Chromatography Solutions

Technical note #021

Glycan Analysis using HILIC with Fluorescence Detection

INTRODUCTION

Monoclonal antibodies (mAbs) are one of the fastest growing areas of therapeutic interest in the biopharmaceutical industry today. They are produced from cell lines and are made up of two heavy and two light polypeptide chains which are joined together by disulphide bonds. The Fab termini of the heavy and light chains contain a variable region which determines the specificity of the mAb for a target antigen (known as the complementary determining region).

mAbs are heavily glycosylated proteins, which means they have oligosaccharide species (glycans) attached at specific locations on the molecule. All mAbs contain at least two glycosylation sites (usually on the heavy chain), as depicted in figure 1, with the presence of glycans affecting the safety and efficacy of the drug. For this reason, the glycosylation pattern must be thoroughly characterised during mAb production to ensure the target drug has been correctly synthesised. In fact, glycosylation is a post-translational modification which is considered a Critical Quality Attribute and therefore should be monitored according to the ICH Q6B guidelines.

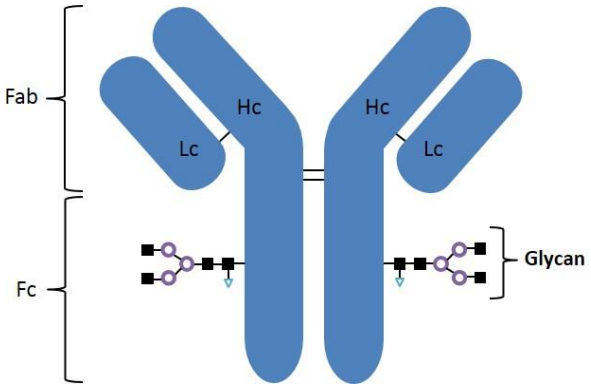

Figure 1: Schematic showing glycosylation regions of a monoclonal antibody

Fc = Crystallisable fragment, Fab = Antigen-binding fragment, Hc = Heavy chain, Lc = Light chain

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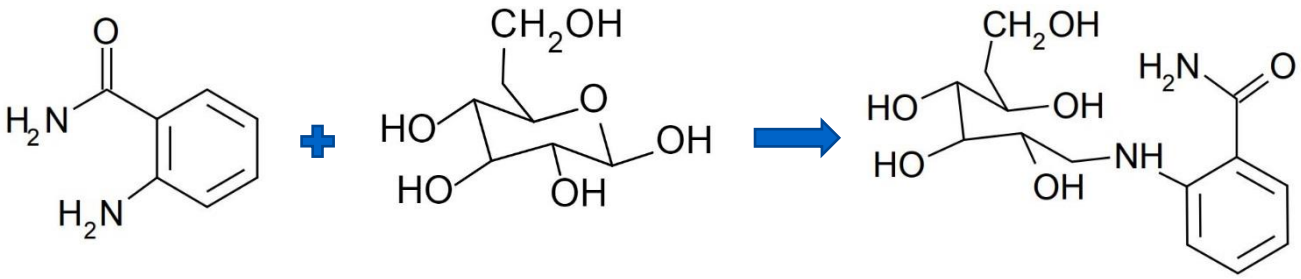
2-AB Labelling

Enzymatic release of the glycan species with PNGaseF allows separation of closely related carbohydrates using HPLC. Since glycans do not contain a chromophore, they must be labelled with a fluorescent compound before being detected. In the application shown in figure 3, 2-aminobenzamide (2-AB) was used as the fluorescent label. Figure 2 shows the formation of a 2-AB labelled glucose monomer. Due to the highly polar nature of released glycans, hydrophilic interaction liquid chromatography (HILIC) is employed for their separation, the details of which are shown in tables 1 and 2.



2-Aminobenzamide | Glucose | 2-AB labelled glucose monomer

Figure 2: Formation of 2-AB labelled glucose monomer

Table 1: Method details

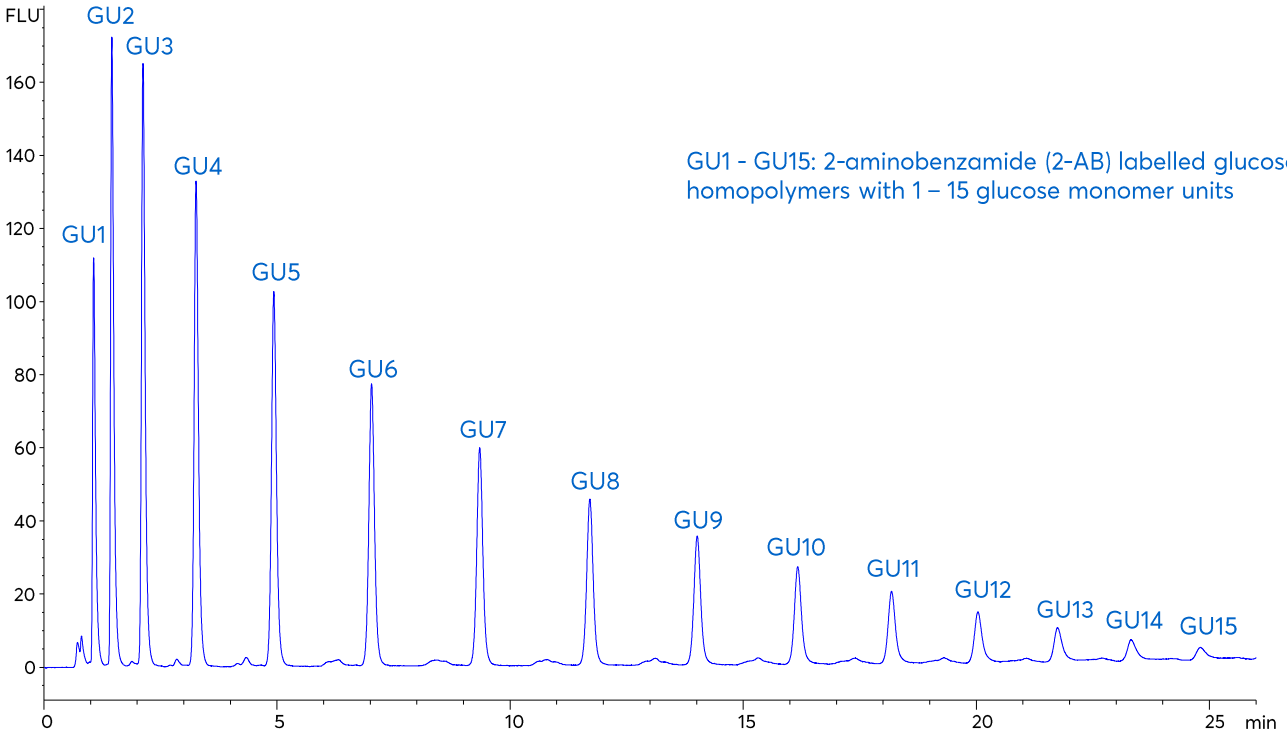
Column	ACE Excel 3 Glycan
Dimensions	150 x 2.1 mm
Part Number	EXL-1116-1502
Mobile Phases	A: 100 mM ammonium formate in H_2O (pH 4.5) B: MeCN
Flow Rate	0.5 mL/min
Injection	1.5 µL
Temperature	55 °C
Detection	FLD, λ_{ex} = 260 nm, λ_{em} = 430 nm
Instrument	VWR Hitachi Chromaster with FLD
Dwell Volume	1950 µL

Table 2: Gradient profile

Time (mins)	%B
0.0	75
24.0	60
24.3	40
24.6	40
24.9	75
45.0	75

HILIC Separation

Figure 3 shows the analysis of a glycan ladder standard containing 15 glucose homopolymers of increasing length. Method details are shown in tables 1 and 2. This method demonstrates the ability of the ACE Glycan phase to separate structurally similar glycan species and can be used as a calibration reference when identifying more complex glycans based on relative retention times.



GU1 - GU15: 2-aminobenzamide (2-AB) labelled glucose homopolymers with 1 – 15 glucose monomer units

Figure 3: Separation of glucose polymers. (ACE Application Note #7160)

ORDERING TABLE

Product	Details	Size	Part number
ACE Excel 3 Glycan	UHPLC column	150 x 2.1 mm	EXL-1116-1502