



CASE STUDY
National Institute for Bioprocessing
Research and Training

Simple, Robust, High Quality Intact Mass Analysis—A Biosimilars Case Study

A world-class facility undertaking cutting-edge research in collaboration with the biopharma industry

The National Institute for Bioprocessing Research and Training (NIBRT) provides training and research solutions for the bioprocessing industry.

NIBRT's mission is to support the bioprocessing industry by providing a unique learning experience for trainees in an environment that replicates the most modern industrial bioprocessing facilities. In parallel, they undertake cutting-edge research in key areas of bioprocessing in collaboration with industry, looking at analytical solutions to help drug product development, manufacture and characterization. The fastest growing class of biopharmaceuticals are monoclonal antibodies (mAbs) and related products and NIBRT's research is aligned with the need for more accurate, high-throughput, simplified analyses to characterize drug products. In this regard, biopharmaceutical production can be followed at every step which can help to correlate process deviations to product structural features and ultimately biological activity.



Biotherapeutic proteins require a panel of analyses in order to be completely characterized: liquid chromatography-mass spectrometry (LC-MS) orthogonal methods are often used to fully assess the critical structural features that are the basis of instability and safety concerns for biologic drug products. Analytical strategies for deciphering lot-to-lot variations need to be in place for already approved drugs, as well as for drugs in development. Providing high quality data and high resolution profiles of biotherapeutics gives the possibility to outline a pattern or ‘fingerprint’ that needs to be matched to ensure similarity and consistency of biological activity.

Analytical approaches providing structural information include:

- **Aggregate analysis**
- **Charge variant profiling**
- **Subunit analysis**
- **Glycan analysis**
- **Intact mass analysis**
- **Peptide mapping**

Choosing the correct analytical approach is increasingly important in the emerging world of the biosimilars. The exponentially growing number of candidate biosimilars undergoing approval highlights the need for quick and accurate characterization methods, which allow structural features to be correlated to biological activity; the final goal being to assess biosimilarity to the originator and thus reduce, as much as possible, the requirement for lengthy and expensive clinical trials.

To achieve quick and confident analysis with high quality and high resolution data, NIBRT decided to implement an Orbitrap-based intact mass analysis solution, comprising:

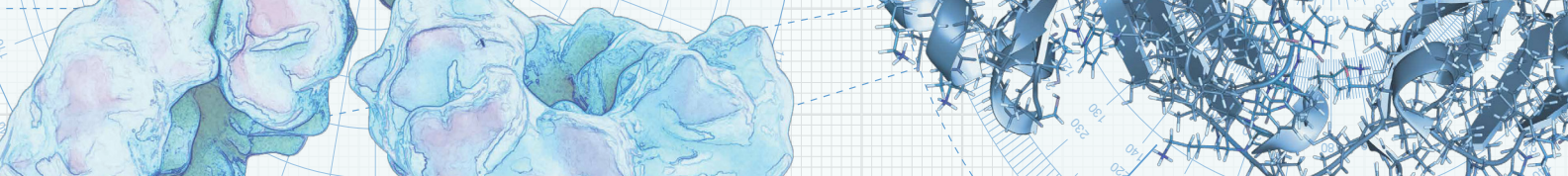
- **Thermo Scientific™ MAbPac™ SEC-1 columns**
- **Thermo Scientific™ MAbPac™ RP columns**
- **Thermo Scientific™ Vanquish™ Flex UHPLC systems**
- **Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software**
- **Thermo Scientific Q Exactive™ Plus Hybrid Quadrupole-Orbitrap Mass Spectrometer with BioPharma Option**
- **Thermo Scientific™ BioPharma Finder™ Software 3.0**

Intact mass analysis can provide information on the quality attributes of mAbs, like glycosylation and lysine truncation. Moreover, intact mass analysis under both denatured and native conditions is a fundamental tool for assessing a candidate biosimilar, to identify any differences quickly and accurately. There is minimal need for sample preparation and data acquisition and spectral deconvolution can be achieved in a short time.



“In NIBRT both research and training aim to support biopharmaceutical companies with a portfolio of state-of-the-art, cutting edge technologies that can cover all aspects of biotherapeutic production and development.”

—Dr. Sara Carillo,
Application Development Team Leader,
NIBRT

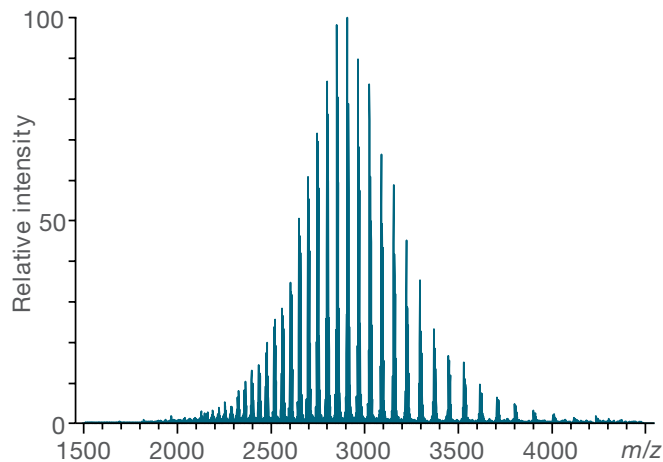


The conventional approach to this analysis is the use of reverse phase (RP) columns as they work with eluents compatible with MS analysis. Nevertheless, under these conditions the eluents cause protein denaturation; as a result, the generated spectrum has a high number of charges (in a range between 20 and 70) and generally appears in the range of m/z 2,000–4,000 for mAbs. Recently, advances have been made in the field of native LC-MS, such as the ability to use these columns with volatile mobile phases as well as improvements in ESI desolvation and large molecule transmission, rendering separation modes such as size exclusion chromatography (SEC) MS compatible. Under these conditions, the resulting spectrum will appear at higher m/z values, generally $m/z > 5,000$. Under native conditions proteins will take on fewer charges due to the more neutral pH of

the mobile phase and occlusion of basic amino acids in the native protein structure. The reduced charge states produced by native MS-based techniques provide increased spectral separation at higher m/z ranges compared to conventional denaturing techniques (Figure 1). The High Mass Range mode within the BioPharma Option available on the Q Exactive Plus is a fundamental tool enabling high resolution spectral acquisition at the high m/z values required for analysis under native conditions.

A holistic solution for intact protein analysis provides quick and easy access to in-depth information on the molecule under investigation, and avoids the occurrence of artificially introduced protein modifications.

RP LC-MS



SEC LC-MS

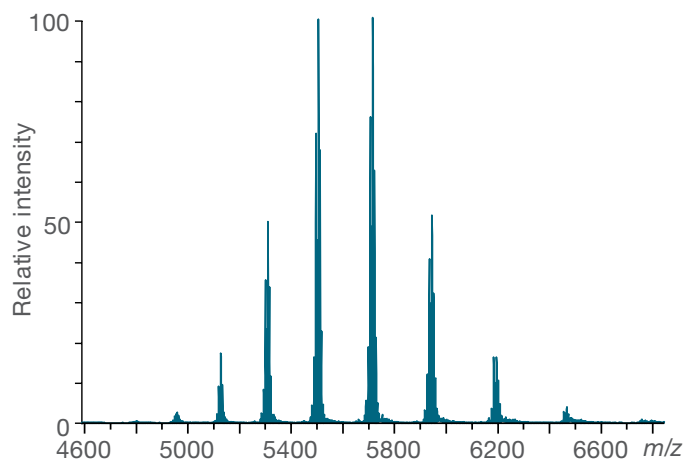


Figure 1. Examples of the charge state envelopes obtained in denaturing (on the left) and native (on the right) intact mass analysis conditions.



“The biotherapeutic market is continuously growing, concurrent with the need for fast, high-throughput methods which are able to return as much information as possible. This intact mass analysis solution clearly meets the industry needs, providing accurate results on mAb glycosylation and major variants in less than one hour.”

—Dr. Sara Carillo,
Application Development Team Leader,
NIBRT

“The Q Exactive Plus mass spectrometer with the BioPharma Option offers high flexibility, high mass accuracy and high spectral resolution for the intact mass analysis of complex samples such as monoclonal antibodies.”

—Dr. Florian Füssl,
Post-Doctoral Researcher,
NIBRT



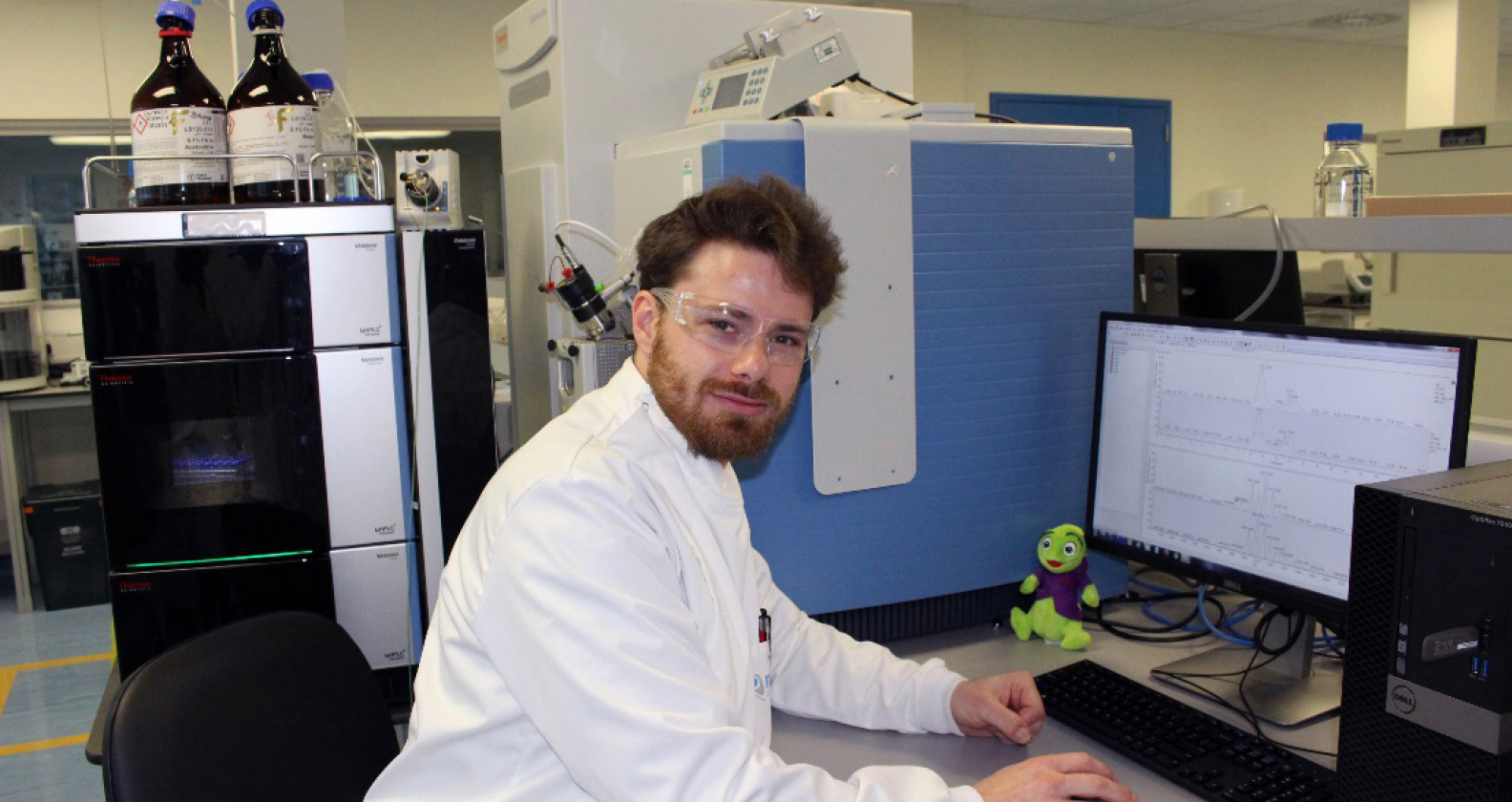
The intact mass analysis solution offered NIBRT advantages in a several key areas:

1. Ideal chromatography for the generation of high quality MS data

- The Vanquish Flex UHPLC systems, in binary and quaternary pump formats, include Thermo Scientific™ SmartInject technology, which offers highly robust and reproducible chromatography over thousands of runs, while being fully biocompatible.
- High quality instrument components minimize the need for maintenance and instrument down-time.
- An adjustable modular organization and compatibility with a variety of chromatographic columns makes the system highly flexible and optimizable for particular experimental needs.
- High pressure resistance enables the use of longer, narrower columns, maximizing chromatographic selectivity and MS sensitivity.
- Excellent chromatographic separations and high peak purity can be prerequisites for the generation of high-quality MS data.

2. Mass spectrometry built for biopharma

- The Q Exactive Plus with BioPharma option is equipped with Orbitrap technology, enabling high resolution, high sensitivity data acquisition.
- Standard, protein, and high mass range (HMR) modes provide user-friendly options for all of the desired characterization applications within the biopharmaceutical sector, including peptide mapping, glycan- and subunit analysis as well as intact protein MS.
- The HMR-mode enables native intact MS of large proteins such as mAbs and related products. Moreover, ion transmission in the HMR mode is optimized for large molecules, maximizing sensitivity and improving data quality.

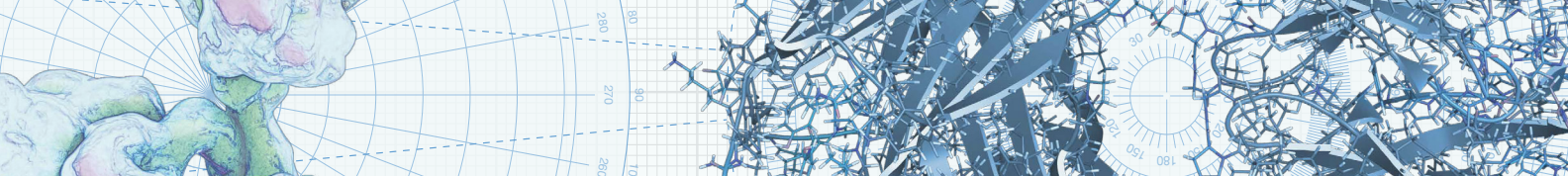


3. A simple path from data to knowledge using BioPharma Finder software

- BioPharma Finder is a powerful software solution for comprehensive MS data analysis of biotherapeutics; catering for peptide mapping, intact protein as well as top-down characterization data and hydrogen-deuterium exchange (HDX) MS data analysis.
- Workflows for intact protein analysis can be customized to fit protein size and analysis type (e.g., native vs denatured) to guarantee optimal deconvolution results.
- The possibility to introduce protein sequences and modifications enables the generation of sequence repositories and automated peak annotation.
- The software also provides a unique Sliding Window deconvolution algorithm, enabling time-resolved, automatic peak identification and relative quantification of species across chromatographic runs.

“Since this intact mass analysis solution has been implemented, it has become our go-to system for fast and confident determination of the main features in our mAb samples—whether these are marketed drug products under investigation or biosimilars under development.”

—Dr. Sara Carillo,
Application Development Team Leader,
NIBRT



A fast and flexible solution for mAb characterization

The interplay of robust chromatography, Orbitrap MS and intelligent data analysis strategies elegantly combines cutting edge technology, high flexibility and ease of use, making this intact protein analysis solution fast and powerful. The possibility to obtain native mass spectral information for large proteins is expanding the chromatographic application range beyond RP chromatography, enabling molecular mass information to be obtained for substances that were previously only detectable via, for example, UV absorption. Irrespective of the chromatography mode used, this solution enables direct and fast comparison of, for example, biosimilars of

a mAb, which is demonstrated in Figure 2. If sequence information for the biopharmaceutical under investigation is available, annotation and comparison can be accomplished within half an hour. This solution has enabled researchers in NIBRT to perform intact protein MS in various modes as a standard procedure. They believe that this is one of the easiest and quickest ways to obtain comprehensive knowledge of biopharmaceuticals with minimal analysis-induced errors.

For more information on the services available from NIBRT, please visit www.nibrt.ie

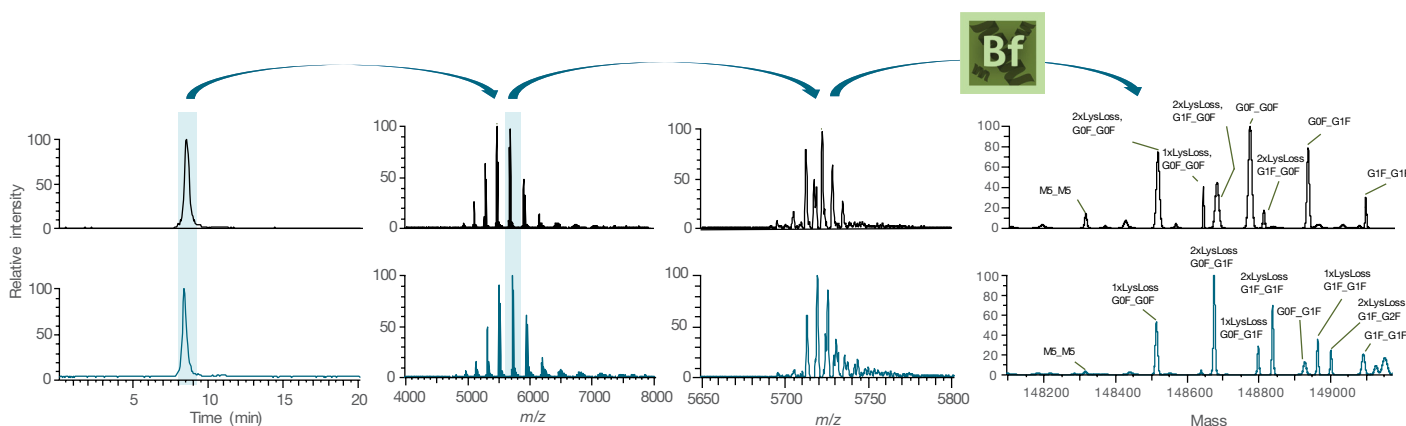


Figure 2. SEC-LC-MS analysis of a monoclonal antibody and its biosimilar candidate. In less than one hour it is possible to obtain accurate information about the main structural features, like glycoform distribution and lysine clipping variants, for the two samples.

Find out more at thermofisher.com/IntactMS

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