nibrt

National Institute for Bioprocessing Research and Training

Contract Research

World Leading Contract Research Services

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About NIBRT

The National Institute for Bioprocessing Research and Training (NIBRT) is a global centre of excellence for training and research in bioprocessing. World leading NIBRT principal investigators and scientific advisors, including Dr Jonathan Bones and Professor Pauline Rudd, continue to drive advancements in the field of bioprocessing analytics through pioneering and innovative research.

About Us

We are a team of characterisation specialists who provide detailed analysis of biologics in line with ICH Q6B and Q5E requirements.

Our highly regarded scientists, renowned for their glycan analysis expertise, are situated in the award winning NIBRT facility with access to a state-of-the-art laboratory equipped with top-of-the-line instrumentation.

Our Story

Professor Rudd has an international reputation for expertise in the fields of glycobiology and glycan analysis. In 2006, her research team was transferred from the Glycobiology Institute, Oxford University to NIBRT, creating the Dublin-Oxford Glycobiology Research Group.

From this group, NIBRT Contract Research launched and began operating as an independent group within the facility.

Our Mission

To exceed our customers' expectations with innovative and bespoke analytical services providing detailed characterisation of their biologics during development and process change.

Our Clients

NIBRT Contract Research has worked with some of the Top 20 global Biopharma companies, SME's, virtual companies and law firms.

At NIBRT every client receives the same high standard of service no matter what their size.

"The NIBRT team's extensive support and quality work was integral in the success of our regulatory submission to the U.S. FDA."

Associate Director, Biologics Development, Horizon Therapeutics

Our Services

Working adjacent to our accomplished team of Principal Investigators carrying out cutting edge, industry aligned research in all areas of biopharmaceutical manufacturing, we are well positioned to support our clients in solving problems at all stages of their product development and production.

"We came across NIBRT Contract Research through our work with a leading expert in the Biotherapeutic characterisation field. From first contact, NIBRT Contract Research has been an outstanding facility to work with. The team at NIBRT Contract Research has been instrumental in pushing the boundaries of our testing needs. They are not only flexible, responsive and a pleasure to work with, they also go several steps further to help us problem solve and develop new ways to test our products and learn more about our systems. NIBRT Contract Research is a top class analytical facility and we will continue to work with them and recommend them to our colleagues going forward. We would use no one else."

North American Law firm specialising in intellectual property

Why Choose Us?

At NIBRT Contract Research we take the time to understand our clients' requirements.

With extensive experience managing complex characterisation projects, our team consistently delivers to the highest standard.

We can provide our clients with:

- Bespoke projects, flexible scheduling and quick response times.
- Clear communication and updates throughout the project lifetime.
- Tailored and detailed reports to ensure full clarity of data and results.
- A subject matter expert and dedicated analysts who offer support throughout the project lifetime.

Our Experience

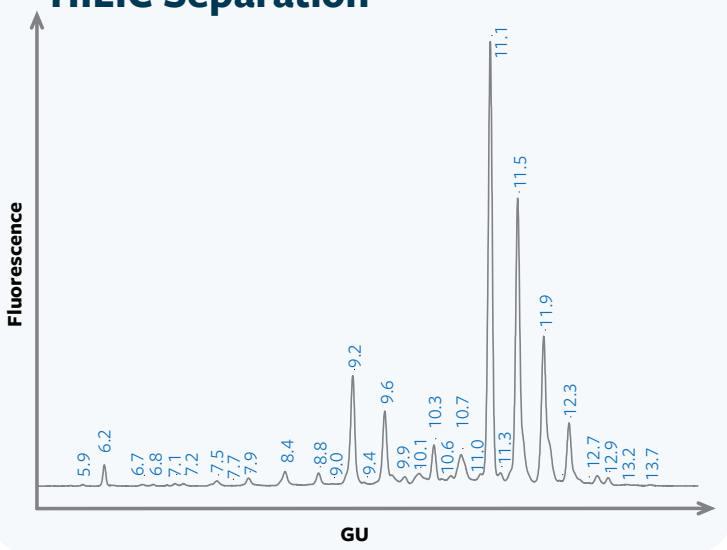
With over ten years' experience we have analysed a variety of proteins and glycoproteins expressed in a range of cell lines and expression systems.

- Monoclonal antibodies
- Gonadotropins
- Fusion proteins
- Erythropoietin
- Interferon
- Enzymes
- Biosimilars

Service Spotlight: *N*-glycan Characterisation

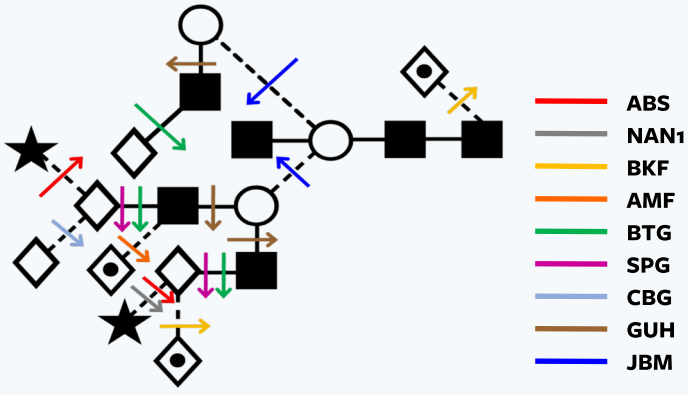
- Many therapeutic proteins are post translationally modified by the addition of *N*- or *O*-linked glycans.
- Glycosylation is considered to be a critical quality attribute (CQA) of biotherapeutics by regulatory authorities.
- Characterising biotherapeutic glycosylation is a requirement under regulatory guidelines (ICH Q5E/Q6B).
- The glycosylation profile can affect the efficacy, immunogenicity and serum half-life of a biotherapeutic.
- Acceptable ranges must be determined as part of the development process and the glycosylation profile monitored and controlled through production.
- Glycosylation of biotherapeutics can be influenced by a number of process-related factors, such as pH, carbon source, dissolved oxygen, temperature during manufacture, and the expression system.
- Our sample preparation involves enzymatic release of *N*-glycans with fluorescent labeling to increase detection sensitivity.
- We use a combination of technologies (HILIC, WAX, LC-MS and exoglycosidase sequencing) to obtain the highest possible level of structural information.
- We provide complete *N*-glycosylation characterisation with confident glycan assignments.

HILIC Separation



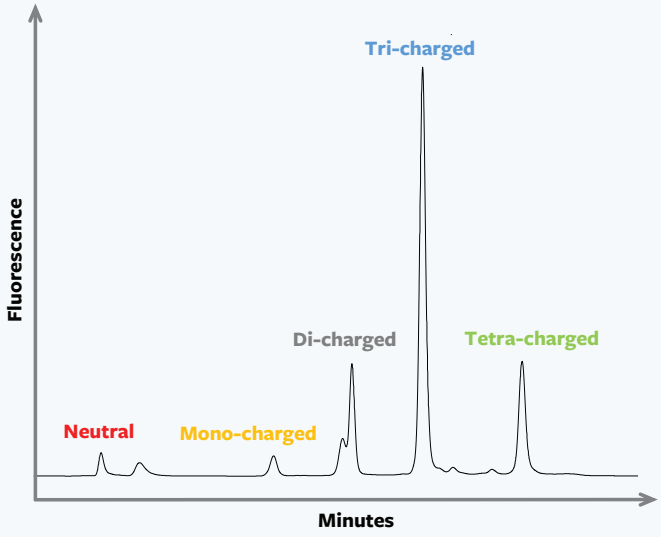
Hydrophilic Interaction Liquid Chromatography (HILIC) separates glycans on the basis of shape, charge and hydrophobic and hydrophilic surfaces. HILIC separation allows for high-resolution separation of complex glycan profiles. Glucose units (GU) are generated using a dextran ladder standard to normalise retention time and to facilitate data analysis.

Exoglycosidase Sequencing



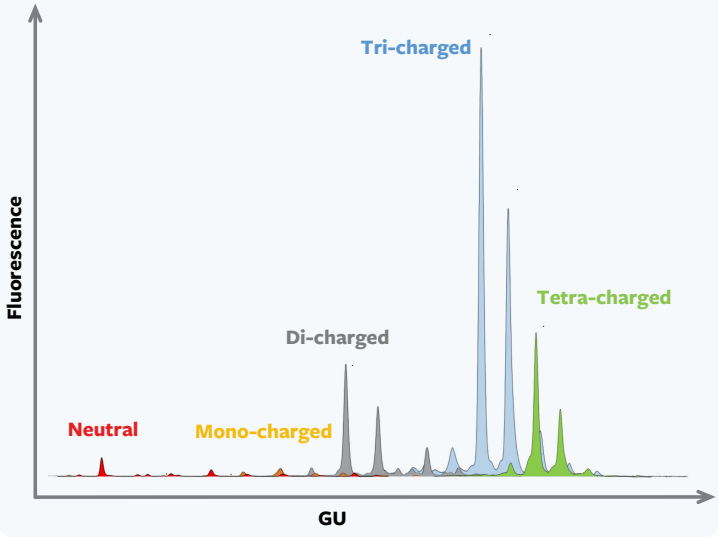
Linkage analysis of glycans is achieved by exoglycosidase enzyme digestion followed by HILIC separation. Exoglycosidase enzymes cleave a terminal monosaccharide with a specific glycosidic linkage. This cleavage yields a characteristic GU shift in the HILIC profile which is used to interpret the data and elucidate the glycan sequence.

WAX Separation



Weak Anion Exchange chromatography (WAX) separates glycans on the basis of the number of charged residues on the glycan. WAX separation allows for the relative quantitation of charged glycans. Sialic acids, phosphates and sulphates exhibit a negative charge and contribute to the glycan charge profile.

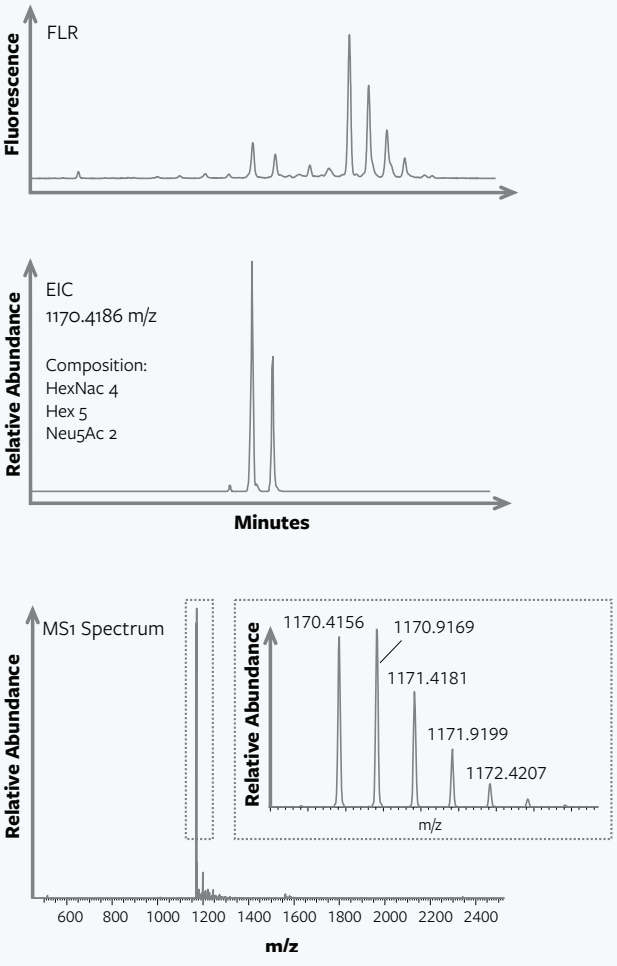
WAX-HILIC Separation



2D-LC with WAX separation in the first dimension and HILIC separation in the second dimension reduces the complexity of highly complex glycosylation profiles.

We use a combination of technologies to obtain the highest possible level of structural information.

Mass Spectrometry



LC-MS with fluorescence detection yields composition information within the HILIC profile and is used as an orthogonal technique to confirm elucidated glycan composition.

ICH Q6B Area: Structural characterisation

Glycan characterisation	
Analysis	**Technique**
***N*- and *O*- glycan characterisation**	*N*-glycans released enzymatically, *O*-glycans released by chemical treatment. Fluorescent labelling of released glycans and analysis by UPLC-FLD (Waters™ Acquity™-FLD) and LC-MS (Thermo Scientific™ Vanquish™-Q Exactive Plus™) or CE LIF (Beckman Coulter™ PA800 plus™). Linkage confirmation by exoglycosidase digestion.
Sialic acid quantitation	DMB labelling of hydrolysed sialic acid and analysis by UPLC-FLD. Quantitation using DMB labelled standards.
Sialic acid linkage relative quantitation	Derivatisation of sialic acids by DMT-MM and analysis by LC-MS.
Site occupancy	Comparison of glycosylated and deglycosylated sample peptide maps by LC-MS.

Protein characterisation	
Analysis	**Technique**
Amino acid sequence	Peptide mapping by LC-MS and bioinformatic analysis against provided protein sequence
N- and C- terminal sequencing	Confirmation of N- and C- terminal amino acids by peptide mapping (detection of blocked N- terminus pyroglutamate/pyroglutamic acid)
	Top down intact mass for orthogonal confirmation
Amino acid composition	Derivatisation and quantitation with AccQ.Tag™ Ultra by UPLC-UV/FLR
Disulfide bonds	Comparison of reduced and non-reduced peptide mapping by LC-MS
Free Thiols	Determination of free thiols using DNTB

ICH Q6B Area: Physicochemical properties

Analysis	Technique
Intact protein molecular weight	Molecular weight determination by RP-LC-MS
	Native MS
Isoform pattern	Profiling of isoforms by various techniques: cIEF peptide mapping and UPLC: IEX, HIC, RP, SEC
Determination of extinction coefficient	Amino acid analysis combined with UV 280nm dilution series

ICH Q6B Area: Process and product related impurities

Analysis	Technique
Aggregate analysis	Determination of aggregates and fragments by SEC, AUC and LC-MS
Molecular variants	Relative quantitation of deamidation, oxidation and other PTM's by peptide mapping LC-MS
	Charge variant analysis by IEX-UPLC and CIEF
	Oxidation by HIC and RP-UPLC
Host Cell Proteins (HCP)	Absolute quantitation of HCP by LC-MS and ELISA
Residual protein A	qPCR using ProteinSEQ™
Host Cell DNA	qPCR using resDNASEQ™
PPG and PEG analysis	Detection of free PPG and PEG by UPLC-CAD
Extractables and Leachables	Detection and quantitation of extracted and leached compounds by ICP-MS, GC-MS and LC-MS.

ICH Q6B Area: Immunochemical Properties

Analysis	Technique
Target antigen binding	Kinetics and affinity/epitope mapping/thermodynamic profiling by SPR (GE Healthcare™ - Biacore T100™)
Effector binding	Measurement of FcγR/FcRn/C1q binding by SPR

ICH Q6B Area: Biological Activity

Analysis	Technique
Cell proliferation	Measurement of thymidine analogue (BruD) incorporation by ELISA (Biotek®-Synergy™ H1) DNA-based measurement of cell cycle (Go/G1, S and G2/M) induction/inhibition using FCM MTT assay with colorimetric readout
Cell death (Autophagy/Apoptosis)	Extrinsic (e.g. Fas) and intrinsic (e.g. caspase activation) apoptosis measurement by FCM (BD™ - FACSMelody™) and ELISA Autophagic flux monitoring by FCM and ELISA
Antibody effector function (ADCC/ADCP)	Mechanism of Action (MOA)-based human FcγRIIIa/FcγRIIa reporter bioassays using luciferase-based detection
Enzyme activity	Kinase/phosphatase/protease activity measurement by FCM and ELISA

Bespoke Analytical Development and Consultancy

Analysis	Technique
Feasibility/pre-validation, verification and qualification	As per client request
Troubleshooting of existing client methods	As per client request
Replication of methods for IP litigation	As per client request
Consultancy	As per client request

Project Process

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