Development of Simple, Rapid & High-Throughput Glycanalytic Methods for Biopharmaceuticals

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Introduction

Over two thirds of biopharmaceutical products are glycosylated proteins. A biopharmaceutical’s glycosylation profile is a critical product quality attribute that impacts on the efficacy and safety of these molecules. The glycosylation profile must be characterised and monitored throughout product development and manufacturing processes. Lectins are bioaffinity proteins that can recognize and bind to specific glycan structures on intact glycosylated biomolecules and can therefore be used for the analysis of these molecules. Currently available lectins are predominantly plant based. These, however, lack specificity, are structurally complex (glycosylated) and difficult to produce recombinantly. GlycoSelect’s Recombinant Prokaryotic Lectins (RPLs) are superior glycoselective molecules that facilitate simple and effective analysis and isolation of intact glycoproteins. RPLs are much more specific, consistent and scalable than their plan counter parts. This project aims to develop a novel analytical platform to advance the rapid analysis of the glycosylation profiles of biopharmaceuticals. Using GlycoSelect RPLs integrated into ForteBio’s biosensor platform we will analyse biopharmaceuticals provided by Allergan Biologics.

Methods

Two biopharmaceutical samples provided by Allergan were analysed using the Octet platform: FSH (follicle stimulating hormone) and Eylea (Fc fusion protein). Samples were tested for terminal β1-4 galactose and sialic acid structures using RPL-Gal1 and RPL-Sia1, respectively. The robustness of the Octet quantitation assay developed at GlycoSeLect for each RPL-Biopharmaceutical pair was assessed at CPI by verifying the linearity, repeatability and percentage spike recovery of each assay.

Results

The linearity of FSH (β1-4 galactose) analysis was determined by the binding rate of two replicates (n=2) at 8 concentration levels: 500 µg/ml, 250 µg/ml, 125 µg/ml, 62.5 µg/ml, 31.3 µg/ml, 15.6 µg/ml, 7.8 µg/ml and 0 µg/ml. The acceptable range of the assay was 7.8 to 500 µg/ml (%CV < 20%).

Six replicates of FSH at 200 µg/ml were measured to determine the repeatability and precision of the Octet method. The concentration results in µg/ml for the six replicates are shown in Figure 4. The standard curve and results for the 6 replicates are shown in Figure 3. The assay demonstrated high levels of repeatability with a %CV of less than 30% for quantitation of the FSH (β1-4 galactose) at 200 µg/ml.

Furthermore, the Octet method demonstrated percentage spike recoveries of 80-120%, high intra-assay linearity and repeatability for both the supplied FSH and Eylea samples with Gal1 and Sia1 RPLs-SAX sensors. These sensors where used in the Octet system to detect terminal β1-4 galactose and sialic acid structures in the FSH and Eylea samples. Figure 6 shows the standard curve obtained for these samples.

Conclusion/Summary

The suitability of a novel high-throughput method for the detection of glycans in purified biopharmaceutical samples has been assessed. The method, which uses the Octet system with Gal1 and Sia1 RPLs-SAX sensors to detect terminal β1-4 galactose and sialic acid structures, demonstrates high intra-assay repeatability and linearity for the supplied FSH and Eylea samples. Percentage spike recoveries of 80-120% indicate that glycan detection is not compromised by the presence of the sample buffer.

This project consortium brought together the expertise and resources of CPI, GlycoSeLect UK Ltd, ForteBio Pall Life Science and Allergan Biologics Ltd, to address the need for new glycoanalytical approaches to meet the needs of the growing biopharmaceuticals market.

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