Analysis of Bovine Serum Albumin (BSA) Protein Digest on a Thermo Scientific Accucore 150-C18, 150 Å Pore Diameter NanoLC Column

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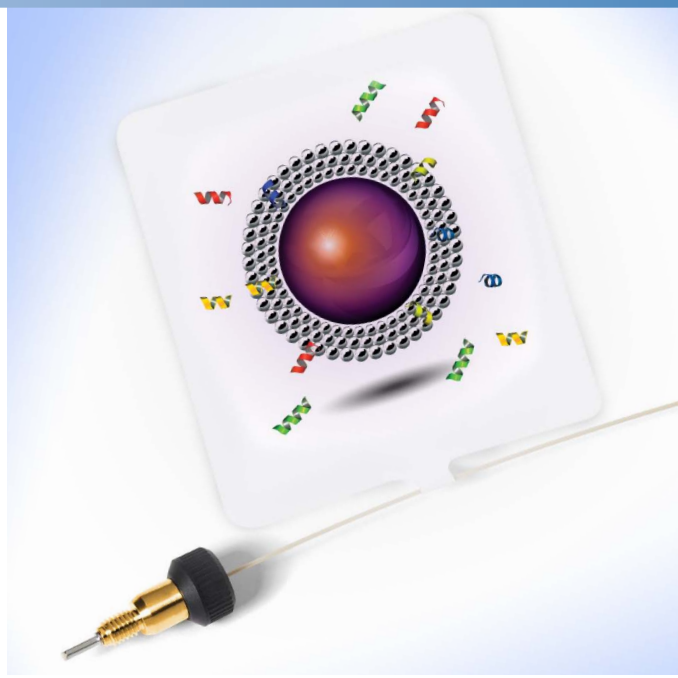
Application Note 20519

Key Words
Accucore 150-C18, nanoLC, bottom-up proteomics, proteins, peptides, fused core, superficially porous, bovine serum albumin (BSA).

Abstract
This application note demonstrates the analysis of trypsin-digested bovine serum albumin (BSA) using a Thermo Scientific Accucore 150-C18 (150 Å pore diameter) nanoLC column.

The analysis was carried out using an acidified water: acetonitrile gradient over 30 minutes generating pressures compatible with conventional nanoLC instrumentation. The data demonstrates excellent peak shape and reproducibility.



In this application note we demonstrate the excellent performance of Accucore 150-C18 nanoLC columns for the chromatographic separation of trypsin-digested BSA.

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. Accucore 150-C18 has been further optimized for the analysis of biomolecules and protein digests by bonding C18 ligands onto the porous outer layer having a pore size of 150 Å.

Conventional bottom-up proteomic samples consist of digested proteins or digested whole cells. This approach leads to high sample complexity, creating a large number of peptide fragments with varying sequence length and hydrophobicity. It is crucial that the chromatographic separation is highly efficient in order to facilitate accurate and reliable identification of all the fragments present in the sample. The 150 Å pore diameter enables larger peptides, resulting from digest protocols, to diffuse more effectively into the stationary phase particle. This leads to improved interaction with the stationary phase surface and as a result increased resolution of these larger peptide fragments compared to smaller pore diameter particles. The combination of solid-core particle technology with wide-pore silica, results in high efficiency separations.

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Experimental Details

Consumables	Part Number
Thermo Scientific National Vials and closures	MSCERT4000-34W
LC-MS CHROMASOLV® water +0.1% formic acid	34673-2.5L-R
LC-MS CHROMASOLV® acetonitrile	34967-2.5L
Proteabio BSA Digest Standard (lyophilized, 500 pmol)	PS-204-3

Separation Conditions		Part Number
Instrumentation	Thermo Scientific Dionex UltiMate 3000 RSLCnano LC system	
Column:	Accucore 150-C18 2.6 µm, 75 µm ID x 150 mm	16126-157569
Mobile phase A:	0.1 % formic acid in water	
Mobile phase B:	80:20 acetonitrile:water	
Injection details:	1 µL	
Loading:	Direct on column loading at gradient start through sample loop	
Flow rate:	300 nL/min	
Gradient:	Time (min)	%B
	0	4
	30	40
	32	95
	34	95
	35	4
	55	4
Backpressure at starting conditions:	198 bar	
Run time:	55 minutes	
Column temperature:	Not controlled	

MS Conditions

Thermo Scientific LTQ-Orbitrap XL mass spectrometer coupled with a Proxeon Nanospray Flex ion source	
Ionization	Nanospray, positive mode
Capillary Temperature	200 ºC
Voltage	2.1 kV
Mass Range	370-1200 m/z
MS Acquisition	MS only
Analyzer type	FTMS

Solutions

Standard preparation:	Digested and lyophilized BSA (500 pmol) was reconstituted in 100 µL of water + 0.1% formic acid to give a 5 pmol/µL solution. The mixture was sonicated for 20 s to ensure full solubilisation with minimal peptide aggregation. A 50 fmol/µL solution was prepared by diluting 10 µL of stock solution in 990 µL of water acidified with 0.1% formic acid.

Data Processing

Software:	Thermo Scientific Xcalibur 2.1

Results

Analysis of BSA digest using the conditions described above leads to elution of tryptic peptides within 36 minutes.

The base peak chromatogram obtained from the separation of 50 fmol of digest is shown in Figure 1.

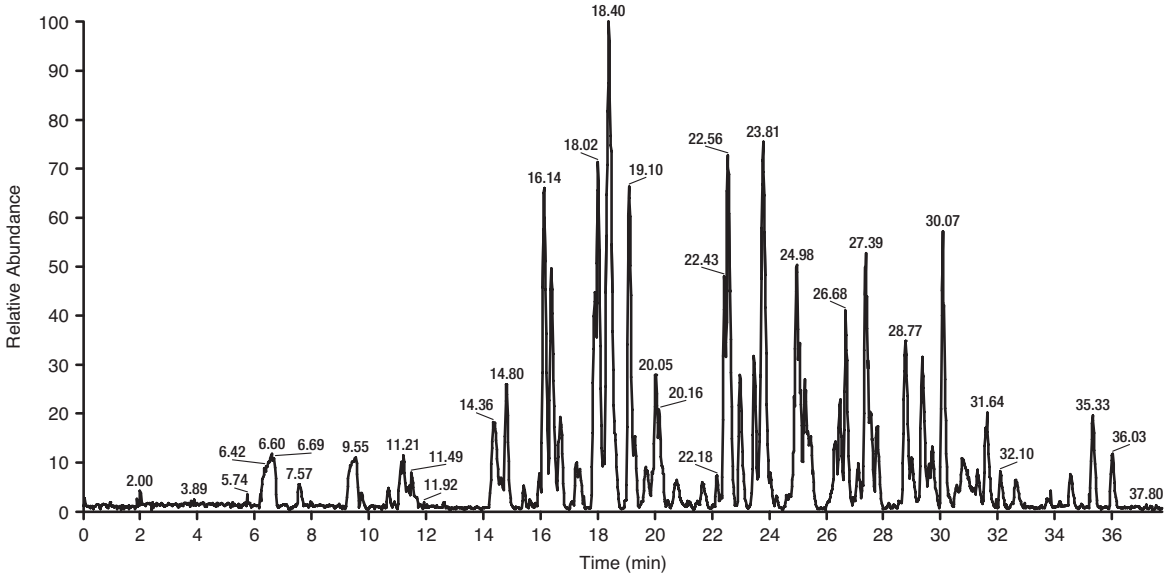

Figure 1: Base peak chromatogram of 50 fmol of digested BSA loaded on an Accucore 150-C18 nanoLC column, 75 µm ID x 150 mm

Reproducibility was assessed by running triplicate analyses (Figure 2, base peak chromatograms). The retention times of a set of twelve peptides from early, middle and late eluting regions were monitored for statistical analysis. In all cases excellent retention time reproducibility was observed, with % RSD values below 0.14 % (Table 1), showing the outstanding reproducibility of Accucore 150-C18 nanoLC columns.

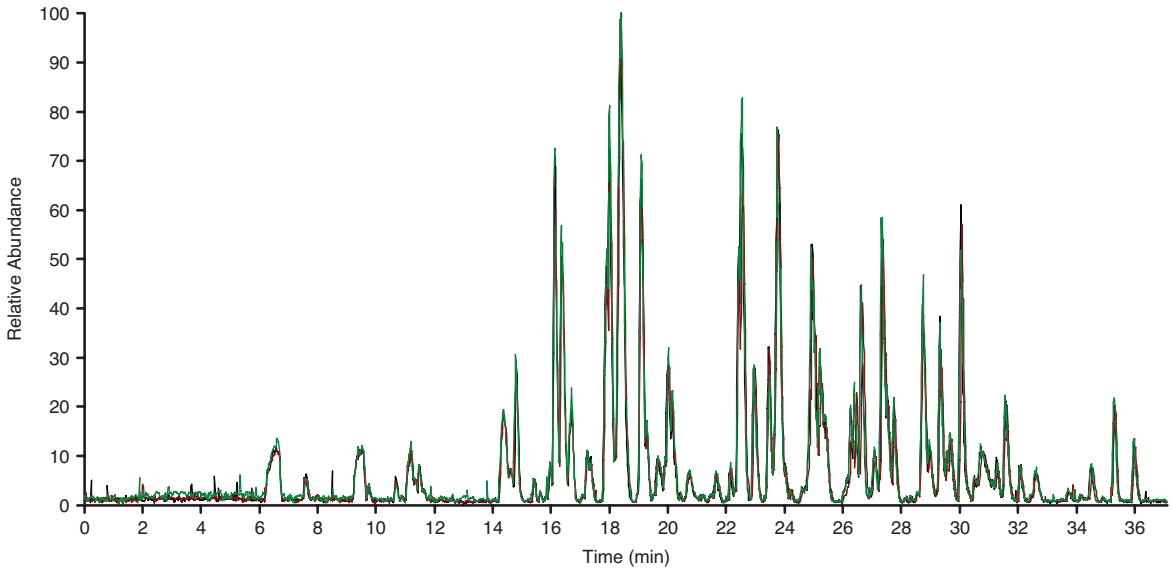

Figure 2: Overlaid base peak chromatogram of triplicate analyses of BSA digests

Peptide m/z	t_R Average (min)	%RSD
424.26	14.36	0.00
722.33	16.13	0.09
461.75	19.10	0.08
653.37	22.42	0.05
547.32	23.77	0.14
480.61	24.95	0.12
582.32	26.64	0.14
995.47	28.74	0.09
507.81	29.34	0.07
740.41	30.05	0.07
768.04	34.51	0.09
784.38	35.99	0.09

Table 1: Retention time t_R average and %RSD values for a set of twelve peptides

Figure 3 shows the extracted ion chromatograms (EIC) of a subset of peptides of the twelve peptides monitored. In all cases the peak shapes of the selected peptides were found to be excellent, with minimal peak tailing.

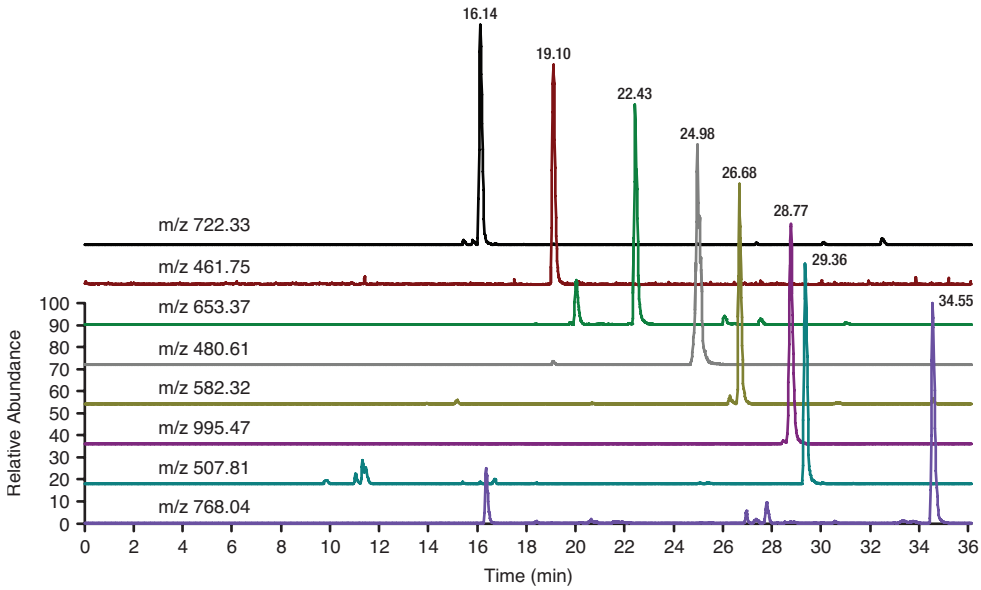

Figure 3: EIC of a set of eight representative peptides from the monitoring set of twelve. In all cases excellent peak shapes were observed

Conclusion

- Accucore 150-C18 nanoLC columns show excellent separation of complex peptide mixtures, making them an ideal choice for bottom-up proteomic workflows
- Excellent run-to-run reproducibility was achieved, showing the robustness and reliability of the columns

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