

Poster Reprint

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# Q-RAI Provides Rapid and Sensitive Data Independent Acquisition for Peptide Quantitation

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Data-independent acquisition (DIA) with high resolution mass spectrometry (HRMS) has grown in popularity for proteomics. Advantages of DIA over traditional data dependent methods are that it is data rich and guarantees fragment information for every ion, even low abundant ions.

Quadrupole Resolved All Ions (Q-RAI) is a high resolution, accurate mass, untargeted data independent MS/MS acquisition mode that collects molecular ion and fragment information for all ionized analytes. The instrument cycle contains a MS1 spectrum of the full acquisition range (i.e.  $m/z$  300-1489) followed by several broad band isolation windows encompassing the whole acquisition range that have collision energy activated (i.e.  $m/z$  300 – 400, CE 20). Fragment spectra are associated only with a narrow band of molecular ions. Thus, Q-RAI allows the simplification of the fragment spectra without the loss of data that can occur with a targeted methodology. Q-RAI allows MS/MS spectra for every ion with high resolution and isotopic fidelity for confident identifications.

Post-translational modifications in protein therapeutics are among those critical quality attributes (CQA) that require sensitive and selective analytical methods. Defining these attributes is challenging and consistency of product quality becomes extremely important. We use Q-RAI for monitoring and quantifying deamidation and oxidation in NIST mAb.

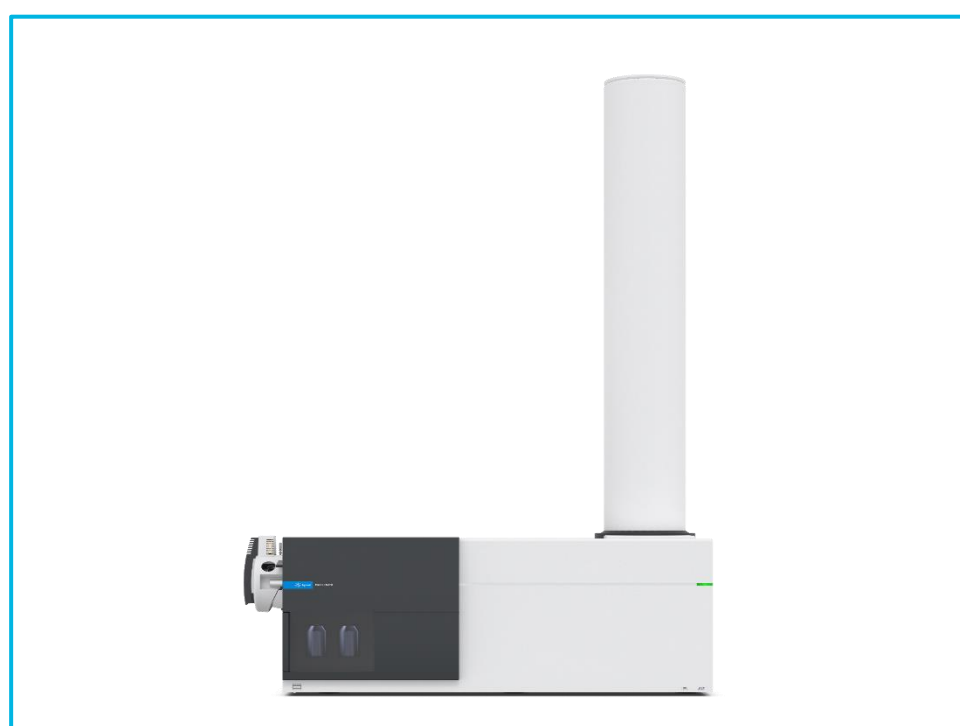


Figure 1. 6546 LC/Q-TOF utilized in the experiment. This system has high resolution and extended dynamic range which is ideal for DIA experiments.

### Sample Preparation

Proteins were reduced, alkylated, and incubated with trypsin overnight (37°C). For forced oxidation, prior to digestion, proteins were incubated with 0, 0.02, 0.05, 0.1, 0.5, and 1% H<sub>2</sub>O<sub>2</sub> overnight. For forced deamidation, proteins were incubated at pH 9 for 0, 1, 2, and 14 days, prior to digestion.

### LC/Q-TOF Conditions

Peptides were separated on a 2.1 x 150 mm AdvanceBio Peptide Mapping column over a 25 minute gradient. Spectral libraries were built from data collected in data dependent mode using iterative MS/MS acquisition with an LC/Q-TOF. Data independent acquisition was performed using Q-RAI mode. Twelve Q-RAI MS windows were used to span a mass range from  $m/z$  300-1489– this was sufficient for selectivity and sensitivity. MS acquisition rate of 8Hz was used and a MS/MS rate of 14 Hz for the Q-RAI windows. These were optimized for optimal sensitivity and to have enough points across the chromatographic peak. Spectral libraries were processed in Spectrum Mill and the overall data analysis was processed with Skyline.

Start $m/z$	End $m/z$	Window Width (amu)	Collision Energy (CE)
300	400	100	20
399	499	100	20
498	598	100	20
597	697	100	20
696	796	100	20
795	895	100	20
894	994	100	20
993	1093	100	25
1092	1192	100	30
1191	1291	100	40
1290	1390	100	40
1389	1489	100	45

Table 1. The twelve windows used in Q-RAI data acquisition. Window sizes and collision energies were optimized. Q-RAI requires a 1 amu overlap between windows. The CE is user defined and was optimized to the peptide size for this experiment. 100 amu window size was optimized for this method, smaller windows are possible but did not enhance the spectra significantly.

### Quality Control of Results

As Q-RAI has never been used for peptide analysis before, it was important to ensure that Q-RAI yielded high quality data. Observing exceptionally low mass error for both MS1 and MS/MS and low *p* and *q*- values from mProphet in Skyline shows that Q-RAI produces excellent data quality for larger ions (Figure 2 and 3).

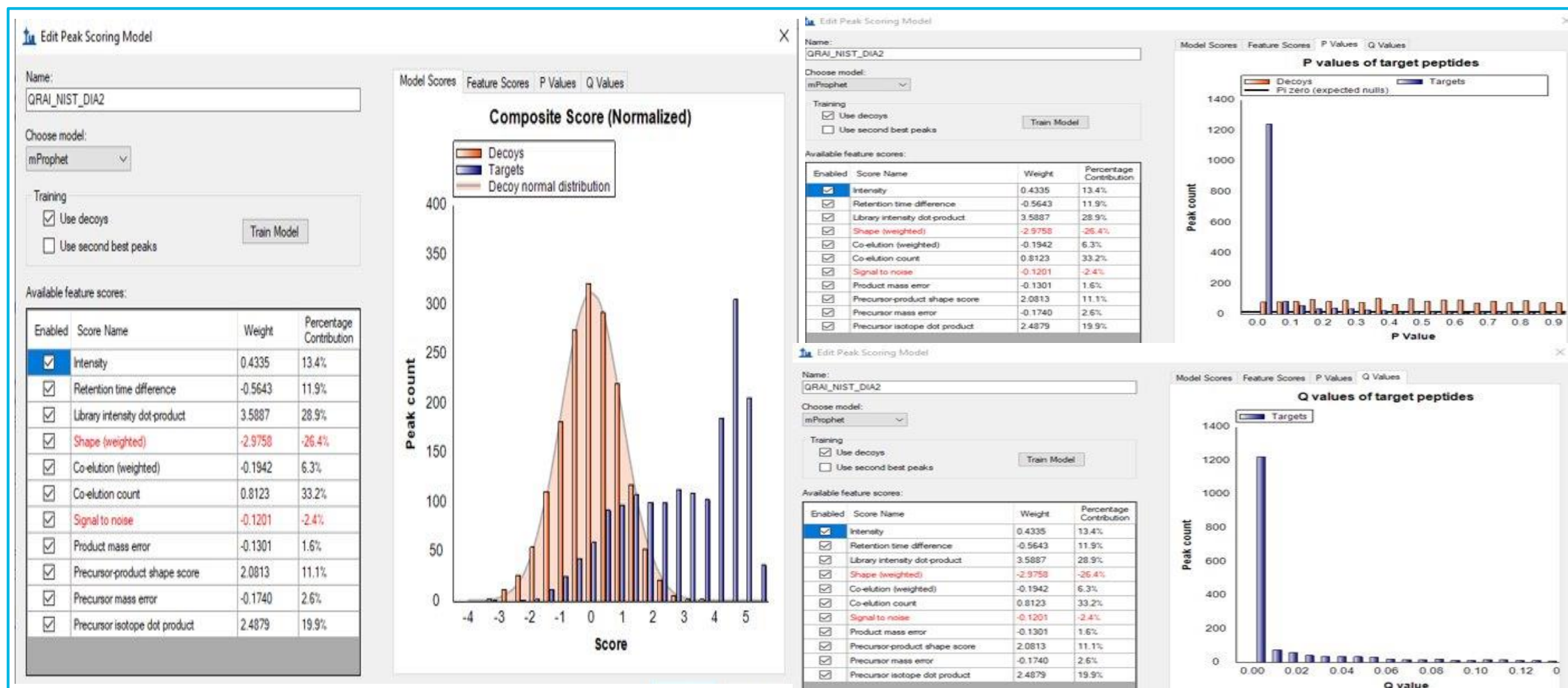


Figure 2. mProphet peak scoring models indicate very low *p* and *q* values for target peptides. These low scores indicate high data quality. The composite score combines co-elution scores, mass error, shape score, intensities, the dot products of the library intensities, and dot products of the Q-RAI data.

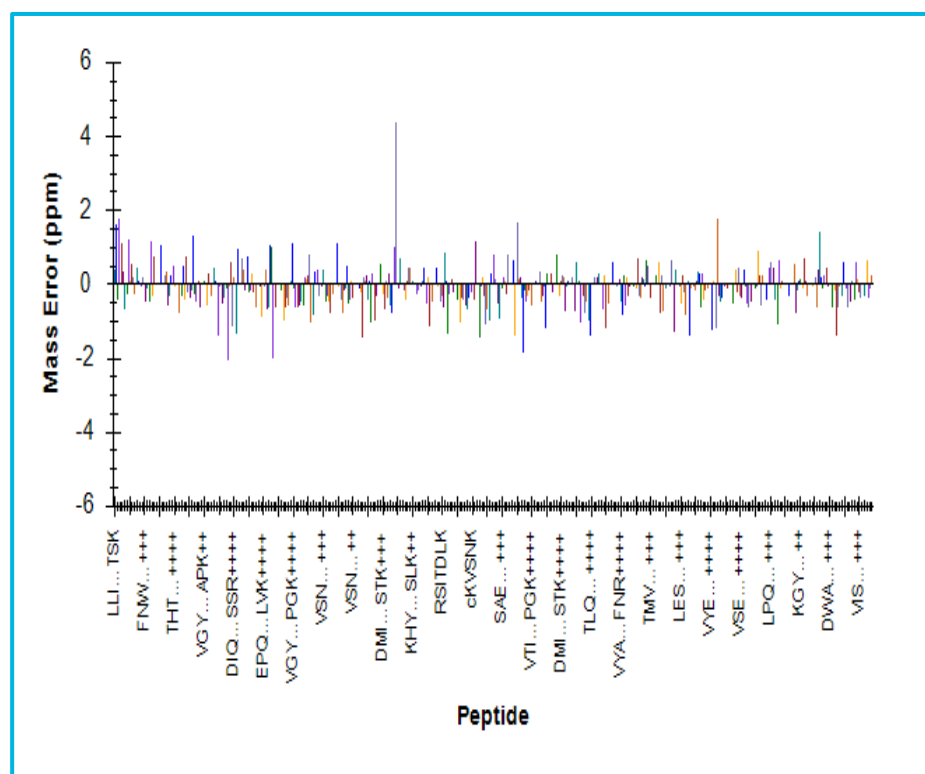


Figure 3. A quality control check determining the mass error of peptides in the heavy chain. Masses include MS/MS fragment ions which do not have internal reference masses in the same spectrum. Thermally stable electronics and flight tube of the 6546 LC/Q-TOF lend to the mass accuracy stability seen in this data.

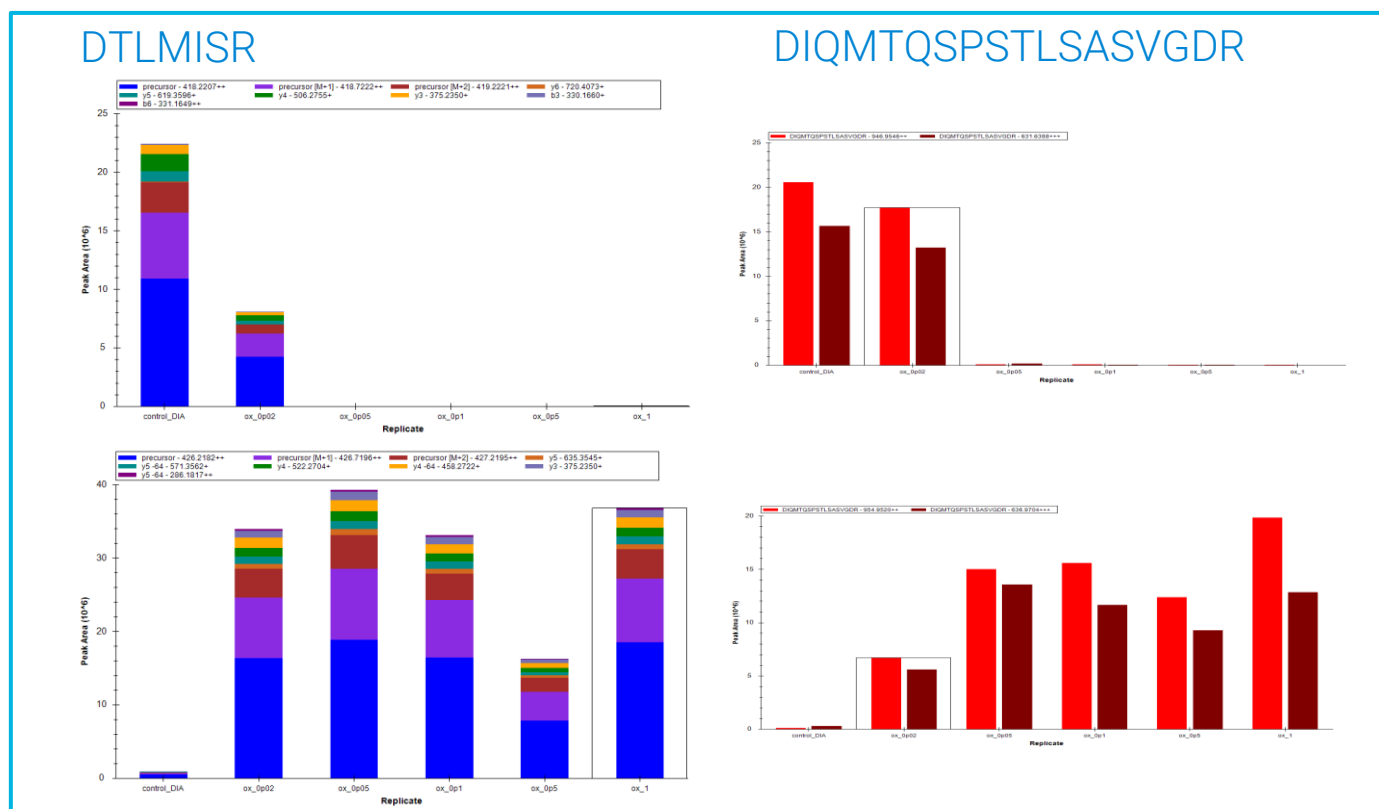


Figure 4. (left) Comparison of the unmodified and oxidized peptide with precursors and product ions. The fragments detected with Q-RAI are proportional in each experiment. Upon further inspection of the 0.05% oxidation data, all peptides were detected at a lower abundance (right) Intensities of all fragments for the 2+ and 3+ charge states which were in different Q-RAI windows but had consistent data between oxidation levels.



## Monitoring Oxidation of Peptides

Oxidation and deamidation are important CQA for protein therapeutics. High oxidation and/or deamidation indicates instability and would render the protein unsuitable. Because oxidation and deamidation levels tend to be low, accurately quantifying the amounts of each with a sensitive method like Q-RAI is very valuable. In addition, the Q-RAI method can provide a peptide map. A peptide map showing high (>95%) or complete sequence coverage is an additional component required to demonstrate viability of the protein therapeutic.

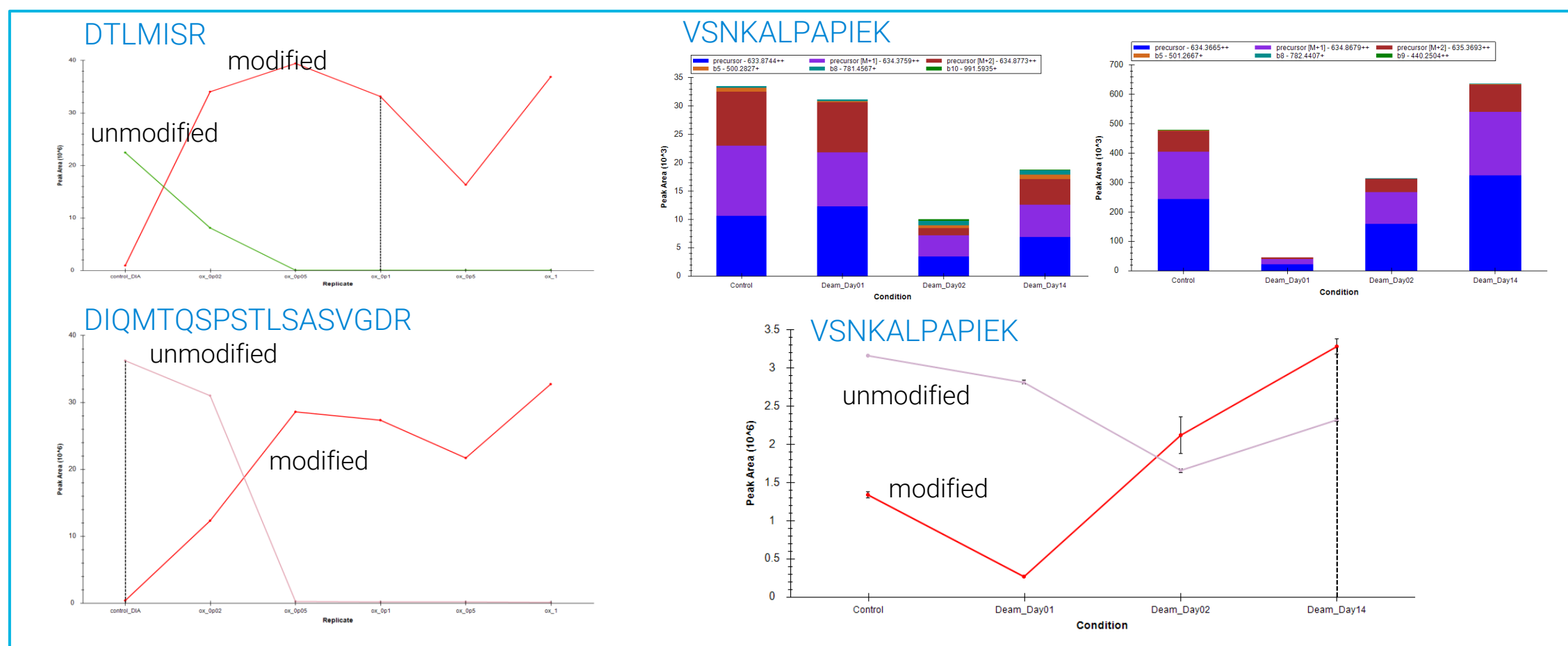


Figure 5. Trends for various peptide modification. Progression and relative quantitation of methionine oxidation (left, top and bottom) and deamidation for VSNKALPAIEK (right, bottom) peptide. Trends fit the expected pattern and were detectable with Q-RAI. Fragments (top, right) were detected and showed good identification.

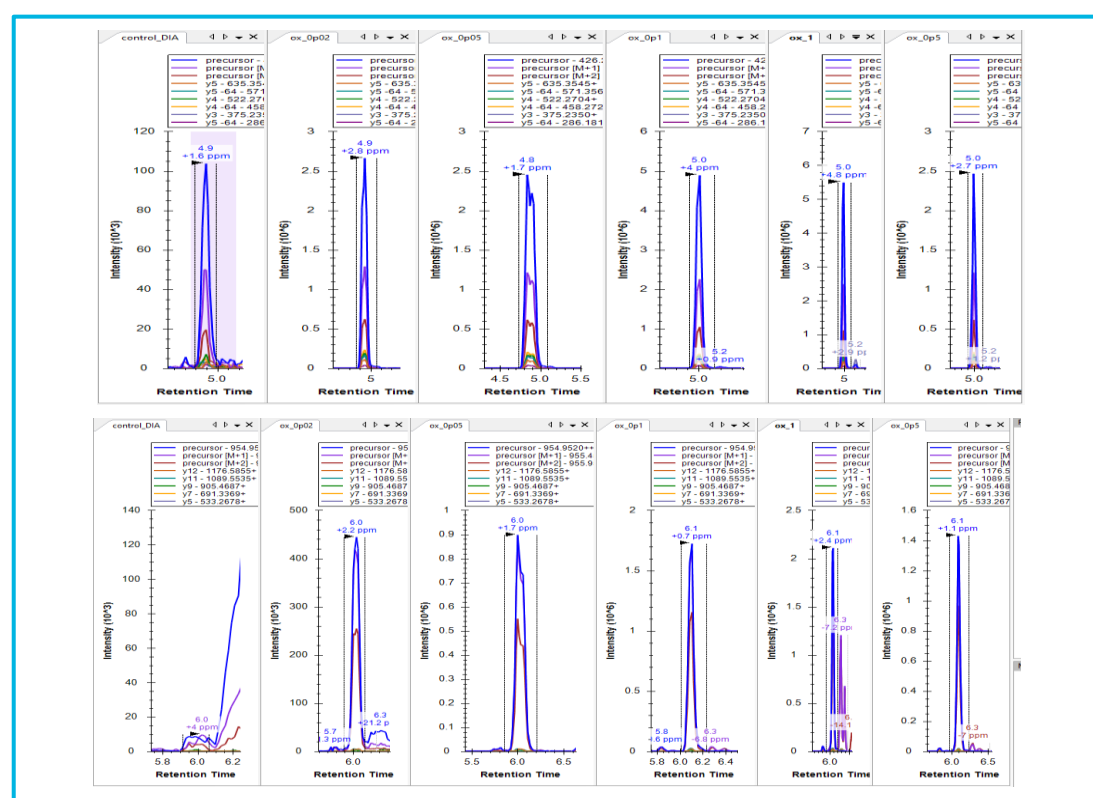


Figure 6. Chromatograms from Skyline showing the progression of the two oxidized peptides. The low amounts of oxidation in the control runs showcase the spectral fidelity and dynamic range of the 6546 LC/Q-TOF. Regardless of intensity the mass error was consistently sub 5 ppm and an identification could be made.

## Conclusions

- Oxidation and deamidation were quantified using Q-RAI, a novel DIA mode which has the sensitivity and selectivity for analyzing CQAs in therapeutics.
- High quality data was obtained with Q-RAI, as shown by the mProphet data and high mass accuracy of precursor and fragment ions.
- Fragment data was proportional in each experiment, demonstrating the reliability and ruggedness of the instrument and method.

## References

- <sup>1</sup>Pino, L.K. et al. *Mass Spectrom. Rev.* 2020. 39(3), 229-244.
- <sup>2</sup>Doerr, A. *Nat. Meth.* 2015. 12, 35.
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