APPLICATION BRIEF 22012

Intact protein analysis on a MAbPac Reversed Phase Column

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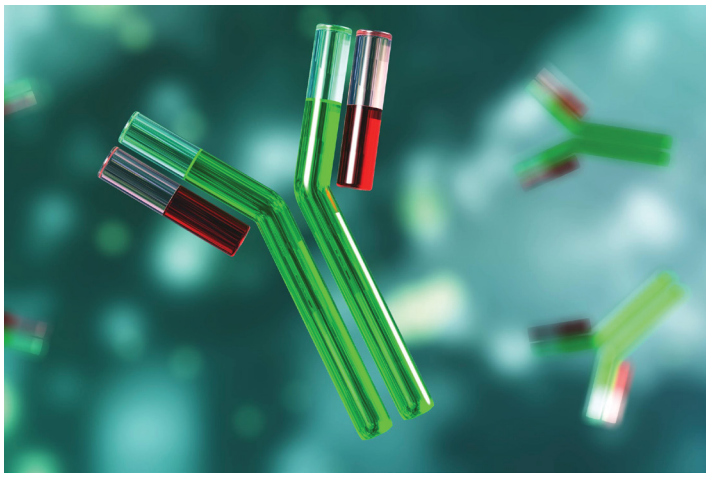

Introduction

Characterization of a protein, biosimilar, antibody, and their isoforms is usually done by peptide mapping analysis of a digested mixture using bottom-up sequencing. This is a powerful technique for obtaining complete coverage, including complex modifications. Unfortunately, in the process, the connectivity and quantitative relationships of isoforms and modifications are lost. An alternate approach is top-down and middle-down analysis of the protein. In top-down analysis, the whole protein is analyzed on the mass spectrometer for molecular weight and limited fragmentation. This allows the analysis of the quantitative aspects of modification to be examined. Middle-down analysis first involves breaking the antibody into light and heavy chains using reduction or top and bottom using specific enzymes. The resulting pieces weighing typically 25–50 kDa are easier to fragment with high coverage. When the analysis is done by liquid chromatography, it is possible to separate and estimate the number of isoforms present. This analysis requires a high-resolution mass spectrometer such as the Thermo Scientific™ Q-Exactive™ Exploris 480, or Thermo Scientific ™ Orbitrap Fusion™ Lumos Eclipse. This protocol describes a short liquid chromatography mass spectrometry (LC-MS) separation of intact proteins and mAbs.

Materials required

- Formic Acid (FA), LC-MS grade
- Acetonitrile (ACN), LC-MS grade
- Water, LC-MS grade
- Thermo Scientific MAbPac RP column, 2.1 × 100 mm, 4 µm (P/N 088647)
- Thermo Scientific™ Pierce™ Intact Protein Standard Mix (P/N A35527)
- NISTmAb Reference Material 8671
- MilliporeSigma SILu™ Lite SigmaMAb Universal Antibody Standard human (P/N MSQC4)
- Thermo Scientific™ ChromaCare™ LC-MS Biologics Flush Solution (P/N MB1241)
- Thermo Scientific™ Vanquish™ UHPLC system
- Orbitrap mass spectrometer

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Protocol

1. Calibrate mass spectrometer according to the manufacturer's directions

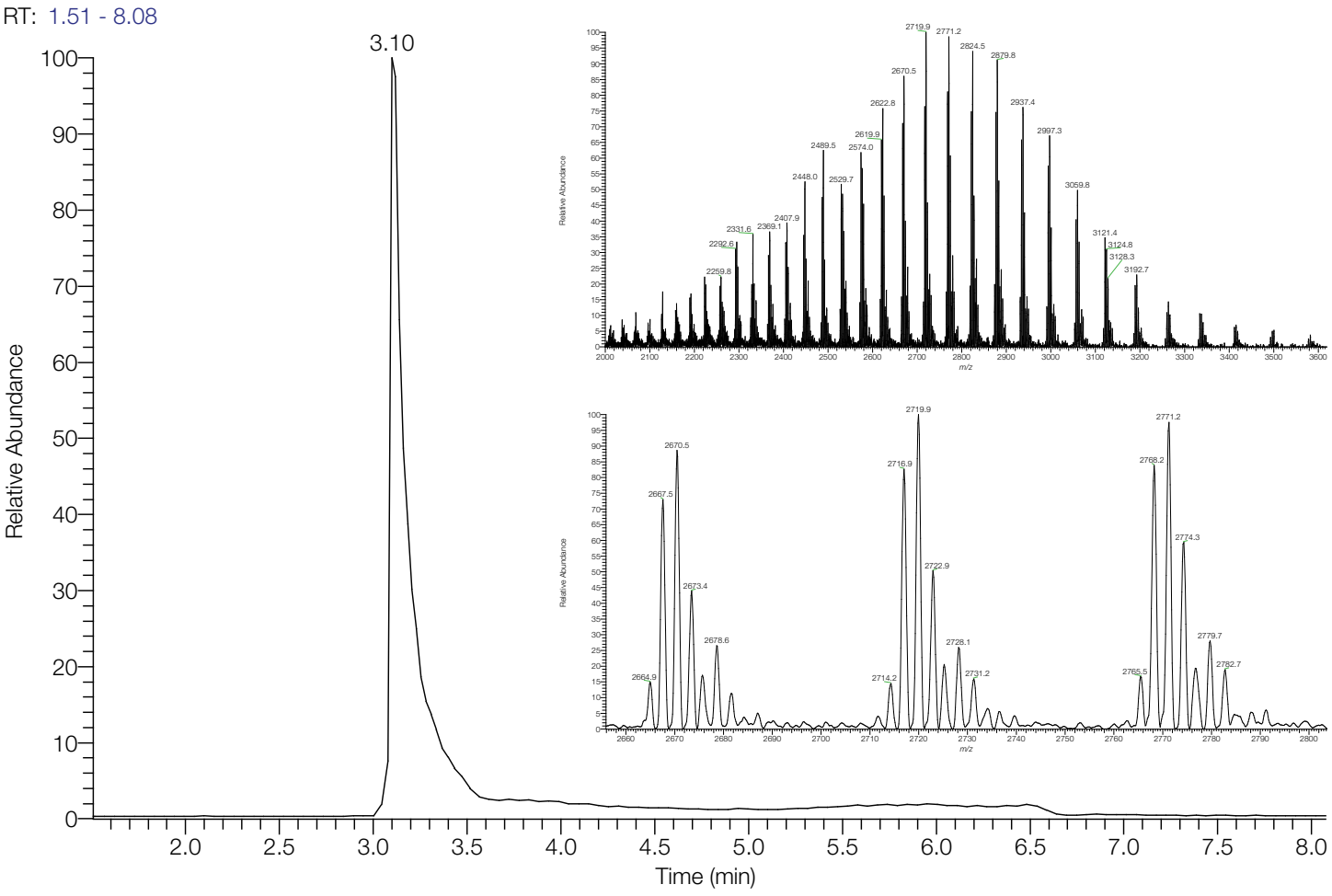
2. Build a tune and instrument method:

   - Set the column temperature to 70 °C (lower temperatures will cause the LC peak to show splitting and tailing)
   - Use the lowest possible resolution setting for full MS to avoid decay of the transient signal in the Orbitrap. Set the scan range from 800–4000 *m/z*
   - Set the microscans to 10 to average transients during data collection prior to Fourier transformation
   - The vaporizer (auxiliary) gas is the most important aspect of getting clean baselines in larger proteins
   - Larger proteins may require the use of in-source CID for complete desolvation if isoforms appear to be adducted
   - Build a short LC gradient at 400 µL/min over 10 minutes from 0.1% FA in water to 0.1% FA in acetonitrile. For mixtures like the Pierce intact standard, the gradient can be changed to start at a higher organic with a shallower gradient for a better separation

3. Load 200 ng to 1 µg of mAb or 50 ng to 200 ng of smaller proteins in 0.1% FA in water. It is suggested to start with either the Pierce mix, NISTmAb, or MilliporeSigma standard which has been proven to work

4. Wash with ChromaCare LC-MS Biologics Flush Solution between injections

5. Deconvolute the averaged spectra using Thermo Scientific™ Biopharma Finder™ software



Representative data of a MAbPac column with peptide standard, showing a good LC peak, a clean full-scan spectrum, and clear glycoforms when the full-scan is zoomed in.

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Related products

Description	Part number
Thermo Scientific™ Vanquish™ Horizon UHPLC System	IQLAAAGABHFAPUMZZZ

Current versions of product instructions are available at
thermofisher.com/chromexpert

Learn more about Bio LC columns and products at
thermofisher.com/biolc

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