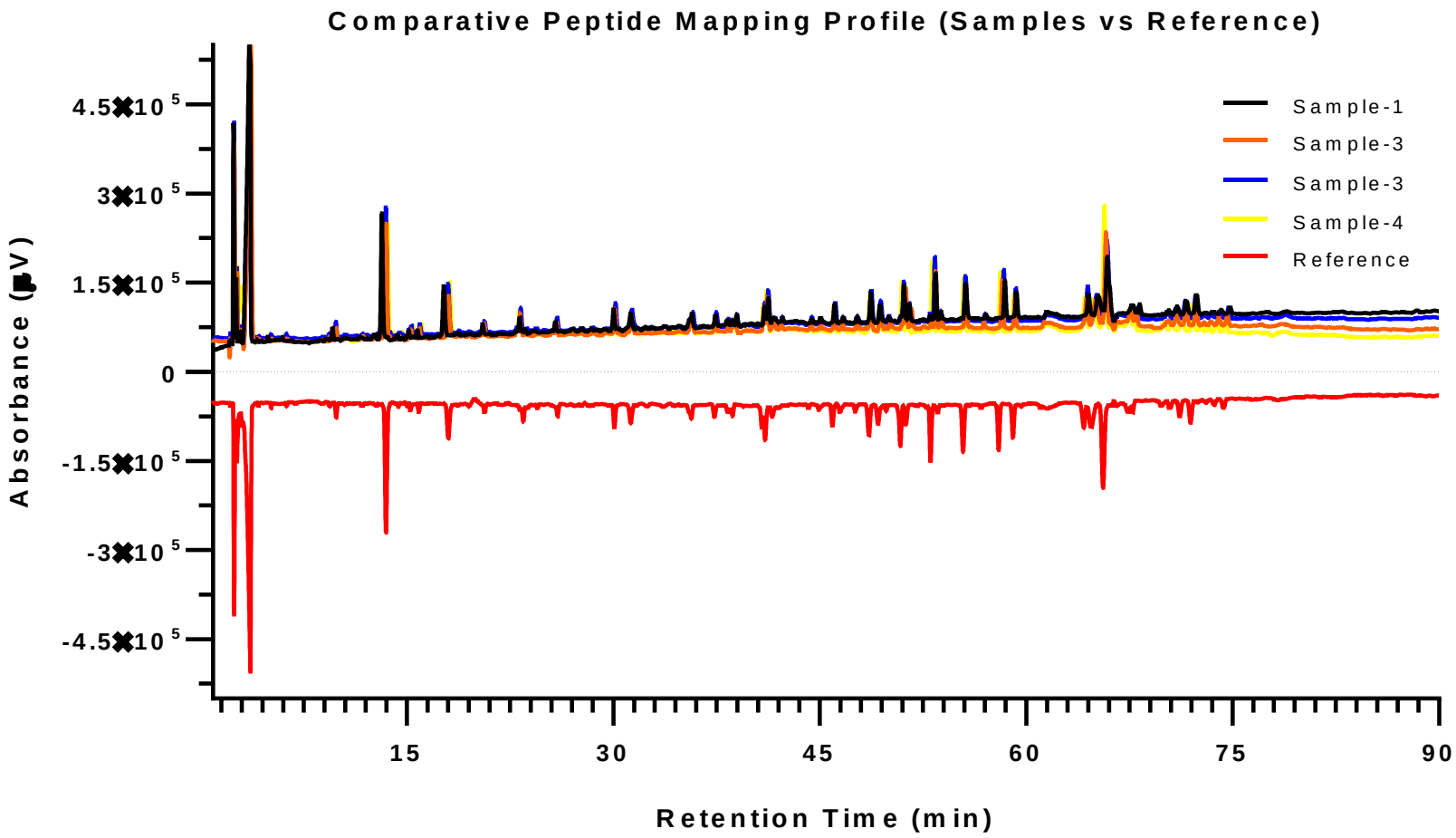


Primary mass matches, however, difference in glycosylated species observed !!!

Peptide-Map Analysis

- Standard and well-established protocols for single and multi protease, in-gel or in-solution, digestions of proteins
- Peptides are separated using RPLC and infused into ESI-QTOF set up for MS and MS/MS analysis
- Obtained masses are compared with the theoretical digest of the proteins using bioinformatic tools and peptide-maps are prepared
- Successfully prepared peptide maps of several (> 30 products) biotherapeutic proteins

Peptide Mapping of Biosimilar mAbs



Peptide Mapping of a Vaccine Product

- Mass data were compared against the theoretical digest of protein Uniprot ID P69899.
- Peptides containing Amino Acids in Red were identified.

MWRPSDSTVYVPPPNPVSKVVATDAYVTRTNIFYHASSSRLLAVGHPYFSIKRANKTVVPKVSGYQYRVFKVVLPDP
NKFALPDSSLFDPTTQRLVWACTGLEVGRGQPLGVGVSGHPFLNKYDDVENSGSGGNPGQDNRVNVGMDYKQT
QLCMVGCAPPLGEHWGKGKQCTNTPVQAGDCPPLELITSVIQDGDMVDTGFGAMNFADLQTNKSDVPIDICGTTC
KYPDYLQMAADPYGDRLFFFLRKEQMFARHFFNRAGEVGEPVPDTLIIKGSGNRTSVGSSIYVNTPSGSLVSSEAQ
LFNKPYWLQKAQGHNNGICWGNQLFVTVVDTTRSTNMTLCASVTTSSTYTNSDYKEYMRHVEEYDLQFIFQLCSIT
LSAEVMAYIHTMNPSVLEDWNFGLSPPPNGTLEDTYRYVQSQAITCQKPTPEKEKPDPYKNLSFWEVNLKEKFSSE
LDQYPLGRKFLLQSGYRGRSSIRTGVKRPAVSKASAAPKRKRAKTKR

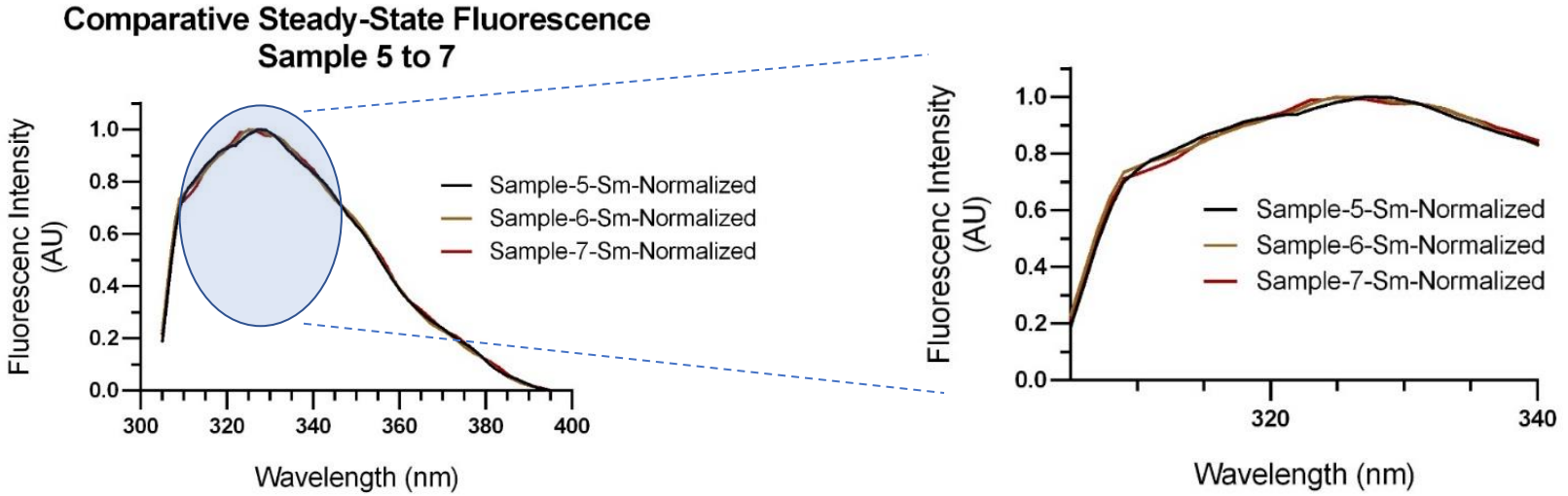
(80% Coverage, 398 / 500 amino acids)

Secondary and Tertiary Structure Analysis

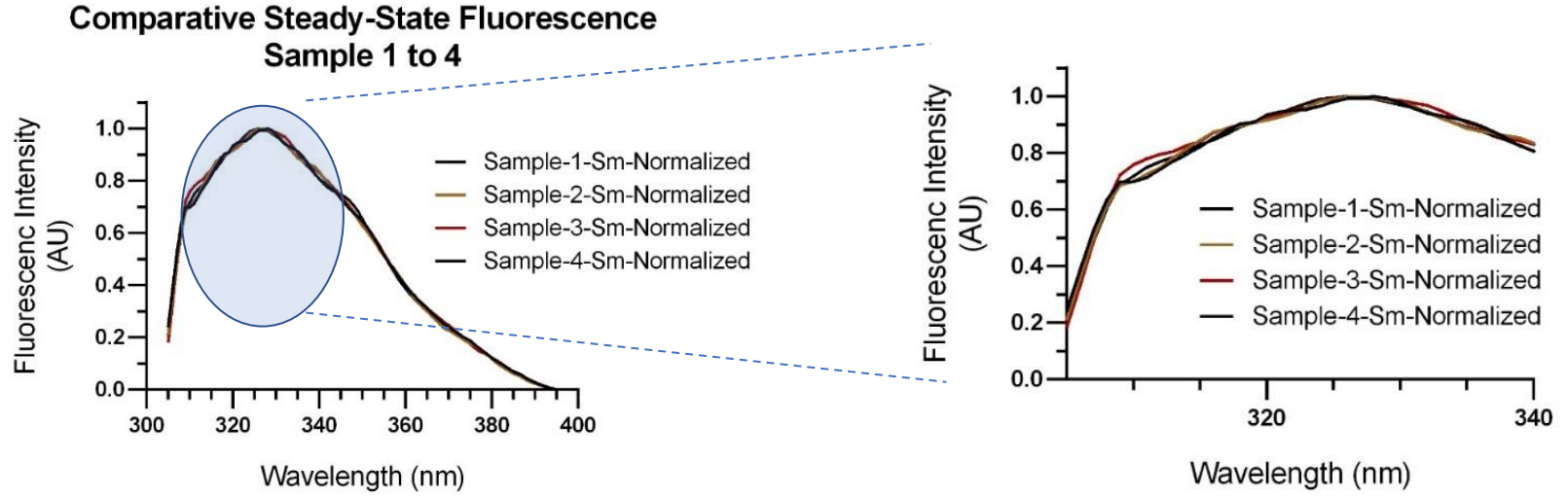
- If needed, samples are de-formulate using Gel-Filtration-Chromatography and then analyzed using Fluorescence and CD Spectroscopy
- Tryptophan Fluorescence Quenching experiments can be performed for in-depth analysis
- Successfully analyzed secondary and tertiary structures of several protein biotherapeutics of innovators and biosimilars

Steady State Fluorescence Spectroscopy

Innovator's Samples →

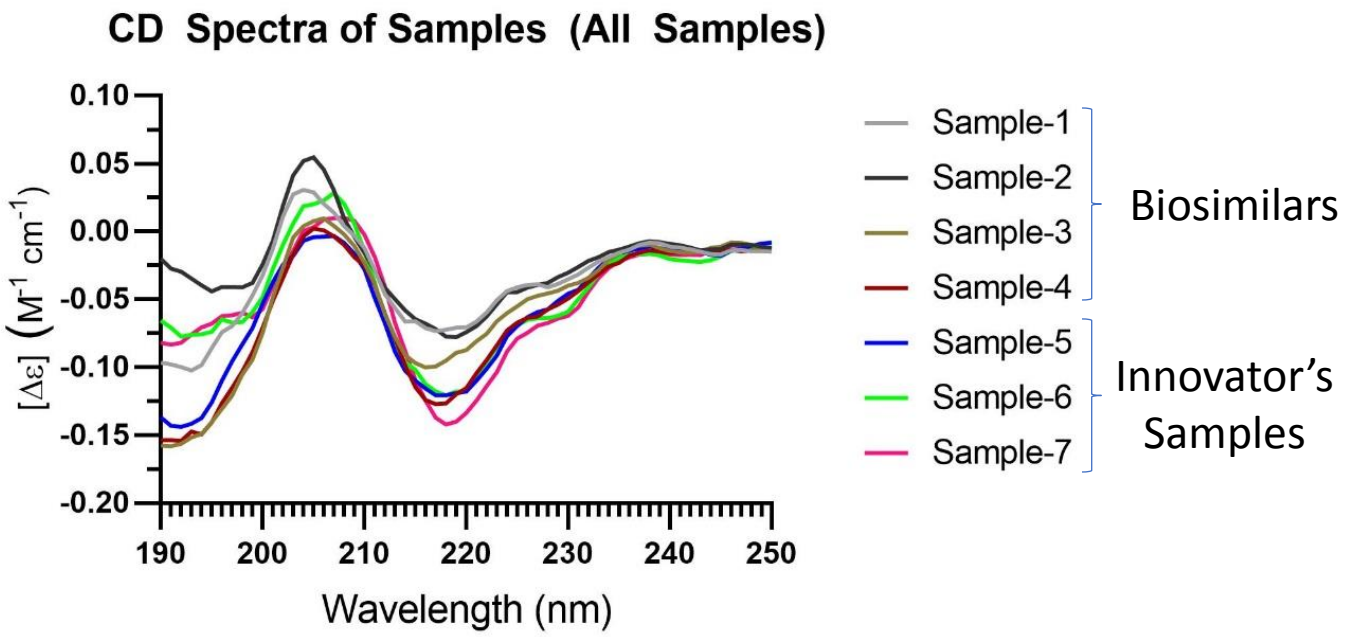


Biosimilars →



No Major Deviation Observed !!!

Circular Dichroism Studies



Sample #	Alpha-Helix	Beta-Sheets	Irregular
# 1	0%	53%	55%
# 2	0%	53%	55%
# 3	0%	53%	55%
# 4	0%	53%	55%
# 5	0%	53%	55%
# 6	0%	53%	55%
# 7	0%	53%	55%

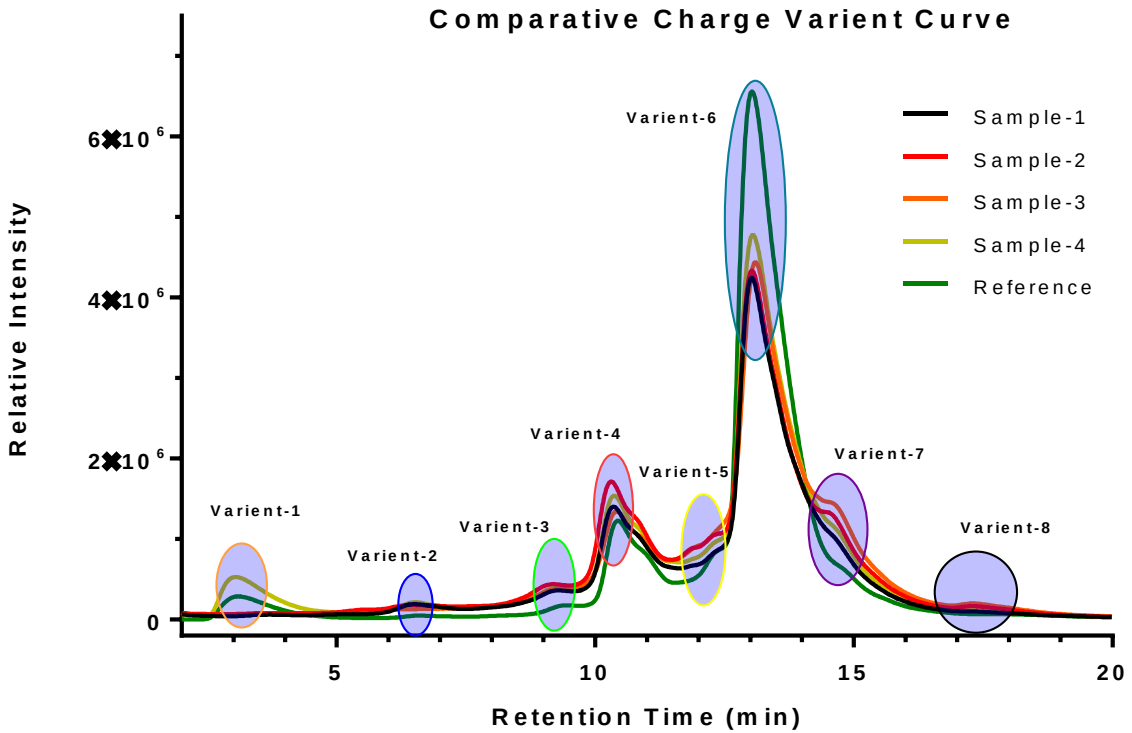
Minor Variations in CD spectra observed, however, No significant Difference in secondary structure components !!!

Charge Variant Analysis

- Charge Variants are analyzed using HPLC-strong-cation exchange chromatography (SCX) and UV/Fluorescence Detectors

- Linear pH gradient is the key to success and well-optimized protocol is utilized for the purpose

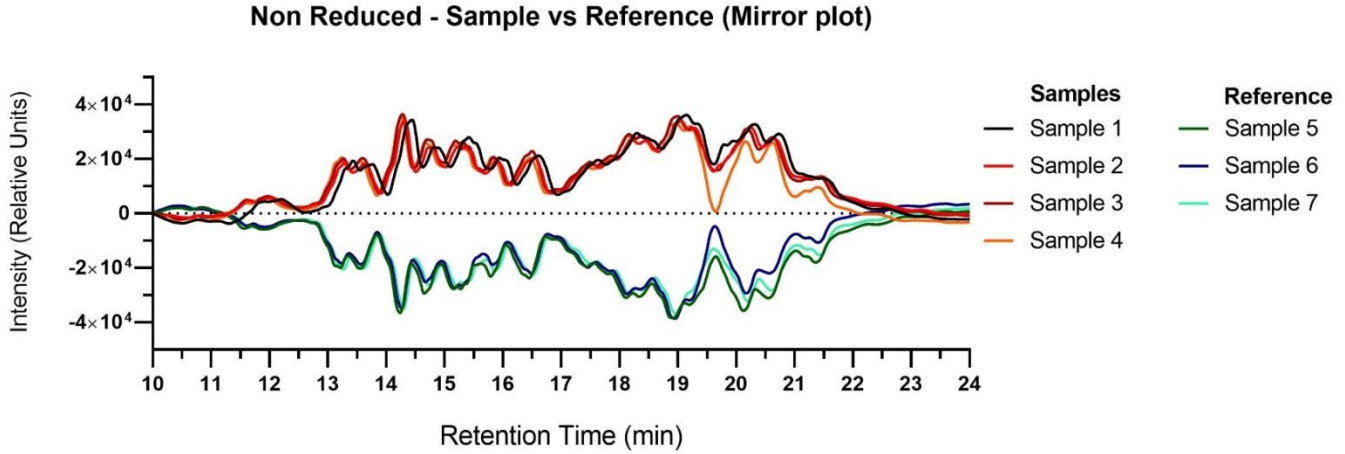
HPLC-SCX-UV Probing of Charge Variants of mAb

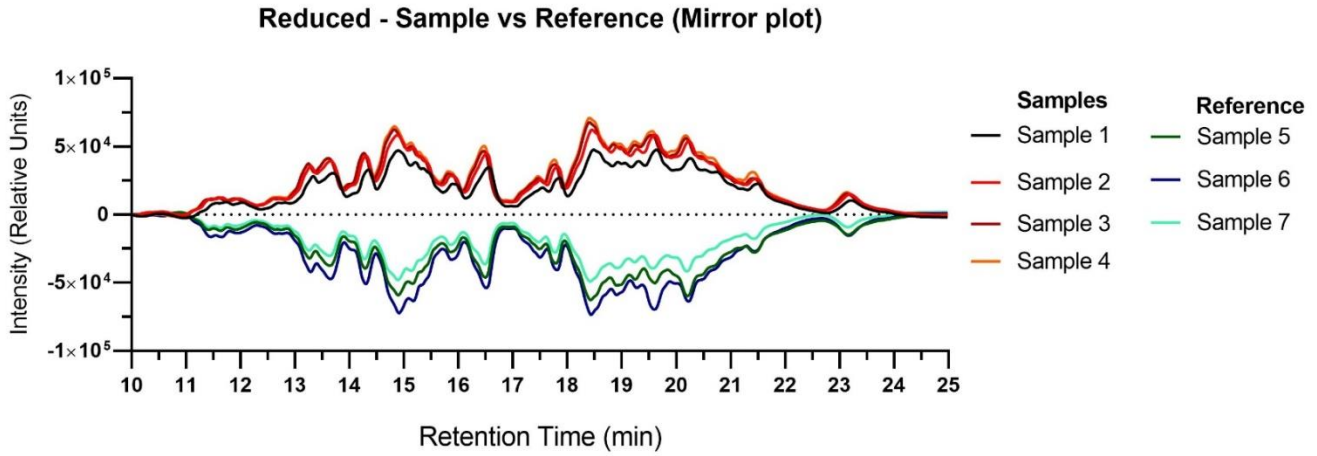


Major difference in percentage contribution of Charge Variants Observed !!!

Disulfide Characterization in mAbs

Typically mAbs are digested with proteases in reduced and non-reduced conditions and obtained peptides are analyzed using LC-UV or LC-MS based workflows.

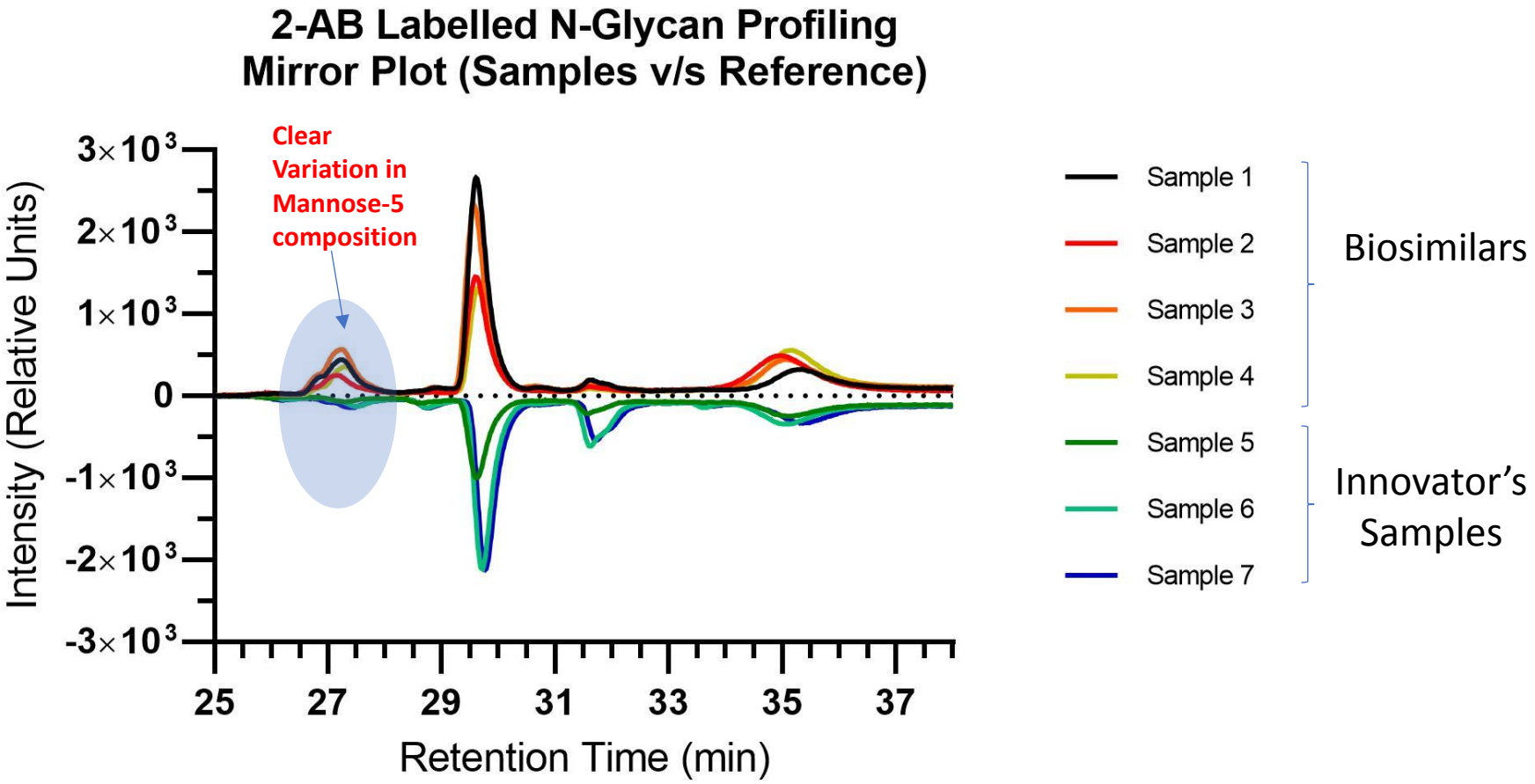




Glycan Profiling

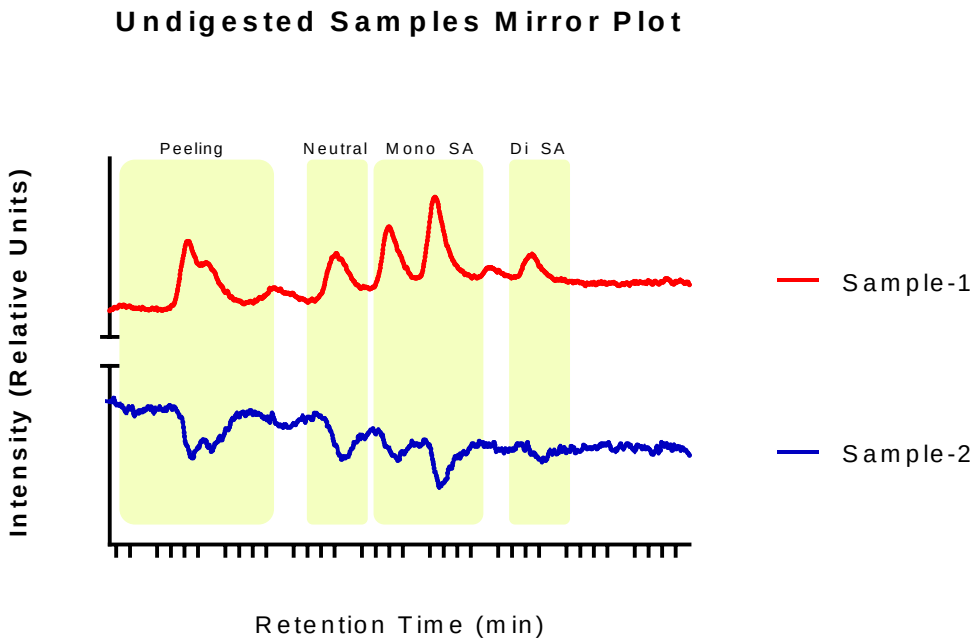
- Standard protocols for N and O Glycan profiling
- Extracted N and O glycans are labelled with 2-AB and analyzed using LC-Flu based workflow and Glycans are identified using mass-spectrometry
- Fluorescence labelled glycans were also analyzed using Fluorophore Assisted Carbohydrate Electrophoresis (FACS)

N-Glycan Profiling of mAbs



Clear Variation in Mannose-5 composition in Biosimilar Samples were observed.

O-Glycan Profiling of mAbs



Species	Sample-1	Sample-2
Peeling	35%	36%
Gal	-	-
Neutral	15%	18%
Mono SA	43%	38%
Di SA	7%	7%

Species were confirmed by ABS and BTG digestion and Mass-Spectrometry.

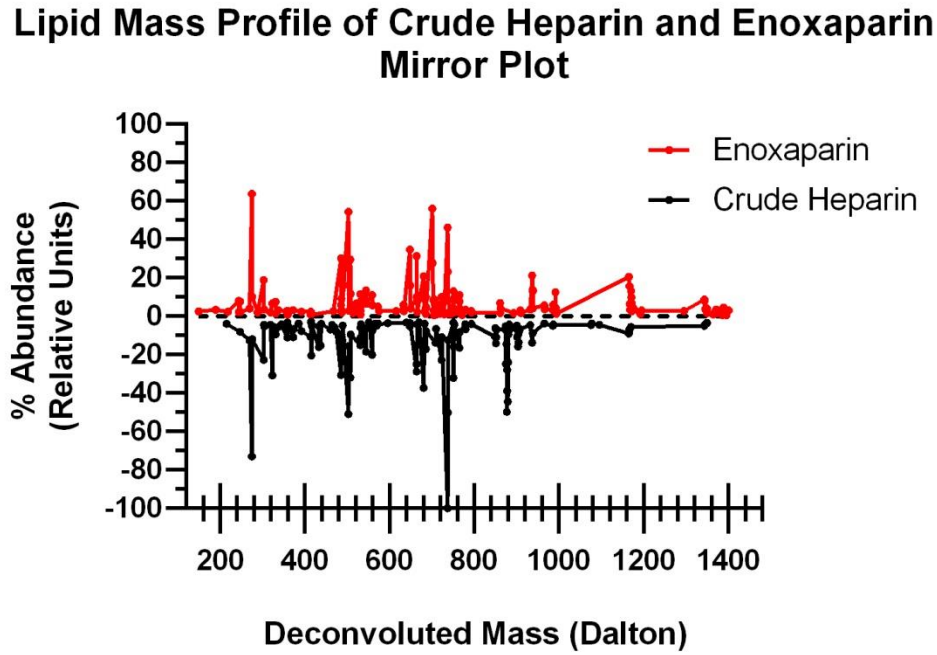
No Major Difference Observed !!!

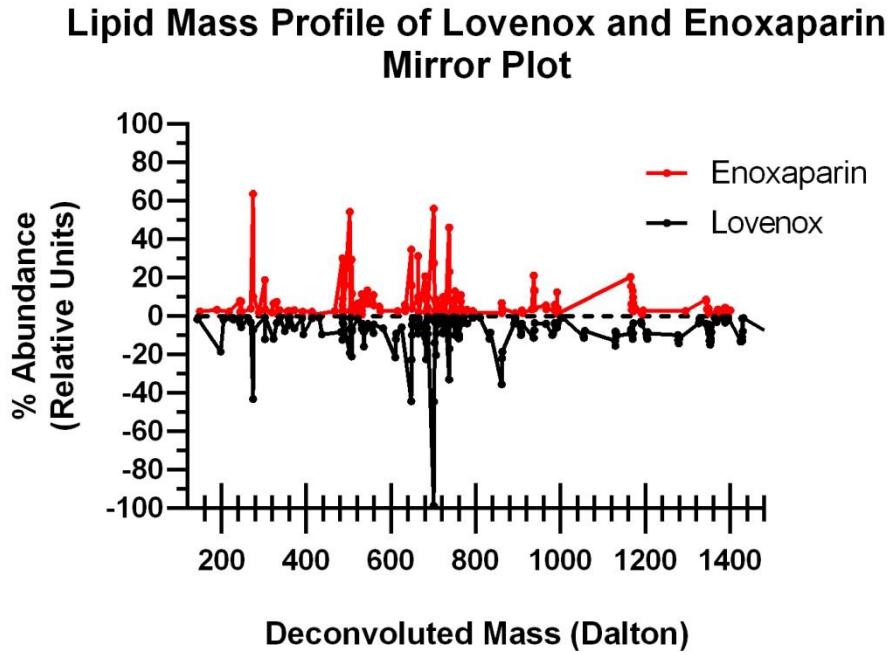
Lipid Analysis

- Mass-spectrometry and Thin-Layer Chromatography are utilized in characterizing the lipid components of the biotherapeutics

- Several lipid analysis method using mass-spectrometry has been standardized in our lab

Comparative Profile of Lipid Impurity in Low Molecular Weight Heparin Products (LMWH)

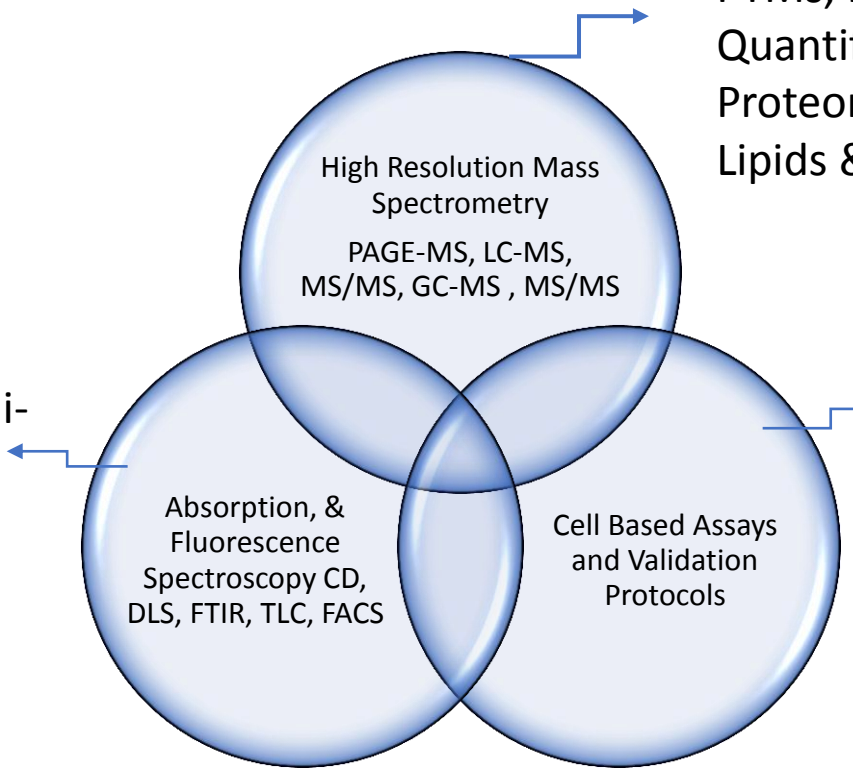




> 70 % Profiled lipid impurity were same between Enoxaparin (Biosimilar) and Lovenox (Innovator). By several supportive experiments it was concluded that lipid impurity profile of biosimilar and innovators product was same.

Our Focus = Protein Analysis

Protein Identification, Peptide Maps PTMs, Host-Cell Impurity, Protein Quantification, Differential Proteomics, Intact mass, Isoform, Lipids & Carbohydrates/Glycans



Secondary and Tertiary Structure, Protein Folding, di-sulfide bridge, Particle Size, Lipid & Carbohydrate Characterization

Immunogenicity, Toxicity, Mechanism of Action, siRNA, Overexpression, Purification

Our Key Strengths

- Globally Competitive Science
- High Ethical and Professional Standards
- Networked Operational Model for Cost-Effectiveness

Our Customer's Profile

East Coast-USA based one of the Largest Pharmaceutical Company

World's Largest Chemical Producer HQ - Germany

Glimpses of Shantani's R&D Center @ Innovation Park

Thank you

Connect for further discussions

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Advancing Technologies and Applications of Proteome Analysis