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Subunit Analysis of Monoclonal Antibodies Using Biozen™ 3.6 µm Intact XB-C8

Helen Whitby, PhD[2], M Christina Malinao[1], Brian Rivera[1], Chad Eichman, PhD[1], and Bryan Tackett, PhD[1]
[1]Phenomenex Inc., 411 Madrid Ave., Torrance, CA 90501, USA
[2]Phenomenex Ltd., Queens Avenue, Hurdsfield Ind. Est., Macclesfiled, Cheshire SK10 2BN UK

Introduction

Monoclonal antibodies (mAbs) are the most common protein therapeutic with an estimated 180 or more mAb drugs on the global market by 2025. Fragmentation of monoclonal antibodies is a Critical Quality Attribute (CQA) which must be measured for all biotherapeutics to assess the purity and integrity of the protein. Fragmentation can occur during expression but also upon storage or metabolism *in vivo*. The fragmentation pattern of a mAb can offer information on its stability making this an essential part of the characterization process. Fragments are formed at sites where the peptide bonds are prone to cleavage and although the factors which influence which sites are most susceptible can be complex, some general fragmentation patterns are typically observed. In this technical note, we employ a forced reduction approach to identify and report scFc and $F(ab')_2$ profiles for two common mAbs through an IdeS digestion. We then look at the fragmentation patterns of a Dithiothreitol (DTT) reduction, which provides separated heavy chain (HC) and light chains (LC).

Impurity profiling and characterization of intact biological subunits present many challenges because of a need to identify small differences between variants. Biozen 3.6 µm Intact XB-C8 and C4 columns are packed with specialized wide pore core-shell particles which facilitate faster mass transfer kinetics for large biomolecules, providing narrower, taller peaks and greater resolving power when analyzing HC / LC, Fc / Fab regions or isoforms, compared to other commercially available fully porous silica-based column solutions designed for mAb analysis. The introduction of high efficiency UHPLC instruments offering low system dispersion coupled with highly efficient core-shell particles has offered significant improvements in the resolution and characterization of antibody fragments.

Sample Preparation

Cetuximab was treated with IdeS enzyme (1 µL per 50 µg mAb) and incubated for 60 min at 37 °C. Rituximab was treated with 10 mM DTT at pH 5.5 for 30 min.

LC Conditions

Columns: Biozen 3.6 µm Intact XB-C8
AdvanceBio RP-mAb 3.5 µm SB-C8

Dimensions: 150 x 2.1 mm

Part No.: 00F-4766-AN

Mobile Phase: A: 0.1 % Trifluoroacetic Acid in Water
B: 0.1 % Trifluoroacetic Acid in Acetonitrile

Gradient:

Time (min)	%B
0	40
1	40
13	60

Flow Rate: 0.5 mL/min

Injection Volume: 1 µL

Temperature: 80 °C

Detection: UV @ 280 nm

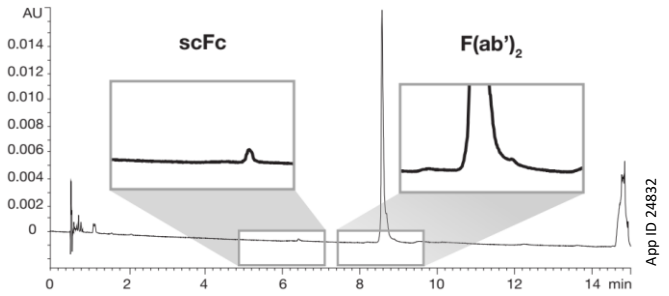
Results and Discussion

For the Ides digestion of Cetuximab, the $F(ab')_2$ was identified along with minor amounts of scFc using a Biozen Intact XB-C8 column (**Figure 1**). Under standard reversed phase gradient conditions, we observed excellent peak shape of the $F(ab')_2$. Analysis of this sample under the same running conditions with the AdvanceBio® RP-mAb SB-C8 column was unable to detect any scFc fragment. Significantly less tailing was also observed in the $F(ab')_2$ fragments with the Biozen Intact XB-C8 column, which allows for improved integration and more accurate results. Notably, both applications were run at an elevated temperature to improve the peak shape of the fragments.

Analysis of both HC and LC variants was also assessed using a DTT reduction of Rituximab (**Figure 2**). After employing the same method conditions, superior separation was observed for the HC variants on the Biozen Intact XB-C8 column. Moreover, this column was able to detect minor amounts of post-translational modifications (HC variants). When using the Advance Bio RP-mAb SB-C8 column the HC variants were not fully resolved.

Figure 1. Subunit Analysis of an IdeS Digestion of Cetuximab.

Biozen 3.6 µm Intact XB-C8



AdvanceBio RP-mAb 3.5 µm SB-C8

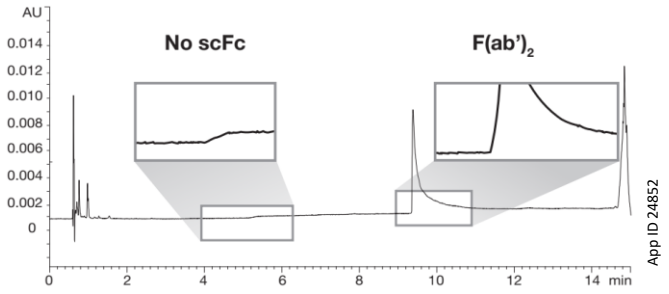
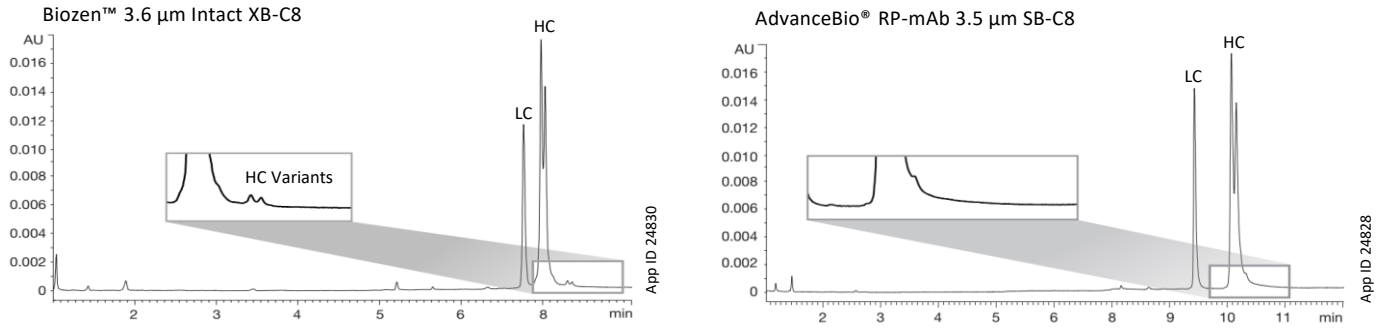


Figure 2. HC / LC Analysis of DTT-reduced Rituximab.



Conclusions

Both chemistries evaluated (Biozen Intact XB-C8 and AdvanceBio RP-mAb SB-C8) are bonded with a C8 ligand. However, the combination of the core-shell particle and the sterically hindered XB-C8 ligand offer better peak shape compared with a fully porous C8 phase. The superior peak shape and resolution observed with the Biozen Intact XB-C8 column was present in both the IdeS digestion of Cetuximab and the DTT reduction of Rituximab. Optimizing the performance of this subunit analysis is pivotal as it serves as a critical quality attribute that needs to be monitored to assess the integrity of the mAb. The wide pore core-shell particle morphology of the Biozen Intact XB-C8 column allows for faster mass transfer kinetics of antibodies in and out of the stationary phase, which reduces the diffusion pathway and improves chromatographic efficiency. The benefits of producing more efficient, narrower peaks is seen with improved resolution for subunit analysis.

Biozen Ordering Information

Biozen Columns (mm)								Biocompatible Guard Cartridges		
	50 x 2.1	100 x 2.1	150 x 2.1	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	for 2.1 mm	for 4.6 mm	Holder
								/3pk	/3pk	ea
Biozen 2.6 µm WidePore C4	00B-4786-AN	00D-4786-AN	00F-4786-AN	00B-4786-E0	00D-4786-E0	00F-4786-E0	00G-4786-E0	AJ0-9816	AJ0-9818	AJ0-9000
Biozen 3.6 µm Intact XB-C8	00B-4766-AN	00D-4766-AN	00F-4766-AN	00B-4766-E0	—	00F-4766-E0	—	AJ0-9812	AJ0-9814	AJ0-9000

Need a different column size or sample preparation format?

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Australia
t: +61 (0)2-9428-6444
auinfo@phenomenex.com

Austria
t: +43 (0)1-319-1301
anfrage@phenomenex.com

Belgium
t: +32 (0)2 503 4015 (French)
t: +32 (0)2 511 8666 (Dutch)
beinfo@phenomenex.com

Canada
t: +1 (800) 543-3681
info@phenomenex.com

China
t: +86 400-606-8099
cninfo@phenomenex.com

Czech Republic
t: +420 272 017 077
cz-info@phenomenex.com

Denmark
t: +45 4824 8048
nordicinfo@phenomenex.com

Finland
t: +358 (0)9 4789 0063
nordicinfo@phenomenex.com

France
t: +33 (0)1 30 09 21 10
franceinfo@phenomenex.com

Germany
t: +49 (0)6021-58830-0
anfrage@phenomenex.com

Hong Kong
t: +852 6012 8162
hkinfo@phenomenex.com

India
t: +91 (0)40-3012 2400
indiainfo@phenomenex.com

Indonesia
t: +62 21 5019 9707
indoinfo@phenomenex.com

Ireland
t: +353 (0)1 247 5405
eireinfo@phenomenex.com

Italy
t: +39 051 6327511
italiainfo@phenomenex.com

Japan
t: +81 (0) 120-149-262
jpinfo@phenomenex.com

Luxembourg
t: +31 (0)30-2418700
nlinfo@phenomenex.com

Mexico
t: 01-800-844-5226
tecnicomx@phenomenex.com

The Netherlands
t: +31 (0)30-2418700
nlinfo@phenomenex.com

New Zealand
t: +64 (0)9-4780951
nzinfo@phenomenex.com

Norway
t: +47 810 02 005
nordicinfo@phenomenex.com

Poland
t: +48 22 104 21 72
pl-info@phenomenex.com

Portugal
t: +351 221 450 488
ptinfo@phenomenex.com

Singapore
t: +65 6559 4364
sginfo@phenomenex.com

Slovakia
t: +420 272 017 077
sk-info@phenomenex.com

Spain
t: +34 91-413-8613
espinfo@phenomenex.com

Sweden
t: +46 (0)8 611 6950
nordicinfo@phenomenex.com

Switzerland
t: +41 (0)61 692 20 20
swissinfo@phenomenex.com

Taiwan
t: +886 (0) 0801-49-1246
twinfo@phenomenex.com

Thailand
t: +66 (0) 2 566 0287
thaiinfo@phenomenex.com

United Kingdom
t: +44 (0)1625-501367
ukinfo@phenomenex.com

USA
t: +1 (310) 212-0555
www.phenomenex.com/chat

All other countries/regions
Corporate Office USA
t: +1 (310) 212-0555
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