Fast UHPLC Analysis of 2AB Labelled N-Linked Glycans of Human IgG with an Agilent 1290 Infinity LC System

Technical Overview

Abstract

HPLC analysis at high pressures using sub-2 µm columns is extensively used in the Biopharma industry. The Agilent 1290 Infinity LC System provides true UHPLC power range up to 2 mL/min at 1,200 bar. This Technical Overview demonstrates the utility of a 1290 Infinity LC System using a < 2-µm analytical column for N-linked Glycan analysis of human IgG.

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Experimental

The UHPLC analysis was performed using an Agilent 1290 Infinity LC System with the following modules

- Agilent 1290 Infinity Binary Pump with integrated vacuum degasser (G4220 A) and 100 µL Jet Weaver mixer
- Agilent 1290 Infinity High Performance Autosampler (G4226A)
- Agilent 1290 Infinity Thermostatted Column Compartment (G1316C)
- Agilent 1290 Infinity Fluorescence Detector (G1321) with a semimicro flow cell.
- HILIC Glycan Amide column, 2.1 × 150, < 2 µm
- OpenLAB Chromatography Data System (CDS) ChemStation C.01.05

Sample

Prozyme GLYKO 2-AB-(Human IgG N linked Glycan Library), GKSB-005

Chromatographic parameters

Parameter	Condition
Mobile phase A	100 mM ammonium formate, pH 4.5
Mobile phase B	Acetonitrile
TCC temperature	60 °C
Injection volume	2 µL with needle wash at the port for 7 seconds
Flow rate	0.5 mL/min and 1 mL/min
FLD detection	Ex- 330 nm, Em- 420 nm

Gradient optimized for fast separation

Time (min)	Solution B (%)	Flow rate (mL/min)
0	75	1.0
18	52.5	1.0
18.15	40	0.5
18.5	40	0.5
18.9	75	0.5
19.05	75	1.0

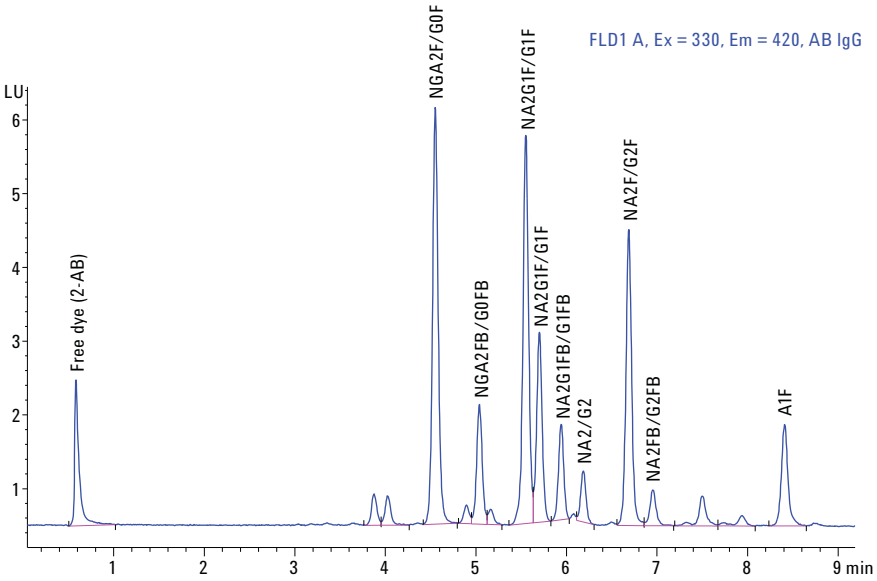

Figure 1. Separation of N-Glycan library from human IgG on an Agilent 1290 Infinity LC System.

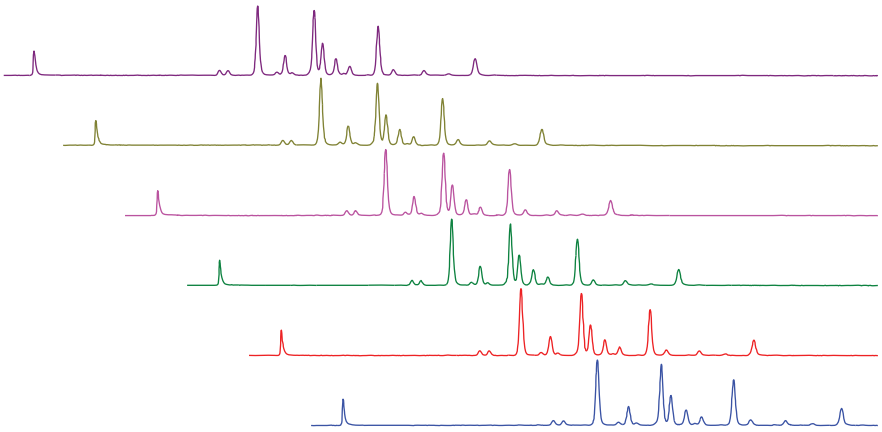

Figure 2. Overlay of six replicates of human N-Glycan library.

Conclusion

- An UHPLC method with FLD detection for fast analysis of N-linked Glycans of human IgG was developed using an Agilent 1290 Infinity Quaternary LC System and sub-2 µm HILIC amide column.
- Separation of all the N-linked Glycans was achieved less than 10 minutes, which is faster than the existing method, which takes about 30 minutes.
- The overlay of six replicates shows the reproducibility of the method.

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