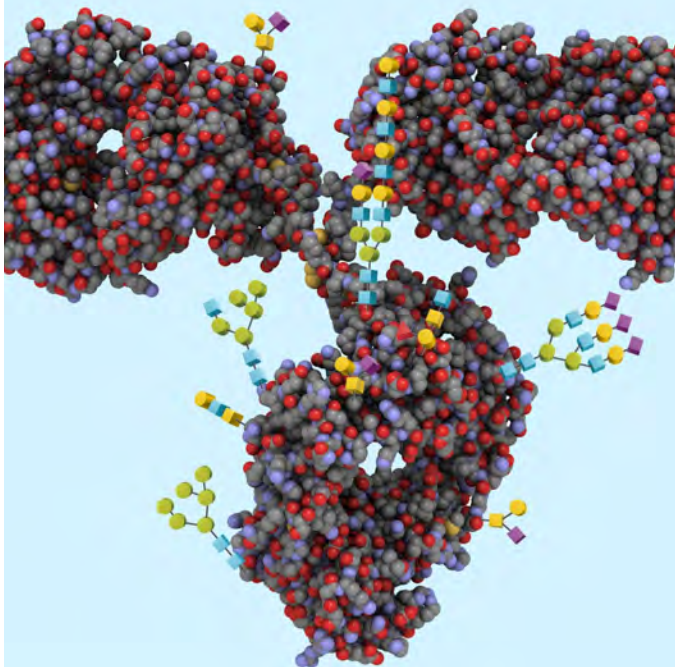
Analysis of Human IgG Glycans on a Solid Core Amide HILIC Stationary Phase

Daniel Spencer[1], Joanna Freeke[2], and Valeria Barattini[2]
[1]Ludger Ltd, Oxford, UK; [2]Thermo Fisher Scientific, Runcorn, UK

Key Words
Accucore 150-Amide-HILIC, Core Enhanced Technology, solid core, amide HILIC, glycans, human IgG, HILIC

Abstract
This application note demonstrates the analysis of human IgG glycans labeled with a fluorescent tag (2-aminobenzamide) by hydrophilic interaction liquid chromatography (HILIC). The separation is carried out with a Thermo Scientific™ Accucore™ 150-Amide-HILIC 150 Å pore diameter solid core HPLC column. The method displays excellent separation at backpressures compatible with conventional HPLC systems.



Introduction
Glycans are oligosaccharides and polysaccharides found on proteins and cell surfaces. These entities play fundamental roles in cellular function by creating a fingerprint tag for the protein they are bound to, which in turn affects cellular activity. Glycans are often key biomarkers for disease states such as cancer. Due to the branching of the chains and post-translational modifications, their structures are very complex. Minor changes in glycan structure can result in dramatic differences in cell function.

It is crucial when analyzing glycans to be able to efficiently separate all isomeric and branching variants present within the sample to achieve maximum structural elucidation. The polarity of the fragments, however, often presents a challenge to chromatographic retention and separation. Smaller fragments are often too polar to be retained by conventional reversed phase methods, and the absence of ionizable groups on many glycans (such as the IgG glycans analyzed here) reduces the usefulness of ion exchange chromatography. For glycans that contain ionizable groups, a mixed mode HILIC/anion exchange column like the Thermo Scientific™ GlycanPac™ AXH-1 column demonstrates a complementary solution for the analysis of charged glycans [1]. Hydrophilic interaction liquid chromatography (HILIC) features increased retention of polar species and has been shown to give good retention of oligosaccharides.

Antibodies have rapidly become a target for bio-pharmaceutical research; accurate structural elucidation is required to control their functioning for the development of bio-therapeutic drugs. In this application note the performance of an Accucore 150-Amide-HILIC HPLC column for the chromatographic separation of human IgG glycans labeled with a fluorescent tag (2-aminobenzamide) is demonstrated.

Accucore HPLC columns use Core Enhanced Technology™ to facilitate fast and highly efficient separations. The 2.6 µm diameter particles are not totally porous, but instead have a solid core and a porous outer layer. The optimized phase bonding creates a series of high coverage, robust phases. The tightly controlled 2.6 µm diameter of Accucore particles results in much lower backpressures than typically seen with sub-2 µm materials. The Accucore 150-Amide-HILIC HPLC phase is designed for the separation of hydrophilic bio-molecules. Hydrophilic interaction liquid chromatography (HILIC) features a partitioning mechanism from an aqueous layer created by water molecules adsorbed on the media surface. Polar analytes are therefore retained in the water

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layer. Additionally, the amide bonded phase on the Accucore 150-Amide-HILIC HPLC column interacts with hydroxyl groups in the analytes via hydrogen bonding, and the larger pore diameter optimizes performance for larger bio-molecules.

Experimental Details

Consumables	Part Number
Fisher Scientific™ HPLC grade water	W/0106/17
Fisher Scientific HPLC grade acetonitrile	A/0627/17
Fisher Chemical™ ammonium formate	A/8050-15
Fisher Scientific™ Optima™ grade formic acid	A117-50

Vials and Closures

Thermo Scientific™ Chromacol™ 9 mm screw thread vial 200 µL, Fused insert-GOLD grade glass(02-FISVG) to be used in conjunction with Thermo Scientific Chromacol 9 mm open top short screw cap 6 mm hole (9-SC(B)-ST1). The high purity glass used for these vials results in extremely low concentration of active sites, therefore minimizing adsorption of basic or highly polar analytes that would otherwise interact with conventional glass surfaces.

Separation Conditions			Part Number
Instrumentation:	Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLC HPLC System equipped with a Thermo Scientific Dionex FLD fluorescence detector		
Column:	Accucore 150-Amide-HILIC, 2.6 µm, 100 × 2.1 mm		16726-102130
Mobile phase A:	Acetonitrile		
Mobile phase B:	50 mM ammonium formate pH 4.4 (prepared from LS-N-BUFFX40, Ludger Ltd)		
Gradient:	Time (min)	% B	
	0	20	
	26	40	
	27	50	
Flow rate:	1 mL/min		
Column temperature:	60 °C		
Backpressure:	300 bar		
Injection details:	5 µL in water, 50 µL loop		
Injection wash solvent:	Acetonitrile / water (78:22 v/v)		
Excitation wavelength:	330 nm		
Emission wavelength:	420 nm		

Sample Preparation

2AB labeled IgG glycans (CAB-IgG-01, Ludger Ltd) were diluted to approximately 2 nmol/mL concentration in HPLC grade water.

Data Processing	
Software:	Thermo Scientific™ Chromeleon™ 7 software

Results

A sample of human IgG glycans was analyzed on an Accucore 150-Amide-HILIC HPLC column. The chromatography is shown in Figure 1 with the details of the labeled peaks contained in Table 1. Symmetrical peak shape and excellent separation of the glycans are observed.

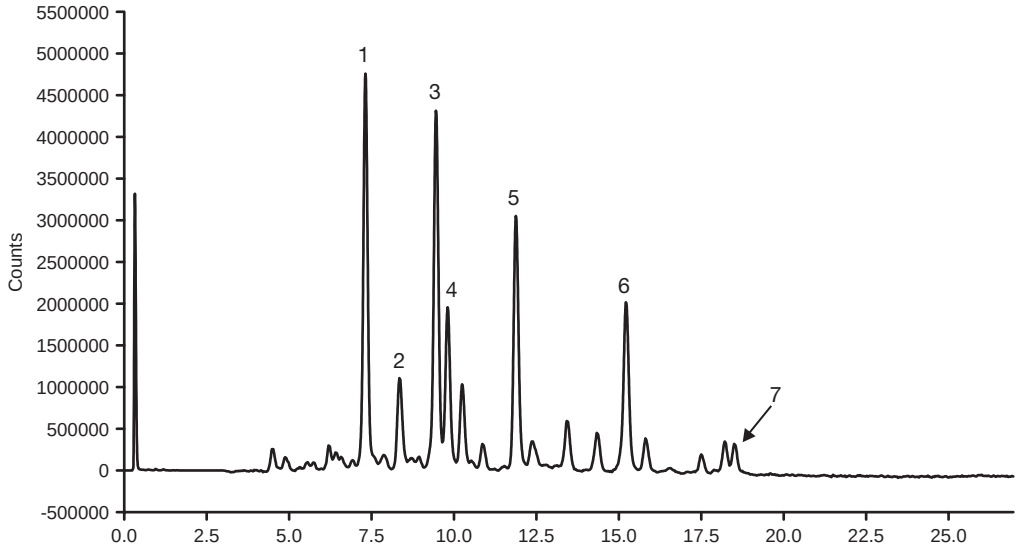

Figure 1: Chromatographic separation of human IgG glycans using an Accucore 150-Amide-HILIC HPLC column

Peak Number	Glycan
1	FA2
2	FA2B
3	FA2G1 (6 arm)
4	FA2G1 (3 arm)
5	FA2G2
6	FA2G2S1
7	FA2G2S2

Table 1: Peak identification for human IgG glycan labeled peaks

Conclusion

- The analysis of 2AB labeled human IgG glycans has been achieved on an Accucore 150-Amide-HILIC HPLC column. The analysis is simple and robust, leading to the separation and detection of 7 individual glycan peaks.
- The solid core technology allows for a highly efficient separation with a system backpressure compatible with conventional HPLC systems (300 bar at gradient apex).
- The Accucore 150-Amide-HILIC column efficiently retains and separates hydrophilic biomolecules.

Reference

[1] Thermo Scientific GlycanPac AXH-1 Product Specification: http://www.dionex.com/en-us/webdocs/114170-PS-GlycanPac-AXH1-Column-PS20695_E.pdf

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USA and Canada +1 800 332 3331
France +33 (0)1 60 92 48 34
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+86 20 83145199 800 810 5118
India +91 22 6742 9494 +91 27 1766 2352
Australia 1 300 735 292 (free call domestic)
New Zealand 0800 933 966 (free call domestic)
All Other Enquiries +44 (0) 1928 534 050

Technical Support
North America +1 800 332 3331
Outside North America +44 (0) 1928 534 440

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