

## Routine Peptide Mapping Analysis using the BioAccord System

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### Abstract

Here we demonstrate how the BioAccord System simplifies routine peptide mapping analysis with integrated analytical workflows purposefully designed to simplify instrument operation, data analysis, and achieve meaningful results.

### Benefits

SmartMS-enabled BioAccord System improves the operational efficiency for routine peptide mapping analysis

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### Introduction

Peptide level analysis of protein modifications is one of the primary analytical methods regularly performed throughout the lifecycle of therapeutic proteins to ensure drug product quality. As biotherapeutic pipelines continue to expand, deploying methods that can assess protein modifications accurately and consistently becomes increasingly important for efficient product development and quality control. This requires analytical platforms that are robust, easy to use, and can be readily deployed in laboratories across an organization. The BioAccord System is such an example; it is comprised of an ACQUITY UPLC I-Class PLUS System configured with an optical detector (TUV or FLR) coupled in-line to the ACQUITY RDa Detector. The compliance-ready software,

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UNIFI Scientific Information System drives the overall system performance while providing automated data acquisition, processing and reporting capabilities. As part of the BioAccord System, integrated workflows have been purposefully designed to improve accessibility of LC-MS-based analyses. This technology brief demonstrates the performance and application of the routine peptide mapping workflow.



**B**

Instrument Setup

Console Navigation

- System
  - NR BSM TUV FTN RDa
  - Binary Solvent Manager
  - ACQUITY RDa Detector
  - TUV Detector
  - Sample Manager FTN
- ACQUITY RDa Detector
  - Summary
  - Setup
  - Maintenance
- Setup
  - Instrument Setup
  - Rear Panel

Instrument Setup

**Instrument Health | Ready** ← instrument status display

Setup Control

▶ Start ■ Stop Reset | To recalibrate in full, click 'Reset' then 'Start' ← simple instrument setup control

Check the fluidics reservoirs contain a sufficient volume before starting instrument setup.

Calibration

<input checked="" type="checkbox"/>	Positive Low mass (50 - 2000 Da)	Success	Nov 12 2018, 17:57:52 GMT
<input checked="" type="checkbox"/>	Positive High mass (400 - 7000 Da)	Success	Nov 12 2018, 17:59:59 GMT
<input checked="" type="checkbox"/>	Negative Low mass (50 - 2000 Da)	Success	Nov 12 2018, 17:48:22 GMT
<input checked="" type="checkbox"/>	Negative High mass (400 - 5000 Da)	Success	Nov 12 2018, 17:49:57 GMT

← on/off calibration control

Figure 1. A) An image of the BioAccord System, it is comprised of an ACQUITY UPLC I-Class PLUS System configured with an optical detector

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*(TUV/FLR) coupled in-line to the ACQUITY RDa Mass Detector.*

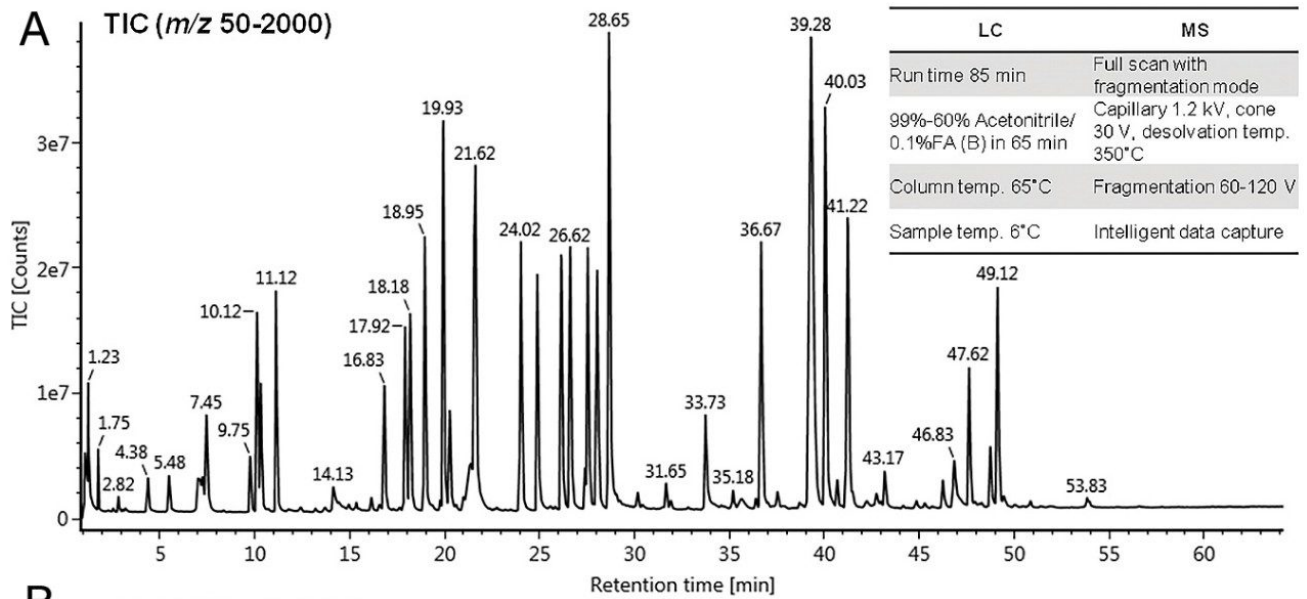
*Figure 1. B) Simplified instrument control and calibration. This page provides access to automated instrument set up and calibration.*

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## Results and Discussion

The BioAccord System introduces the ACQUITY RDa Detector. The ACQUITY RDa is a high performance SmartMS system engineered with a smaller laboratory foot print and intelligent system setup, calibration, and optimization (Figure 1A) for easy deployment in a variety of lab settings. As part of this instrument design, the BioAccord System contains many user-friendly features to increase accessibility and instrument uptime including automated instrument mass calibration and system readiness check. When the instrument requires calibration, it will notify the operator and can be easily initiated with the “one-click” calibration feature (Figure 1B). The integrated informatics system constantly monitors and logs the instrument health status for maintained performance.

The integrated UNIFI informatics platform of the BioAccord System is comprised of an analytical workflow for peptide analysis. This peptide mapping workflow is a fully automated informatics solution dedicated for robust protein sequence confirmation (Figure 2A and B), PTM identification, and relative quantitation of protein modifications. Figure 3A shows a processed chromatogram with peptide peaks labeled for tryptic peptides of mAb tryptic digestion standard (p/n 186009126). The raw spectrum of the largest tryptic peptide (T15, MW = 6713.3144 Da) from the heavy chain shows well resolved monoisotopic peak (Figure 3B). In one of its modes of operation, the ACQUITY RDa is capable of generating structurally informative fragment ions, adding more confidence to peptide assignments (Figure 3C). This unique feature is achieved by alternating MS scans (one with low energy voltage, and one with high energy voltage ramping) during data acquisition. As a result, the instrument can be operated in MS only or full scan with fragmentation (data independent acquisition) modes with collision induced fragmentation. The overall peptide mapping workflow allows scientists to confirm sequence coverage, achieve peptide level PTM characterization and determine the extent of quality attributes of the protein drug in single or batch processed samples (Figure 4).



**B** Sequence Coverage

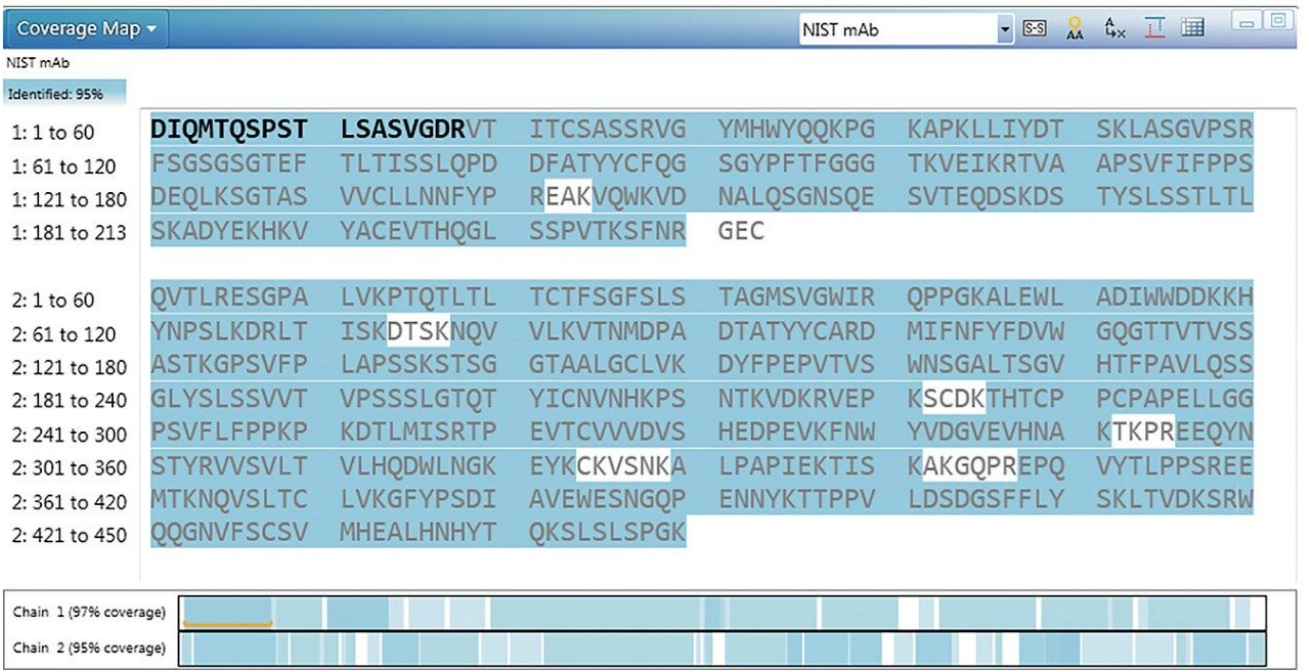
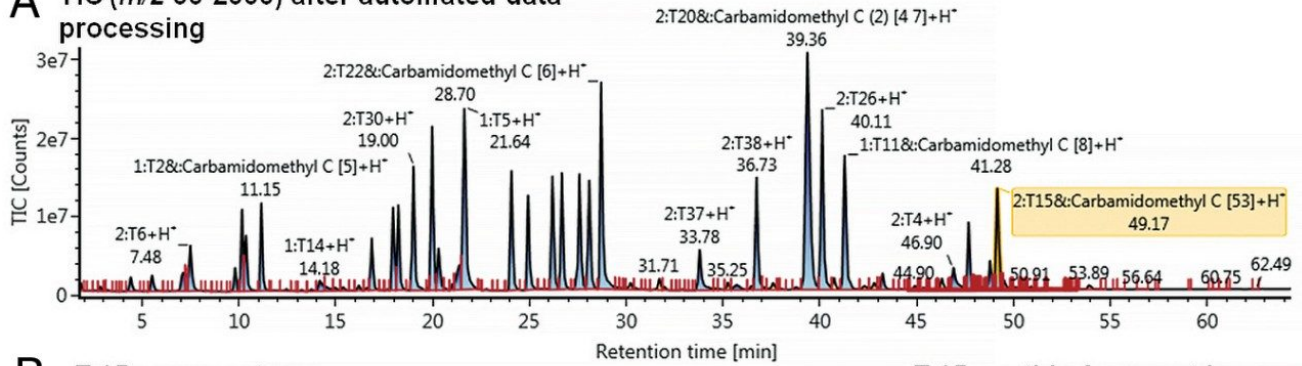
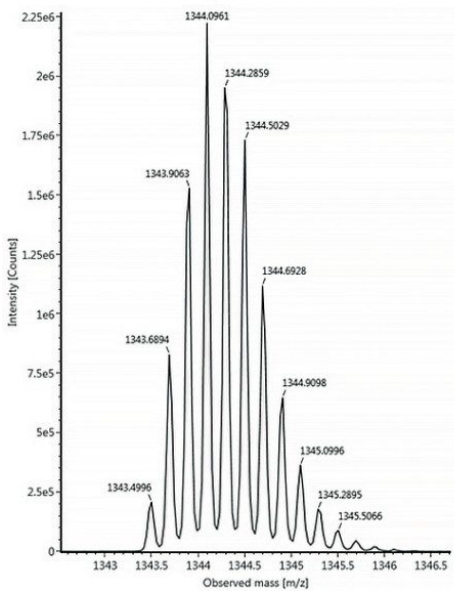


Figure 2. A) Total Ion Chromatogram (TIC) of NIST mAb tryptic peptides. B) The sequence coverage of mAb Tryptic Digestion Standard (97% sequence coverage for light chain, 95% for heavy chain). The assignments are based on <10 ppm mass tolerance, with a minimum of 3 primary fragment ions per peptide. The sequence coverage shown here is 95%.

**A** TIC ( $m/z$  50-2000) after automated data processing



**B** T:15 raw spectrum



T:15 peptide fragment ions

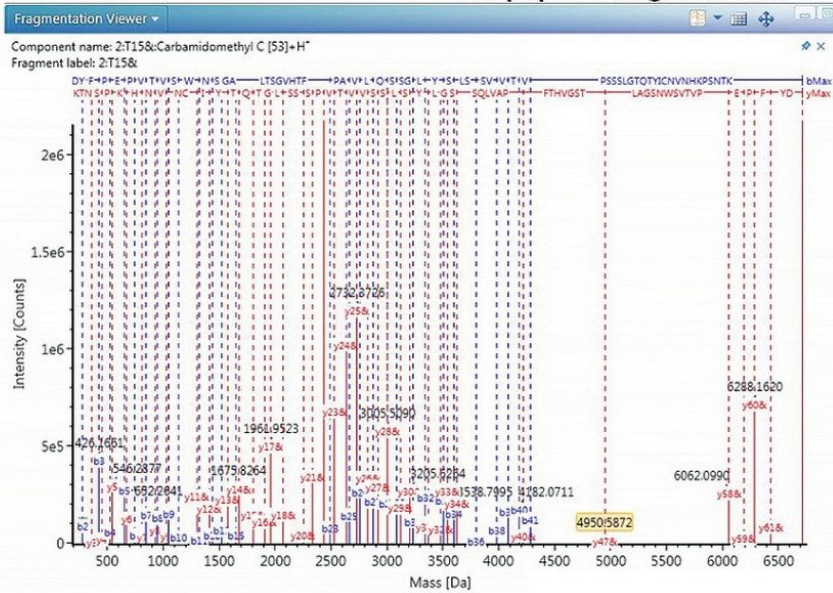


Figure 3. A) The figure shows the processed chromatogram with labeled tryptic peptides of mAb Tryptic Digestion Standard. The T15 peptide from heavy chain of this protein standard is highlighted (MW = 6713.3144 Da). B) The MS spectrum shows the raw data for  $m/z$  1343.6655 (+5) peak and C) the final labeled fragmentation spectrum for T15 peptide with an impressive 64 fragment ions for sequence verification.

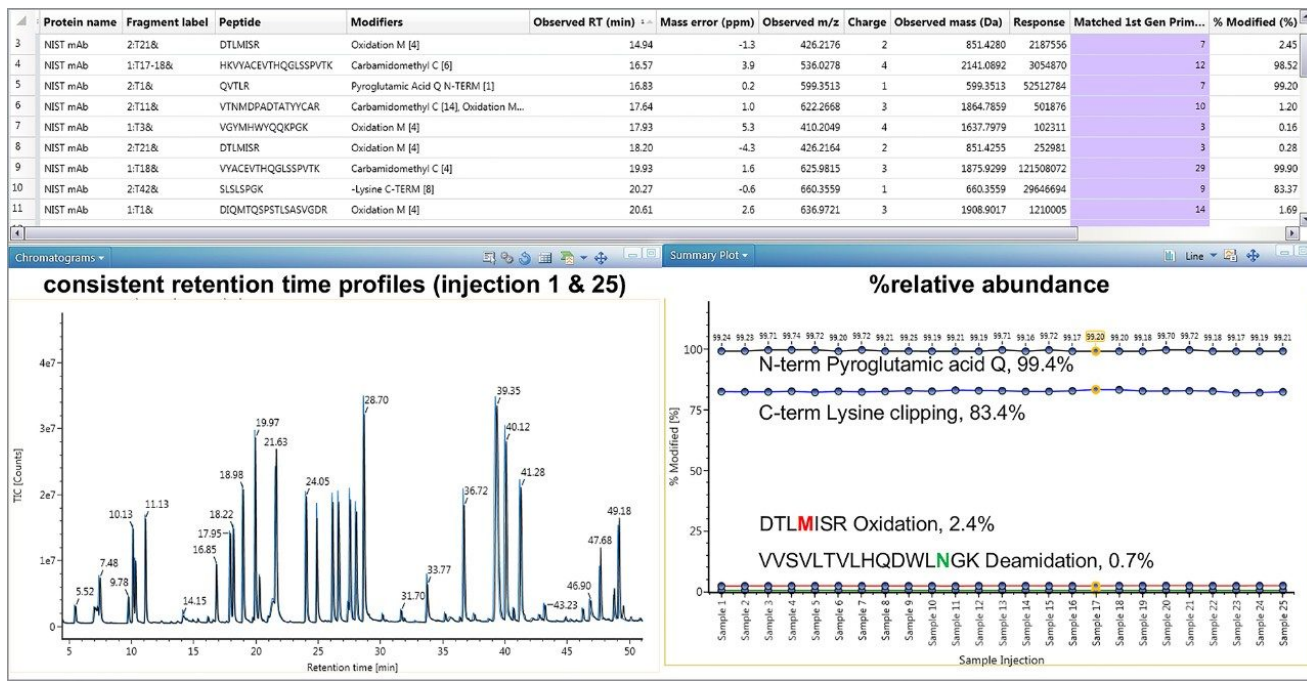


Figure 4. The peptide mapping workflow of the BioAccord System provides PTM identifications. The figure shows the review panel following automated data processing. The component table contains: sequence information, m/z and neutral mass, mass tolerance, %rel. abundance as well as fragmentation information for each peptide. The summary table provides a concise view of selected peptide modifications across all samples. The consistency in retention times is shown in the bottom left panel using TIC's for injections 1 and 25.

## Conclusion

The BioAccord System is purposefully designed to improve accessibility of routine therapeutic protein characterization and monitoring for late stage development. With its design features such as simple instrument set up, calibration, and software guided instructions, scientists can utilize the system to its full potential. One of the three streamlined workflows supported by this system is routine peptide mapping, which is used to confirm sequence coverage as well as monitor quality attributes of drug product. The workflow is seamlessly integrated by the compliance-ready software UNIFI and can be used to improve confidence of peptide mapping analyses without compromising productivity in regulated environments.

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## Featured Products

BioAccord LC-MS System for Biopharmaceuticals <<https://www.waters.com/waters/BioAccord-LC-MS-System-for-Biopharmaceuticals/nav.htm?cid=135005818>>

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