Application Note 20593

Analysis of 2-aminobenzamide Labeled Dextran Ladder on a Solid Core HPLC Column

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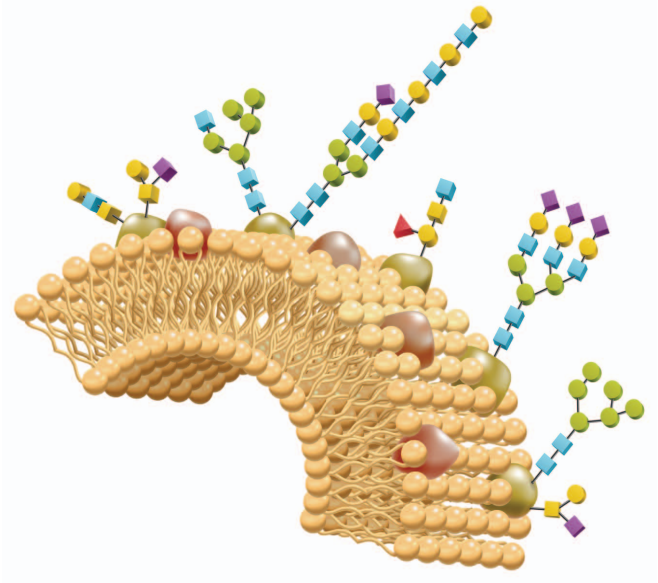
Key Words

Accucore 150-Amide-HILIC, HILIC, glycomics, proteomics, glycoproteins, peptides, glycopeptides, glycans, biomolecules, fused core, superficially porous, 150 Å, dextran, glucose, sugars

Abstract

This application note demonstrates the analysis by HILIC chromatography of a dextran ladder labeled with a fluorescent tag (2-aminobenzamide). The separation is carried out with a Thermo Scientific™ Accucore™ 150-Amide-HILIC (150 Å pore diameter solid core) HPLC column. The method is simple, robust and features excellent separations at backpressures compatible with conventional HPLC systems.



Introduction

Accucore HPLC columns use Core Enhanced Technology™ to facilitate fast and high efficiency separations. The 2.6 µm diameter particles are not totally porous, but rather 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. Accucore 150-Amide-HILIC is designed for the separation of hydrophilic biomolecules. Hydrophilic Interaction 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. Additionally, the amide bonded phase on Accucore 150-Amide-HILIC silica interacts with hydroxyl groups in the analytes via hydrogen bonding and the 150 Å pore diameter optimizes performance for larger molecules.

Glycans are oligosaccharides and polysaccharides bound to 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 in order to achieve maximum structural elucidation. The polarity of the fragments, however, often presents itself as a challenge with regards to chromatographic retention and separation. Smaller fragments are often too polar to be retained by conventional reversed phase methods, whilst the absence of ionizable groups renders ion exchange chromatography redundant. Hydrophilic Interaction Chromatography (HILIC) features increased retention of polar species and has been shown to give good retention of oligosaccharides.

In this application note we demonstrate the excellent performance of an Accucore 150-Amide-HILIC HPLC column for the chromatographic separation of a dextran ladder sample, labeled with a fluorescent tag (2-aminobenzamide).

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Experimental Details

Consumables	Part Number
Fisher Scientific HPLC grade water	W/0106/17
Fisher Scientific HPLC grade acetonitrile	A/0627/17
Fisher Chemicals ammonium formate	A15080153
Fisher Scientific OPTIMA grade formic acid	A117-50
LudgerPure™ 2-AB labeled Glucose Homopolymer	CAB-GHP-30
FinnPippette Kit 1	4700870
Thermo Scientific National 11 mm Crimp Top TPX Insert Vial	C4012-15
11 mm Aluminum Crimp Vial Closure, Red PTFE/White Silicone Septum	C4011-4A

Solutions

Preparation of Ammonium Formate, 50 mM, pH 4.5

A 1M stock solution of ammonium formate was prepared by dissolving 31.9 g of salt in 0.5 L of water. The solution was placed in an ultrasound bath for 30 minutes until full dissolution of the salt was achieved. A 50 mL aliquot was dissolved in 950 mL of water and the pH adjusted to 4.5 using formic acid.

Preparation of 2-AB Labeled Glucose Homopolymer

The sample was dissolved in 300 µL of water and lightly vortexed to ensure full dissolution. No further sample preparation was applied prior to analysis.

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 x 2.1 mm	16726-102130
Mobile phase A:	acetonitrile	
Mobile phase B:	50 mM ammonium formate, pH 4.5	
Gradient:	Time (minutes)	%B
	0	20
	40	50
	45	50
	45.5	20
	50	20
Flow rate:	0.5 mL/min	
Backpressure at starting conditions:	110 bar	
Run time:	50 minutes	
Column temperature:	60 °C	
Injection details:	2 µL to 5 µL of sample	
Injection wash solvent:	80:20 (v/v) acetonitrile:water	
Fluorescence detector acquisition parameters:	330 nm excitation wavelength; 420 nm emission wavelength; acquisition start 3 min after gradient start	

Data Processing

Software:	Thermo Scientific Dionex Chromeleon v7.0 Chromatography Data System

Results

The analysis of 2-AB labeled dextran ladder was carried out on an Accucore 150-Amide-HILIC HPLC column. The chromatography is shown in Figure 1. At least 11 homopolymers were clearly identified. Excellent resolution factors were found, with average Rs values of 9.97 for the first 5 peaks and 3.39 for peaks 6-10 (In both cases the European Pharmacopoeia formula was applied to the calculation).

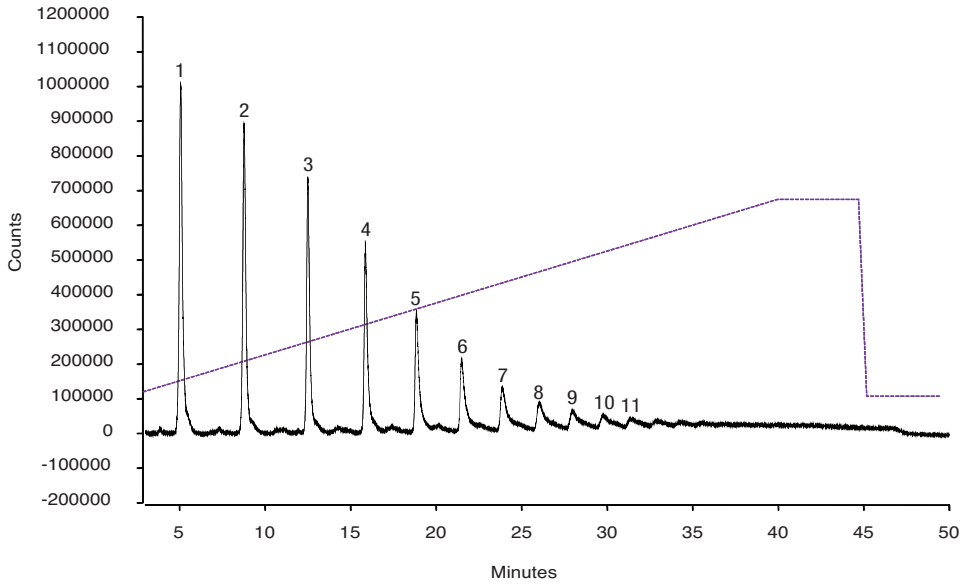
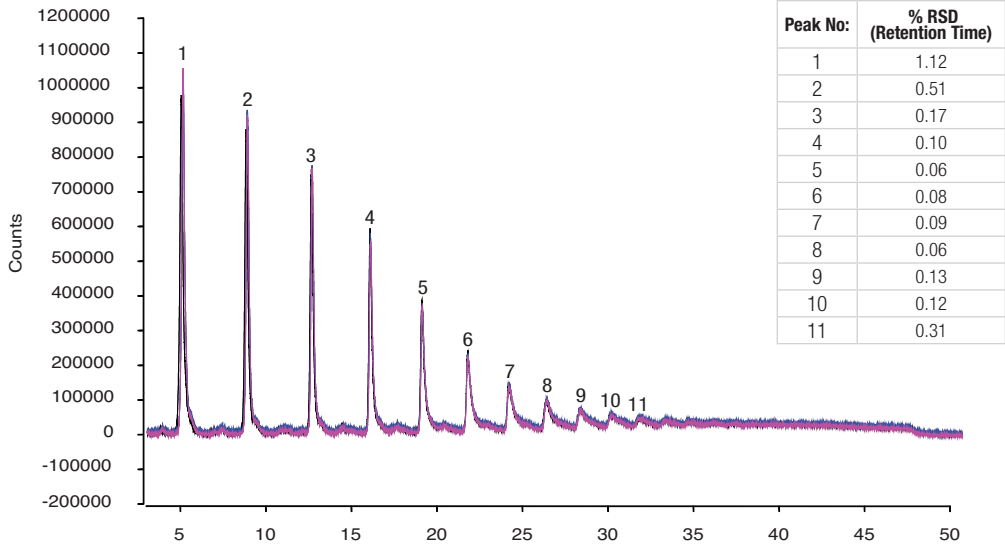


Figure 1: 2-AB dextran ladder on Accucore 150-Amide-HILIC column. The separation is achieved using a HILIC gradient of 50 mM ammonium formate, pH 4.5

Column run-to-run reproducibility was probed by running multiple repetitions of the analysis. Figure 2 shows the overlay of three replicate analyses. As can be seen from the inset table, excellent %RSD values were found across the whole sample, demonstrating the excellent column performance and robustness.



Peak No:	% RSD (Retention Time)
1	1.12
2	0.51
3	0.17
4	0.10
5	0.06
6	0.08
7	0.09
8	0.06
9	0.13
10	0.12
11	0.31

Figure 2: Overlay of triplicate runs of 2-AB labeled dextran ladder. Excellent reproducibility was found, further shown by the outstanding % RSD values (top right inset).

To further establish the resolution power of Accucore 150-Amide-HILIC columns, a larger injection volume (5 µL of sample) was applied in order to elucidate the number of higher order dextran homo-polymers retained and resolved. A further 10 entities were clearly observed, as can be seen in Figure 3, where we have zoomed-in on the later stage of the gradient.

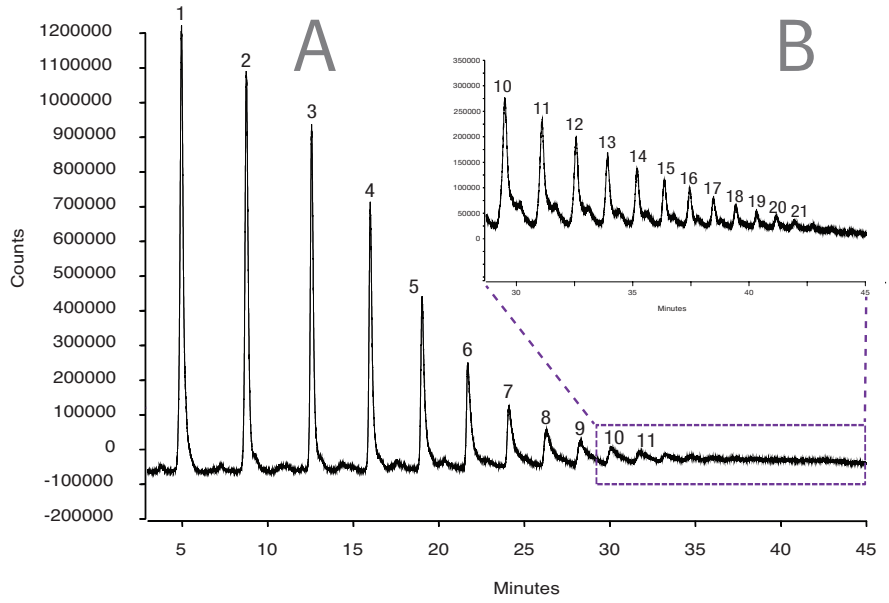


Figure 3: 2-AB dextran ladder separation. (A) 2 µL injection of sample, where 11 glycans were separated. (B) 5 µL injection of sample, zoomed-in to the later part of the gradient rise. A further 10 glycans were detected.

Conclusion

- The analysis of 2-AB labeled dextran ladder has been achieved on Accucore 150-Amide-HILIC. The analysis is simple and robust, leading to the separation and detection of at least 21 glycans.
- The solid core technology allows for a highly efficient separation with a system backpressure compatible with conventional HPLC systems (160 bar at gradient apex).
- Accucore 150-Amide-HILIC efficiently retains and separates hydrophilic biomolecules.

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