Application Note 20518

Analysis of Intact Proteins on a Thermo Scientific Accucore 150-C4 150 Å Pore Diameter NanoLC Column

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Key Words

Accucore C4, top down proteomics, proteins, fused core, superficially porous, 150 Å, nanoLC, ovalbumin, insulin, cytochrome C, myoglobin, carbonic anhydrase

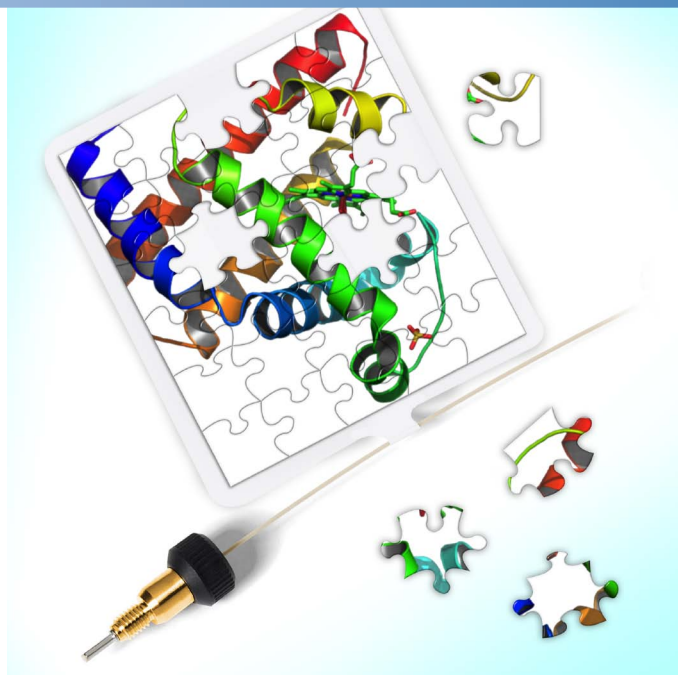
Abstract

This application note demonstrates the analysis of proteins intact using a Thermo Scientific Accucore 150-C4 (150 Å pore diameter) nanoLC column. The analysis of five proteins (ranging in mass from 6 to 45 kDa) is carried out in 15 minutes with pressures compatible with conventional nanoLC instrumentation.

Introduction

Accucore™ HPLC columns use Core Enhanced Technology™ to facilitate fast and high efficiency separations. The 2.6 µm diameter particles 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. For the analysis of large biomolecules the Accucore pore size has been further optimized and a C4 phase with reduced hydrophobic retention has been prepared. This 150 Å pore size enables the effective analysis of molecules unable to penetrate into smaller diameter pores and the low hydrophobicity C4 phase results in protein separation by hydrophobicity.

Currently, proteins analysis is carried out either using the intact protein or, after enzymatically digesting the proteins on the peptide level. The latter approach leads to greatly increased sample complexity, which results in challenging data interpretation requiring deconvolution of the peptide fragments produced. Analysis of proteins at the intact level is preferred for the reduced sample complexity as well as the additional global protein information available.



Developments in MS technology have enabled detection and analysis of intact proteins and this is being utilized in 'top-down' proteomics approaches. These approaches rely heavily on separation of proteins prior to MS analysis. In this application note we demonstrate the excellent performance of an Accucore 150-C4 nanoLC column for the chromatographic separation of five proteins (6-45 kDa).

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Experimental Details

Consumables	Part Number
Fisher Scientific MS grade water	W/0112/17
Thermo Scientific Pierce LC-MS grade acetonitrile	TS-511001
Fisher Scientific Pierce LC-MS grade formic acid	PI-28905
Liquid handling hardware: FinnPippette Kit 1	4700870
11 mm Chromacol Snap Cap 200 µL vial, fused insert – GOLD grade glass	02-FIRVG
11 mm Chromacol Snap Cap 6 mm hole	11-PSN(B)-ST1
Insulin, cytochrome c, myoglobin, carbonic anhydrase and ovalbumin standards	

Separation Conditions		Part Number
Instrumentation:	Thermo Scientific EASY nLC II and Thermo Scientific Dionex UltiMate 3000 Rapid Separation Four Channel Variable Wavelength Detector (3 nL flow cell)	
Column:	Accucore 150-C4, 2.6 µm, 150 x 75 µm	16526-157569
Mobile phase A:	0.1 % formic acid in water	
Mobile phase B:	0.1 % formic acid in acetonitrile	
Gradient:	time	% B
	0	0
	1	30
	11	60
	12	95
	15	95
Flow rate:	300 nL/min	
Backpressure (100% aqueous):	204 bar	
Run time:	15 minutes + equilibration time	
Column temperature:	40 °C	
Injection details:	0.25 µL 2 pmol/µL solution	
UV detector wavelength:	214 nm	

Solutions	
Standard preparation:	A 1 mg/mL solution of each protein was prepared in water. These solutions were combined, with the resultant solution diluted to a final concentration of 2 pmol/µL.

Data Processing	
Software:	Thermo Scientific Chromeleon 7

Results

Under these conditions five proteins covering the mass range of 6 to 45 kDa can be separated on an Accucore 150-C4 nanoLC column in less than fifteen minutes with backpressures compatible with conventional nanoLC equipment. The chromatography is shown in Figure 1, with all of the proteins being well resolved. The identities of the proteins analyzed, their retention times, the percentage relative standard deviation (%RSD) in the retention time over six replicate injections and peak width are summarized in Table 1.

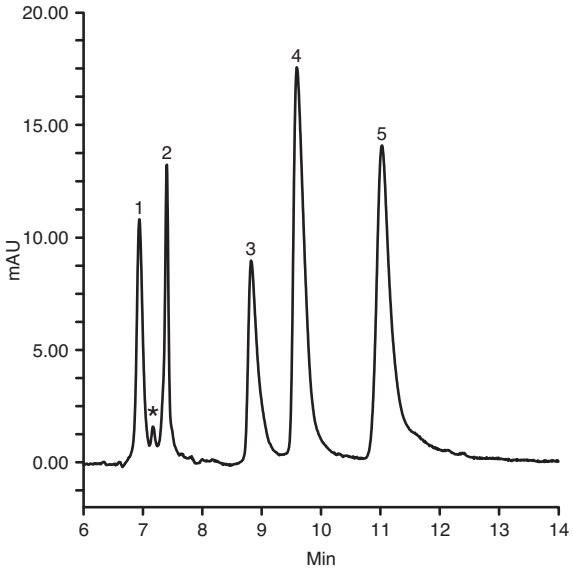

Figure 1: UV chromatogram for five proteins separated on an Accucore 150-C4, 150 mm x 75 µm nano HPLC column (blank injection subtracted to compensate for the change in baseline with acetonitrile concentration). The proteins are listed in Table 1, * indicates an impurity from carbonic anhydrase.

Peak Number	Protein	t_R minutes	% RSD	Peak width at half height / minutes
1	Cytochrome C	6.94	0.18	0.10
2	Insulin	7.39	0.20	0.06
3	Myoglobin	8.82	0.15	0.16
4	Carbonic Anhydrase	9.60	0.27	0.20
5	Ovalbumin	11.03	0.11	0.23

Table 1: List of proteins analyzed, average retention time (t_R), percentage relative standard deviation (% RSD) in retention time over six replicate injections and peak width at half height

Conclusion

- Accucore 150-C4 column shows excellent separation of five test proteins of differing mass (6-45 kDa) within 15 minutes
- Good peak shape is observed for all proteins
- Backpressure is compatible with a conventional nanoLC system

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