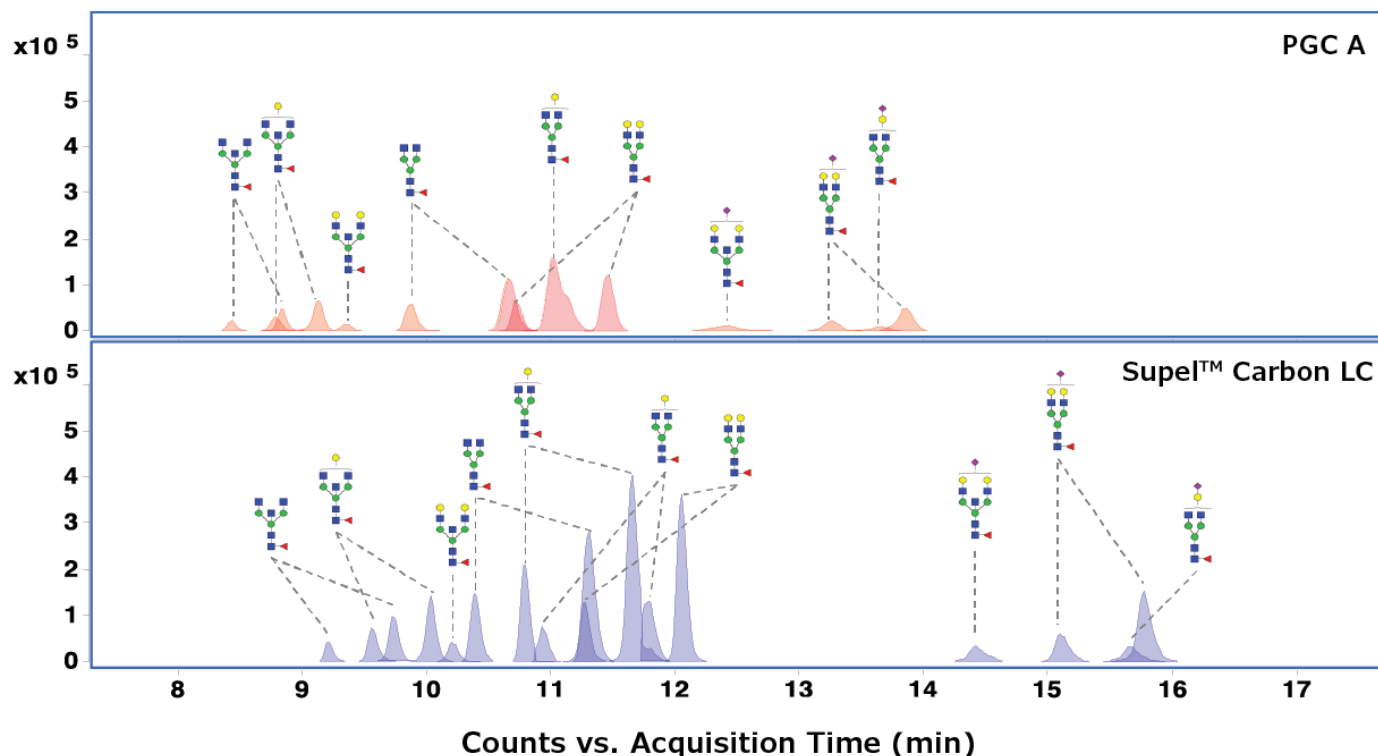


# UHPLC-MS Analysis of Released N-Glycans from Human IgG on a Supel™ Carbon LC Column

## Introduction:

Glycans are molecules consisting of monosaccharides linked through glycosidic bonds that are attached to proteins through certain amino acids. From a biotherapeutic perspective, glycans play an important role in ensuring that the protein interacts with the appropriate cell to elicit its therapeutic effect. The glycan profile of a biotherapeutic is a critical quality attribute that must be reported to regulatory agencies prior to the drug being authorized for use.

Since glycans are polar in nature, it is challenging to analyze these compounds by reversed-phase chromatography; therefore, hydrophilic interaction liquid chromatography (HILIC) with derivatization has been the method of choice for years. This application demonstrates the use of Supel™ Carbon LC, a column packed with porous graphitic carbon (PGC) particles, to resolve released glycans from human IgG under reversed-phase conditions without derivatization. Supel™ Carbon LC is also compared to an alternative PGC column, demonstrating higher efficiencies over the competing column.



Data courtesy of Prof. Hyun Joo An, Chungnam National University, South Korea

## Conditions

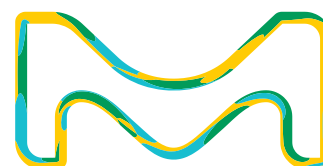
Column	Supel™ Carbon LC, 10 cm x 2.1 mm I.D., 2.7 µm with guard cartridge, 2 cm x 2.1 mm I.D., 2.7 µm
Mobile Phase	[A] 97:3 Water (0.1% (v/v) Formic acid): Acetonitrile (0.1% (v/v) Formic acid); [B] 10:90 Water (0.1% (v/v) Formic acid): Acetonitrile (0.1% (v/v) Formic acid)
Gradient	Hold at 3% B for 2 min; 3% B to 16% B in 8 min; 16% B to 40% B in 8 min; 40% B to 60% B in 2 min; 60% B to 100% B in 2 min; hold at 100% B for 8 min
Flow Rate	0.3 mL/min
Column Temp.	40 °C
Detector	MSD
Injection	1.0 µL
Sample	Released N-glycans from human IgG, 3 µg on column, water

## Conclusion:

This application note described the use of Supel™ Carbon LC to resolve a mixture of glycans released from human IgG under reversed phase conditions without derivatization. Excellent resolution was achieved between the glycan species which allows for accurate quantitation by MS. In addition, as compared to an alternative PGC column, sensitivity was more than doubled, and peak widths were half as wide on Supel™ Carbon LC, indicating this column is an excellent alternative to HILIC-based glycan methods or methods employing other PGC columns.

Product information	Cat. No.
Supel™ Carbon LC, 10 cm x 2.1 mm I.D., 2.7 µm	59986-U
Supel™ Carbon LC Guard Cartridge, 2 cm x 2.1 mm I.D., 2.7 µm	59982-U
Water, for UHPLC, suitable for MS	900682
Acetonitrile, for UHPLC, suitable for MS	900667
Formic acid, 98 – 100%, LiChropur™	5.33002

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Millipore® Sigma-Aldrich® Supelco® Milli-Q® SAFC® BioReliance®