

APPLICATIONS

Comparison of UHPLC Particles for Intact Mass Subunit Analysis of Monoclonal Antibodies

Dawn Chen, Ivan Lebedev, Brian Rivera, and Chad Eichman
Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501, USA

Overview

With many regulatory characterization requirements in biotherapeutic development, one of the essential requirements is intact liquid chromatography-mass spectrometry (LC-MS) by reversed phase chromatography. This technique rapidly provides data for monoclonal antibody (mAb) primary sequence confirmation, impurity identification, and heterogeneity information. The mAb can be further simplified by reducing to yield heavy chain and light chain. These subunits can then be analyzed by high resolution MS detection such as a time of flight (TOF) instrument to provide more insight on sample heterogeneity and glycoform identity that otherwise would not be determined at the intact level.

Because peak shapes are prioritized for intact mass LC methods, a relatively ballistic gradient (i.e. gradient slopes exceeding 5% organic per column volume) must be implemented to ensure that good peak shapes are maintained. The resulting Gaussian peak shape should facilitate the spectral acquisition of the MS. Although less of a concern at the intact level, when analyzing subunits, the separation may be adversely affected when running steep gradient slopes. Namely, a loss of resolution between the subunits may result in poorer spectral quality, because of partial coelution.

As such, the use of high efficiency UHPLC particles, i.e. sub-2 μm fully porous or more recently, superficially porous particles, have been more common for intact mass subunit MS analysis. Although both sub-2 μm fully porous particles and superficially porous particles can obtain acceptable resolution between mAb heavy chain and light chain, peak shapes may be less desirable using the sub-2 μm particle. This may impact both the spectrum quality, as well as the characterization of different variants. Utilizing a [bioZen™ 2.6 \$\mu\text{m}\$ WidePore C4](#) column compared to a sub-2 μm C4 allows for separation advantages for subunit analysis.

Figure 1 shows the separation obtained using a sub-2 μm fully porous particle for reduced NIST mAb. Separation of light chain and heavy chain is acceptable, though there is some peak tailing observed with the heavy chain fragment, even with the high gradient slope for the chromatographic method (7.9 % B/column volume).

Conversely, in **Figure 2**, we can observe the separation of reduced NIST mAb giving better peak shapes for both heavy and light chain. Further, a later eluting impurity, presumed deamidated variant because of the similar spectra obtained relative to the main peak, is separated.

In summary, the use of steep gradient slopes limits the separation when analyzing mAb subunits by intact mass. High efficiency particles could be adequate for this separation but the use of [bioZen 2.6 \$\mu\text{m}\$ WidePore C4](#) core-shell superficially porous particles may be optimal in instances where efficiency is required for detection and separation of low level impurities.

LC-MS Conditions

Column: [bioZen 2.6 \$\mu\text{m}\$ WidePore C4, 400 Å](#)

Fully porous 1.7 μm C4, 300 Å

Dimensions: 100 x 2.1 mm

Part No.: [00D-4786-AN](#) (WidePore C4)

Mobile Phase A: 0.1 % Formic acid in Water

Mobile Phase B: 0.1 % Formic acid in Acetonitrile

Gradient Program: 5-90 % B in 6.5 minutes

Flow-rate: 0.3 mL/min

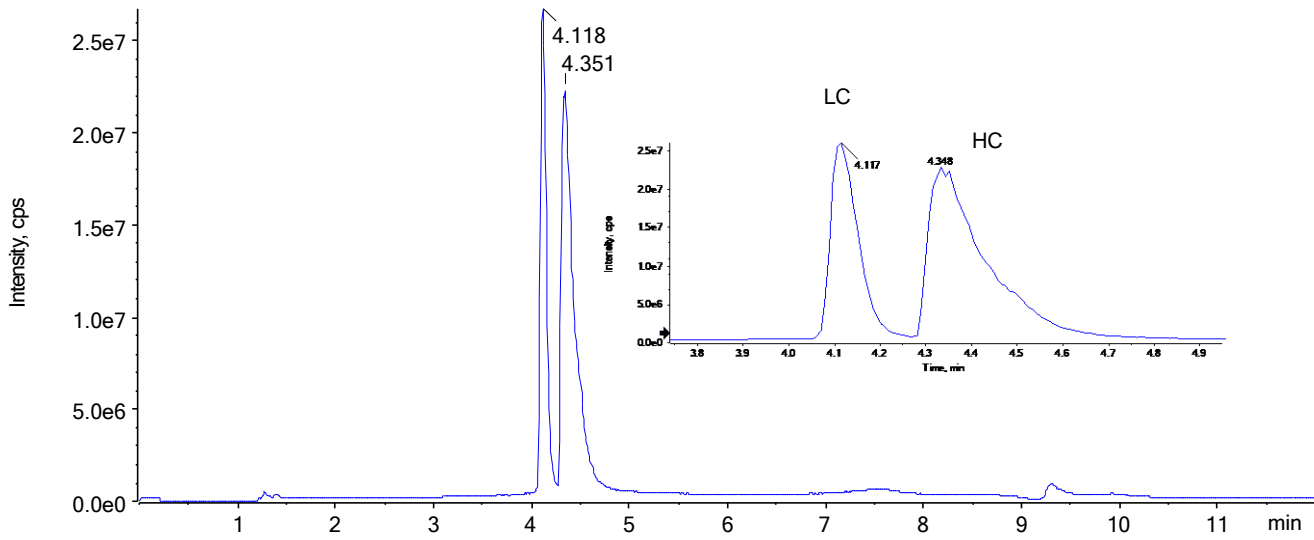
Temperature: 80 °C

Detection: QTOF

Injection: 2 μL

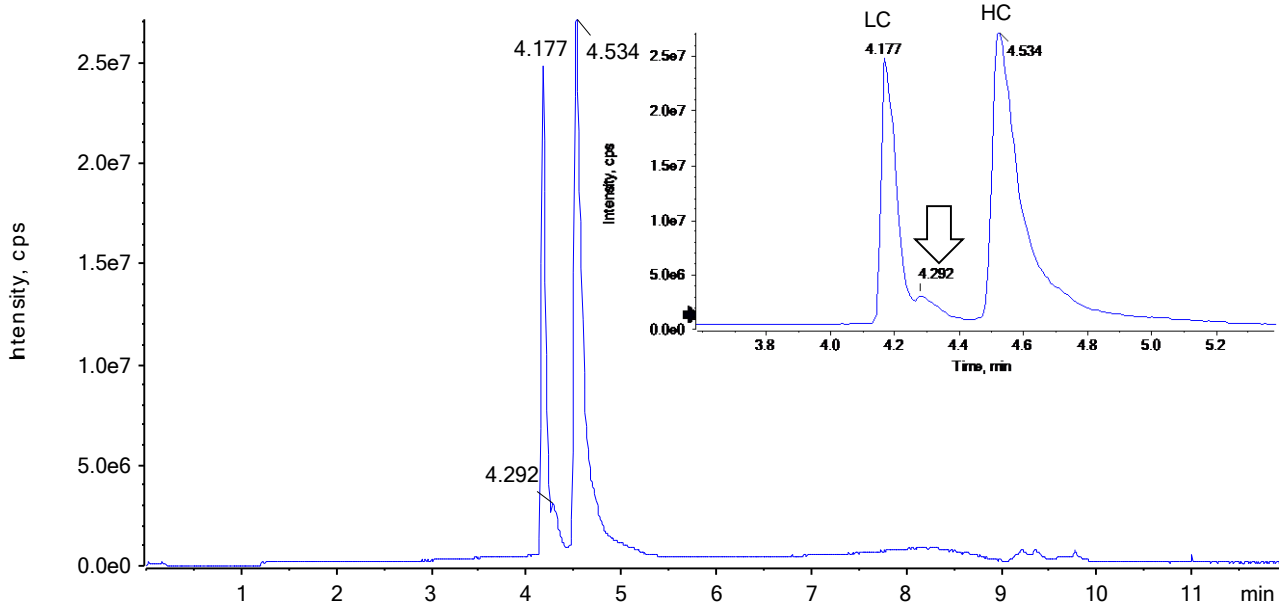
Sample: NIST mAb, 1 mg/mL

Figure 1. Separation of NIST mAb Heavy and Light Chain on sub-2 μm , Fully Porous C4, 300Å



App ID 25871

Figure 2. Separation of NIST mAb Heavy and Light Chain on bioZen[™] WidePore C4, 400 Å



App ID 25870

APPLICATIONS

Need a different column size or sample preparation format?

No problem! We have a majority of our available dimensions up on www.phenomenex.com, but if you can't find what you need right away, our super helpful Technical Specialists can guide you to the solution via our online chat portal www.phenomenex.com/ChatNow.

Australia

t: +61 (0)2-9428-6444
auiinfo@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

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

India

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

Ireland

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

Italy

t: +39 051 6327511
italiainfo@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 (12) 881 0121
pl-info@phenomenex.com

Portugal

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

Singapore

t: +65 800-852-3944
sginfo@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

United Kingdom

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

USA

t: +1 (310) 212-0555
info@phenomenex.com

🌐 All other countries/regions

Corporate Office USA
t: +1 (310) 212-0555
info@phenomenex.com

www.phenomenex.com

Phenomenex products are available worldwide. For the distributor in your country/region, contact Phenomenex USA, International Department at international@phenomenex.com

Terms and Conditions

Subject to Phenomenex Standard Terms and Conditions, which may be viewed at www.phenomenex.com/TermsAndConditions.

Trademarks

bioZen is a trademarks of Phenomenex..

Competitor comparisons are not representative of all applications.

FOR RESEARCH USE ONLY. Not for use in clinical diagnostic procedures.

© 2020 Phenomenex, Inc. All rights reserved.