

# Identification of Glycosylated Peptides from Data Dependent Neutral Loss Scans Using a Linear Ion Trap Mass Spectrometer

Gargi Choudhary, Jae Schwartz, and Diane Cho, Thermo Fisher Scientific, San Jose, CA  
Edited by: Wendy Schaeffer, Thermo Fisher Scientific, San Jose, CA

## Introduction

Glycosylation is an important post-translational modification associated with many proteins that have a regulatory function. Several LC-MS/MS approaches have been used for the analysis and structural elucidation of glycoproteins. Most commonly, a glycoprotein is enzymatically digested, resulting in fragments that are fractionated by reversed phase-LC. The peptide fractions may be analyzed by either on-line MS/MS, or collected and analyzed off-line by MALDI. Peptides that do not correspond to predicted masses may represent glycosylated forms. These peptides are subsequently treated with a glycosidase to cleave off the oligosaccharide. The difference in the mass following a cleavage is used to infer the carbohydrate constituents. Generally, this method does not allow determination of the oligosaccharide structure or of the exact site of its attachment to the peptide. Glycoproteins can be challenging to analyze because they are generally present in low concentration in cells. In addition, glycopeptides are often hydrophilic and do not bind well to reversed-phase columns commonly used in peptide analysis, making determination of the position of elution difficult. This report describes a method for using a Data Dependent Neutral Loss experiment and the high sensitivity MS<sup>n</sup> capabilities of the Thermo Scientific LTQ linear ion trap mass spectrometer to characterize glycoproteins.

## Goal

To develop a selective and sensitive LC-MS/MS method for unambiguous identification and characterization of glycoproteins.

## Experimental Conditions

### Sample Prep

Ribonuclease B was reduced with dithiothreitol (DTT), alkylated with iodoacetic acid, and then enzymatically modified.

### HPLC

The digested protein was separated on a 100 × 0.15 mm column packed with 5 μm C-18 stationary phase. The Thermo Scientific Surveyor™ MS Pump was used with the following gradient conditions:

Solvent A: water/0.1% formic acid

Solvent B: acetonitrile/0.1% formic acid

Gradient: 5% to 60% B in 20 min.

60% to 80% B in 2 min.

80% B for 5 min.

### Mass Spectrometry

Eluted peptides were analyzed on an LTQ linear ion trap mass spectrometer, equipped with a NanoSpray ion source, operated at 1.7 kV spray voltage and 150°C

heated capillary temperature. A Data Dependent Neutral Loss experiment was performed with a Normalized Collision Energy™ of 25% and a neutral loss mass width of 0.2 u. The Data Dependent acquisition parameters are shown in Figure 1.

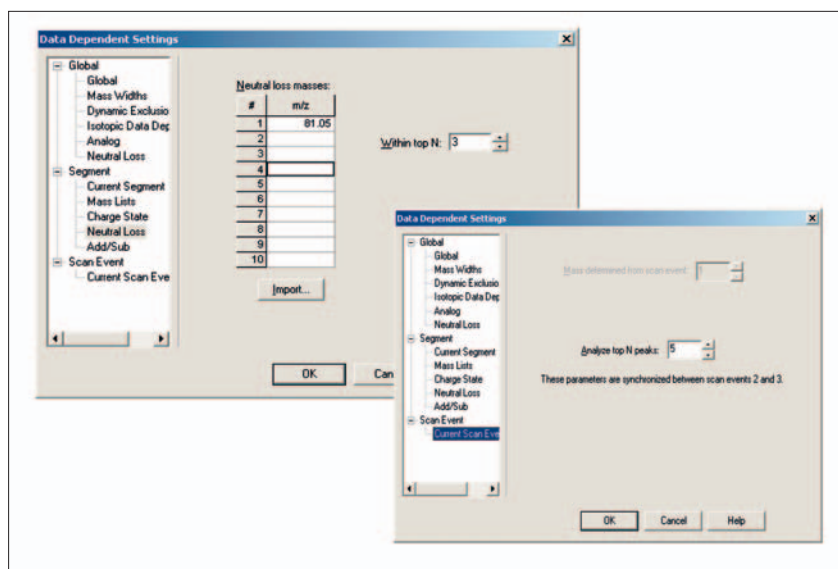


Figure 1: Data Dependent parameters for analysis of enzymatically modified ribonuclease B

## Key Words

- LTQ™
- Data Dependent™
- Glycosylation
- MS<sup>n</sup> Sensitivity
- Neutral Loss

## Results and Discussion

When glycopeptides are fragmented in tandem mass spectrometry, they typically exhibit neutral losses corresponding to the mass of a monosaccharide moiety. Table 1 shows common monosaccharide components of glycoproteins and their corresponding neutral loss masses. High mannose glycoproteins such as ribonuclease B show losses of 162.1 u for a singly-charged ion, or 81.05 u for a doubly-charged ion. Therefore, data-dependent settings were chosen to trigger an MS<sup>3</sup> scan when a peptide showing a neutral loss of 81.05 u is detected.

<i>Sugar</i>	<i>+1</i>	<i>+2</i>	<i>+3</i>
Mannose (hexose)	162.1	81.05	54.0
HexNAc	203.2	101.6	67.7
NANA	291.3	145.6	97.1
HexNAc+Hex	365.3	182.6	121.8

Table 1: Neutral loss markers for glycopeptides

Figure 2 shows a flow chart of scan events in the Data Dependent Neutral Loss experiment. First, an MS survey scan is taken. The five most intense peaks from the survey scan are chosen for MS/MS scans. If an MS/MS scan detects a neutral loss of 81.05 u, and this ion is among the three most intense peaks, an MS<sup>3</sup> scan is triggered. When the MS<sup>3</sup> scans are complete, or if no neutral loss is detected, the process begins again with another MS survey scan.

Figure 3 shows the base peak chromatogram generated by LC-MS/MS analysis of the ribonuclease B digest using the LTQ mass spectrometer. The position of elution of the glycopeptides was determined from the MS<sup>3</sup> scans. A total of 19 MS<sup>3</sup> scans were acquired, of which 13 corresponded to the observed glycoforms of ribonuclease B. This illustrates the specificity of the method. All five known glycoforms of ribonuclease B were successfully identified.

Figures 4 through 8 show representative MS/MS and MS<sup>3</sup> spectra for each of the five glycoforms of ribonuclease B. All spectra shown are single spectra, illustrating the ultra-high sensitivity of the LTQ mass spectrometer.

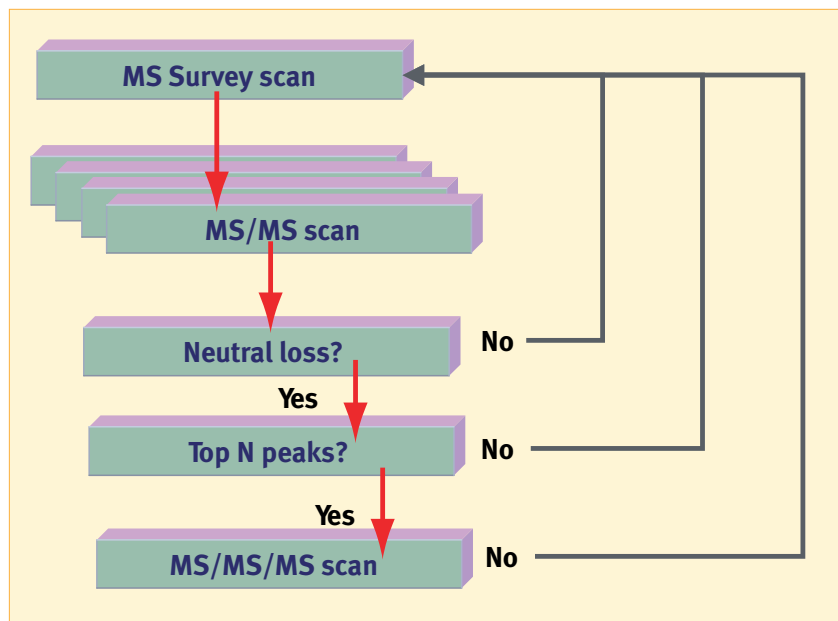


Figure 2: Flow chart of scan events in the Data Dependent Neutral Loss experiment

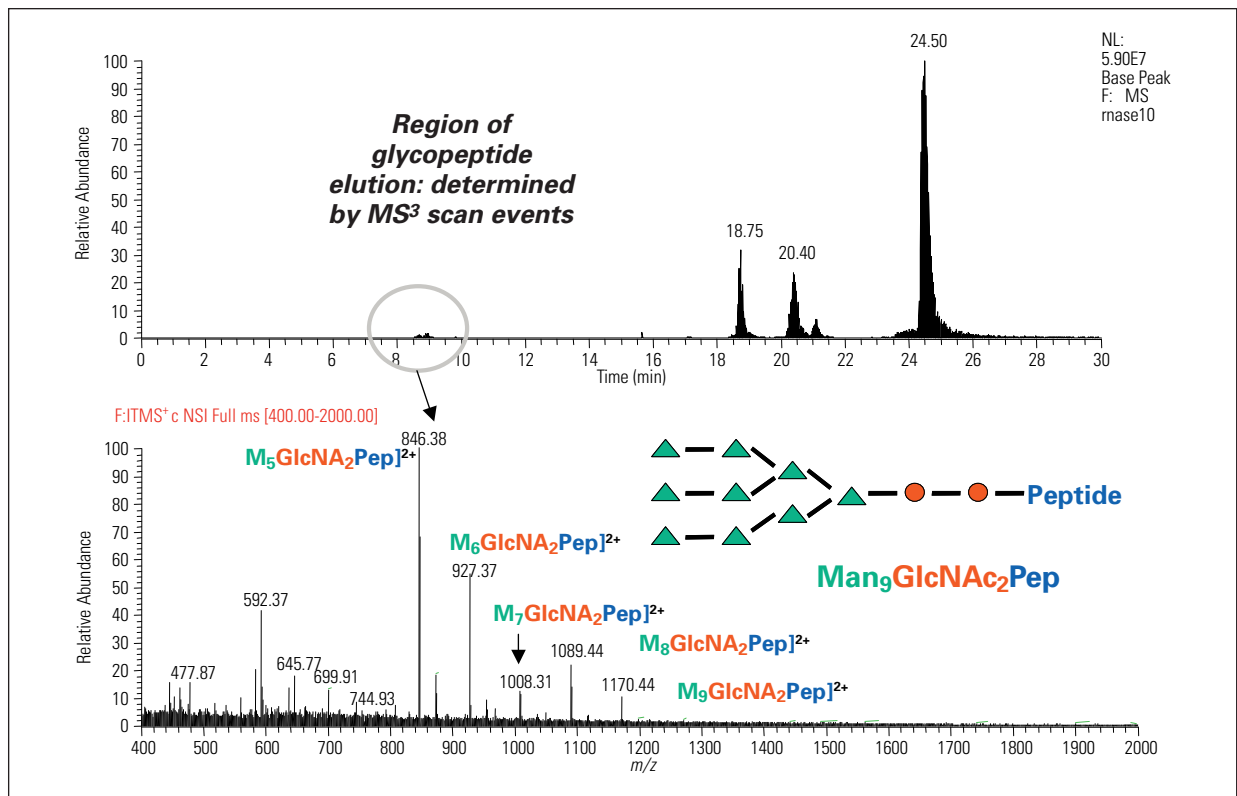


Figure 3: Base peak chromatogram generated by LC-MS/MS analysis of enzymatically modified ribonuclease B

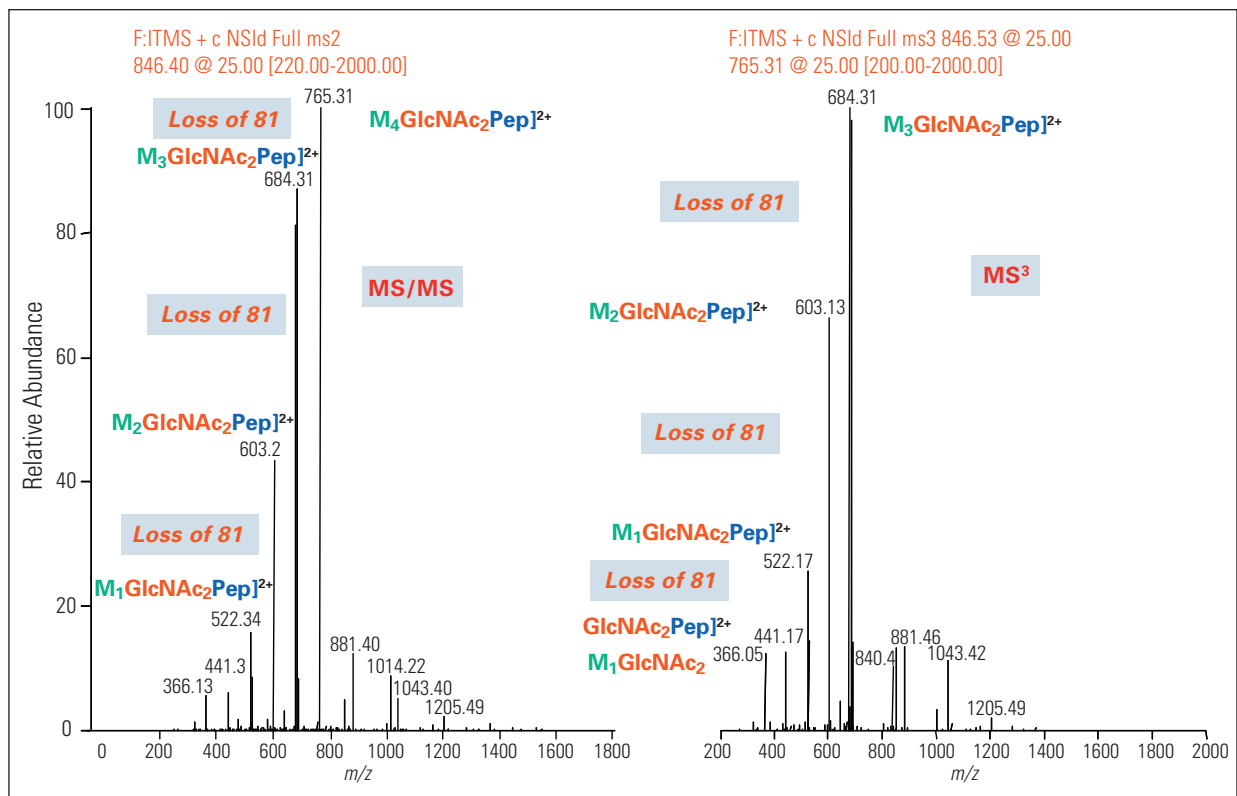


Figure 4: Single scan MS/MS and MS<sup>3</sup> spectra of the five mannose glycoform of ribonuclease B

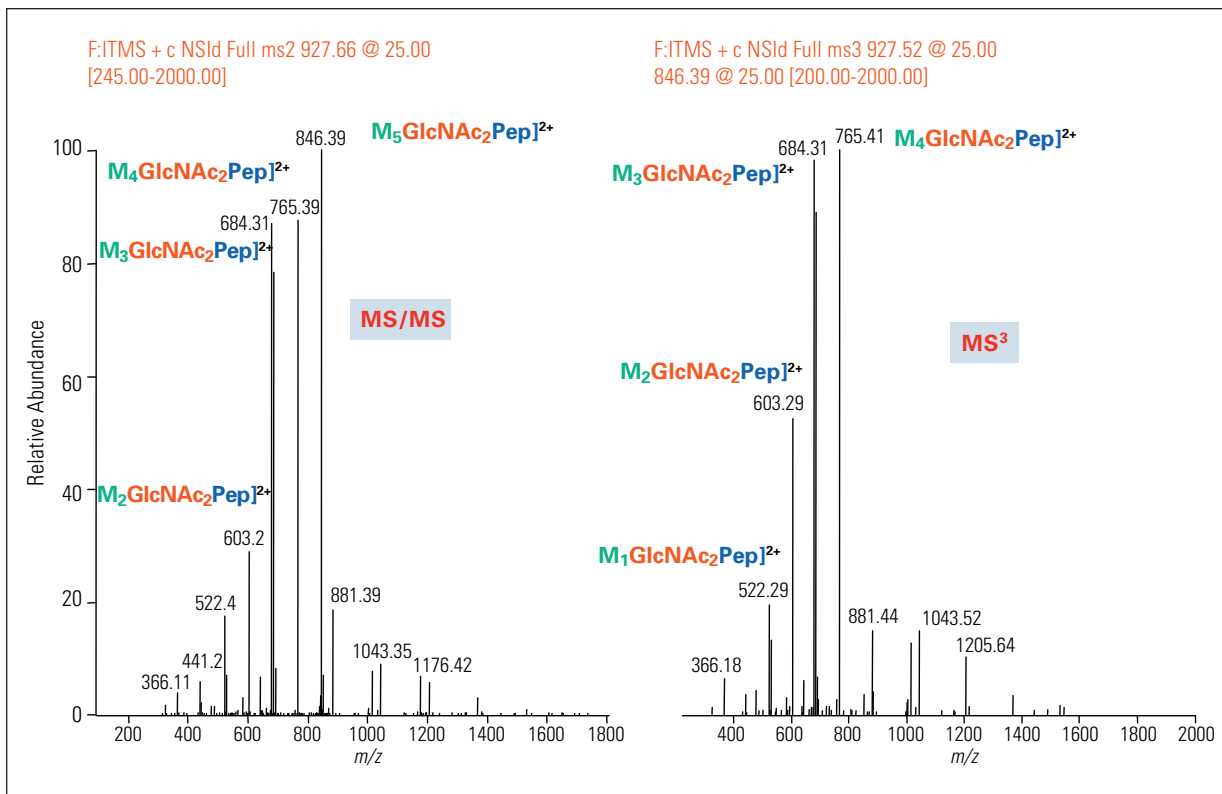


Figure 5: Single scan MS/MS and MS<sup>3</sup> spectra of the six mannose glycoform of ribonuclease B

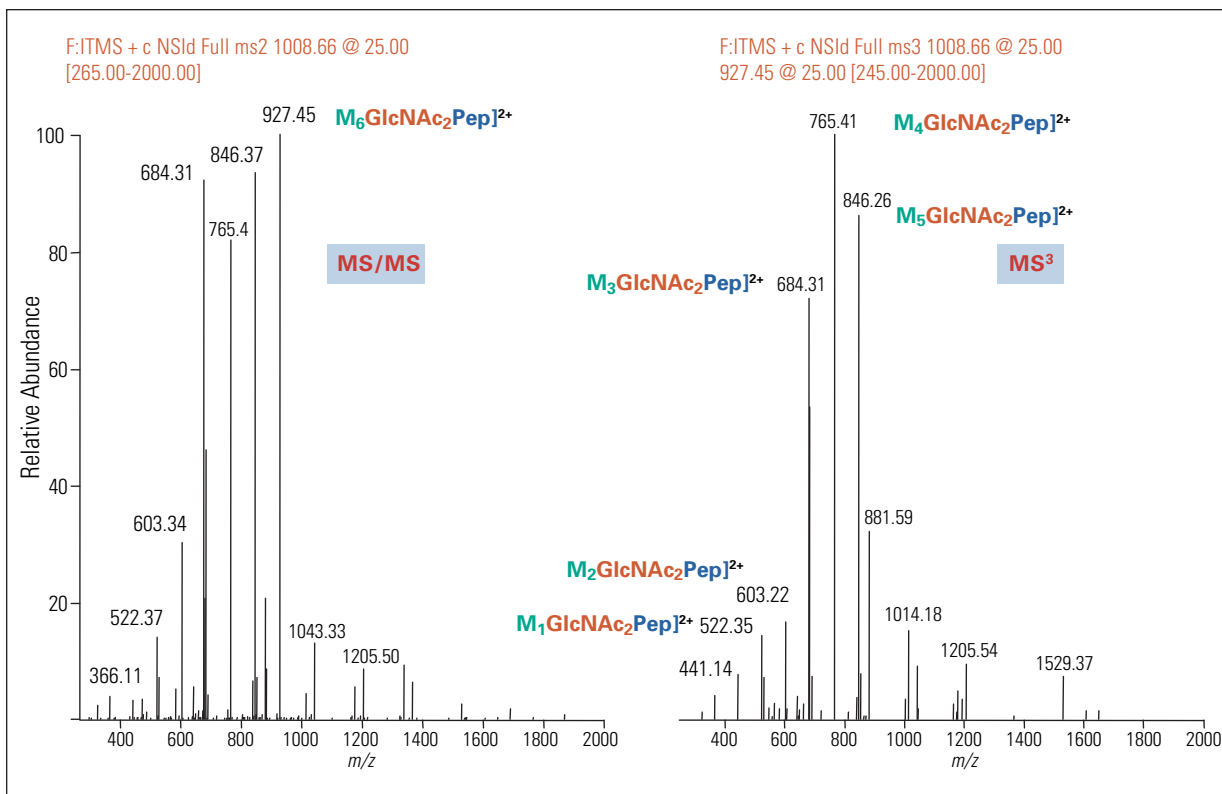


Figure 6: Single scan MS/MS and MS<sup>3</sup> spectra of the seven mannose glycoform of ribonuclease B

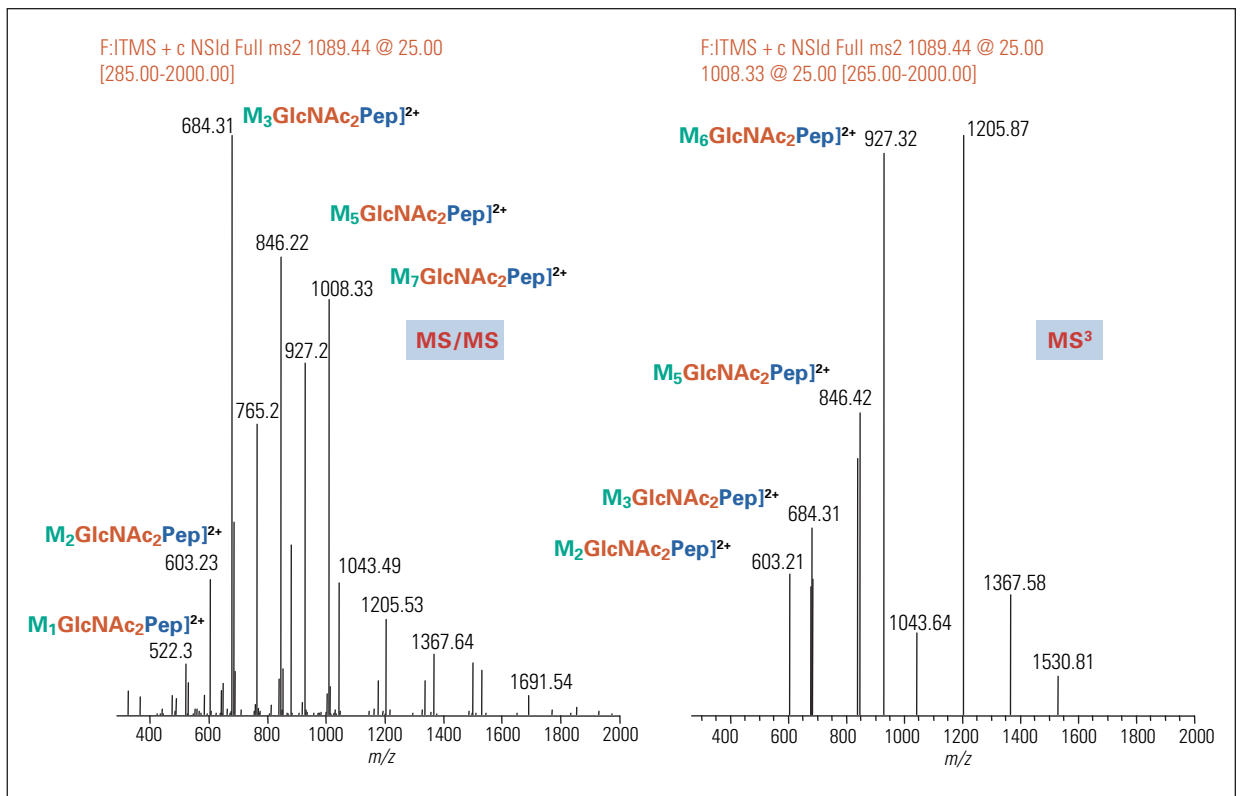


Figure 7: Single scan MS/MS and MS<sup>3</sup> spectra of the eight mannose glycoform of ribonuclease B

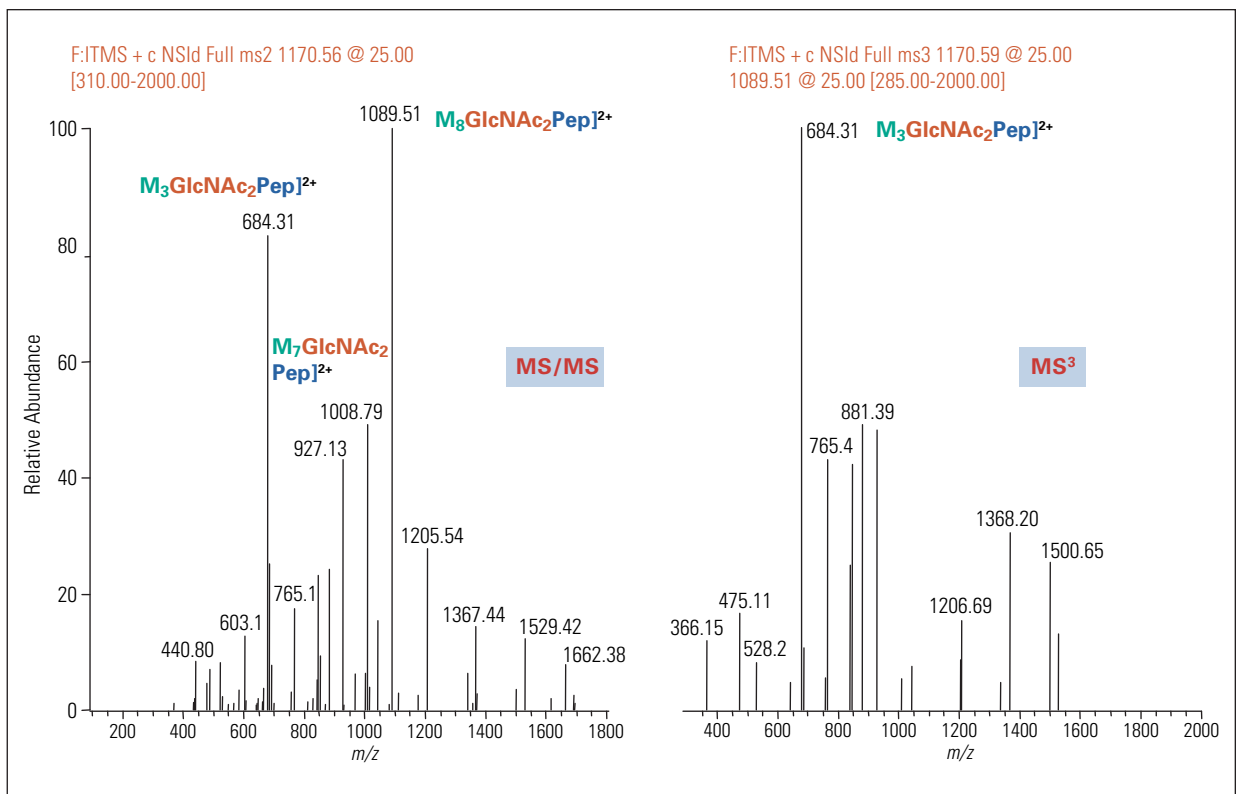


Figure 8: Single scan MS/MS and MS<sup>3</sup> spectra of the nine mannose glycoform of ribonuclease B

## Conclusion

These results demonstrate that the ultra-high sensitivity and high spectral quality offered by the LTQ mass spectrometer make it ideal for the analysis of enzymatically modified glycoproteins. The Data Dependent Neutral Loss experiment presented here enables determination of the position of elution, whereas the high sensitivity MS/MS and MS<sup>3</sup> spectra generate information-rich data for structural elucidation of the various glycoforms of ribonuclease B.

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