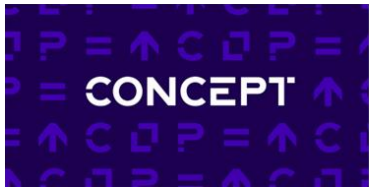
Charge variant microheterogeneity explored via icIEF and offline MS analysis of infliximab biosimilars

Anna Mulligan, Nora Crushell, Sara Carillo, Jonathan Bones

CE Pharm, 18th September 2024

NIBRT Overview



- Unique facility dedicated to address the training and research needs of the global biopharmaceutical industry based in Dublin, Ireland.
- Competency based training experience in an environment that replicates modern industrial bioprocessing facilities.
- Research with impact – developing solutions to address real challenges faced within the biopharmaceutical industry.
- Facility expansion for advanced therapy research and training and new early-stage development facility opened in 2023.

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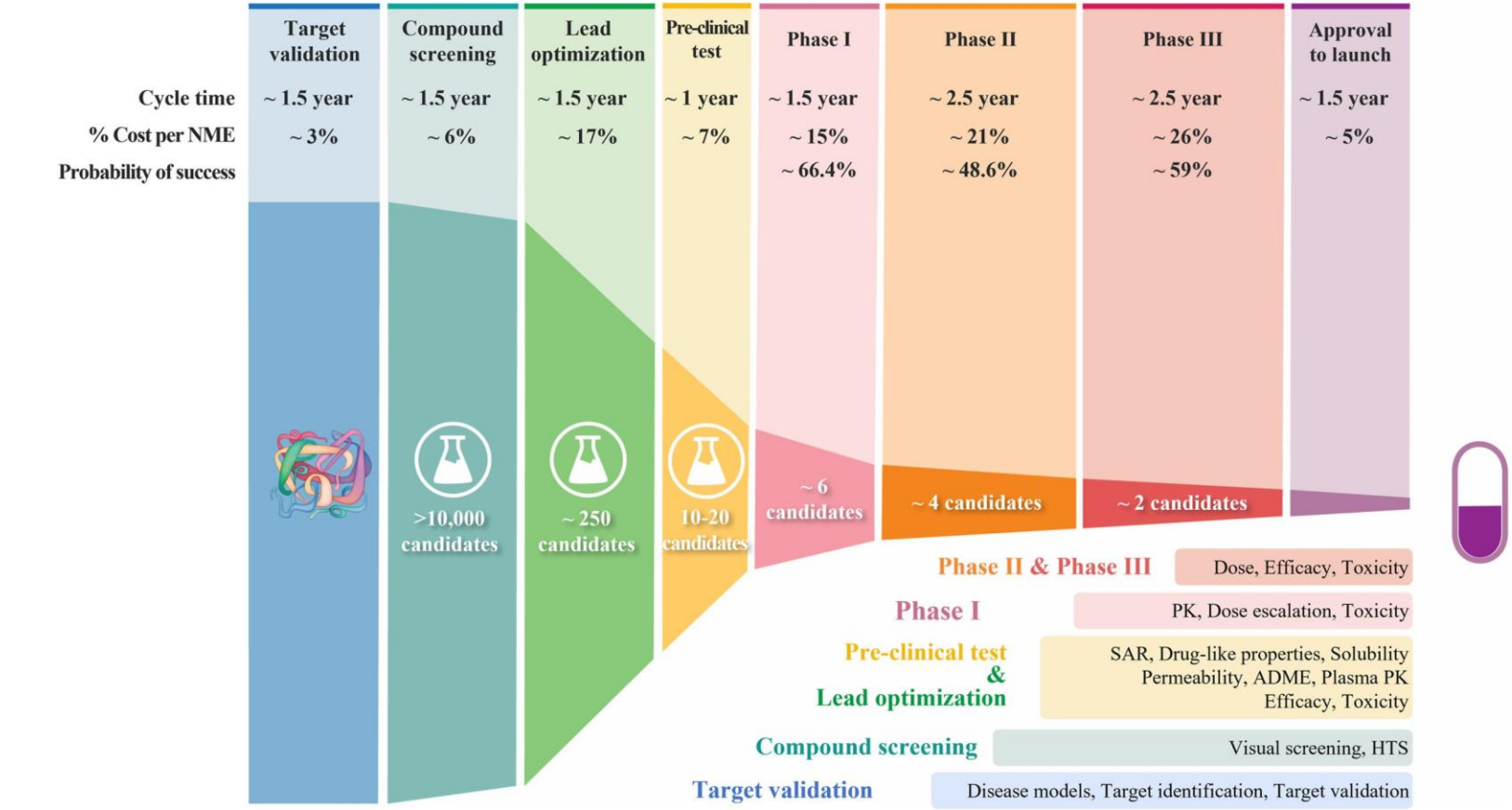
Drug Development Process



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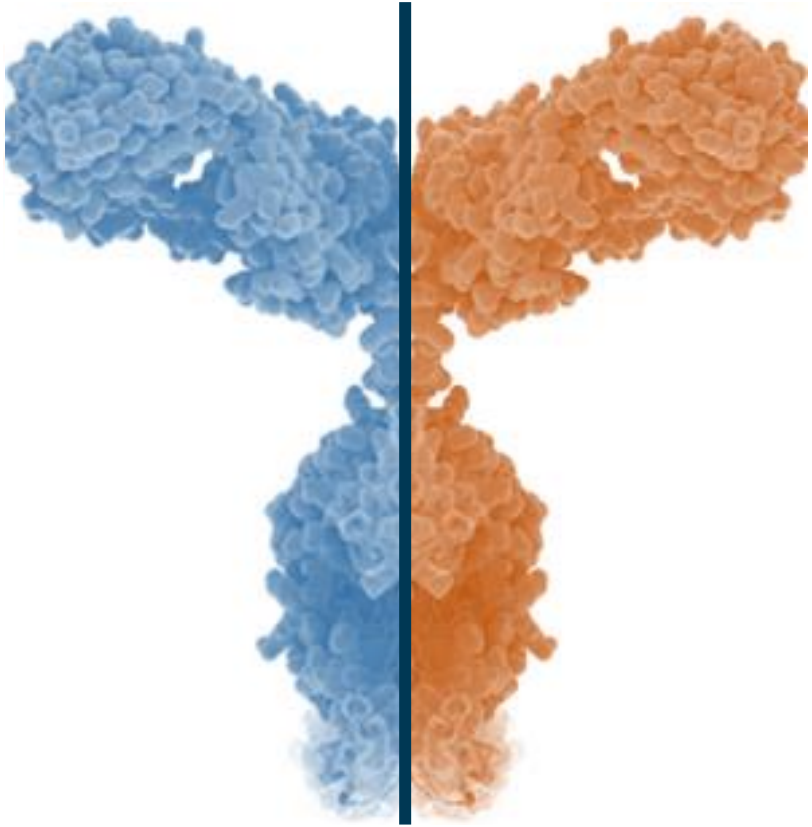
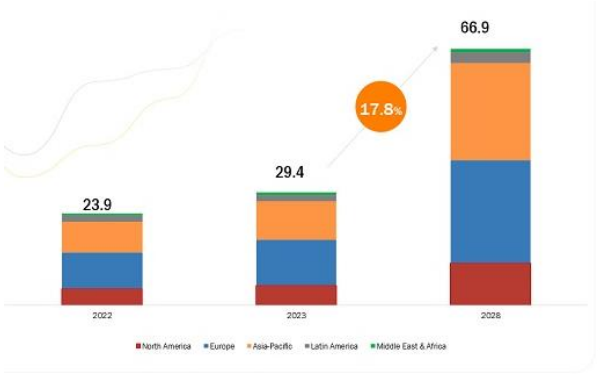
What is a Biosimilar?

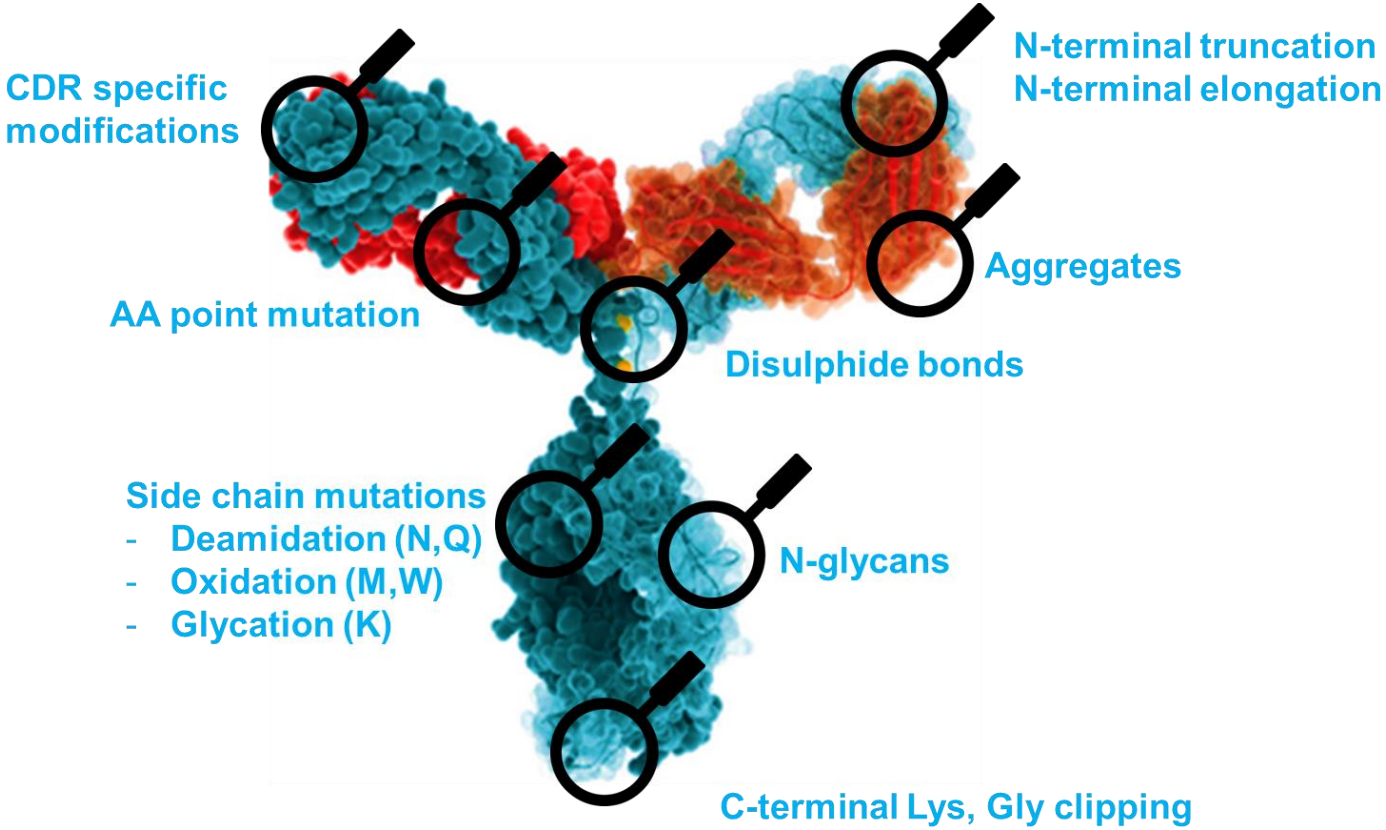


“A biosimilar is a biologic that is highly similar to and has no clinically meaningful differences in terms of safety, purity, and potency (safety and effectiveness) from an existing FDA-approved biologic, called a reference product.”

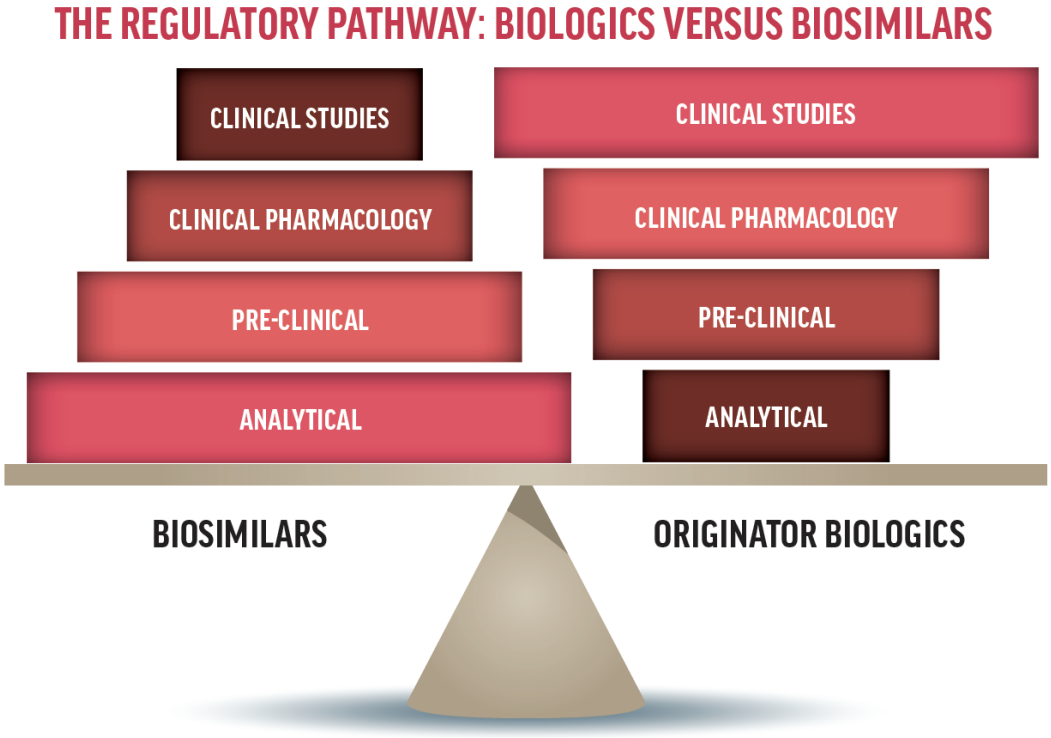
https://www.fda.gov/drugs/biosimilars/review-and-approval



Biopharmaceutical Complexity



The importance of analytical assays



Identify source of difference: CQA or PQA?

"Levels of basic variants in all SB5 batches (8.6-10.9%) were below the quality ranges of USHumira (17.5-30.2%) and EU-Humira (18.1-27.8%). Levels of acidic variants in all SB5 batches (22.6-25.6%) were above the quality ranges of US-Humira (11.9-18.7%) and EU-Humira (11.4 19.5%). The Sponsor provided characterization and biological activity data showing that acidic variants were enriched in sialylated N-glycans, which are discussed under bullet 3. Basic variants had high levels of C-terminal Lys and α-amidated C-terminal proline residues of the heavy chain, which are not CQAs because C-terminal lysine is cleaved in serum and proline amidation of the C-terminus of monoclonal antibodies does not impact the Fc-mediated effector function. Acidic and basic variants had similar activities to the main product in both the TNF-□ binding assay and the ADCC assay. Therefore, the differences seen in acidic and basic variants do not preclude a determination that SB5 and US-Humira are highly similar or a determination that the analytical part of the scientific bridge was established."

Hadlima, BLA Product quality review. 2019

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Which are the tools available?

Although recent advancements are providing depth characterization with fewer, data-rich assays (mass spectrometry, multi-attribute method), performing routine assays is still a regulatory requirement for drug development, testing and batch release.

Charge variant analysis must be performed in order to provide a biosimilarity assessment.

icIEF is the gold standard for charge variant analysis.

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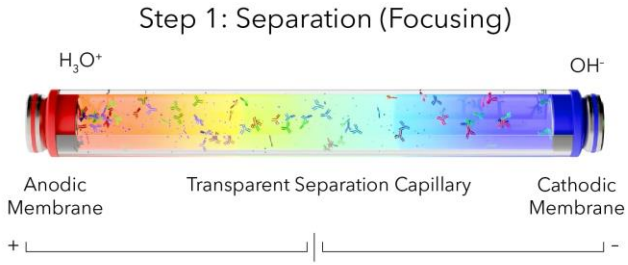
icIEF: Maurice Flex



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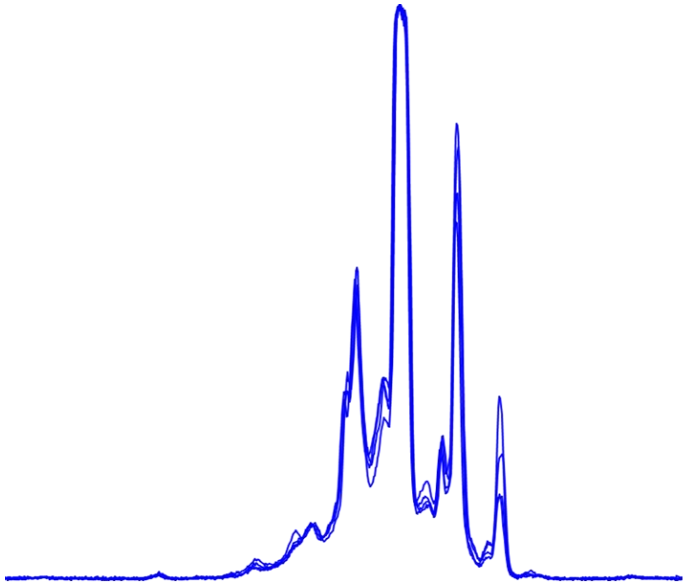
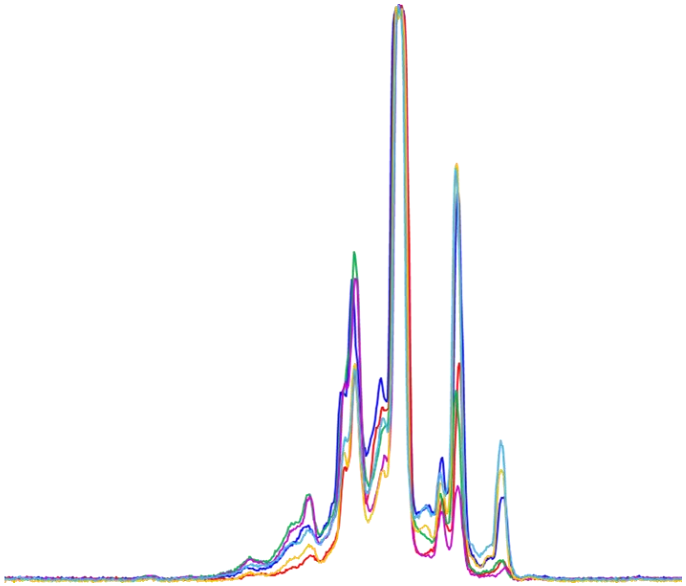
icIEF: Adalimumab batches vs biosimilars





Separation Method
1 min @ 1500 V, 10 min @ 3000 V
Sample load: 55 sec
Detection: 5 exposures (Absorbance 0.005 sec, Fluorescence 3 sec, 5 sec, 10 sec, 20 sec)

Master Mix
0.35 % Methyl Cellulose
10 mM Arg
4 % pH 3-10, 4% pH8-10.5 ampholytes
20 % SimpleSol
1 %(v/v) pI marker 6.14, 1 % (v/v) pI marker 9.50

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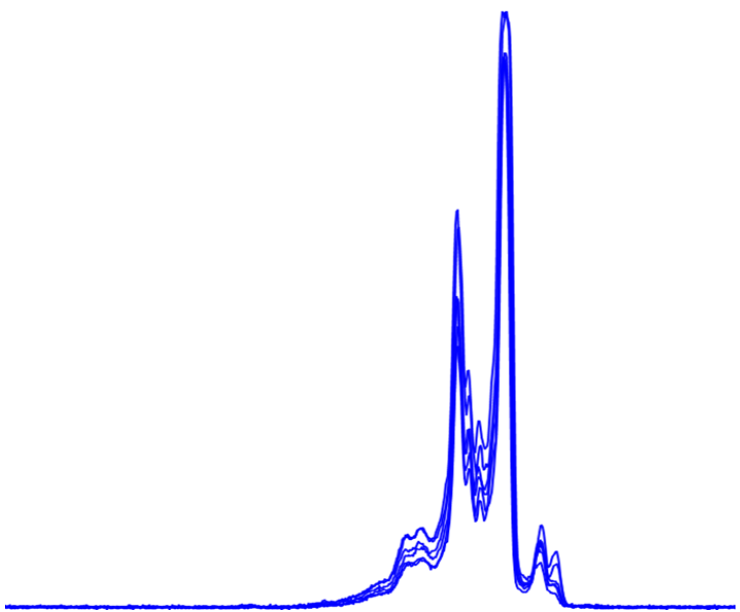
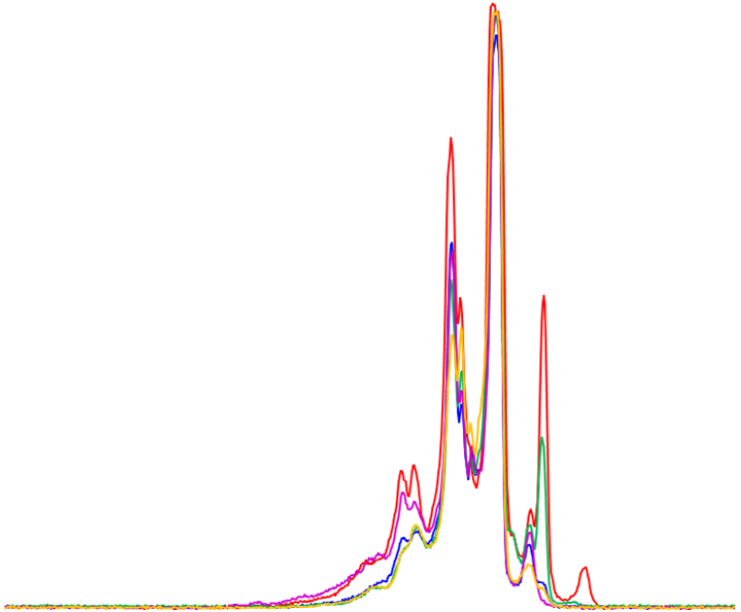
icIEF: Trastuzumab batches vs biosimilars





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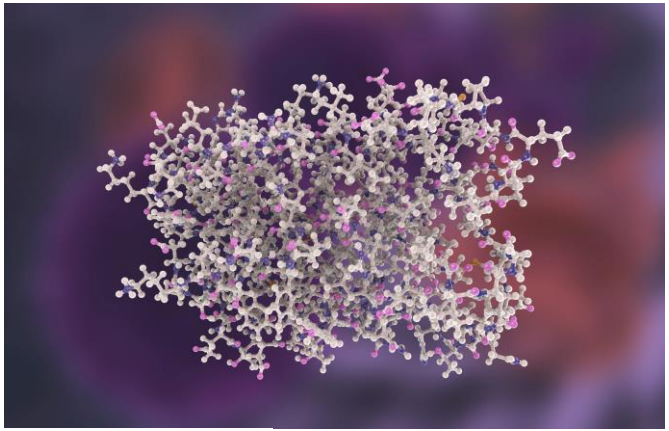
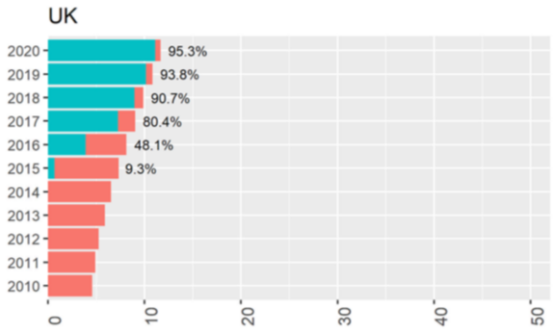
Infliximab



Copyright: Kateryna_Kon - Fotolia



Infliximab is an anti-TNFα chimeric monoclonal antibody used against inflammatory diseases (Crohn's disease)

MoA: it binds to TNFα inhibiting its binding to the receptor of immune cells

Remicade (Janssen) was first approved by FDA in 1998 and by EMA in 1999

Biosimilars started to appear on the market in 2013. 8 biosimilars now present on the market.

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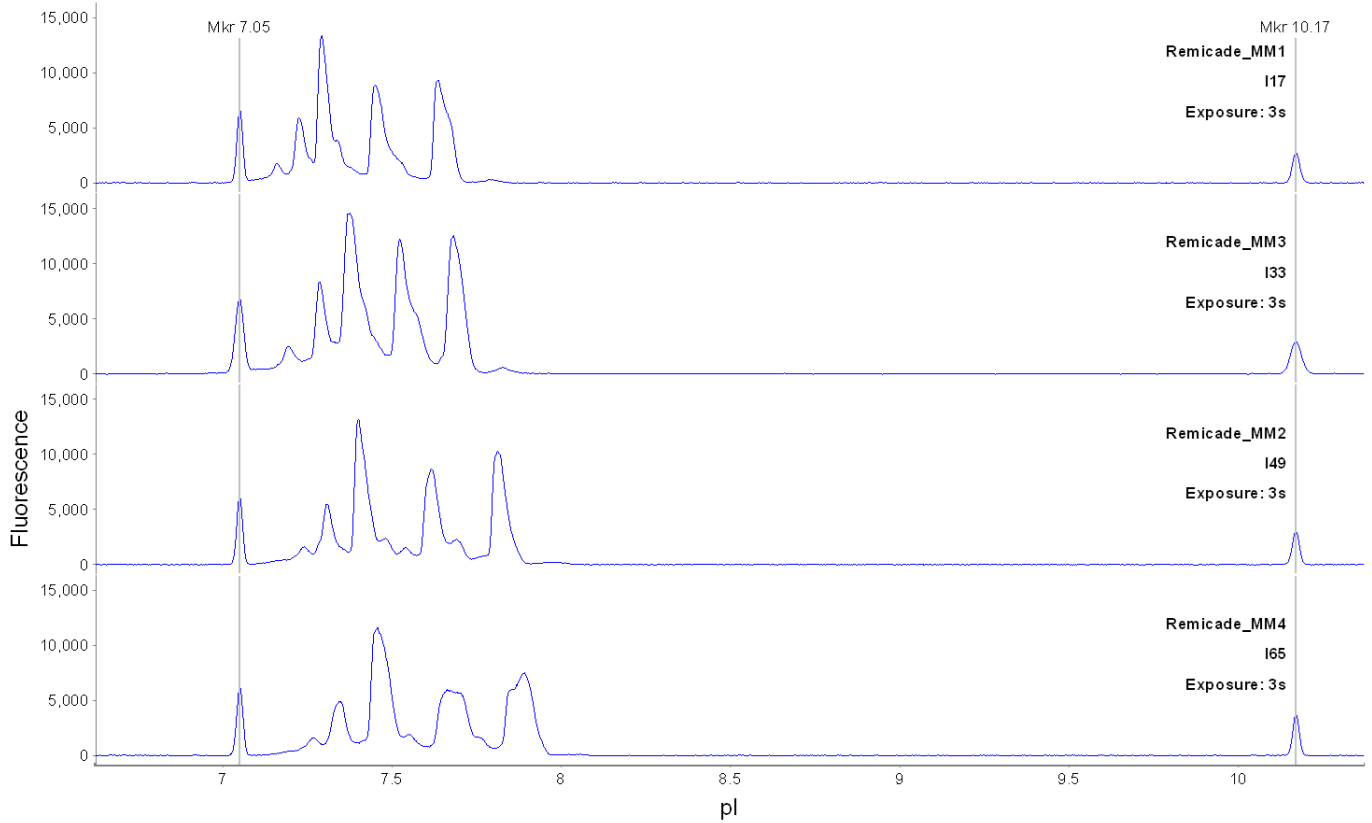
Method Optimization: Protein Solubilizer



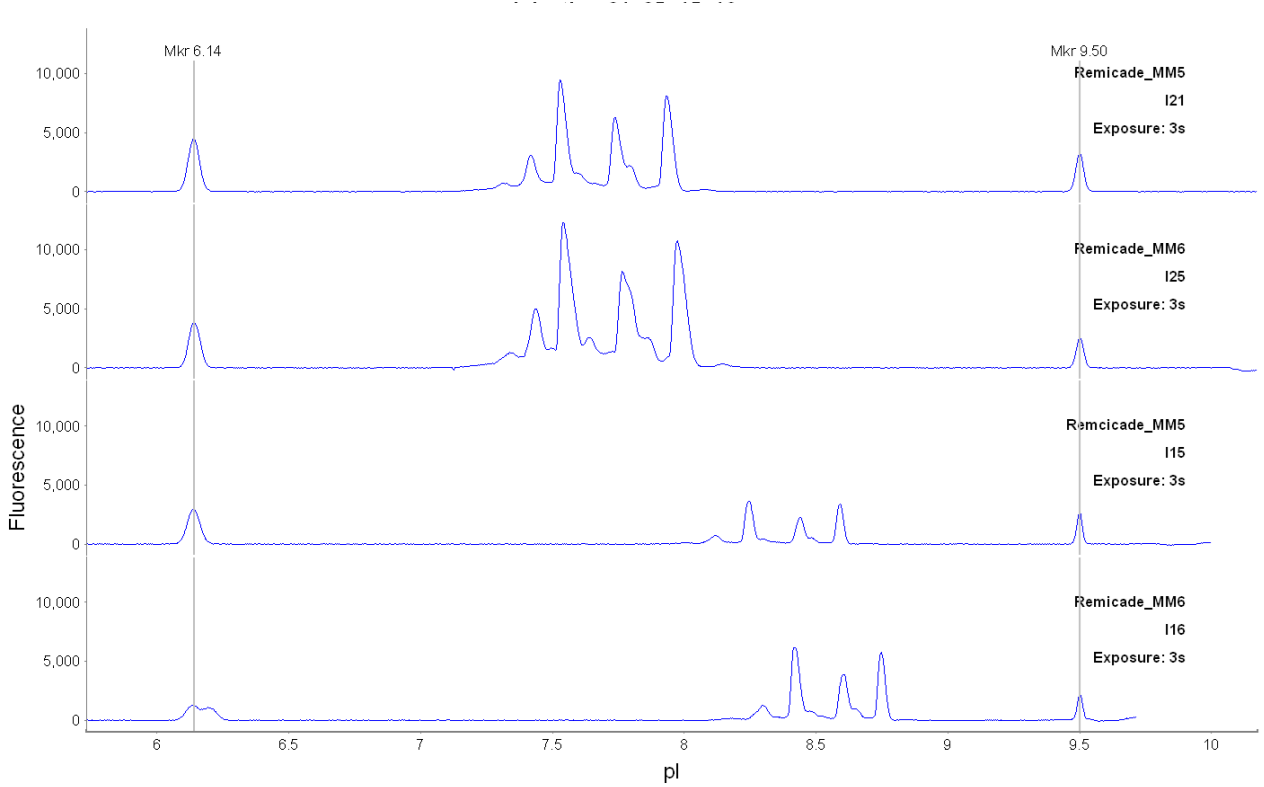
3 M Urea

3.75 M Urea

20% SimpleSol

30% SimpleSol

icIEF – Originator Method Optimization: Ampholytes



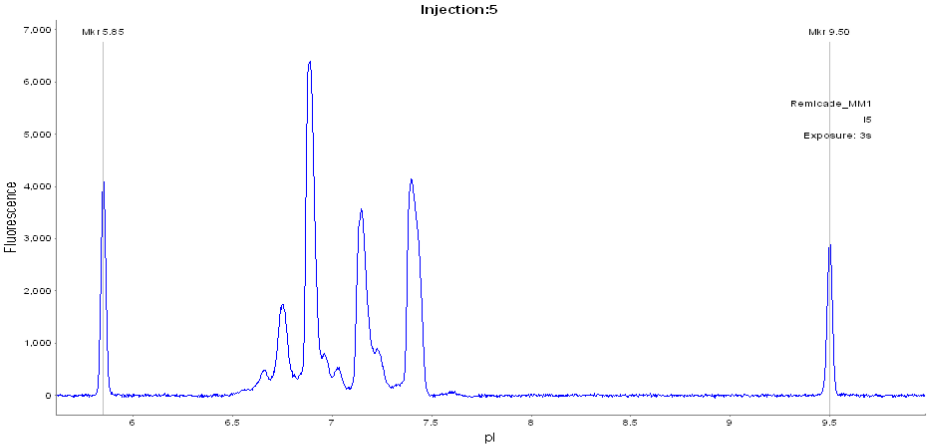
pH 3-10 4%

pH 3-10 8%

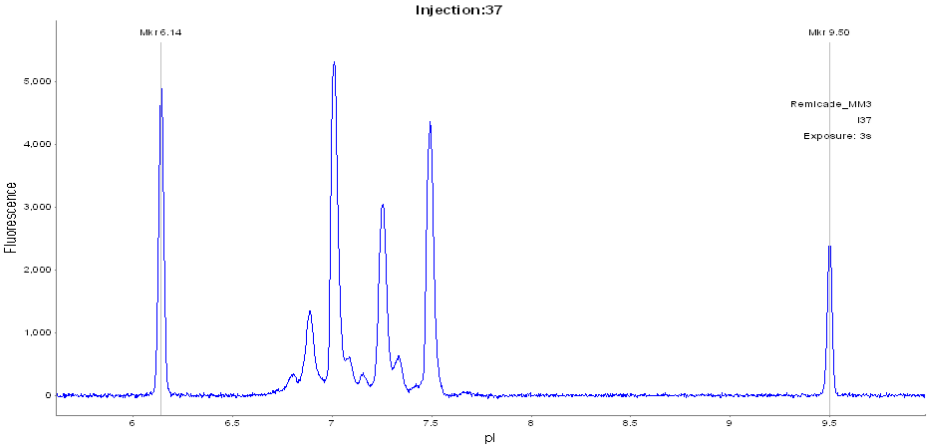
pH 3-10 4%, pH 5-8 4%

pH 3-10 4%, pH 5-8 8%

Method Optimization: pI markers

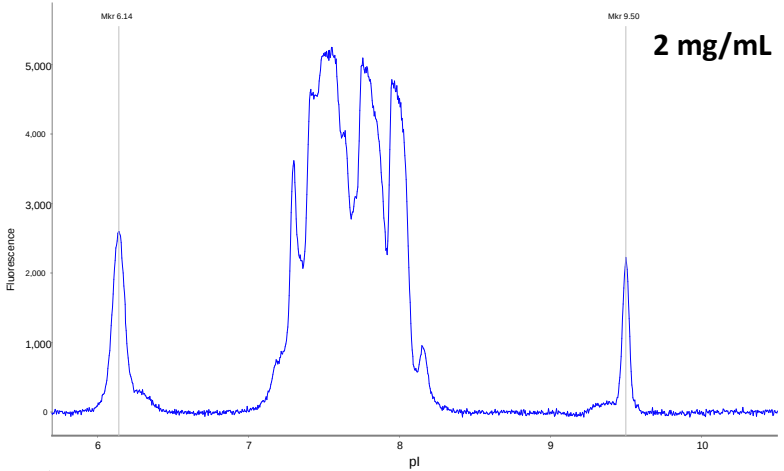


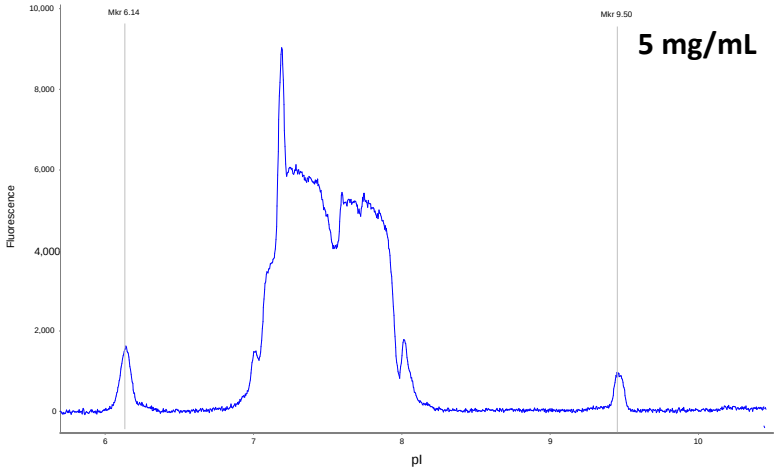
5.85, 9.50

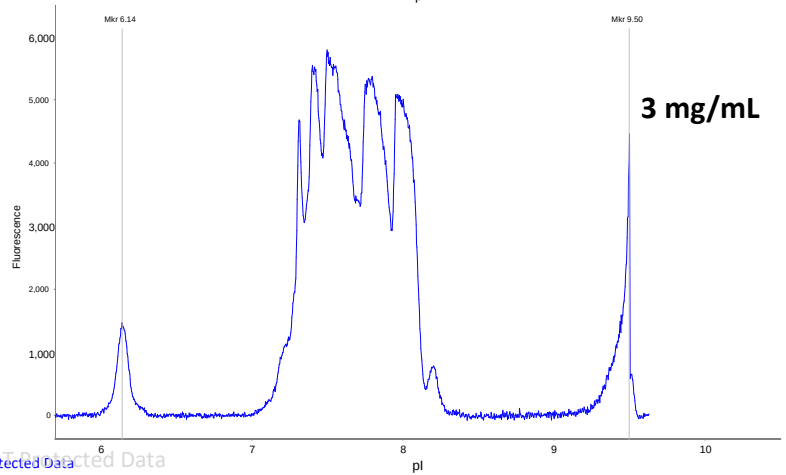


6.14, 9.50

Method Optimization: Fractionation







2 mg/mL initial concentration was selected to preserve resolution and peaks purity.

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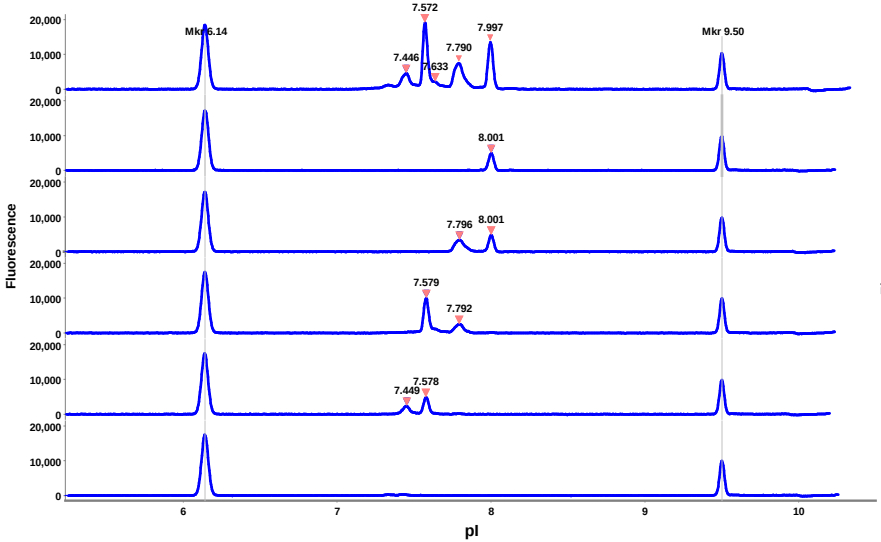
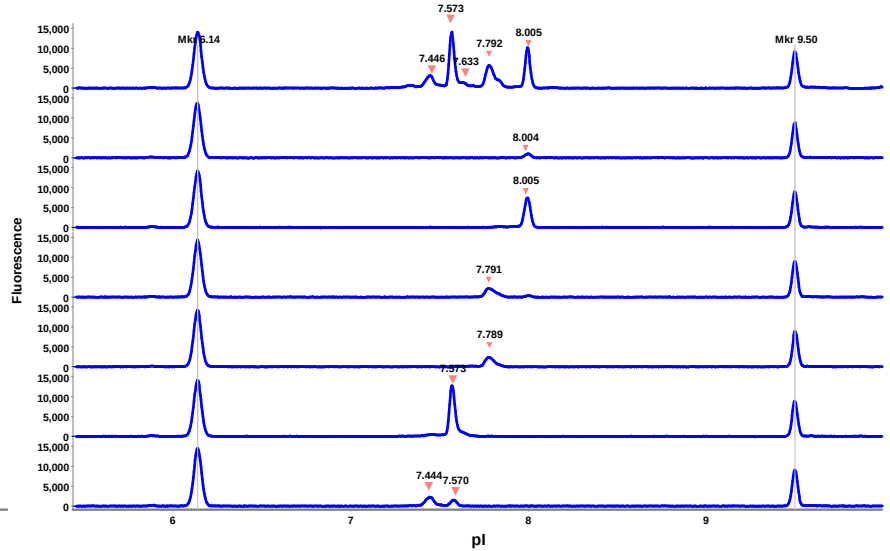
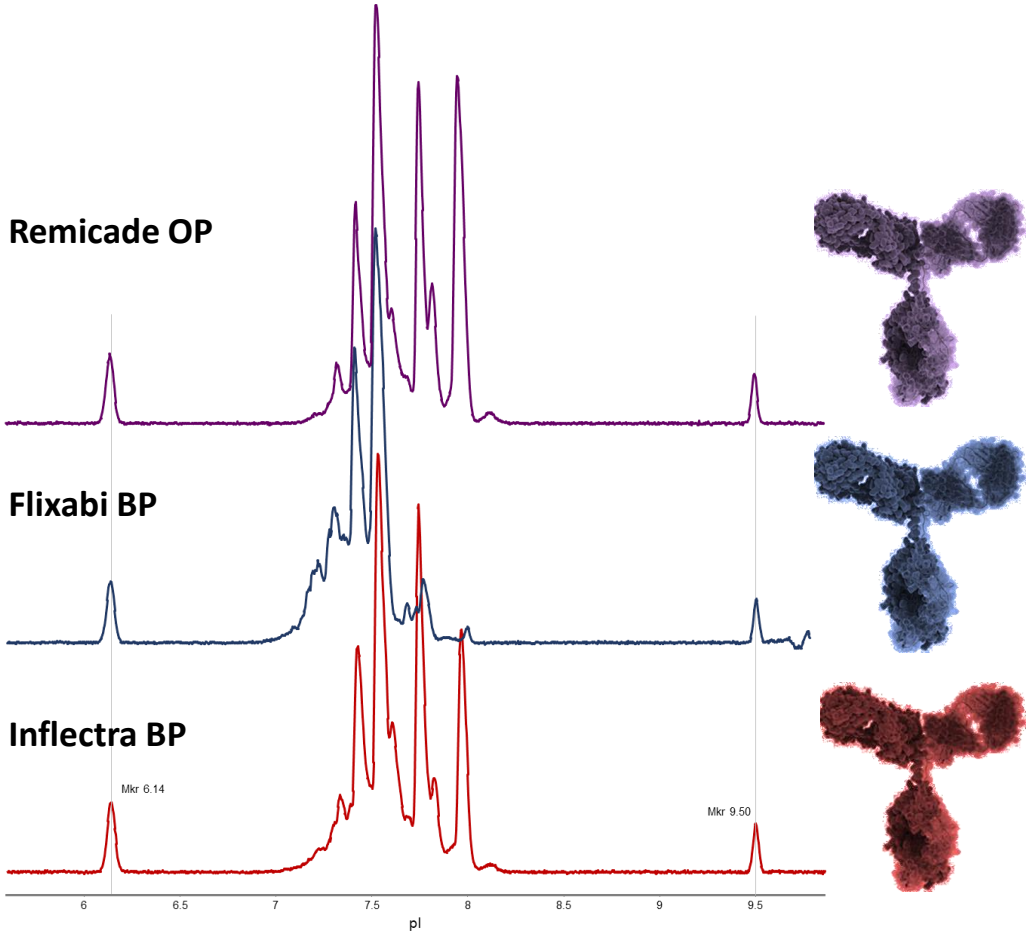
Fraction verification: Refocusing step off/on

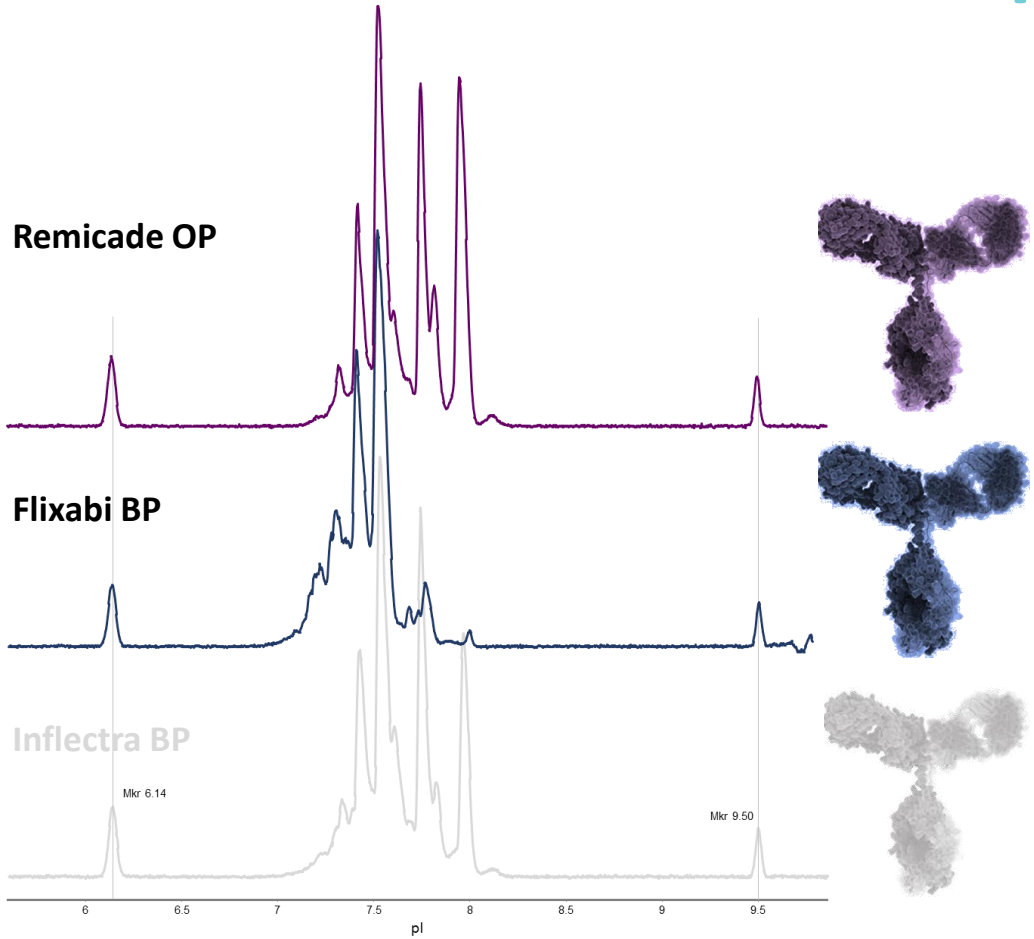




Infliximab biosimilars comparison



Infliximab biosimilars comparison

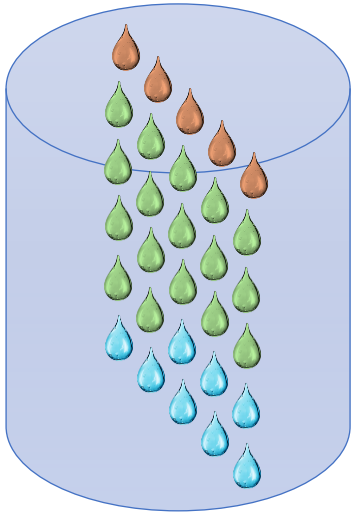


Master Mix

0.35% MC
4% pH 3-10 ampholytes
50 mM Arg
20% SimpleSol
1.5% pI markers 6.14, 9.50

Separation	10 min 500 V, 10 min 1000 V, 25 min 1500 V
Fluorescence detection	0.2 sec
Sample Load	20 sec
Mobilisation	30 min 1500 V
Refocus	5 min 1500 V
Fractionation	25 s, 1500 V
pI Mrkers	6.14, 9.50

Offline peak ID analysis

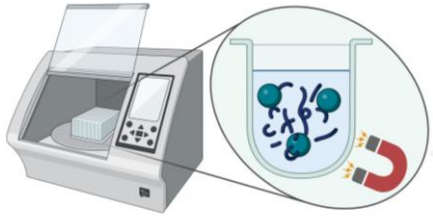


Intact native MS
CE-MS

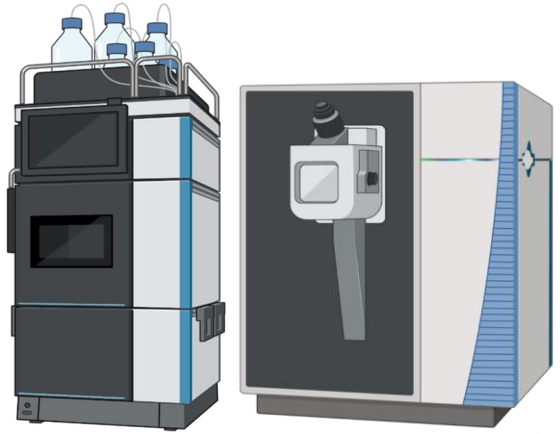
908 Devices ZipChip® CVA kit
3x dilution in CVA diluent, 10 µL on chip, 2 nL injection

Thermo Scientific™ Orbitrap Exploris™ MX mass detector

Peptide mapping nLC-MS/MS



Automated protein clean up (SP3) and tryptic digestion on Kingfisher™ Duo Prime



Reduction → Alkylation → SP3 clean up → Trypsin digestion → nLC-MS/MS

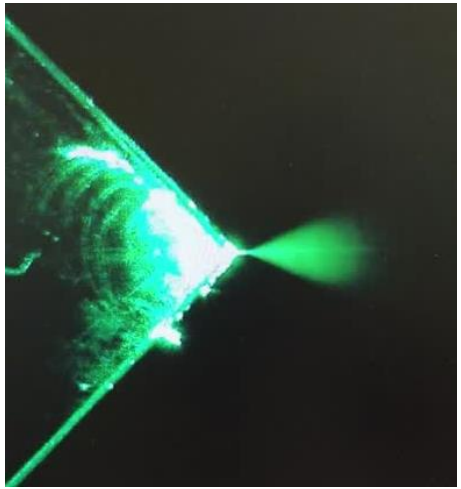
icIEF Fraction verification

EASY-Spray PepMap Neo UHPLC C18 on Thermo Scientific™ Vanquish Neo hyphenated to Orbitrap Exploris™ 480 Mass Spectrometer

Fraction verification: Off-line CE-MS analysis

thermo scientific

908devices



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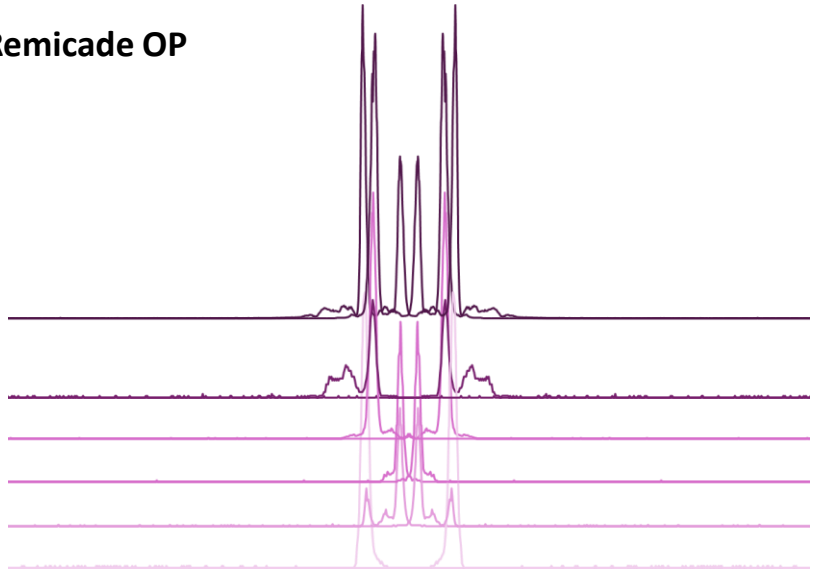
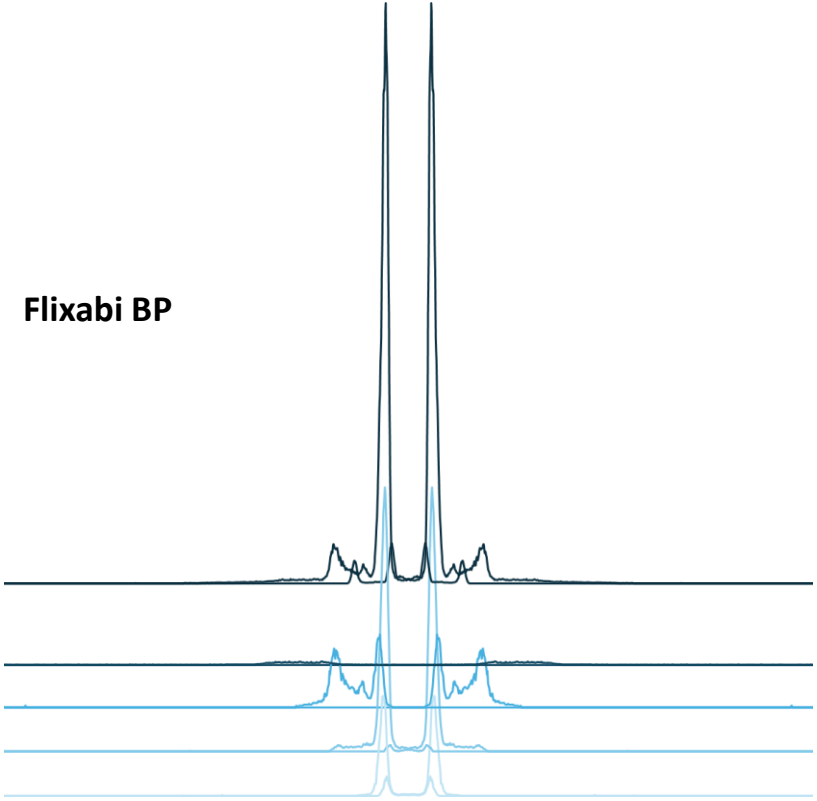
Fraction verification: Off-line CE-MS analysis

Remicade OP



Flixabi BP



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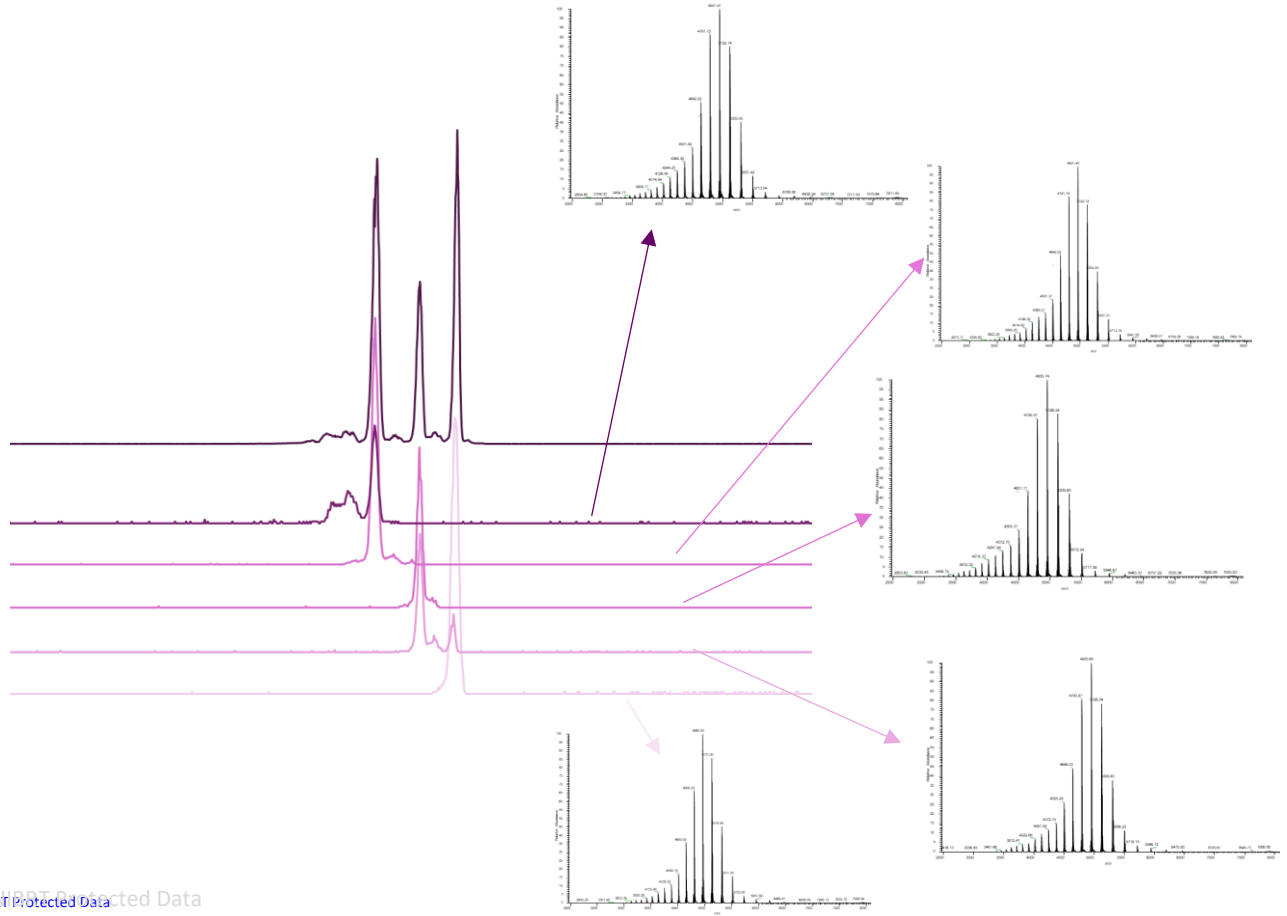
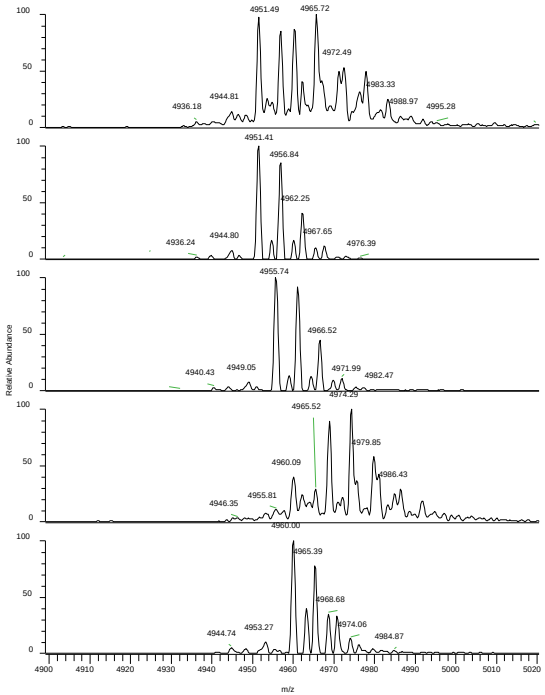
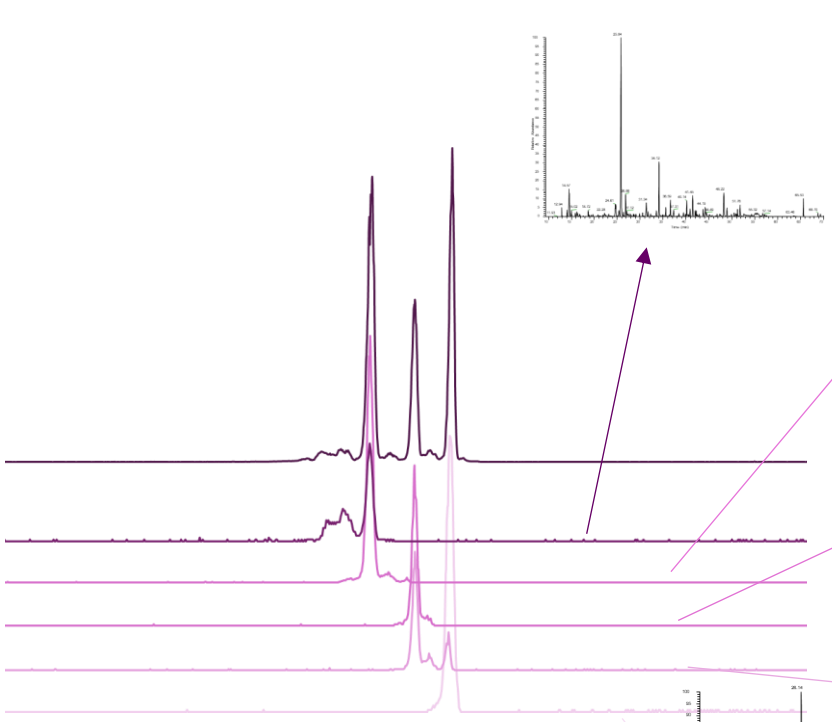
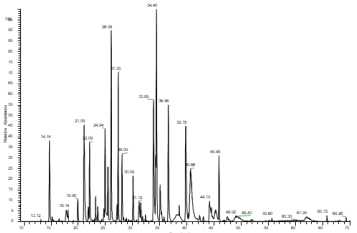
Fraction verification: Off-line CE-MS analysis

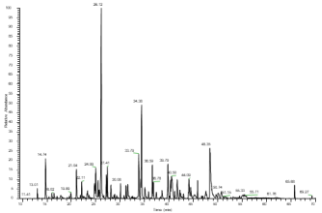


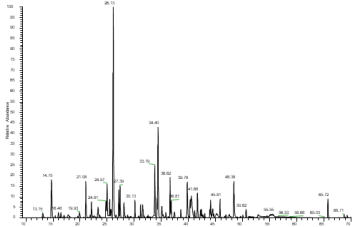


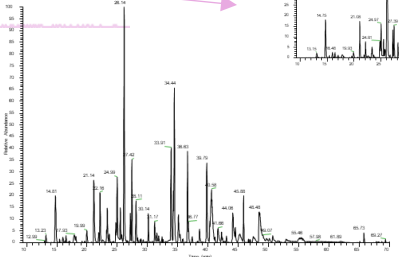
Fraction verification: Off-line peptide mapping

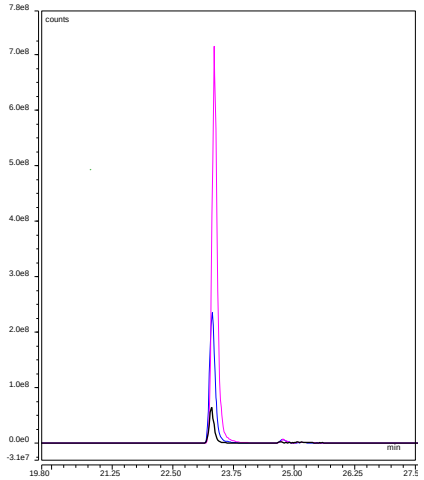






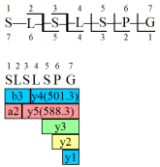




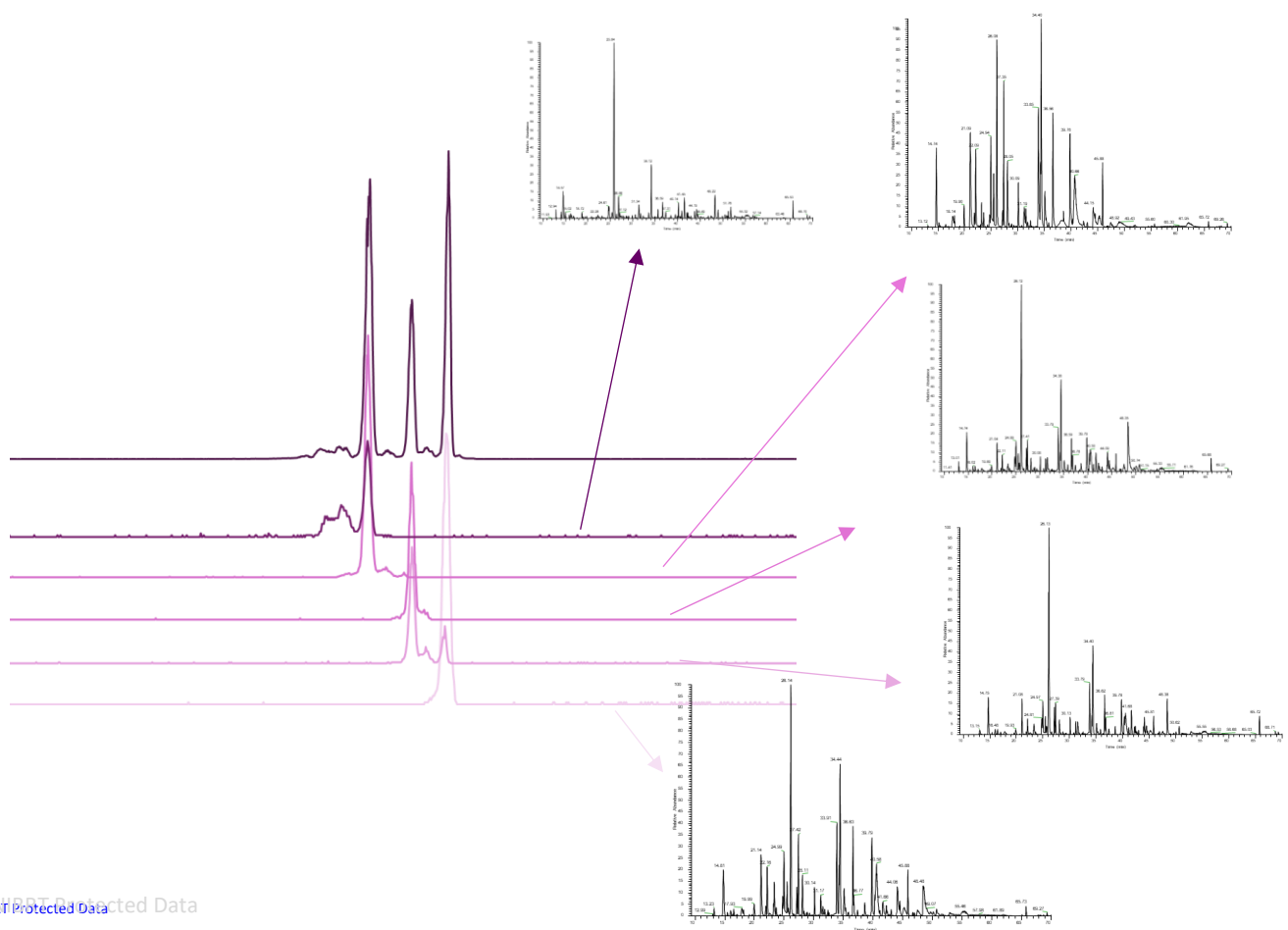


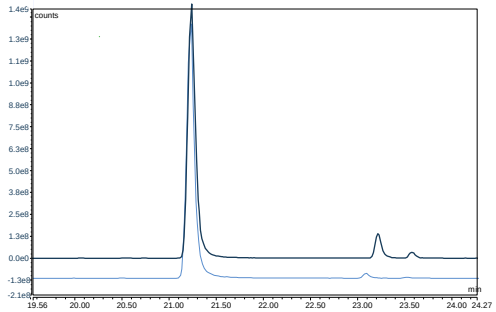
SLSLSPG(G7+Lys) (2+)

Average Structural Resolution = 1.2 residues



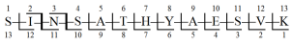
Fraction verification: Off-line peptide mapping

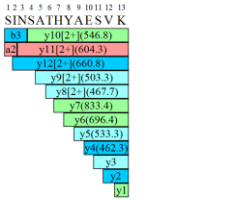




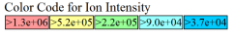
SIDSATHYAESVK (3+)

Average Structural Resolution = 1.0 residues





Color Code for Ion Intensity



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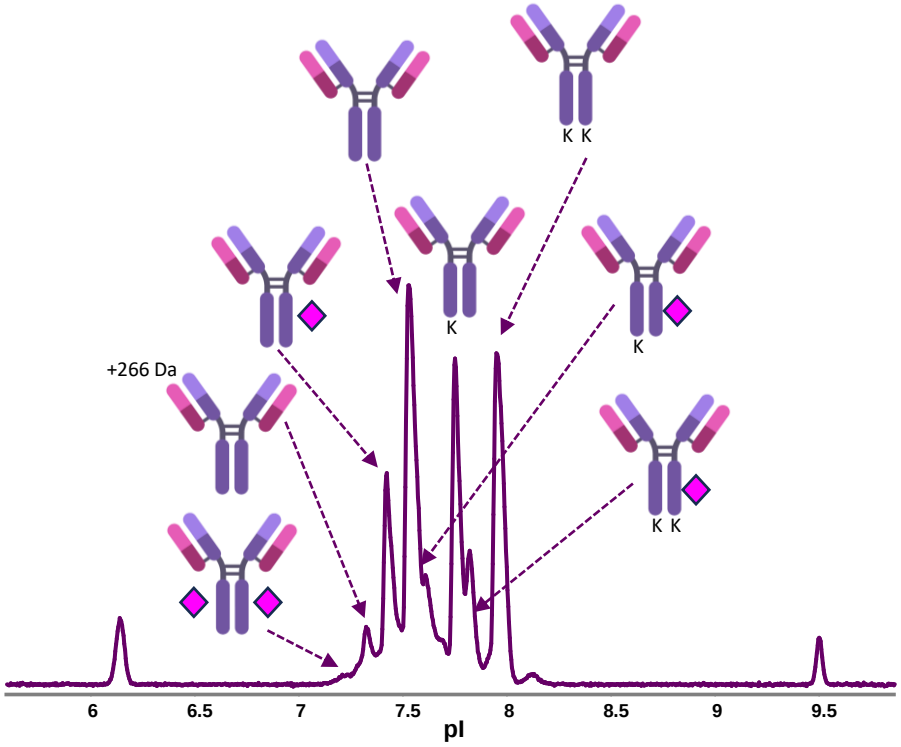
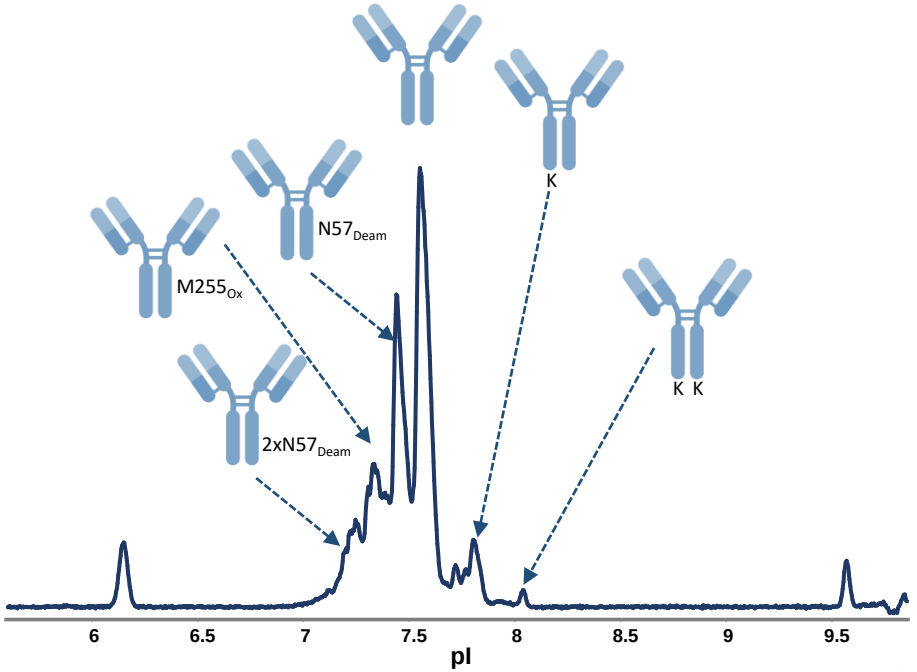
Remicade vs. Flixabi





Conclusions

- Charge variant analysis remains a key analytical tool to solve the complex structural heterogeneity of biotherapeutics.
- icIEF is a robust and reliable platform for charge variant analysis and the fractionation feature of the new MauriceFlex™ allows direct offline MS analysis using both intact and bottom-up approach.
- Although the differences highlighted in infliximab biosimilars are not clinically meaningful, the icIEF fractionation allowed to confidently distinguish acidic variants generated from sialylated N-glycans and acidic variants derived from deamidation events in the CDR region.
- The fractionation of species not allowing easy identification with commonly known modifications allows further studies to be carried for complete analysis.

NIBRT Protected Data

Acknowledgements

NIBRT:
Anna Mulligan, Nora Crushell, Jonathan Bones

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Science Foundation Ireland sfi For what's next

nibrt National Institute for Bioprocessing Research and Training