Glucose Homopolymer (GHP) – a System Suitability and a Reference Standard for Glycan Analysis using Liquid Chromatography

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During glycan analysis using hydrophilic interaction liquid chromatography (HILIC) platform there are two main analytical goals: 1) *to have a reliable and reproducible analytical system* and 2) *to identify your analytes with accuracy and confidence*. Therefore, it is good practice to run a regular system suitability check and to implement reference standards within your analysis. To meet both of these requirements, we use Glucose Homopolymer (GHP) standard at Ludger. This standard gives a characteristic ladder profile from monomeric glucose units up to approximately 20-mers of glucose oligosaccharides, dependent upon the chromatographic conditions employed. The elution position of each peak in this ladder is expressed as a glucose unit (GU).

Use of GHP as the system suitability standard

GHP ladder can be used as a system suitability standard (See figure 1) to ensure that the liquid chromatography (LC) system is fit for purpose. The criteria which must be met are the following:

- Does the GHP profile match the profile shown in the CofA of the standard?
- Are the peaks symmetrical and well resolved? Do the peaks match the regular distribution pattern?
- Do at least two GHP profiles overlay?

Common problems encountered when assessing system suitability can include the following; asymmetrical peaks may indicate compromised column quality or column aging. Whilst, irregular elution patterns could result from insufficient equilibration and/or various issues with LC hardware components (i.e. pumps, buffer system, blockages etc.)

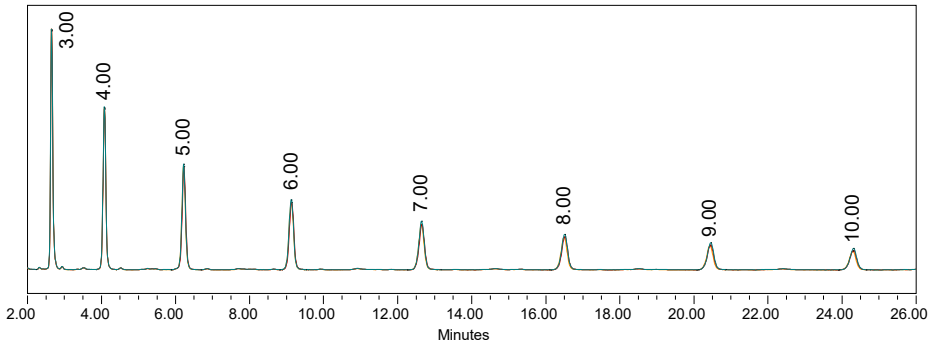

Figure 1: *Triplicate overlay of Ultra-high performance liquid chromatography (UHPLC) chromatograms from 2-AB labelled GHP Ladder [CAB-GHP-30] run on a HILIC column. Each peak is labelled with their corresponding GU value.*

Use of GHP as the reference standard

GHP ladder can also be used as a reference standard (See figure 2) to assign GU values to peaks in the released glycan pool by comparison with the ladder. These GU values are reproducible and predictive as each monosaccharide in a glycan contributes a set increment to the GU value. This allows for primary assignment of structure by comparison of GU values for unknown glycans with glycan standards whose GU values are in databases or reported in the literature (https://glycostore.org/displayCollection/Ludger).
It is important to remember that the proposed identity of a glycan must be confirmed with further orthogonal structural analysis (e.g. MS or exoglycosidase sequencing).

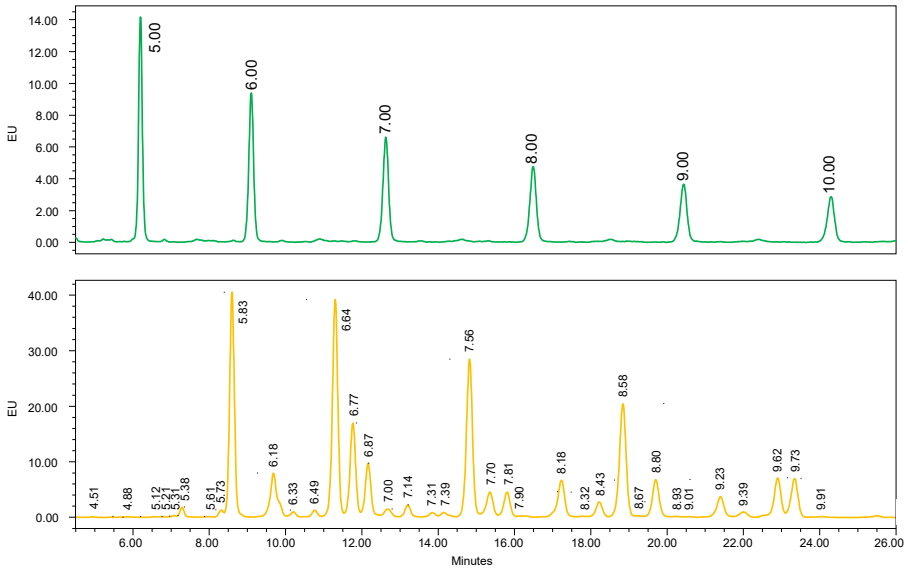

Figure 2: *Stack plot of HILIC UHPLC chromatograms from 2-AB labelled GHP (top panel) used as a reference standard for analysis of 2-AB-labelled glycans released from human IgG [GCP-IgG-100U] (bottom panel).*

GHP standards labelled with 2-AB [CAB-GHP-30], 2-AA [CAA-GHP-30], and procainamide [CPROC-GHP-30] are available at Ludger to match your glycan analysis strategy. These standards are supplied in amounts that are suitable for 30 LC injections. For any enquiries, to request a quote or place an order please contact info@ludger.com