

## Hyphenating Size Exclusion Chromatography to High Resolution Mass Spectrometry - Bispecific Antibody

Dr. Laurence Brill, Brian Rivera, and Dr. Bryan Tackett  
Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA

### Overview

Size Exclusion Chromatography (SEC) is a technique focused on separating biomolecules based on their size in solution and is particularly useful for quantitating size variants and high molecular weight aggregates. The hyphenation of SEC to high resolution mass spectrometry (HRMS) has enabled characterization of biologics. This non-denaturing, so-called “Native MS” is especially useful for higher order structure (HOS) of monoclonal antibodies (mAbs).

In this application, we demonstrate the use of SEC-HRMS for the characterization of a bispecific antibody, Emicizumab. **Figure 1** shows the total ion chromatogram for Emicizumab. Good peak shape is observed, and the high molecular weight aggregate is separated, even using a relatively short column (150 mm). **Figure 2** shows the deconvoluted spectrum for the main peak. Glycoforms are identified with good mass accuracies, with most being less than 10 ppm.

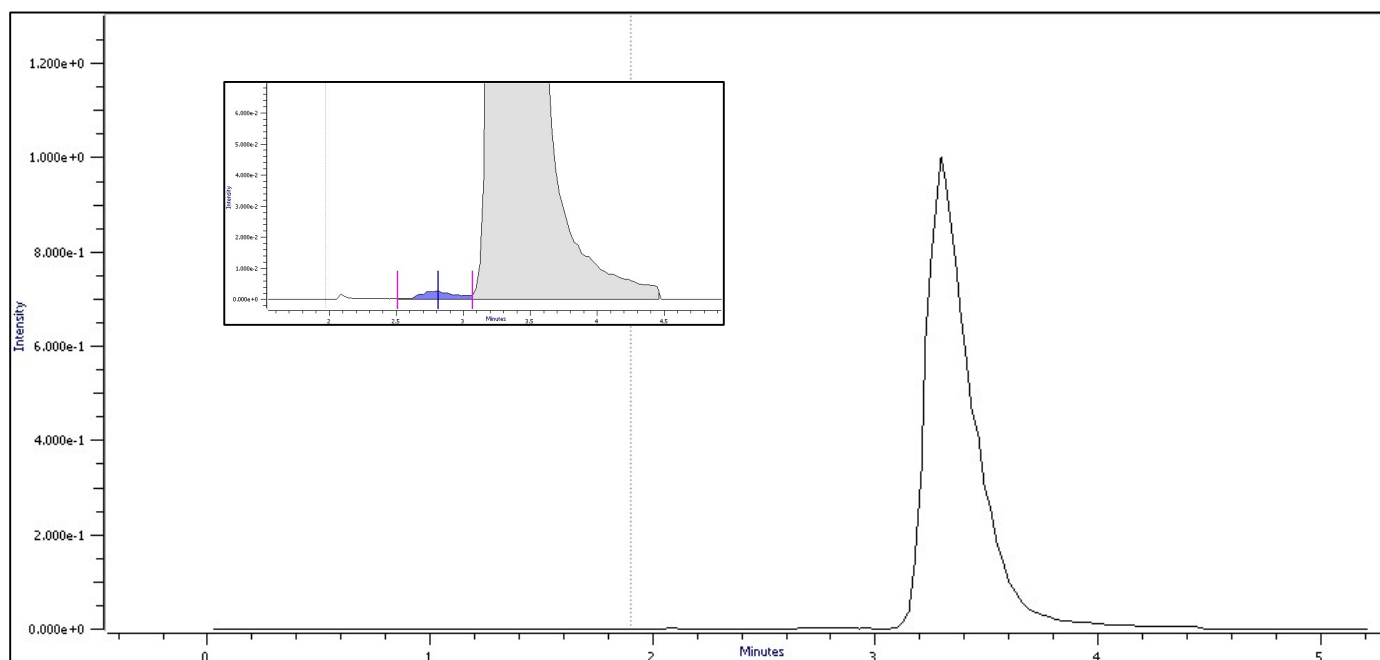
### LC Conditions

**Column:** Biozen™ 1.8 μm dSEC-2, 200 Å  
**Dimensions:** 150 x 2.1 mm  
**Part No.:** [00F-4787-AN](#)  
**Mobile Phase:** 200 mM Ammonium Acetate  
**Flow Rate:** 90 μL/min  
**Injection Volume:** 2.5 μL  
**Temperature:** 25 °C  
**Detection:** HRMS  
**Sample:** Emicizumab, 10 mg/mL

**Figure 3** shows the deconvoluted spectrum for the high molecular weight peak, showing mostly dimer. The raw spectrum shows some denaturing of the mAb, which is likely dissociation in the gas phase. Dimer for the most abundant glycoform (G0F/G0F/G1F/G1F) is less than 40 ppm.

In summary, SEC-HRMS is a powerful technique that can provide valuable characterization data, including confirming primary sequence and glycoforms, as well as confirmation of dimer and other high molecular weight species.

**Figure 1. Total ion chromatogram (TIC) for Emicizumab, 25 μg**



App ID 26625



Figure 2. Deconvoluted Spectrum, Main Peak - Emicizumab

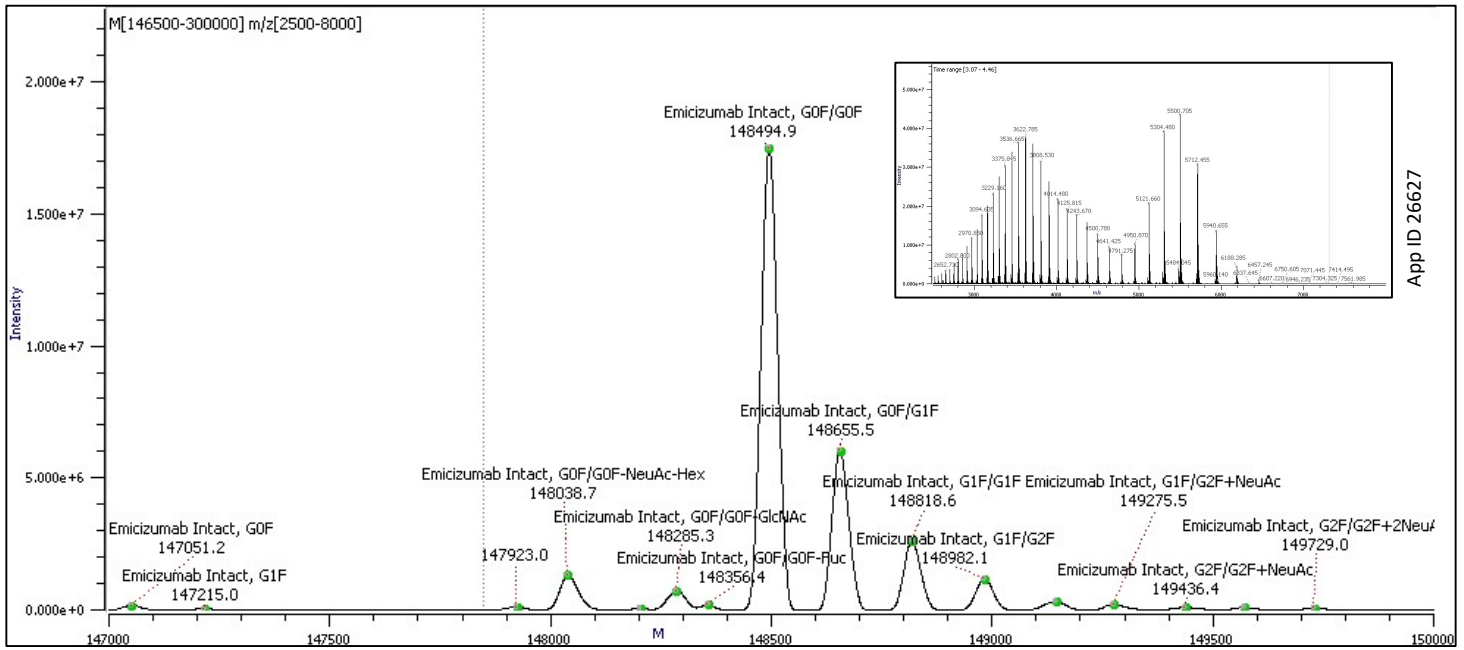
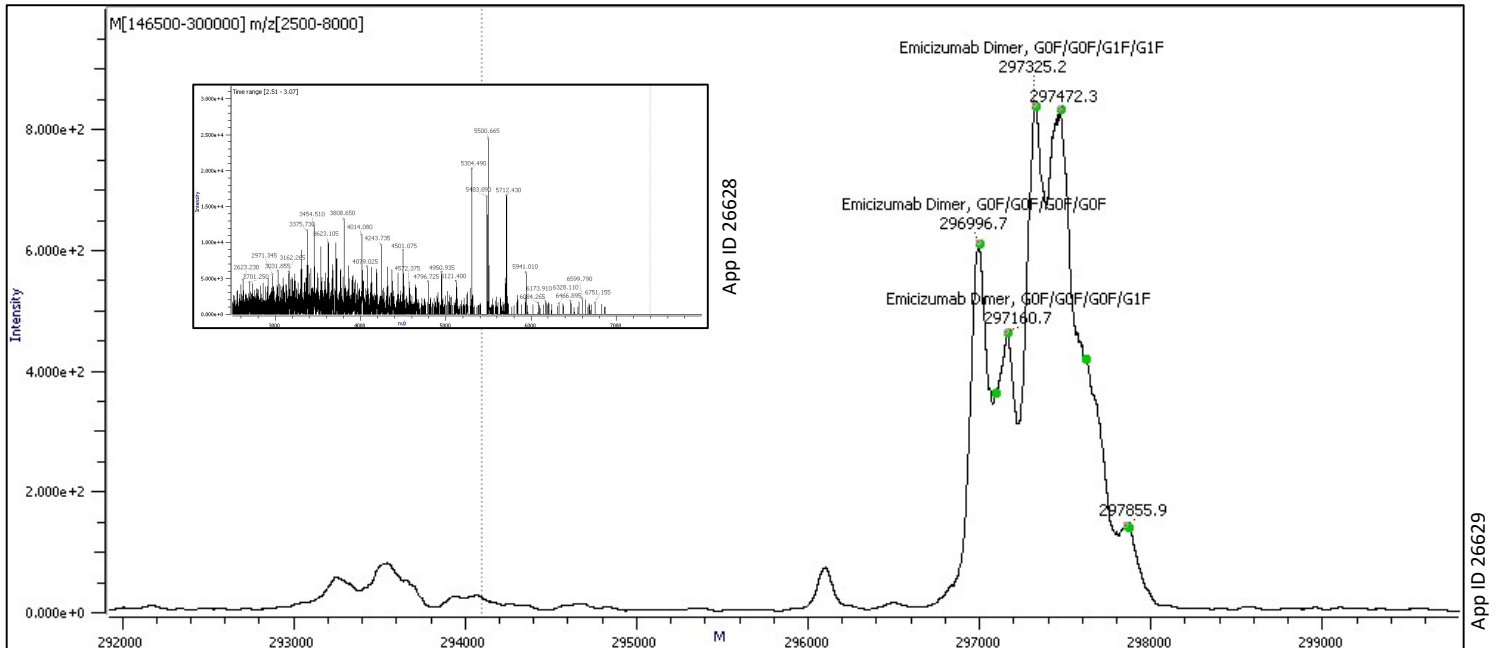


Figure 3. Deconvoluted Spectrum, Dimer/High Molecular Weight Aggregate Peak - Emicizumab



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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 5010 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: +65 800-852-3944  
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  
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