Application Note 20597

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

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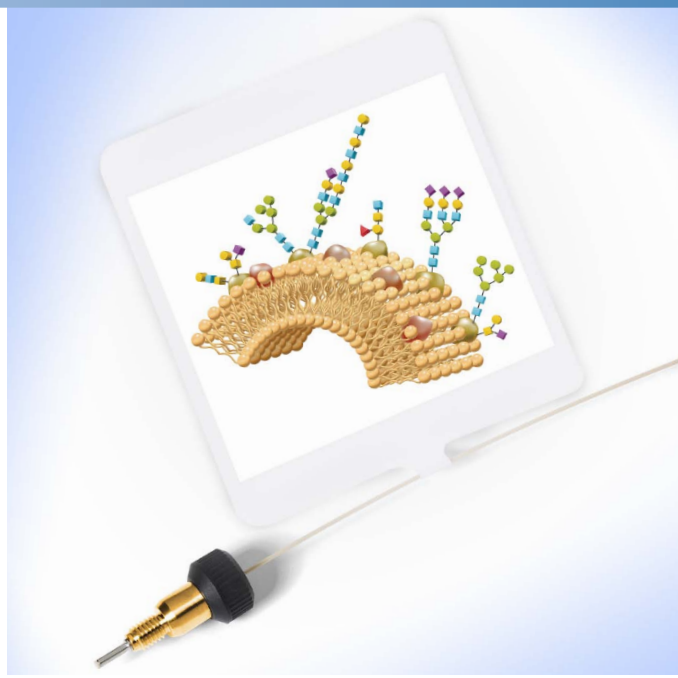
Key Words

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 2-aminobenzamide. The separation is carried out with a Thermo Scientific™ Accucore™ 150-Amide-HILIC (150 Å pore diameter solid core) nanoLC column. The method is simple, robust and features excellent separations in conditions compatible with MS detection.



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. The Accucore 150-Amide-HILIC nanoLC column 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 interact with this layer and are therefore retained. Additionally, the amide bonded phase on Accucore 150-Amide-HILIC 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 oligo-saccharides.

In this application note we demonstrate the excellent performance of an Accucore 150-Amide-HILIC nanoLC column for the chromatographic separation of a dextran ladder sample, labeled with 2-aminobenzamide. The method is simple and uses analytical conditions compatible with MS detection.

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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
FinnPippette Kit 1	4700870
Thermo Scientific Chromacol 9 mm screw thread vial 200 µL, Fused insert-GOLD grade glass	02-FISVG
9 mm open top short screw cap 6 mm hole	9-SC(B)-ST1
Dextran Ladder, from Leuconostoc mesenteroides, Sigma-Aldrich	D3818-200UG
Glycoprofile™ 2-AB Labelling Kit, Sigma-Aldrich	PP0520

Preparation of 2-AB Labeled Dextran Ladder

The dextran ladder sample was labeled according to the instructions provided in the Glycoprofile 2-AB Labelling Kit. A 10 µL aliquot was further diluted in 90 µL of acetonitrile and used for analysis.

Separation Conditions		Part Number
Instrumentation:	Thermo Scientific EASY 1000 nLC System equipped with a Thermo Scientific Dionex VWD UV detector. Column heating applied with a Thermo Scientific Hot Pocket	92016
Column:	Accucore 150-Amide-HILIC 2.6 µm, 150 mm x 75 µm	16726-157569
Mobile phase A:	98:2 (v/v) acetonitrile: water	
Mobile phase B:	2:98 (v/v) acetonitrile: water	
Analytical column equilibration :	5 µL at 280 bar	
Sample pick-up:	0.5 µL at 20 µL/min	
Sample loading:	1 µL at 280 bar	
Gradient:	Time (minutes) %B	
	0 0	
	50 50	
	58 50	
Flow rate:	200 nL/min	
Backpressure at gradient starting conditions:	60 bar	
Run time:	58 minutes	
Column temperature:	40 °C	
Injection wash solvent:	98:2 (v/v) acetonitrile: water	
UV detection:	240 and 330 nm	

Data Processing	
Software:	Thermo Scientific Dionex Chromeleon Chromatography Data System

Results

The analysis of 2-AB labeled dextran ladder was carried out on an Accucore 150-Amide-HILIC nanoLC column. The chromatography is shown in Figure 1. At least 18 homopolymers were clearly identified. Excellent resolution factors were found, with average Rs values of 10.22 for the first 5 peaks, 4.30 for peaks 6-10 and 2.91 for peaks 10-18 (In all cases the European Pharmacopoeia formula was applied to the calculation).

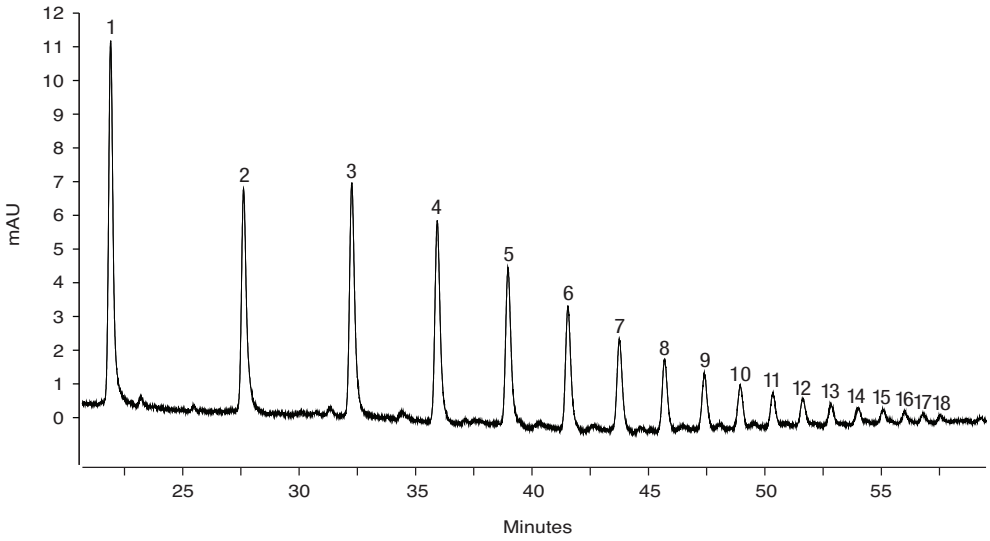

Figure 1: 2-AB dextran ladder at 330 nm on Accucore 150-Amide-HILIC nanoLC column. The separation is achieved using simple aqueous HILIC gradient with no pH adjustment.

Column run-to-run reproducibility was probed by running multiple repetitions of the analysis (n=3). The %RSD values for the retention times of 10 representative peaks are summarized in Table 1. As can be seen, excellent reproducibility was found across this range.

Peak number	Retention Time (minutes)	% RSD value
1	22.03	0.64
2	27.74	0.38
3	32.37	0.29
4	36.05	0.31
5	39.09	0.31
6	41.65	0.29
7	43.88	0.33
8	45.82	0.33
9	47.55	0.39
10	49.12	0.57

Table 1: % RSD values for retention times of the first 10 peaks of 2-AB dextran ladder. Excellent values are observed throughout

Conclusion

- The analysis of 2-AB Labelled dextran ladder has been achieved on an Accucore 150-Amide-HILIC nanoLC column. The analysis is simple and robust, leading to the separation and detection of at least 18 glycans.
- The solid core technology allows for a highly efficient separation with low system backpressure (100 bar at gradient apex), thus eliminating the requirement for high-pressure compatible nanoLC instruments. Furthermore, the conditions applied are compatible with MS detection for full characterization and quantification studies.
- Accucore 150-Amide-HILIC nanoLC columns efficiently retain and separate hydrophilic biomolecules.

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