Waters™

應用手冊

BioAccord™ LC-MS System Enhancements for Improved Native MS Analysis

Samantha Ippoliti, Ying Qing Yu, Scott Berger

Waters Corporation, Milford MA

Published on May 27, 2025

Contact Sales

Application Brief

This is an application brief and does not contain a detailed Experimental section.

Abstract

This application brief demonstrates the significance of recent enhancements to the BioAccord LC-MS System for biopharmaceutical analysis workflows requiring native mass spectrometry (MS) analysis. The extension of the mass detection window on the BioAccord System's ACQUITY™ RDa™ Detector, now reaching up to *m/z* 9000 in positive ion mode, enables more effective MS analysis of larger protein complexes and protein conjugates,

broadening the system's applicability for characterization, attribute monitoring, and quality control for these emerging biopharmaceutical modalities.

Benefits

- Extended mass detection up to *m/z* 9000 in positive ion mode widens the acquisition mass range sufficiently for analysis of larger protein species such as monoclonal antibody (mAb) aggregation, noncovalent protein complexes, and native MS analysis of antibody conjugates with higher drug-to-antibody ratio (DAR) values.

Introduction

The BioAccord LC-MS System (Figure 1), originally launched in 2019, is a high-resolution compact benchtop time-of-flight mass spectrometry (TOF MS) system engineered for robust biopharmaceutical analysis. It has demonstrated capability for the analyses of intact mAbs and proteins,[1–2] peptide mapping,[3–4] antibody-drug conjugates (ADCs),[5] released glycans,[6–7] oligonucleotides,[8–9] and emerging biotherapeutic modalities such as mRNA[10–11] and viral vectors.[12] Key features such as automated instrument setup, workflow-based analyses, and the compliance-ready waters_connect™ Informatics Platform facilitate rapid adoption by analysts— regardless of their MS experience levels— across discovery, development, manufacturing, and QC/release organizations.

Figure 1. BioAccord™ LC-MS System.

The BioAccord System incorporates a source design that is optimized for gentler ionization, making it particularly effective for native MS applications, where the preservation of the protein tertiary structure is critical. Examples of native MS analysis that are becoming more routine for biopharmaceutical analysis include: the structural assessment of non-covalent protein complexes or conjugates and for pairing with nondenaturing chromatographic techniques such as ion exchange chromatography (IEX)[13–14] and size exclusion chromatography (SEC).[5]

Notably, raw combined mass spectra differ significantly between denatured and native MS conditions (Figure 2). For example, under native conditions (Figure 2B), the MS signal for the National Institute of Standards and Technology (NIST) mAb Reference Material 8671 appears with fewer charge states at higher *m/z* values (corresponding to lower charge states) vs. the typical denaturing LC-MS pattern (Figure 2A). This difference reflects the reduced chargeable surface area of folded proteins compared to their denatured counterparts. The

original BioAccord System supported a positive ion *m/z* range of up to 7000, which proved sufficient for most mAb analysis, denaturing or native. However, as biopharmaceutical development continues to push the boundaries of innovation with more complex molecules and conjugates, the BioAccord System has been enhanced to offer an extended mass range now up to *m/z* 9000, providing greater flexibility for the analysis of larger or more complex protein species.

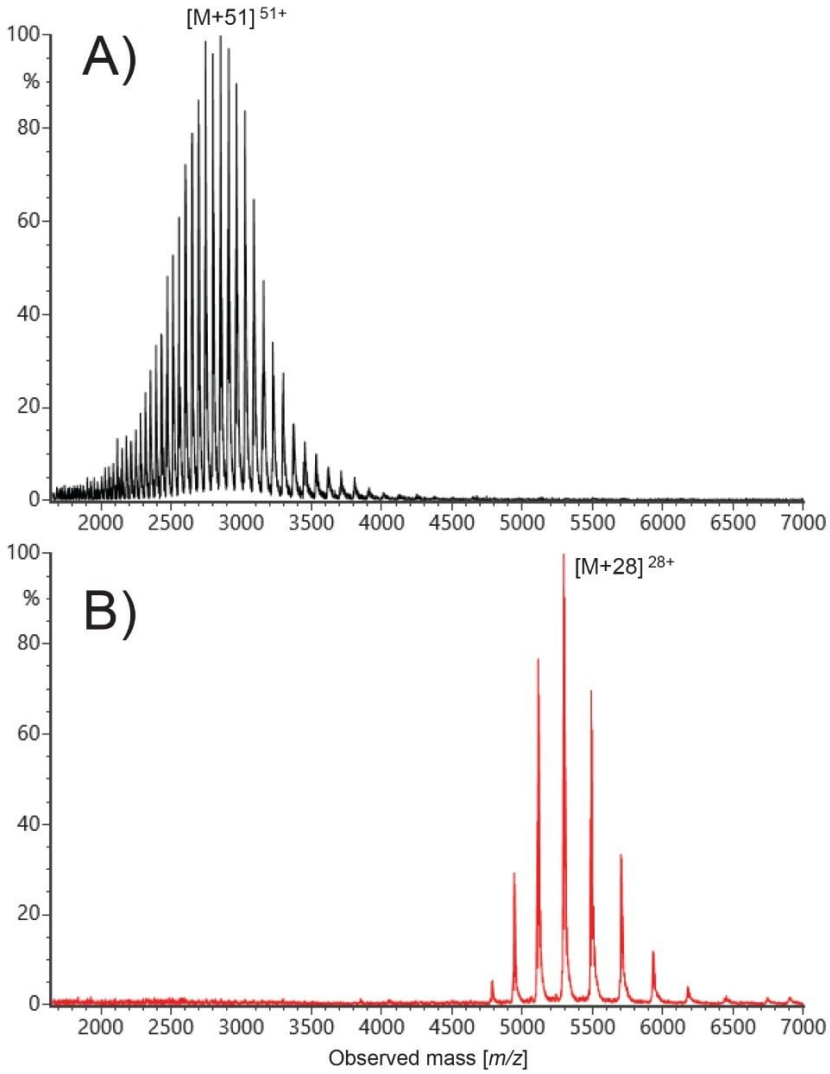

Figure 2. (A) Denaturing RPLC-MS vs (B) Native SEC-MS spectra of NIST mAb Reference Material 8671.

Results and Discussion

For this study, native SEC-MS was carried out on an ACQUITY UPLC™ Protein BEH™ SEC Column, 200 Å, 1.7 µm, 2.1 x 150 mm (p/n: 186008471 <https://www.waters.com/nextgen/global/shop/columns/186008471-acquity-uplc-protein-beh-sec-column-200a-17--m-21-mm-x-150-mm-1-.html>) maintained at 30 °C using a 10-minute isocratic flow of 50 mM ammonium acetate, pH 6.8. The ACQUITY RDa Detector was set to collect MS data in electrospray ionization (ESI) positive mode, comparing two mass ranges: 1) High Mass Range (HMR): *m/z* 400-7000 and 2) the new Extended Mass Range (EMR): *m/z* 400-9000. Calibration of both modes can be accomplished with the existing standard BioAccord calibration solution, also useful for lower *m/z* mode system calibrations.

The protein samples of interest in this case were yeast alcohol dehydrogenase (ADH), a known multimeric protein, and a stressed infliximab sample which contains a small percentage of high molecular weight (HMW) species detectable by SEC-MS.

yADH is a cytosolic protein that naturally self-associates into a tetrameric complex.[15] Using the standard HMR (*m/z* 400–7000) acquisition mode, the ACQUITY RDa Detector readily identified ADH tetramer (~148 kDa) and dimer species (~ 74 kDa) (Figure 3A). Applying the EMR (*m/z* 400-9000) mode, the additional octamer[16] (~ 296 kDa) was observed (Figure 3B), demonstrating the capability for analysis of larger non-covalent protein complexes and aggregates.

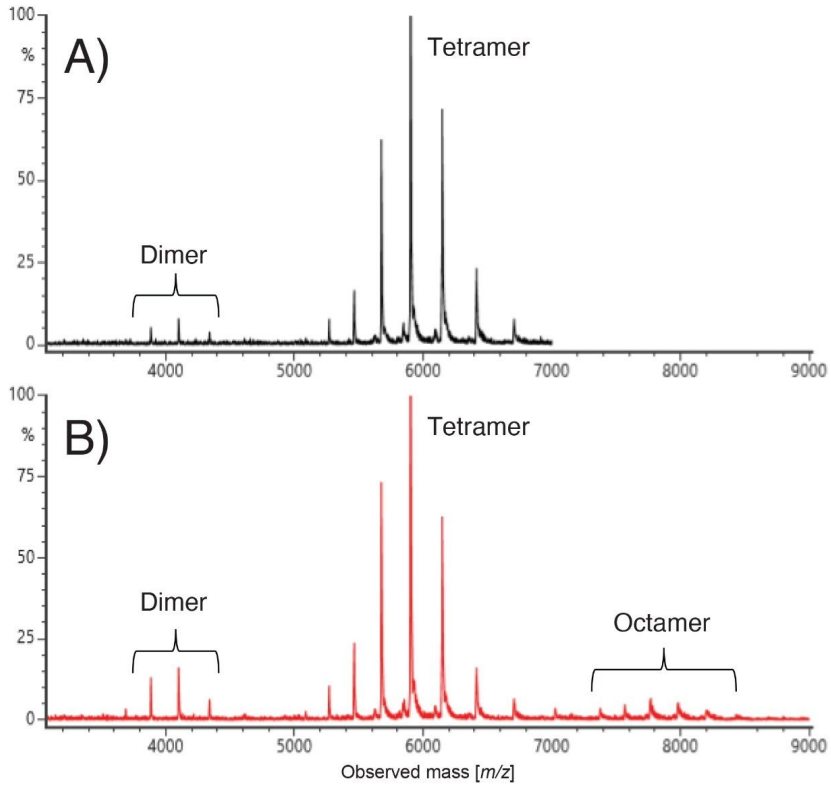

Figure 3. Combined Native SEC-MS spectra for yeast alcohol dehydrogenase (ADH) acquired with A) High Mass Range (m/z 400–7000) and B) Extended Mass Range (m/z 400–9000) acquired on the BioAccord LC-MS System.

In an additional set of experiments, a sample of infliximab was stressed by freeze/thaw cycles in the attempt to induce aggregation. A small percentage of HMW species was detected in the UV280 chromatogram (Figure 4A, highlighted in red). This HMW species was also observed by mass detection on the BioAccord System (Figure 4B inlay, red spectrum) and compared with the spectrum from the monomer peak (Figure 4A inlay, black spectrum). The observed HMW (dimer) species appears in the *m/z* 6500-8500 range, enabled by the EMR mode of the ACQUITY RDa Detector, and has a mass consistent with the expected mass for the dimer of infliximab (Figure 4B, bottom panel). This dimer species would not have been detectable under the original HMR settings.

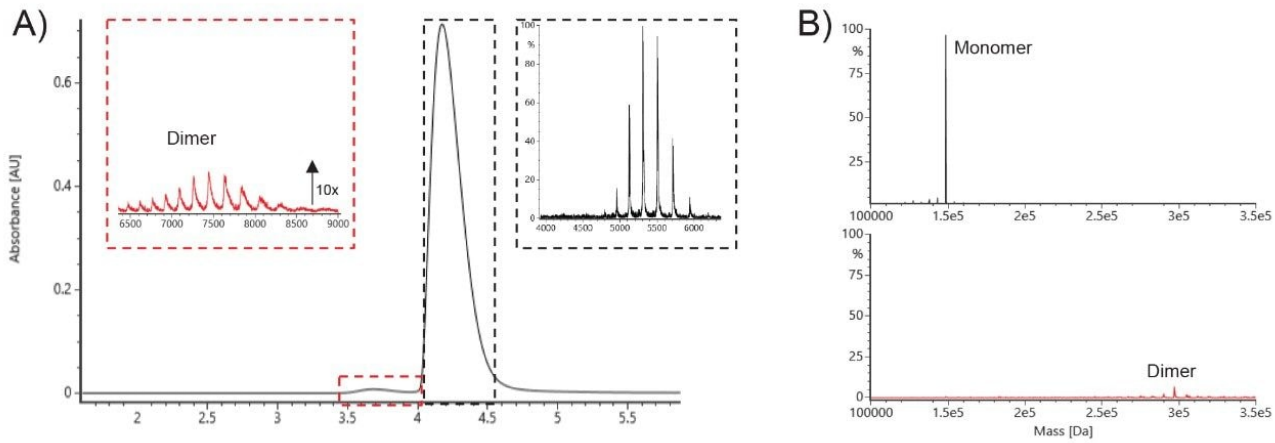

Figure 4. Native SEC-MS analysis of freeze/thaw stressed infliximab sample. High Molecular Weight (HMW) species was observed in the UV chromatogram (Panel A) and by mass detection (red spectra, inset of Panel A), in addition to the later eluting monomer (black spectra). The HMW peak was confirmed mAb dimer species following deconvolution (Panel B).

Conclusion

The extension of the mass detection window on the BioAccord System to *m/z* 9000 in positive mode allows for effective analysis of higher molecular weight species or those shifted to higher *m/z* by conjugation or LC inlet conditions. This study showed successful detection of ADH protein higher-level multimers and a mAb dimer species resolved via native SEC-MS analysis. The EMR mode of the BioAccord System directly addresses growing industry demands for characterizing larger protein complexes, protein conjugates with increasingly complex payloads (***e.g.*** ADCs or antibody-oligonucleotide conjugates), and for the study of aggregation prone therapeutics.

References

1. Shion H, Yu YQ, Chen W. Enabling Routine and Reproducible Intact Mass Analysis When Data Integrity Matters. Waters Application Note 720006472 <https://www.waters.com/nextgen/global/library/application-notes/2019/enabling-routine-and-reproducible-intact-mass-analysis.html> . Rev Oct 2020.

2. Ippoliti S, Young J, McNally K, Hanna C, Yu YQ, Prater B, Wrona MW, Harry E. Similis Bio- Streamlined mAb Subunit LC-MS Workflow for Multiple Attribute Monitoring of Biosimilar mAb Candidates During Bioprocessing and Development. Waters Application Note 720007997. < https://www.waters.com/nextgen/global/library/application-notes/2023/similis-bio-streamlined-mab-subunit-lc-ms-workflow-for-multiple-attribute-monitoring-of-biosimilar-mab-candidates-during-bioprocessing-and-development.html> Aug 2023.

3. Ranbaduge N, Du M, Yu YQ, Chen W. Establishing an Integrated Peptide Attribute Profiling and Monitoring Workflow for Improved Productivity and Confidence. Waters Application Note 720006602 < https://www.waters.cm/nextgen/global/library/application-notes/2019/integrated-peptide-attribute-profiling-monitoring-workflow-for-improved-productivity-confidence.html> . Jun 2019.

4. Muriithi B, Ippoliti S, Finny A, Addepalli B, Lauber MA. Clean and Complete Protein Digestion with an Autolysis Resistant Trypsin for Peptide Mapping. *Journal of Proteome Research*. 2024. 23 (11), 5221-5228.

5. Shion H, Yu YQ, Chen W. Analysis of Antibody Drug Conjugates (ADCs) by Native Mass Spectrometry on the BioAccord System. Waters Application Note 720006570 < https://www.waters.com/nextgen/global/library/application-notes/2019/analysis-of-antibody-drug-conjugates-adcs-by-native-mass-spectrometry-on-the-bioaccord-system.html> . May 2019.

6. Zhang X, Reed C, Shion H, Alley WR, Birdsall RE, Yu YQ. Released N-linked Glycan Analysis Using the BioAccord System. Waters Application Note 720006474 < https://www.waters.com/nextgen/global/library/application-notes/2019/released-n-linked-glycan-analysis-using-bioaccord.html> . Jan 2019.

7. DeLaney K, Htet Y, Yu YQ. Comparison of Released N-Glycans in Biosimilar mAb Drug Products Using the BioAccord LC-MS System. Waters Application Note 720008041 < https://www.waters.com/nextgen/global/library/application-notes/2023/comparison-of-released-n-glycans-in-biosimilar-mAb-drug-products-using-the-bioAccord-lc-ms-system.html> . Oct 2023.

8. Doneanu CE, Knowles C, Fox J, Harry E, Yu YQ, Fredette J, Chen W. Analysis of Oligonucleotide Impurities on the BioAccord System with ACQUITY Premier. Waters Application Note 720007301 <

https://www.waters.com/nextgen/global/library/application-notes/2021/analysis-of-oligonucleotide-impurities-on-the-bioaccord-lc-ms-system-with-acquity-premier.html> . Jul 2021.

9. Doneanu CE, Fox J, Harry E, Knowles C, Yu YQ, Fredette J, Chen W. Intact Mass Confirmation Analysis on the BioAccord LC-MS System for a Variety of Extensively Modified Oligonucleotides. Waters Application Note 720007028 <https://www.waters.com/nextgen/global/library/application-notes/2020/intact-mass-confirmation-analysis-on-the-bioaccord-lc-ms-system-for-a-variety-of-extensively-modified-oligonucleotides.html> . Oct 2020.

10. D'Esposito RJ, Doneanu CE, Gastall H, Berger SJ, Yu YQ. RNA CQA Analysis using the BioAccord LC-MS System and INTACT Mass waters_connect Application. Waters Application Note 720008130 < https://www.waters.com/nextgen/global/library/application-notes/2023/rna-cqa-analysis-using-the-bioaccord-lc-ms-system-and-intact-mass-waters-connect-application.html> . Nov 2023.

11. Nguyen JM, Chan S, Roy B, Gilar M, Robb B, Chen W, Lauber MA. Rapid Analysis of Synthetic mRNA Cap Structure Using Ion-Pairing RPLC with the BioAccord LC-MS System. Waters Application Note 720007329 < https://www.waters.com/nextgen/global/library/application-notes/2021/rapid-analysis-of-synthetic-mrna-cap-structure-using-ion-pairing-rplc-with-the-bioaccord-lc-ms-system.html> . Aug 2021.

12. Zhang X, Koza SM, Yu YQ, Chen W. Optimizing Adeno-Associated Virus (AAV) Capsid Protein Analysis Using UPLC and UPLC-MS. Waters Application Note 720006869 < https://www.waters.com/nextgen/global/library/application-notes/2020/optimizing-adeno-associated-virus-aav-capsid-protein-analysis-using-uplc-and-uplc-ms.html> . May 2020.

13. Ippoliti S, Schmudlach A, Lauber MA, Yu YQ. Online IEX-MS of mAb Charge Variants Using a BioResolve SCX mAb Column, IonHance XC-MS pH Concentrates, and BioAccord System. Waters Application Note 720006672 <https://www.waters.com/nextgen/global/library/application-notes/2019/online-iex-ms-mab-charge-variants-bioresolve-scx-mab-column-ionhance-cx-ms-ph-concentrates-and-bioaccord-system.html> . Sept 2019.

14. Birdsall RE, Koshel BM, Yu YQ. Accelerating Charge Variant Analysis of Biotherapeutics with the BioAccord System. Waters Application Note. 720007706 <https://www.waters.com/nextgen/global/library/application-notes/2022/accelerating-charge-variant-analysis-of-biotherapeutics-with-the-bioaccord-system.html> . Aug 2022.

15. Mallis CS, Zheng X, Qiu X, McCabe JW, Shirzadeh M, Lyu J, Laganowsky A, Russell DH. Development of

Native MS Capabilities on an Extended Mass Range Q-TOF MS. *Int J Mass Spec*. 2020. 458: 116451.

16. Wang Y, Schubert M, Ingendoh A, Franzen J. Analysis of non-covalent protein complexes up to 290 kDa using electrospray ionization and ion trap mass spectrometry. *Rapid Comm. Mass Spec*. 2000. 14 (1), 12-17.

Featured Products

BioAccord LC-MS System <https://www.waters.com/nextgen/global/products/mass-spectrometry/mass-spectrometry-systems/bioaccord-lc-ms-system.html>

ACQUITY RDa Detector <https://www.waters.com/nextgen/global/products/mass-spectrometry/mass-spectrometry-systems/acquity-rda-detector.html>

waters_connect Informatics Platform <https://www.waters.com/nextgen/global/products/informatics-and-software/waters_connect/waters-connect-informatics-platform.html>

720008813, May 2025