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

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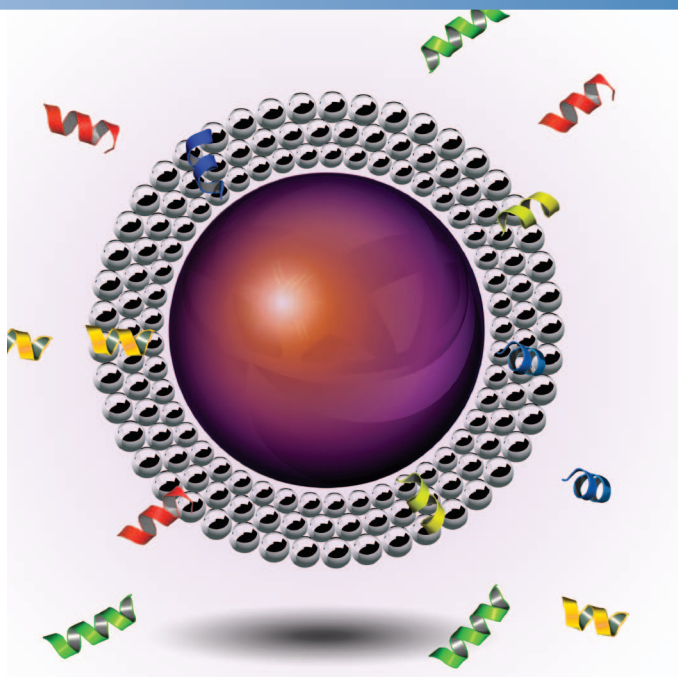
Key Words
Accucore C18, bottom-up proteomics, proteins, peptides, fused core, superficially porous, 150 Å, bovine serum albumin (BSA)

Abstract
This application note demonstrates the analysis of trypsin-digested BSA using a Thermo Scientific Accucore 150-C18 HPLC column (150 Å pore diameter). The analysis is carried out using an acidified water:acetonitrile gradient over 40 minutes, with pressures compatible with conventional HPLC instrumentation. The data shows high peak capacity with excellent reproducibility.

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 columns have been further optimized for the analysis of biomolecules and protein digests by bonding C18 ligands onto the porous outer layer, which has a pore size of 150 Å.

The sample preparation for a standard bottom-up proteomics experiment involves enzymatic digestion of proteins or even complex mixtures of proteins, such as a whole cell lysate. Breaking down an already complex sample mixture into a large number of peptide fragments of varying size and hydrophobicity presents an analytical challenge. It is crucial that the chromatographic separation is highly efficient with high peak capacity in order to achieve accurate and reliable identification of all the fragments present in the sample. The 150 Å pore diameter enables larger peptides to penetrate more efficiently into the stationary phase particle. This leads to improved interaction with the stationary phase surface and as a result increased resolution compared to a narrow pore diameter particle. The combination of Core Enhanced Technology with wide-pore silica, results in very high efficiency separations using conventional HPLC systems.



Developments in MS technology have enabled detection and analysis of very low abundance species within complex peptide mixtures. However, greater confidence in peptide identification can be achieved through the use of higher resolving power stationary phases.

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

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Experimental Details

Consumables	Part Number
Fisher Scientific HPLC grade water	W/0106/17
Fisher Scientific HPLC grade acetonitrile	A/0626/17
Fisher Scientific Optima formic acid	A117-50
Proteabio BSA Digest Standard (lyophilized, 500 pmol)	PS-204-3
Liquid handling hardware: FinnPippette Kit 1	4700870
Vials and closures	MSCERT4000-34W

Separation Conditions			Part Number
Instrument:	Thermo Scientific Accela HPLC System		
Column:	Accucore 150-C18, 2.6 µm, 100 x 2.1 mm		16126-102130
Mobile phase A:	0.1 % formic acid in water		
Mobile phase B:	0.1 % formic acid in acetonitrile		
Gradient:	Time (min)	%B	
	0	5	
	5	5	
	45	50	
	46	95	
	50	95	
	51	5	
	60	5	
Flow rate:	0.2 mL/min		
Backpressure at starting conditions:	170 bar		
Run time:	60 minutes		
Column temperature:	40 °C		
Injection details:	3 µL 5 pmol/µL solution		
Injection wash solvent:	water + 0.1% formic acid		

MS Conditions

Thermo Scientific LCQ-Deca XP ion-trap mass spectrometer	
Ionization	ESI positive mode
Sheath Gas Flow Rate	60
Aux Gas Flow Rate	20
Capillary Temperature	300 ºC
Voltage	4.5 kV
Cap V	30 V
Mass Range	300-1500 m/z

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 20s to ensure full solubilisation with minimal peptide aggregation.

Data Processing

Software:	Thermo Scientific Xcalibur 2.0 SR2

Results

Analysis of BSA digest in the conditions described above leads to elution of tryptic peptides within 30 minutes.

The base peak chromatogram obtained for this separation is shown in Figure 1.

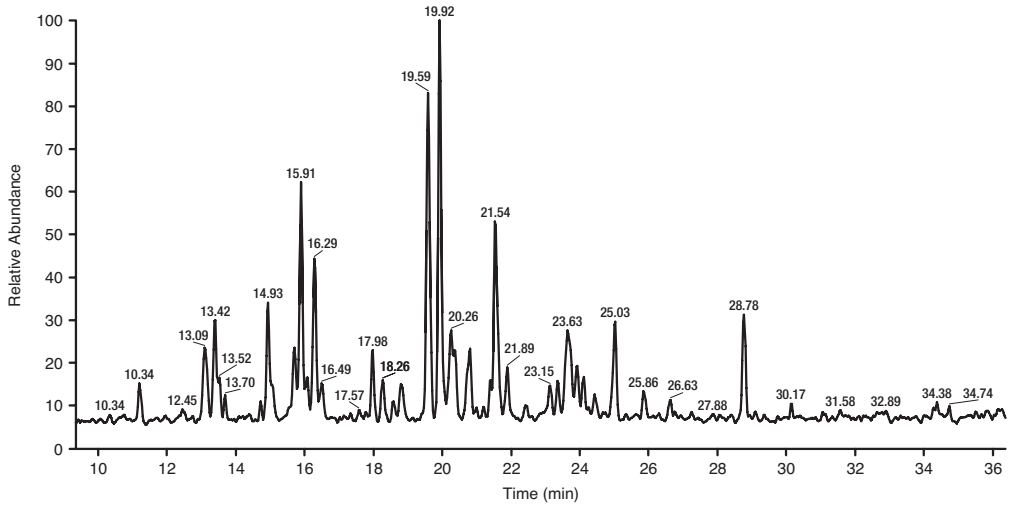

Figure 1: Base Peak chromatogram of digested BSA on Accucore 150-C18, 100 x 2.1 mm HPLC column

Retention time reproducibility was assessed by running triplicate analyses (Figure 2, base peak chromatograms). The retention times of a set of eight peptides from early, middle and late eluting regions were monitored for statistical analysis. In all eight cases excellent retention time reproducibility was observed, with %RSD values below 0.4 % (Table 1).

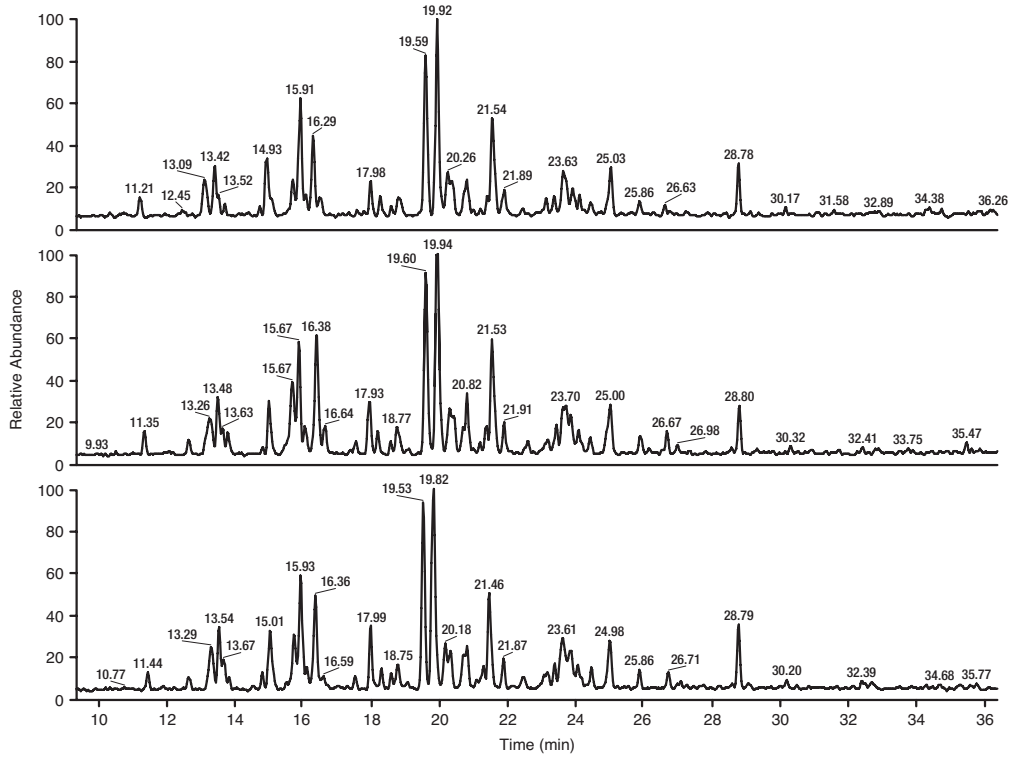

Figure 2: Base peak chromatogram of triplicate analyses of BSA digests

Peptide m/z	Retention time Average (min)	%RSD
395.33	14.97	0.37
523.73	15.90	0.19
656.13	16.29	0.29
547.73	19.57	0.19
628.00	20.23	0.32
708.87	23.15	0.15
534.67	23.66	0.20
625.73	25.01	0.12

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

Resolution Power – Peak Capacity

For many analysts running complex digested samples, the peak capacity (i.e. the number of peaks that can be resolved within a gradient run) is a crucial parameter, which ultimately influences the number of peptide identification hits.

Peak capacity is conventionally calculated as follows [1]:

$$n_c = 1 + \left(\frac{t_g}{W}\right)$$

Where n_c = Peak capacity

t_g = gradient time

W = average peak width 10% height

The peak widths at 10% height were measured for peptides listed in Table 1; in all cases these were found to be excellent, with an average width of 10.8 s (Figure 3). A peak capacity calculation based on this data set resulted in an average value of 225, indicating the high resolution power of Accucore 150-C18 HPLC column.

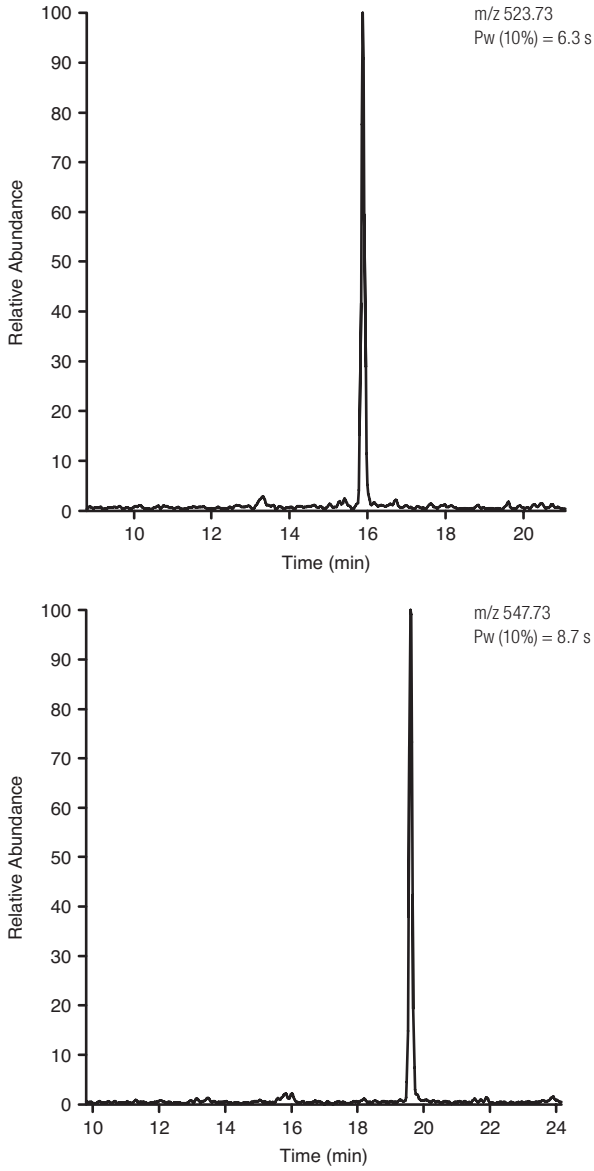

Figure 3: EIC for two peptides in the monitoring set of eight. In both cases excellent peak widths are found

Conclusion

- Accucore 150-C18 HPLC columns shows excellent separation of BSA digest fragments
- High peak capacity observed for a complex peptide digest
- Outstanding run-to-run reproducibility
- Backpressure compatible with conventional HPLC systems

References

[1] Wang X., Peak Capacity Optimization of Peptide Separations in Reversed-Phase Gradient Elution Chromatography (2006), 78 (10), 3406-3416

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