Exploring the Effect of Mobile Phase Concentration on Sensitivity of Size Exclusion-Mass Spec (SEC-MS) for Monoclonal Antibodies using a bioZen™ SEC-3 Column

M. Christina Malinao and Helen Whitby Ph.D.
Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501

Overview

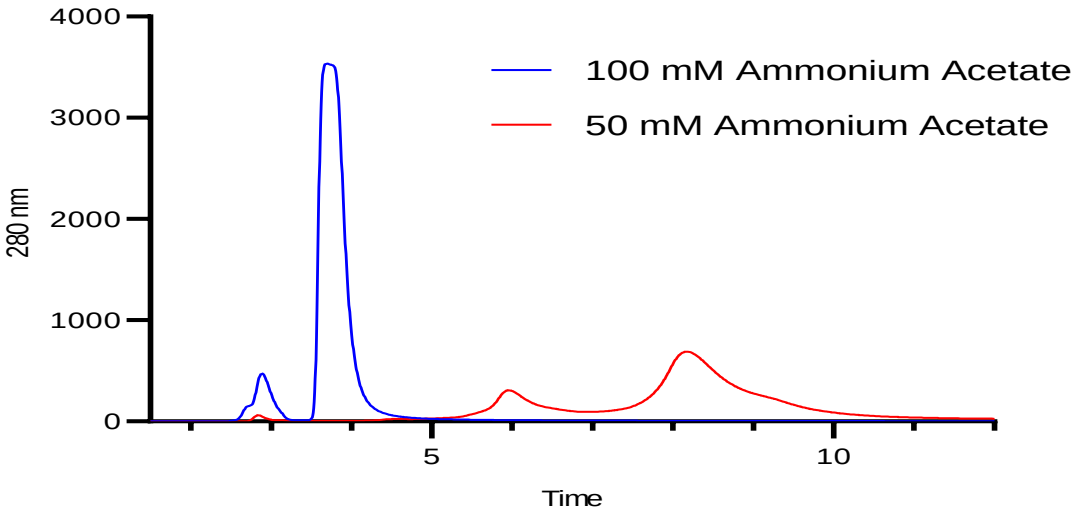
In recent years the trend of drug design and development has switched from small molecule targets to biotherapeutics as the next generation of therapies in order to address the short comings of the small molecules. Monoclonal antibody (mAb) aggregates are one significant class of these large molecules that have significant implications in safety, immunogenicity, and efficacy for therapeutic drug development. Size exclusion chromatography (SEC) is one of the laboratory methods used to determine the extent of aggregation of a protein, a critical quality attribute required for all therapeutic mAbs. In addition, SEC can detect low molecular weight (LMW) species including protein fragments. Native mass spectrometry (MS) using electrospray ionization allows proteins to be analyzed in non-denaturing volatile buffers. When this is done using controlled pH, ionic strength and ionization the conformation of the protein in the solution phase is maintained to allow it to remain intact during transmission into the gas phase. The use of intact native MS is an important technique to study non-covalent protein interactions, including those observed in reversible aggregate species.

In this application note we look at the impact that mobile phase concentration has on the sensitivity of SEC-MS when used to analyze intact mAb's under non-denaturing conditions. The SEC analysis of Trastuzumab is used to demonstrate the loss in MS sensitivity when working at high buffer concentrations. Trastuzumab was analyzed using the bioZen 1.8 µm SEC-3, 300Å column with an ammonium acetate mobile phase. Higher resolution of the monomer and dimer peaks can be achieved using a higher concentration buffer of 100mM however this resulted in lower sensitivity of the mass spec data. When working at 50 mM, the signal from the mass spec was found to be significantly higher but this was at the expense of resolution and separation of the monomer from dimer were compromised due to significant peak broadening. Overall, care should be taken to balance mobile phase formulation to maximize mass spec sensitivity while maintaining chromatographic performance.

LC Conditions

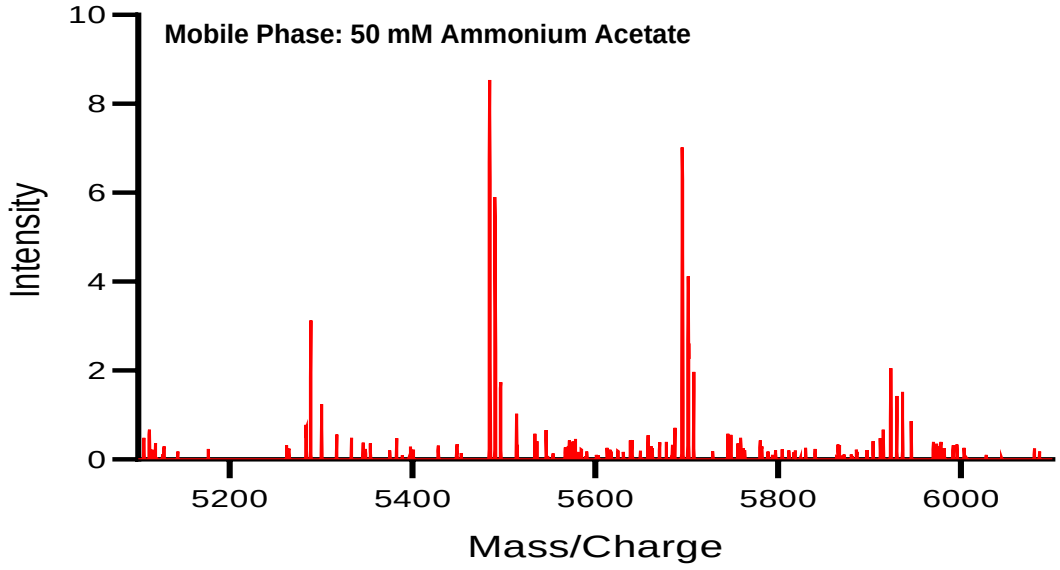
Column:	bioZen 1.8 µm SEC-3
Dimension:	50 x 2.1 mm
Part No.:	00B-4772-AN
Recommended Guard:	SecurityGuard™ ULTRA
Guard Cartridge Part No.:	AJ0-9851
Guard Holder Part No.:	AJ0-9000
Mobile Phase:	A: 50 mM Ammonium Acetate B: 100 mM Ammonium Acetate
Flow Rate:	50 µL/min
Temperature:	25 °C
Detector:	QTOF (SCIEX® X500B)
Sample:	Trastuzumab

DAD-280 nm Trastuzumab



App ID: 25583

Mobile Phase: 50 mM Ammonium Acetate



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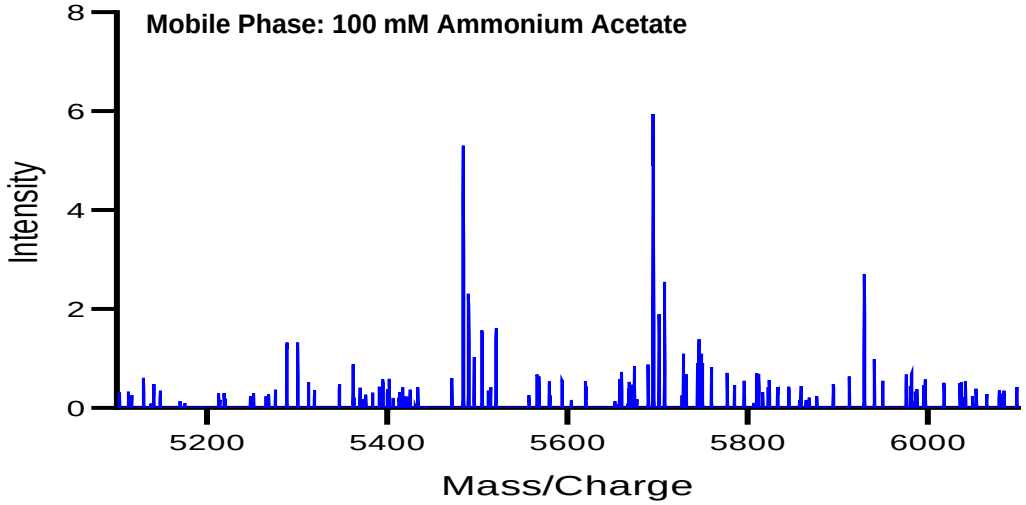
Mobile Phase: 100 mM Ammonium Acetate



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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

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

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

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