Systematic Analysis of Drug Glycosylation Critical Quality Attributes (GCQAs)

Louise Royle, Radoslaw P. Kozak, Li Phing Liew, Robert J. Emery and Daryl L. Fernandes

Ludger Ltd. Culham Science Centre, Oxfordshire, OX14 3EB, United Kingdom email: rad.kozak@ludger.com

Introduction

Glycosylation can have a significant effect on the clinical safety and efficacy of biopharmaceuticals. Issues with glycans have caused great financial, legal and regulatory problems for those companies who have not dealt effectively with their product's glycosylation. Regulatory authorities are now tightening the requirements for biopharmaceutical companies to characterise, control and compare the glycosylation of their therapeutics. However, measurement and control of drug glycans can be difficult to achieve due to their complexity and heterogeneity. Consequently, changes in glycosylation are the major cause of batch variability for most glycoprotein therapeutics[*Ref 1*].

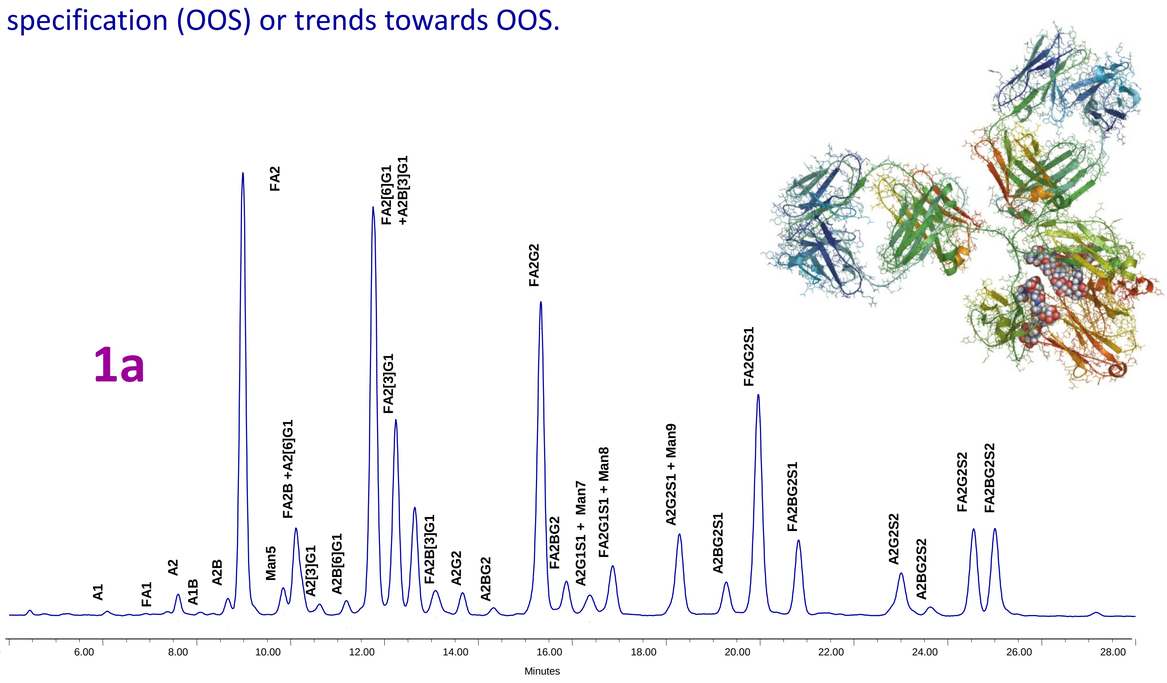
At Ludger we use a systematic approach to greatly reduce the risks of suffering from problems with glycosylation. This system aligns with current and emerging regulatory guidelines from FDA, EMA and ICH and has three broad steps:

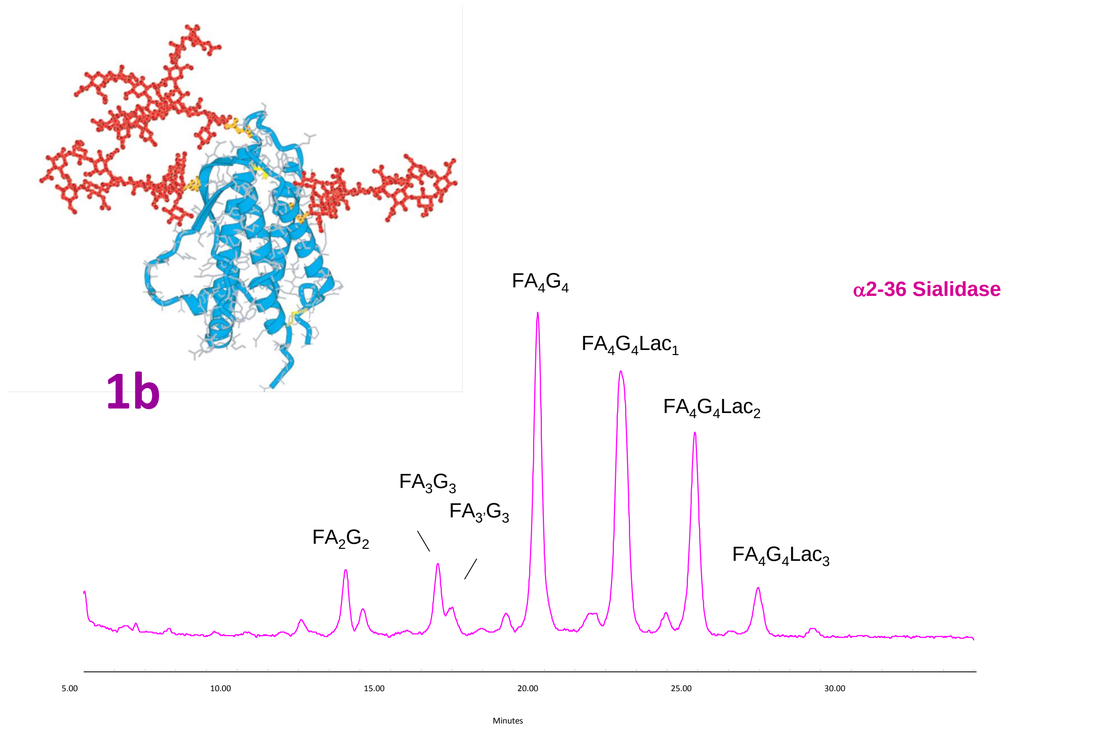
- **GCQAs.** Specification of Glycosylation Critical Quality Attributes (GCQAs) (i.e. those glycosylation parameters that most influence the drug product's safety and efficacy profiles).
- **Glycoprofiling.** Implementation of appropriate, affordable glycoprofiling modules to measure the GCQAs throughout the drug's life cycle.
- **Interpretation and Corrective Action.** Interpretation of the glycoprofiling data and taking appropriate action if the product falls out of specification (OOS) or trends towards OOS.

Analysis of Critical Quality Attributes

A wide range of analytical techniques are available for glycan structure analysis and profiling - but it can be difficult to select the most appropriate of these modules for a particular drug. The types of glycoprofiling analyses required may also change at different points during a drug's life cycle. For example a wide range of complementary methods may be required for initial characterisation of glycosylation and identification of the GCQAs (e.g. monosaccharide analysis, sialic acid analysis, HILIC-LC, full exoglycosidase sequencing, WAX-HPLC, MALDI-MS of neutral glycans, MALDI-MS of permathylated sialylated glycans….), but once they have been identified a simpler, faster method may be sufficient for quality control. To reduce regulatory problems it is crucial that reliable, robust, validated methods are used.

We present here some straightforward strategies that drug developers and biomanufacturers can use for detection and quantification of common biopharmaceutical GCQAs such as sialylation, core fucosylation, antennary composition, lactosamine extensions, alpha-galactose and *N*-glycolyl-sialic acid. The emphasis is on methods that allow compliance with emerging drug regulations from the FDA and EMA [*Refs 1-3*].





F= core fucose α1-6 linked to the inner GlcNAc; Ax, number of antenna (GlcNAc) on trimannosyl core; A2, biantennary with both GlcNAcs as β1-2 linked; A3, triantennary with a GlcNAc linked β1-2 to both mannose and the third GlcNAc linked β1-4 to the α1-3 linked mannose; A3', triantennary with a GlcNAc linked β1-2 to both mannose and the third GlcNAc linked β1-6 to the α1-6 linked mannose; A4, GlcNAcs linked as A3 with additional GlcNAc β1-6 linked to α1-6 mannose; Gx, number (x) of linked beta galactose on antenna; B, bisecting GlcNAc linked β1-4 to β1-3 mannose; Gx, number (x) of linked beta galactose on antenna; [3]G1 and [6]G1 indicates that the galactose is on the antenna of the α1-3 or α1-6 mannose; Gax, number (x) of linked alpha galactose; Sx, number (x) of sialic acids linked to galactose; Lacx, number (x) of lactosamine (Gal-GlcNAc) extensions.

Figure 1. N-Glycan Structure Determination.

(1a). HILIC-UPLC data of 2AB-labelled N-glycans released from IgG. Structures have been assigned by a rage of orthoganal methods including exoglycosidase sequencing and mass spectrometry. The HILIC-UPLC profile allows the determination of several important antibody GCQAs. In particular, the anti-inflammatory and Fc effector functions of IgG are dependent on a range of glycosylation attributes: core fucosylation; sialylation; bisecting GlcNAcs; differently galactosylated glycans (G0:G1:G2); and high mannose type vs. complex glycans.
(1b) HILIC-UPLC analysis 2AB labelled N-glycans released from EPO, following digestion with α2-3,6 sialidase allows identification and quantification of bi-, tri- and tetra-antennary glycans as well as glycans with lactosamine extensions. Biopharmaceutical drug functions can be dependent on a wide range of glycosylation attributes including N-glycan branching (bi-, tri- or tetra-antennary) or degree of lactosamine extensions. These lactosamine epitopes can bind to high affinity galectins which could affect drug function and/or clearance.

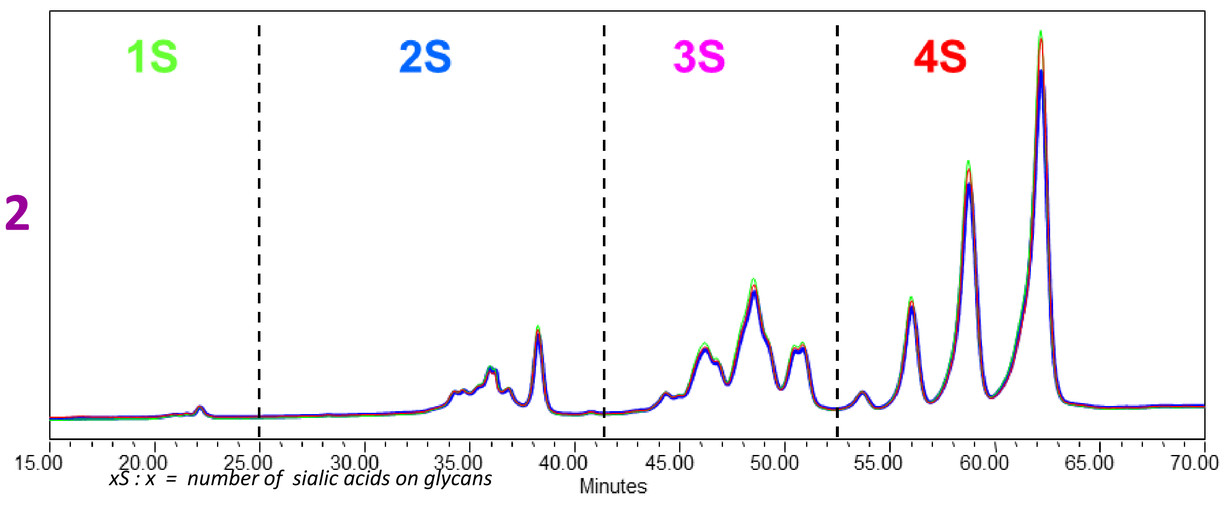


Figure 2. Degree of Sialylation.

The degree of sialylation can impact the function of and biological half-life of biopharmaceuticals such as EPO, as neutral glycans are cleared by asialoglycoprotein receptors in the liver. WAX-HPLC analysis on a LudgerSep-C3 column of the released 2AB labelled N-glycans provides charge profiles for comparability studies.

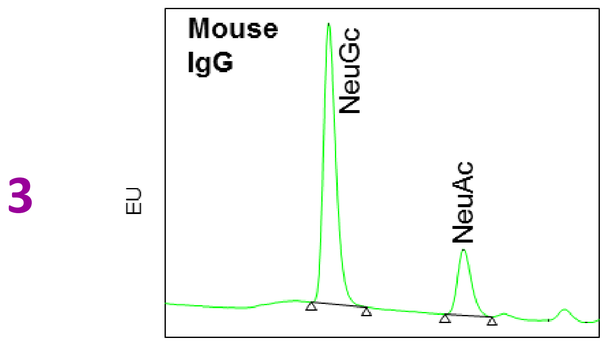


Figure 3. Human vs. Non-Human Sialic Acids.

NeuGc is a non-human sialic acid. It's presence can lead to potential adverse reactions and neutralisation of the drug by anti-NeuGc antibodies. The amounts of sialic acids can be quantified by RP-HPLC on a LudgerSep-R of the DMB labelled released sialic acids.

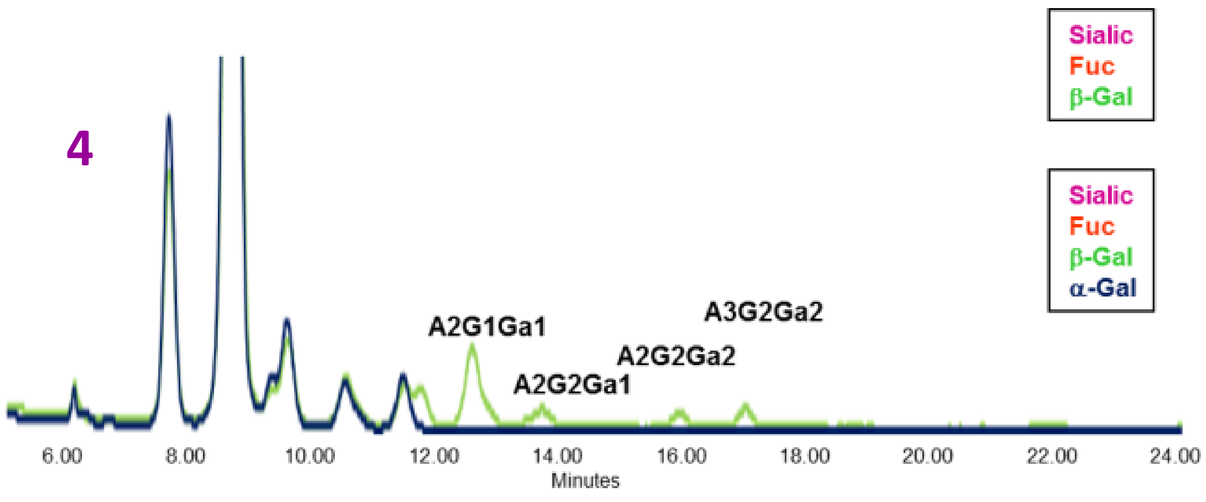


Figure 4. Non-Human-Alpha-galactose.

Galactose α1-3 linked to beta galactose is a non-human glycan epitope. It's presence can lead to potential adverse reactions and neutralisation of the drug by anti-alpha-galactose antibodies. HILIC-UPLC profiles of the released 2AB labelled N-glycans after removal of fucoses, sialic acids and beta galactoses, before and after alpha-galactosidase are compared in order to measure the amounts of glycans that carry the Galα1-3Gal epitope on a MAb.

References.

1. Fernandes, DL (2012) '*A Systematic Approach to Optimisation and Comparability of Biopharmaceutical Glycosylation Throughout the Drug Life Cycle*' in Subramanian, G (Ed) Biopharmaceutical Production Technology' published by Wiley-VCH Verlag GmbH & Co KGaA
2. Fernandes, DL (2012) '*Assessing Comparability of Glycosylation between a Biosimilar Antibody and the Innovator Product*', Antibodies Asia 2012, Shanghai, China
3. Tharmalingam T, Adamczyk B, Doherty MA, Royle L, Rudd PM. (2013) '*Strategies for the profiling, characterisation and detailed structural analysis of N-linked oligosaccharides*' Glycoconj J. **30**:137-146