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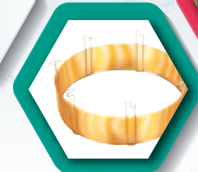
TUDO PARA CROMATOGRAFIA



Facilitando o seu Sucesso

Tips from our Protein Separation ZenMasters

N-Glycan Analysis

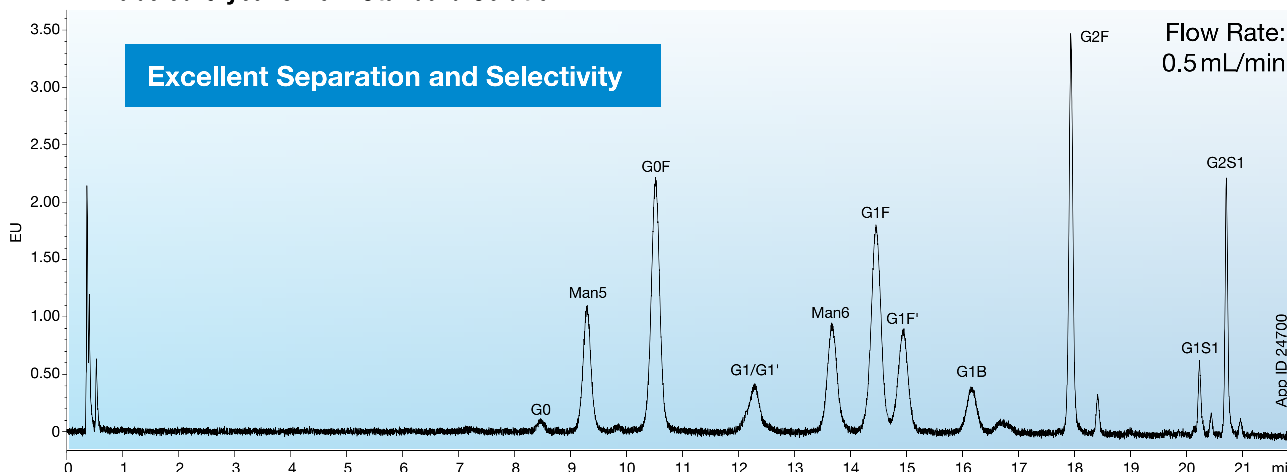
Chad Eichman, Ph.D.
BioPharm Global Marketing Manager

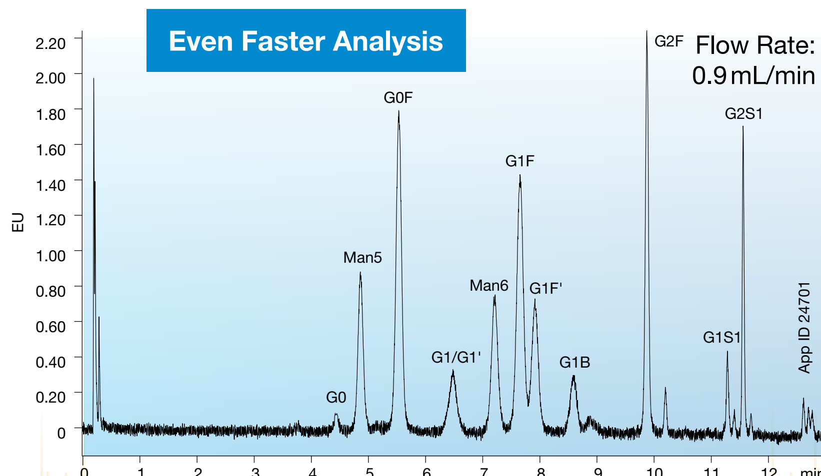
I'm working with mAbs and looking at common fucosylated, neutral biantennary glycans. Most of the methods I see are for the Human IgG library - the glycosylation patterns I see are nowhere near this complex, where do I start?

There are typically favorable glycosylation profiles; depending on the desired effect of the therapeutic, glycosylation strongly influences efficacy, clearance, and even immunogenicity.[1] As such, it is critical to monitor several key glycans, including high mannose (Man-5, Man-6) depending on the cell line used. High mannose tends to improve clearance. Others to monitor include afucosylated glycans for effector function, and sialylated glycans which affect antibody dependent cell cytotoxicity (ADCC), amongst other things.[2] Moreover, "critical pairs" for a mAb expressed in one cell line, purified by a method, will be completely different than another mAb expressed in the same cell line.

Although methods reported for Human IgG library are a good starting point, for robust methods, care should be taken into gradient optimization to separate out critical pairs. In most instances, separation of high mannose species from afucosylated glycans (e.g. Man-5 and G0), in addition to isomers (G1F(3) and G1F(6) are most common. Of course, selection of a selective stationary phase is also critical.

2-AB Labeled Glycans from Standard Solution





Conditions for both columns:

Column: bioZen 2.6 µm Glycan
Dimensions: 100 x 2.1 mm
Part No.: 00D-4773-AN
Mobile Phase: A: 100 mM Ammonium Formate, pH 4.5
B: Acetonitrile
Gradient:

Time (min)	% B
0	76
16	72
25.9	40
27.2	40
27.3	76
30	76

Flow Rate: As noted
Tempertaure: 50 °C
Detection: FLD ex/em 330/420 nm
Sample: Human IgG Glycan Library

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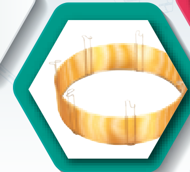

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How do I look at terminal sialic acid for my glycoprotein? Better yet, can I use one column for sialylated glycans and neutral, biantennary glycans?

Sialylated glycans are of critical importance for fusion proteins and clearance, reduced ADCC for mAbs, and anti-inflammatory effects for immunotherapy applications.[2] Further, some biotherapeutics such as recombinant erythropoietin is heavily glycosylated, with much more complexity and sialylation than mAbs which are typically only mono- or di-sialylated.

Depending on the column chemistry used, HILIC may not give the proper selectivity of different sialylated glycans. Alternatively, a mixed-mode anion-exchange column or DMB labeling might be necessary.

However, when using a phase chemistry with good selectivity for sialylated glycans adjustment of the gradient might be necessary for tri- and tetra-antennary glycans. With this adjustment, most sialylated glycans can be separated. MS can then be used for identification of N-glycolneuraminic acid and N-acetylneuraminic acid, the former being found in non-human mammals with the potential of immunogenicity.

1. Higel, F.; Seidl, A.; Sorgel, F.; Fiess, W. "N-Glycosylation heterogeneity and the influence on structure, function and pharmacokinetics of monoclonal antibodies and Fc fusion proteins." European Journal of Pharmaceutics and Biopharmaceutics, vol. 100, 2016, pp. 94–100., doi:10.1016/j.ejpb.2016.01.005.

2. Liu, L. "Antibody Glycosylation and Its Impact on the Pharmacokinetics and Pharmacodynamics of Monoclonal Antibodies and Fc-Fusion Proteins." Journal of Pharmaceutical Sciences, vol. 104, no. 6, 2015, pp. 1866–1884., doi:10.1002/jps.24444

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