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Peptide Mapping Using Intelligent Data Capture on Vion IMS QTof

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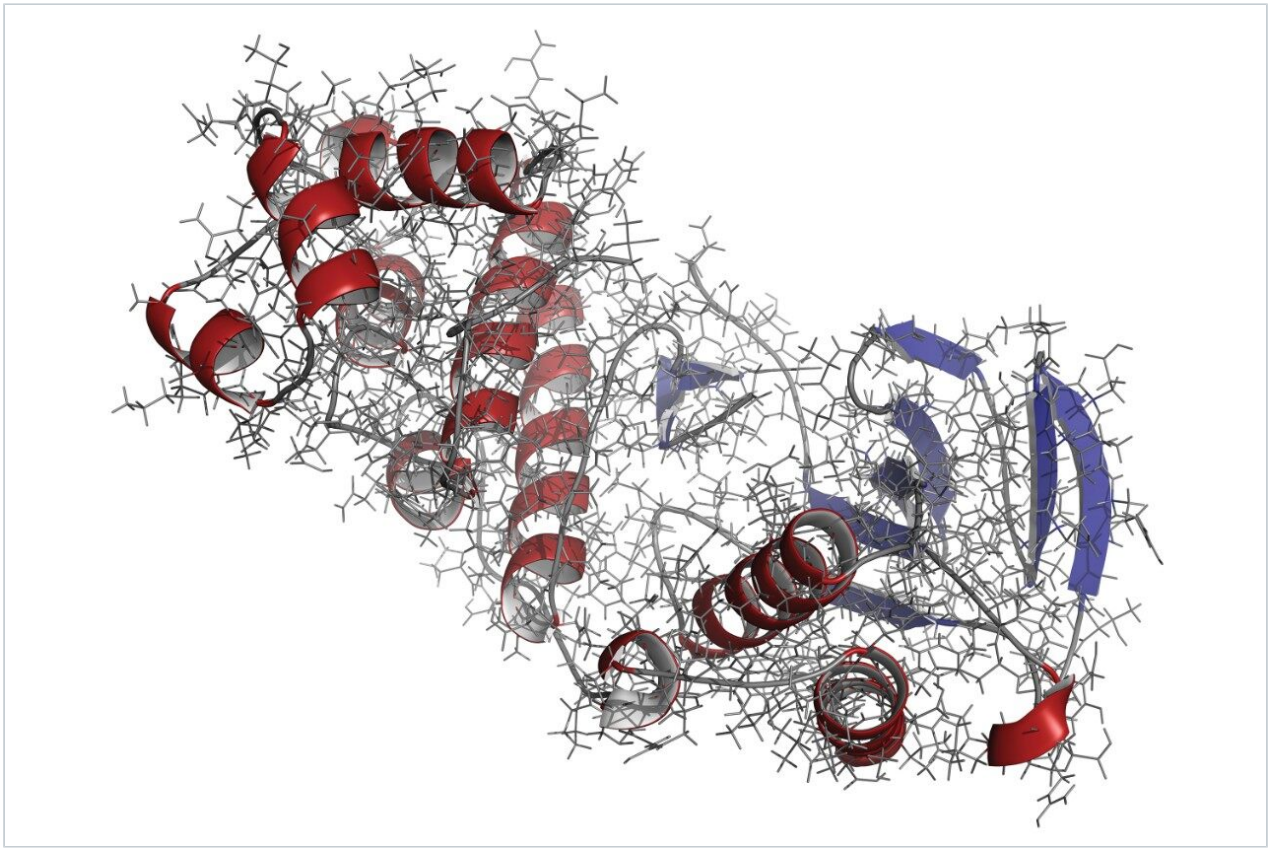


This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This application brief demonstrates the impact of enabling Intelligent Data Capture (IDC) for noise and concomitant data file size reductions in LC-MS peptide mapping analysis.

Benefits

Real-time file size and noise reduction to speed up data processing and provide high quality peptide mapping results.

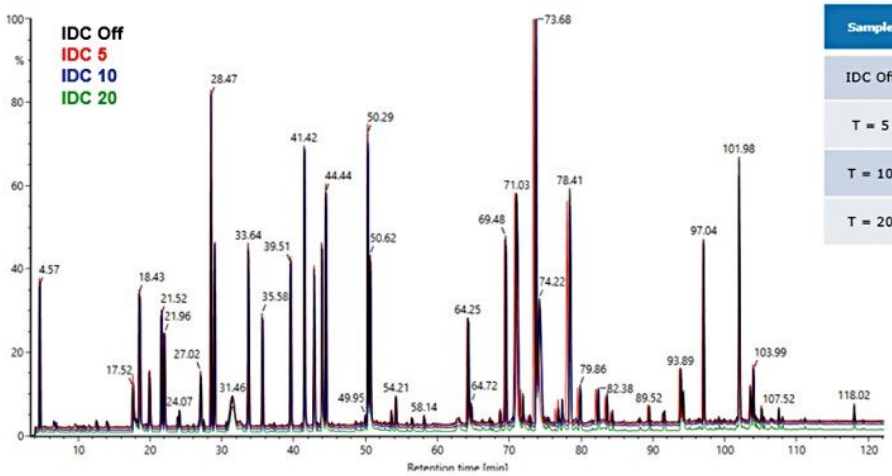
Introduction

LC-MS has become an essential technique in the analysis of biomolecules because it provides very detailed and comprehensive data to support characterization. Peptide mapping for LC-MS analyses typically require long LC gradient methods using full MS scan, which generate large files that can quickly fill up PC hard drives. In addition, unwanted chemical and electronic background noise can unnecessarily increase the file size and prolong data processing time, as the peak picking algorithm needs to distinguish real peaks from background signals. Waters has introduced a real-time data reduction algorithm called Intelligent Data Capture (IDC) that can automatically reduce file size without compromising the data quality.[1] In this study, we show the value in enabling IDC on the Vion IMS QTof Mass Spectrometer for peptide mapping using the LC-MS^E acquisition method (data-independent acquisition with alternate low and high collisional energy scans).

Results and Discussion

To assess the impact and potential benefits of IDC, the mAb Tryptic Digest Standard (p/n: 186009126) was used as a model for a peptide mapping experiment. This reduced, alkylated, tryptic digest of the NIST mAb was injected in triplicate in a typical reversed-phase LC-MS/MS experiment (LC and MS conditions) either with IDC disabled or enabled with a setting of either 5, 10, or 20 counts. Figure 1 shows an overlay of the total ion chromatogram (TIC) for each of these four conditions. All profiles are comparable, only that the overall

baseline is lowered as the IDC setting increases. This is due to the reduction in data originating from noise. Figure 1 also shows the file sizes and percent reduction for each of these conditions. By enabling IDC with a setting of only five counts, we observe almost 50% reduction in data file size.



Sample	Total File Size (Mb)	% File Size Reduction	Processing Time
IDC Off	2,325.8	(0%)	4 min, 30 sec
T = 5	1,491.5	-46%	3 min, 45 sec
T = 10	956.3	-59%	3 min, 0 sec
T = 20	301.1	-87%	2 min, 10 sec

Figure 1. TIC overlay of mAb Tryptic Digest Standard injections with IDC off and IDC enabled with thresholds of 5, 10, and 20. The associated table shows total file size, percent reduction, and data processing time with UNIFI Scientific Information System.

Of equal importance, we need to confirm that none of the real sample data is lost due to the use of IDC. Comparison of the final, processed, sequence coverages for NIST mAb light chains and heavy chains remain the same for all four conditions (92% and 90%, respectively). Raw data quality and peak assignment were also evaluated for each condition for peptides of both high and low abundance. Figure 2A shows the MS spectra for a low-abundance, oxidized peptide from the light chain (DIQMTQSPSTLSASVGDR), roughly 2% relative to the unmodified peptide. Figure 2B shows a zoom of the 2+ charge species for this ion at *m/z* 636.97. Figure 3 highlights the MS^E fragmentation data for this oxidized species. Notice in the y6 ion (Figure 3B), the background noise is reduced as the IDC threshold is increased. From this MS^E data, a positive identification for this low abundant peptide is possible with all conditions tested.

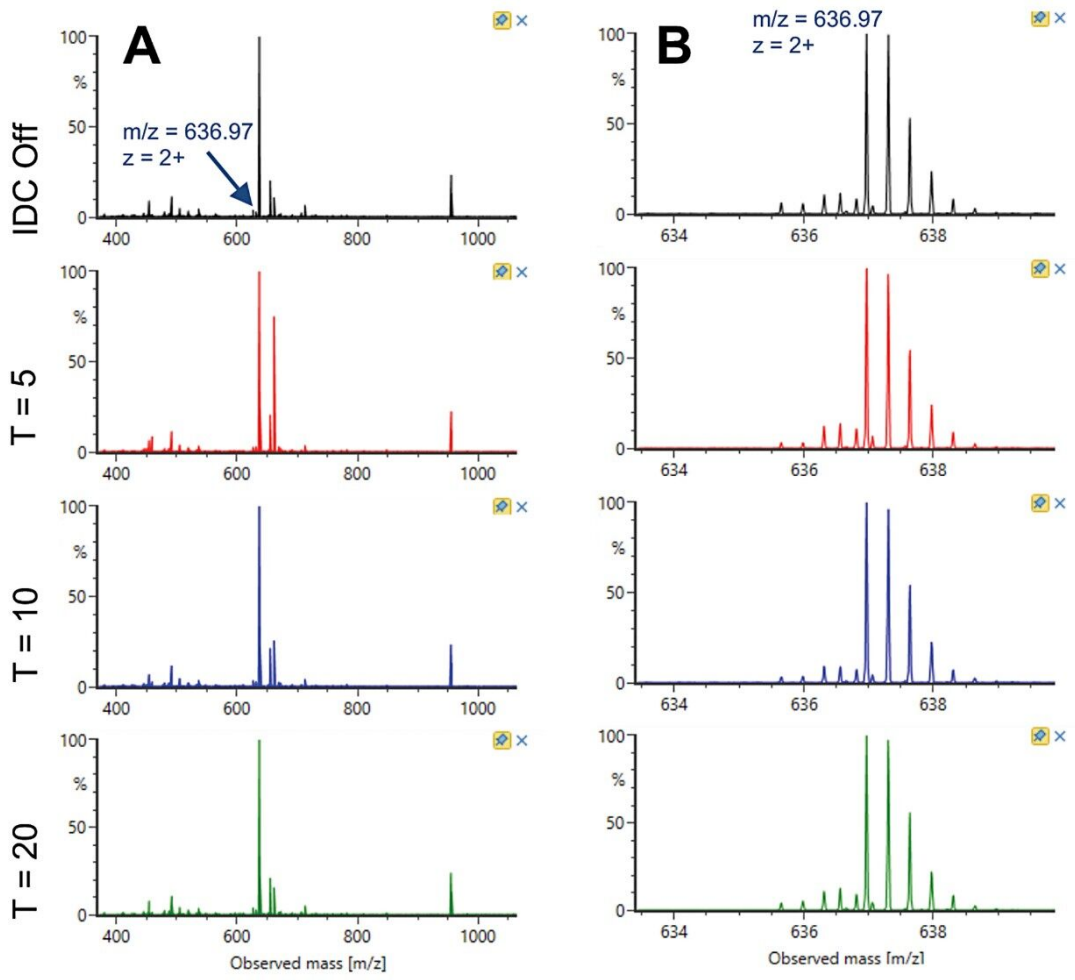


Figure 2. MS spectra for oxidized light chain peptide DIQMTQSPSTLSASVGDR (A) and zoom of 2+ charge state (B) at m/z 636.97.

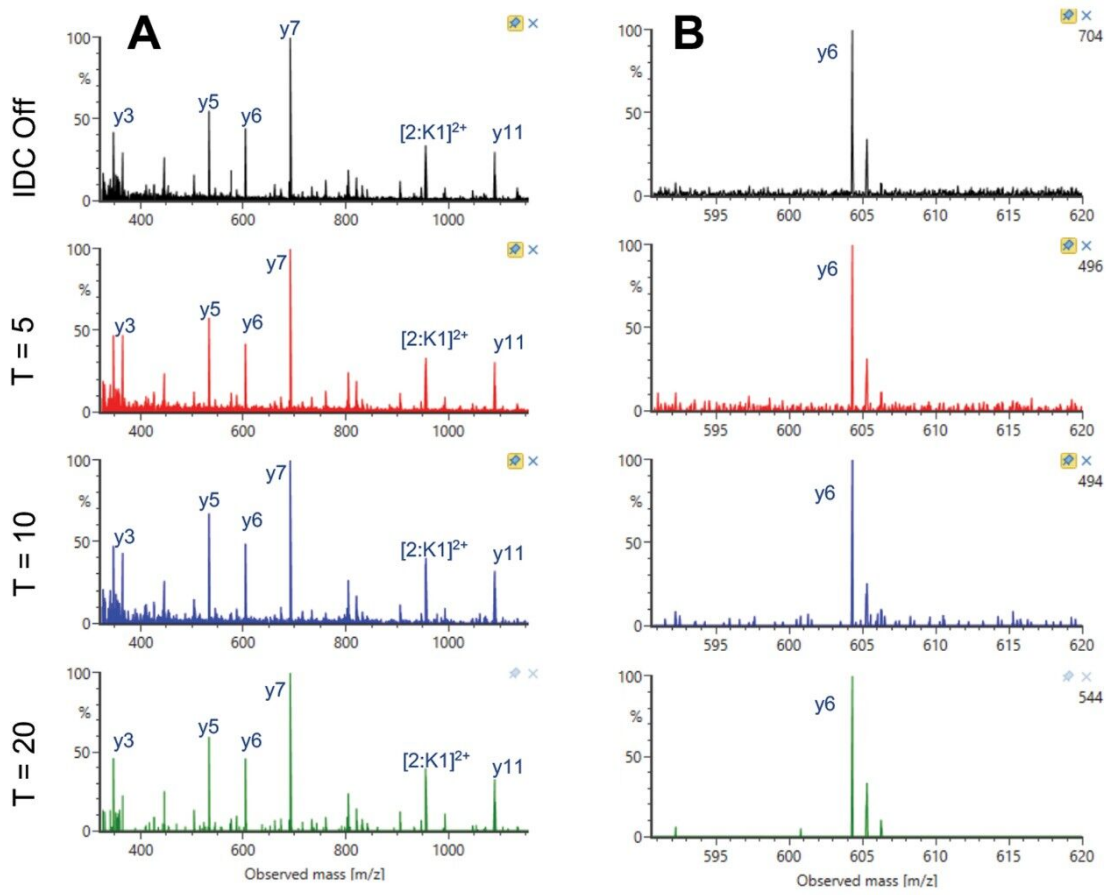


Figure 3. MS^E fragmentation for oxidized light chain peptide DIQMTQSPSTLSASVGDR (A), and focus on y6 ion (B).

Conclusion

Data file size is a very important challenge for analytical scientists, both because of data storage constraints as well as data processing time. In this study, we observe that the application of IDC into peptide mapping workflows significantly reduces data file size and cleans up MS spectra, while retaining the full integrity of the results. This will release some of the burden of processing large complex data files, allowing scientists to spend precious time and energy on moving projects forward.

References

1. Mortishire-Smith, R.; Richardson, K.; Denny, R.; Hughes, C. Intelligent Data Capture: Real-Time Noise Reduction for High Resolution Mass Spectrometry. Waters White Paper, 720006567EN (2019).

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Vion IMS QTof Ion Mobility Quadrupole Time-of-flight Mass Spectrometry <https://www.waters.com/134845751>

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