

A HILIC Method for the Analysis of Bovine Fetuin Glycans

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Key Words

Accucore 150-Amide-HILIC, Core Enhanced Technology, amide HILIC, bovine fetuin glycans, HILIC, 150 Å pore size

Abstract

This application note demonstrates the analysis of bovine fetuin 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 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 reduces the usefulness of ion exchange chromatography. For glycans that contain ionizable groups (such as the bovine fetuin glycans analyzed here), 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, with separation based on charge in addition to hydrophilicity [1]. Hydrophilic interaction liquid chromatography (HILIC) features increased retention of polar species and has been shown to give good retention of oligosaccharides.



In this application note the excellent performance of an Accucore 150-Amide-HILIC HPLC column for the chromatographic separation of bovine fetuin 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 biomolecules. Hydrophilic interaction liquid chromatography features a partitioning mechanism from an aqueous layer created by water molecules adsorbed on the

media surface. Polar analytes are retained in this water 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 molecules such as peptides and proteins.

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:	<table border="1"> <thead> <tr> <th>Time (min)</th> <th>% B</th> <th>Flow rate (mL/min)</th> </tr> </thead> <tbody> <tr> <td>0</td> <td>20</td> <td>1.0</td> </tr> <tr> <td>26</td> <td>40</td> <td>1.0</td> </tr> <tr> <td>27</td> <td>50</td> <td>1.0</td> </tr> </tbody> </table>	Time (min)	% B	Flow rate (mL/min)	0	20	1.0	26	40	1.0	27	50	1.0
Time (min)	% B	Flow rate (mL/min)											
0	20	1.0											
26	40	1.0											
27	50	1.0											
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												

Solutions

2AB labeled bovine fetuin glycans (CAB-Fetuin-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 bovine fetuin 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 was observed.

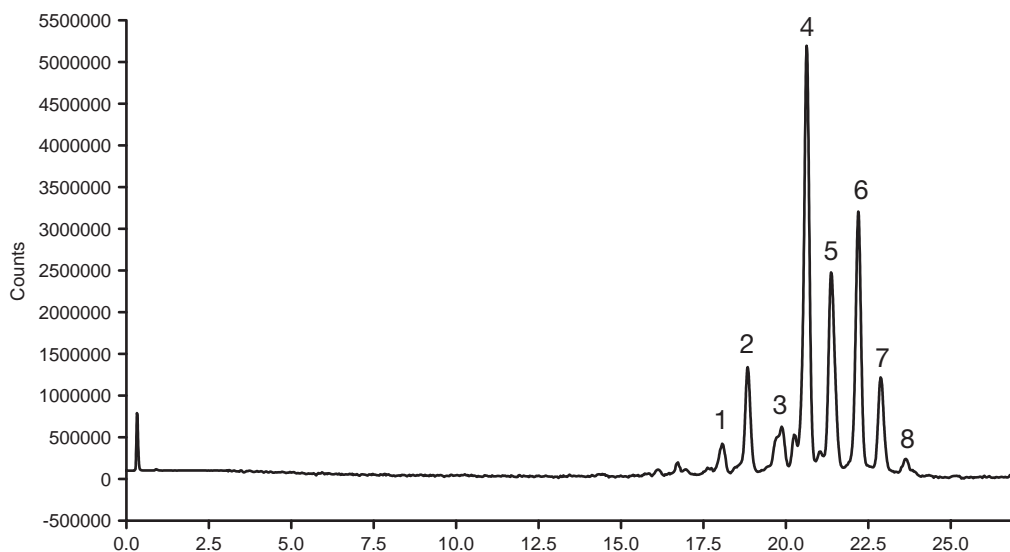


Figure 1: Chromatographic separation of bovine fetuin glycans using an Accucore 150-Amide-HILIC HPLC column

Peak Number	Glycan
1, 2	A3G2S2, A3G3S1, A3G3S2
3	A3G3S2, A3G2S3
4	A3G3S3, A3G3S4
5, 6	A3G3S3, A3G2S4
7	A3G3S3, A3G3S4
8	A3G3S3, A3G3S4

Table 1: Peak identification for bovine fetuin glycan labeled peaks. Identical labels in separate peaks indicate the separation of structural variants.

Conclusion

- The analysis of 2AB labeled bovine fetuin 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 8 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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