Application Note 20570

Reversed-Phase Analysis of a Monoclonal Antibody on an Accucore 150-C4 HPLC Column

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Key Words

Accucore 150-C4, protein, therapeutic protein, monoclonal antibody, antibody, biopharmaceutical, pharma, core enhanced technology, solid core, alemtuzumab, campath, MAb

Abstract

This application note demonstrates the analysis of a monoclonal antibody (MAb) using a Thermo Scientific Accucore 150-C4 HPLC column. The analysis is carried out within 20 minutes at pressures compatible with conventional HPLC instrumentation.

Introduction

Accucore™ HPLC columns use Core Enhanced Technology™ to facilitate fast and highly efficient separations. The 2.6 µm diameter particles are not totally porous but 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 the 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 effective analysis of molecules that are unable to penetrate small pore sizes, and the lower hydrophobicity allows elution of hydrophobic proteins that are too strongly retained on C18.

Biotherapeutic proteins are a growing class of pharmaceutical drugs. Monoclonal antibodies are the most established members of this class, with highly specific interactions and good biological compatibility. The interaction of an antibody with an antigen occurs at the fragment antigen binding (FAb) region, which is flexibly linked to the fragment crystallizable (Fc) region. Monoclonal antibodies are commonly analyzed, both in the intact form and after cleavage into the FAb and Fc regions, by ion exchange chromatography and size exclusion chromatography. The resolution of variants by size exclusion chromatography requires significant differences in size, while ion exchange chromatography mainly separates charge variants. Additional methods are required to characterize antibodies and therapeutic proteins to prove product purity and to monitor

production of the active protein. The Accucore 150-C4 HPLC column offers an alternative selectivity based on hydrophobicity, which provides orthogonal high-resolution separations that may be more sensitive to small hydrophobic mutations. However, in this application note we demonstrate the excellent performance of an Accucore 150-C4 HPLC column for the analysis of a Campath® monoclonal antibody (alemtuzumab), which is used to treat chronic lymphocytic leukemia.

Unlike ion exchange and standard SEC, reversed-phase techniques are compatible with mass spectrometry. Therefore, this analysis can be coupled to high-resolution mass spectrometry systems to confirm the accurate molecular weight of the target product and identify variants.

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Experimental Details

Consumables	Part Number
Fisher Scientific HPLC grade acetonitrile	A/0626/17
Millipore® purified water (18 MΩ)	
Fisher Scientific trifluoroacetic acid (TFA)	A116-50
Thermo Scientific Chromacol 9 mm screw thread vial 200 µL, Fused insert-GOLD grade glass	02-FISVG
Thermo Scientific Chromacol 9 mm open top short screw cap, 6 mm hole	9-SC(B)-ST1

Separation Conditions			Part Number
Instrumentation:	Thermo Scientific Dionex UltiMate U3000 Ti, including WPS-3000PL autosampler, TCC oven, VWD3400 detector with 2.5 µL micro cell, and LPG 3400MB pump		
Column:	Accucore 150-C4 2.6 µm, 100 x 2.1 mm		16526-102130
Mobile phase A:	0.1 % TFA in acetonitrile / water (2.5:97.5 v/v)		
Mobile phase B:	0.1 % TFA in acetonitrile / water (90:10 v/v)		
Gradient:	Time (min)	% B	
	0.0	10	
	15.0	70	
	15.1	100	
	16.0	100	
	16.1	10	
	19.0	10	
Flow rate:	300 µL/min		
Run time:	19 minutes		
Column temperature:	70 °C		
Column backpressure:	160 bar		
Injection volume:	15 µL		
Injection wash solvent:	10:90 acetonitrile / water		
UV detector wavelength:	214 nm		

Solutions	
Sample concentration:	0.2 mg/mL
Sample preparation:	Diluted in 0.1% TFA in acetonitrile / water (2.5:97.5 v/v) Campath monoclonal antibody FAb fragment from Campath monoclonal antibody

Data Processing	
Software:	Thermo Scientific Dionex Chromeleon 6.8 SR11

Results

The analysis of a Campath MAb was carried out on an Accucore 150-C4 HPLC column. The chromatography is shown in Figure 1. The intact 150 kDa Mab appears as a sharp symmetrical peak at 9 minutes retention time.

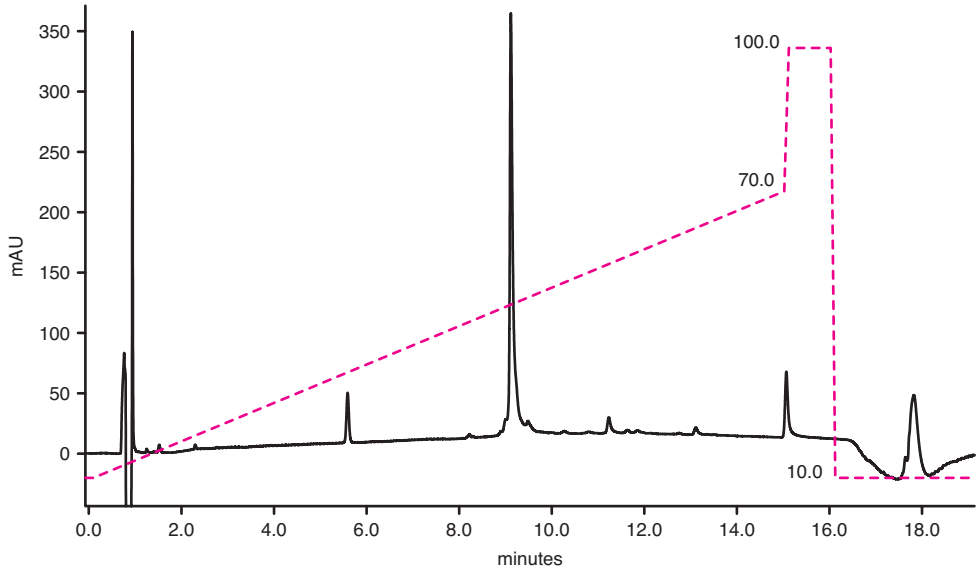

Figure 1: Campath MAb analysis on an Accucore 150-C4 HPLC column

After cleavage, the FAb region was analyzed and gave a very sharp peak (Figure 2). The chromatogram shows several minor variant peaks (Figure 3), which are easily seen in the presence of the major Fab fragment peak.

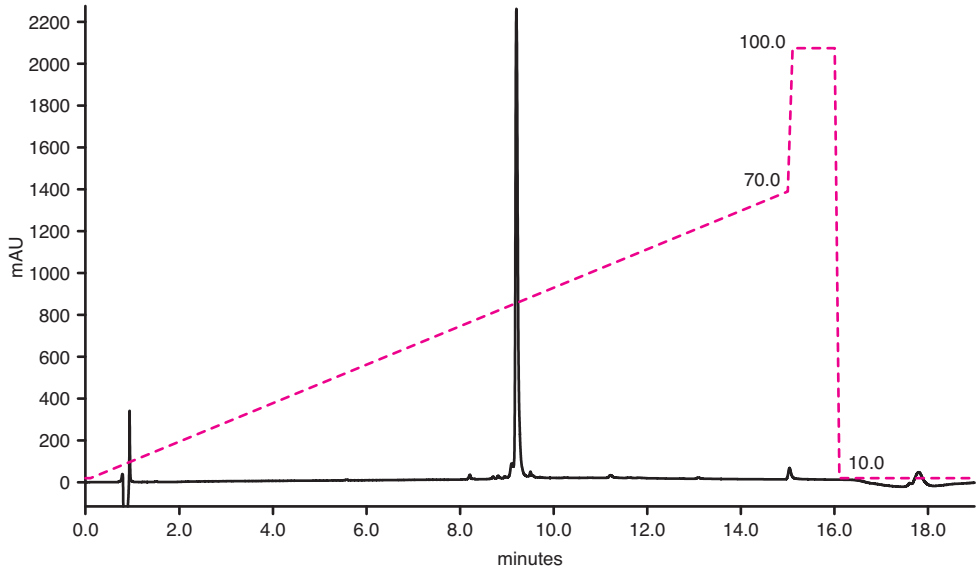

Figure 2: Campath FAb analysis on an Accucore 150-C4 HPLC column

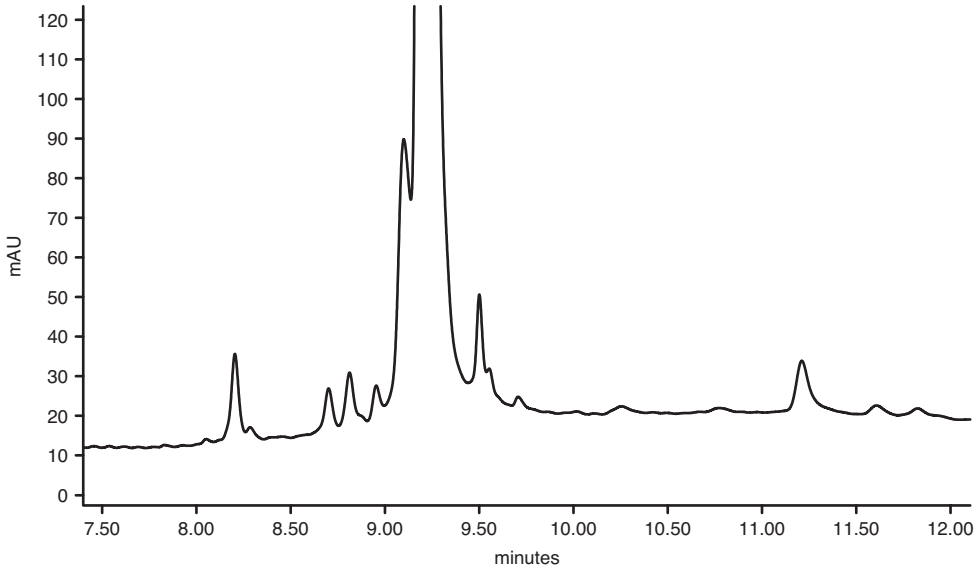

Figure 3: Expanded region around the main peak in Figure 2, showing the resolution of several low level minor impurities

Conclusion

- We have shown a novel separation of monoclonal antibodies on Accucore 150-C4 columns. The solid core technology allows for fast, high-efficiency separation of the entire antibody as well as the fragmented region. Several low-level impurities resulting from fractionation were also resolved.
- The backpressure (160 bar) is compatible with a conventional HPLC system.
- This reversed-phase separation technique is compatible with mass spectrometry, and therefore provides a method to introduce intact proteins to high resolution mass spectrometry for accurate molecular weight determination.

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