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Intelligent Data Capture (IDC) Enables Optimal Xevo G2-XS Data Acquisition and Processing for Multi-Attribute Method (MAM) Studies

Nilini Ranbaduge, Ying Qing Yu

Waters Corporation

Abstract

The application note demonstrates the benefits of applying an acquisition-centric data reduction algorithm, intelligent data capture (IDC), on the Xevo G2-XS System for Multi-Attribute Method (MAM) studies. The Xevo G2-XS System (QTof MS), often used in characterization of drug products, can also be applied for routine monitoring of product quality attributes (PQAs) and critical quality attributes (CQAs) at the peptide level. To support the current efforts in developing sensitive and robust Multi-Attribute Methods (MAM), we have shown how IDC can be utilized to improve Peptide MAM data quality without compromising quantitative fidelity.

IDC is enabled on the waters_connect informatics platform via an intuitive IDC software switch, and selection amongst default IDC levels. The results shown here confirm the fitness of IDC-enabled data relative to IDC Off acquisition, revealing the superiority of IDC-enabled data for routine MAM analysis. The IDC algorithm significantly minimized the number of false positives in new peak detection (NPD) while maintaining comparable %modification levels. This significantly reduces the required efforts of analysts to manually validate these new

peaks and enables streamlined operation of MAM workflows in regulated environments. It was found that the default IDC setting of 15 showed the optimal performance for MAM analysis of a stressed NISTmAb sample. This level of data reduction considerably reduced the data file size by over 90% and accelerated data processing four-fold, supporting general efficiency gains for MAM assay deployment and subsequent data management.

Benefits

- Efficient compliant-ready workflow method for peptide MAM analysis
- Improved new peak detection, reducing the need analyst interventions
- Reduced raw data file size and data processing times
- No impacts to attribute quantification results

Introduction

The Multi-Attribute Method (MAM) is an emerging LC-MS based analysis approach for directly measuring the product and critical quality attributes that are essential to maintaining drug molecule's quality profile. MAM has gained popularity due to many qualities such as: increased laboratory efficiency due to multiplexed attribute analysis to support product ID confirmation, monitor targeted attributes and impurities, and to detect new peaks in support of purity analysis. However, translating MAM assays in biopharmaceutical development to assays that can be routinely performed in regulated environments requires a more thorough investigation of LC-MS platform, informatics, and workflows to ensure accuracy and robustness.

The Xevo G2-XS QTof MS System for MAM has many benefits in biopharmaceutical development since the instrument methods can be rolled over from attribute characterization to attribute monitoring without method transfer or re-optimization. Equipped with broader range of MS capabilities for MS/MS ion fragmentation, this system can enable further investigation of any new peaks that are detected and provide confident assignment of the lowest level product attributes.

Maximizing the utility of Peptide MAM App in waters_connect, the intelligent data capture (IDC) noise reduction algorithm that was first released for the BioAccord System has been made available for the Xevo G2-XS System. The IDC functionality enables real time data reduction and minimizes chemical and electronic noise to reduce the

file size and improve data processing quality and speed.[1,2] This application note confirms the benefits of IDC on Xevo G2-XS QTof MAM data in comparison to "IDC Off" data collection. Targeted monitoring of peptide level attributes and new peak detection (NPD) was used to confirm data quality of the noise reduced data and quantify the benefit of IDC on reducing the files sizes and data processing times.

Experimental

Sample Description

System suitability sample: MassPREP peptide mixture (p/n: 186002337 < https://www.waters.com/nextgen/global/shop/standards--reagents/186002337-massprep-peptide-mixture.html >).

Control sample/ negative control: mAb Tryptic digestion Standard (p/n: 186009126 < https://www.waters.com/nextgen/global/shop/standards--reagents/186009126-mab-tryptic-digestion-standard.html>).

Spiked control sample: mAb tryptic digestion standard spiked in with 15 heavy labeled peptides.

Stressed sample: NISTmAb reference material 8671 was incubated at pH 8.0 in 50 mM Tris buffer at 40 °C for 14 days. At the end of 14 days the sample was reduced, alkylated, and tryptic digested. The digest was acidified and diluted in 0.1% formic acid to a final concentration of 0.1 µg/µL before the analysis.

LC Conditions

LC system:	ACQUITY UPLC H-Class PLUS Bio System
Detection:	Tunable UV, ESI+ MS
Vials:	QuanRecovery with MaxPeak HPS Vials (p/n: 186009186)

Column(s):	ACQUITY Premier Peptide CSH C_{18} Column (p/n: 186009489)
Column temperature:	60 °C
Sample temperature:	6 °C
Injection volume:	10 µL
Flow rate:	0.2 mL/min
Mobile phase A:	0.1% Formic acid in water
Mobile phase B:	0.1% Formic acid in acetonitrile

Time (min)	Flow (mL/min)	%A	%B	Curve
0.00	0.200	99.0	1.0	Initial
3.00	0.200	99.0	1.0	6
78.00	0.200	65.0	35.0	6
85.70	0.200	15.0	85.0	6
93.00	0.200	15.0	85.0	6
100.70	0.200	99.0	1.0	6
120.00	0.200	99.0	1.0	6

Mobile phase A: H_2O, 0.1% FA Mobile phase B: Acetonitrile, 0.1% FA

MS Conditions

MS system:	Xevo G2-XS System

Ionization mode:	ESI+
Acquisition mode:	MS^E
Acquisition range:	*m/z* 50–2000
Capillary voltage:	1.2 kV
Collision energy:	60–120 V
Cone voltage:	20 V

Data Management

Informatics:	waters_connect with Peptide MAM and UNIFI Apps

Results and Discussion

The intelligent data capture setting is enabled through a dialog box under the MS instrument parameters of the UNIFI app. As shown in Figure 1, the IDC settings that control the extent of denoising of the data are in a drop-down menu containing three default settings (15, 10, and 5) alongside a custom setting option. We have evaluate the effects of all three default IDC parameters on peptide MAM data, monitoring %peptide attribute levels and new peak detection capabilities (NPD). Reduction of instrument and chemical noise artifacts in MS data has been known to significantly reduce files sizes, also reducing time spent on data processing. This was measured using data processed through the waters_connect Peptide MAM application.

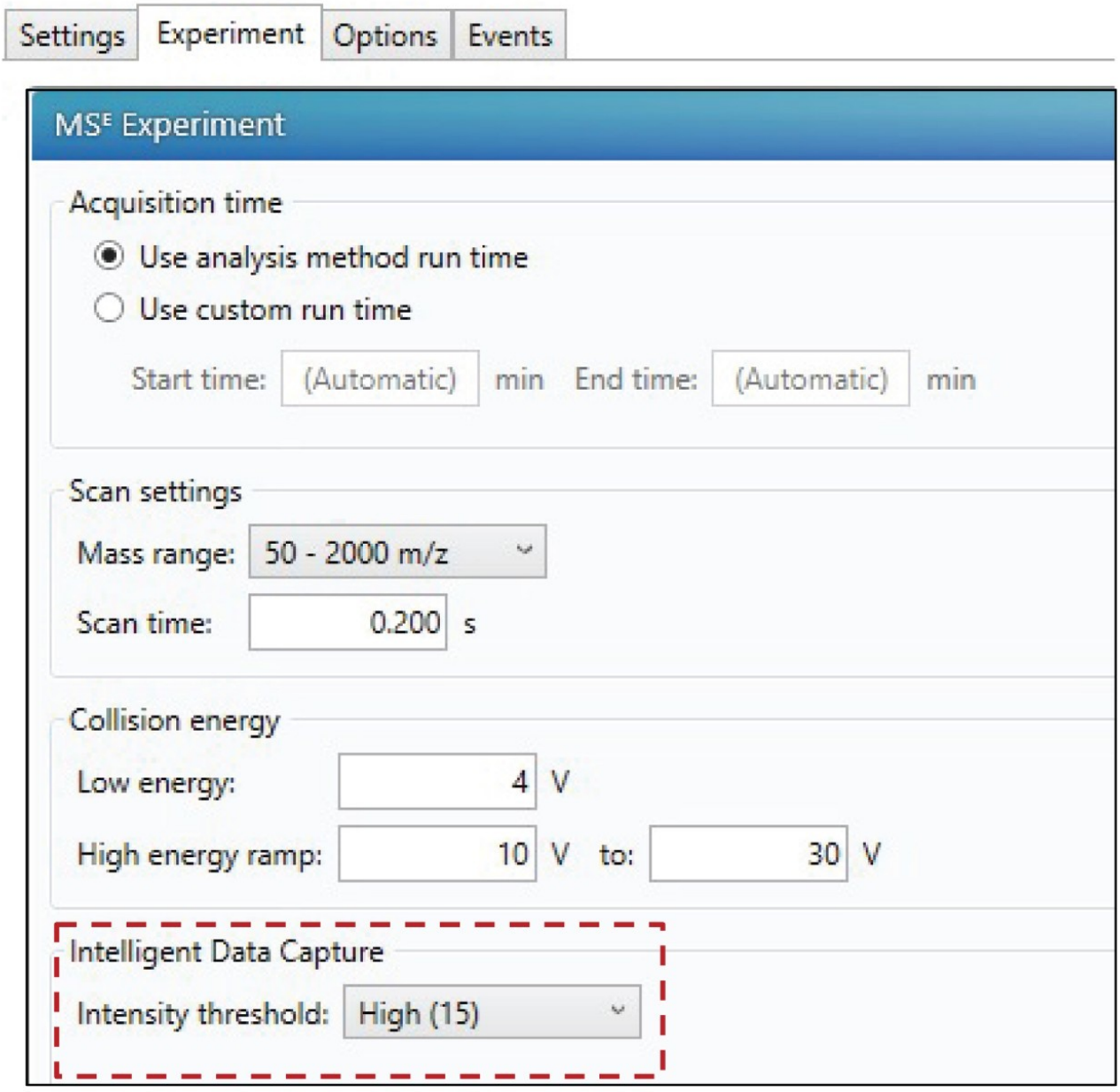

Figure 1. The intelligent data capture (IDC) feature within the MS method provides 4 default noise reduction levels of increasing noise reduction capability: Off, 5, 10, and 15, along with a custom setting option for a user specified threshold.

Identical %modification levels generated with IDC

Optimizing the benefits of IDC for peptide MAM, we acquired data for a NIST mAb digest using IDC thresholding at 15, 10, 5, and 0 (off) levels. The data was processed in the Peptide MAM App to determine the %modification levels of 30 selected quality attributes. Figure 2A reveals the results for HC T25: EEQYNSTYR glycopeptides. Glycopeptides are relatively low intensity peptides that could be disproportionately affected by improper application of data reduction algorithms. The IDC-enabled and IDC Off acquired data, generated consistent %modification profiles for selected glycopeptides, including the lower levels detected for the T25 unmodified (0.53–0.58%) and Man5 T25 (1.26–1.13%) peaks. The Man5 glycopeptide represents only 0.08% of the base peak peptide intensity. Further, IDC maintained MS spectral quality (Figure 2B) for the Man5 spectra acquired with IDC settings of 15 and 0 (off). Both MS spectra show consistent isotope distribution for the +2 charge state peak at *m/z* 1203.2711. Relative to the IDC Off data, IDC 15 data shows the reduced base line noise levels resulting in higher quality spectra and reduced data file size.

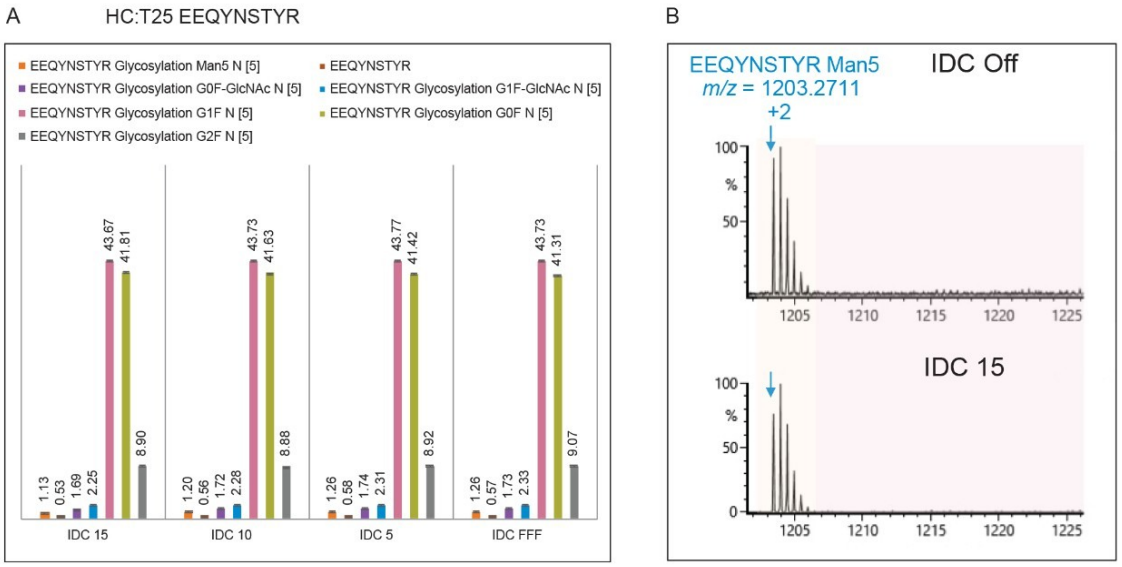

Figure 2. Glycopeptide Attribute Profiling of NISTmAb. (A) Relative %modification levels of selected glycopeptides and the unmodified EEQYNSTYR HC:T25 peptide are shown. The low level Man5 attribute was detected from MS1 data acquired at all IDC levels with data acquired in duplicate injections for each IDC condition (shown in orange). (B) MS spectra for the low level Man5 attribute glycopeptide have comparable isotopic pattern for data acquired from IDC at 15 and IDC Off.C

High quality new peak detection (NPD) data

NPD is performed following targeted peptide attribute monitoring during MAM data processing. The goal is to identify new or significantly changed peaks in an experimental sample compared to a reference control sample (Figure 3A). These “new” peaks may represent unexpected product variants or product/process related impurities whose profile is altered during drug development process. However, manual verification of each new peak costs time and resources, particularly when encountered in regulated quality and manufacturing environments where a new peak could also trigger costly Out of Specification (OOS) investigations and product to market delays. Equipped with real-time noise reduction IDC-enabled acquisition could eliminate potential false positive peaks due to LC-MS acquisition artifacts. As demonstrated in Figure 3, the experiment evaluated three levels of processing, IDC 15, IDC 5, and, IDC Off data acquisition. The resulting data sets were filtered for new peaks using common default parameters (Figure 3A). IDC 15 processing (Figure 3B) presented no new peaks in negative control, 15 new peaks in spiked in control (positive control), and 136 peaks in a stressed mAb sample. The NPD data for the spiked sample matched the 15 spiked heavy isotope labeled peptides described within experimental section *(*Figure 3C). In comparison, the IDC 5 acquired data showed much higher levels of new peaks compared to IDC 15 *(*Figure 3D), and contained multiple false positive identifications (Figure 3D). This higher false positive observation was even more pronounced in IDC Off acquired data (not shown), clearly demonstrating the ability to retain all true new peaks, while eliminating false positive results.

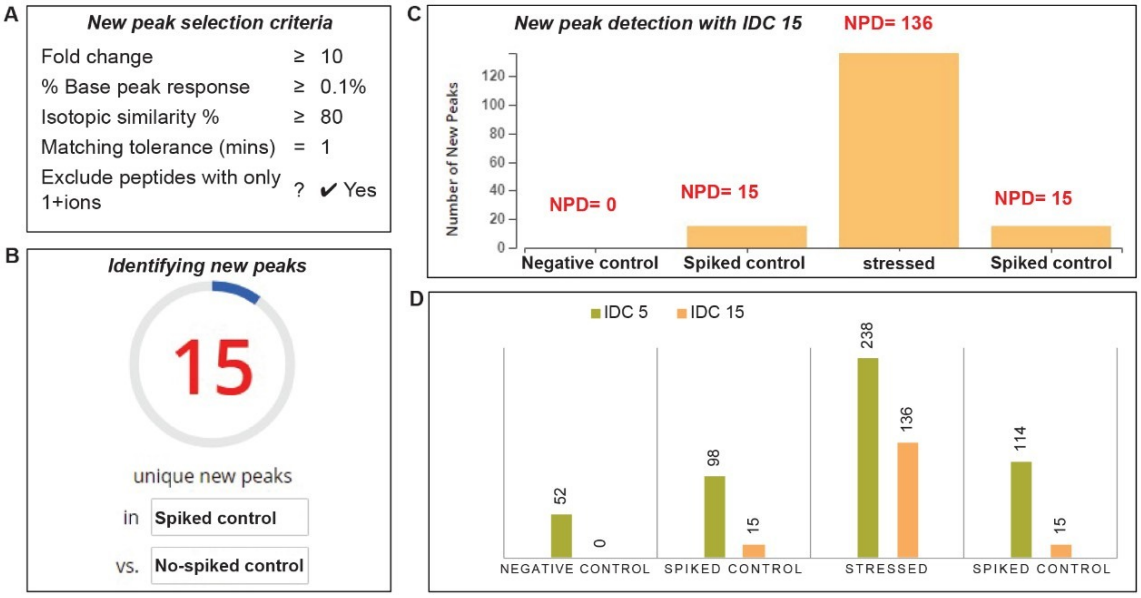

Figure 3. New peak detection (NPD) for Xevo G2-XS based MAM. The filtering criteria (Panel A) used in waters_connect Peptide MAM App is shown here to generate the result of 15 "new" peaks (Panel B) for binary comparison of 15-peptide spiked sample vs a non-spiked control sample. The new peaks detected for spiked and stressed mAb samples compared to the control sample (Panel C) is shown for IDC 15 acquired data. The superiority of NPD data for IDC 15 vs IDC 5 acquisition was established with the demonstration of higher false positives from Peptide MAM App processing for the weaker noise reduction setting.

Reduced data file sizes and processing times

Large file sizes and data sets from complex MAM studies may strain data management systems unnecessarily. Intelligent data capture enabled data acquisition will provide a solution through real-time data noise processing and generating reduced raw data files size. This is done by eliminating the majority of chemical and mechanical noise that distracts, rather than enhances processing of the true peak signals that contribute to meaningful results. By enabling IDC in peptide MAM data acquisition at selected IDC 5, 10, and 15 levels, we managed to reduce the data file sizes significantly (Figure 4A) compared to IDC Off acquisition (From IDC "Off" to IDC 5 shows a 65% file size reduction, and more than 90% reduction at the IDC 15 setting). As a result, the data processing speed for peptide MAM data was accelerated compared to IDC Off acquisition (Figure 4B). At IDC level 15, where optimal data noise reduction was observed, the total data processing times were reduced by 75%,

and for a data set of 2 injections, over 3 hours of processing time was saved compared to IDC Off acquired data.

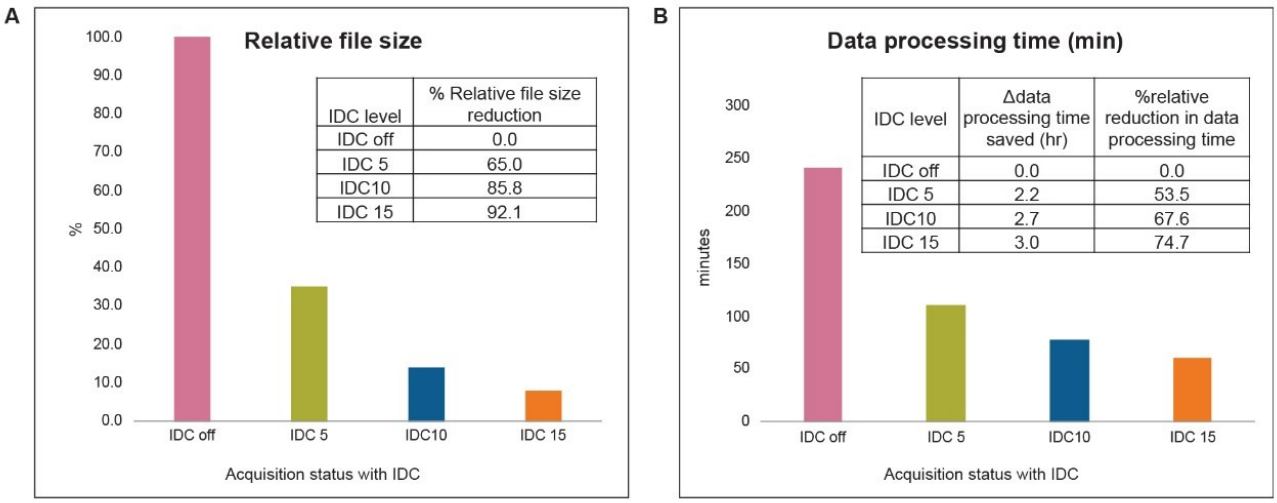

Figure 4. The average file size (A) is dramatically reduced with progressive application of more stringent IDC settings during data acquisition. Data file size was reduced by 92% between the off setting and IDC 15 based acquisition. (B) Peptide MAM App processing times for two injections were correspondingly reduced by as much as 75% at IDC 15 level compared to IDC Off.

Conclusion

The application of intelligent data capture (IDC) on the Xevo G2-XS QTof operated on waters_connect was shown to benefit many aspects of Peptide MAM analysis. The generation of higher quality MS data, while dramatically reducing data file size and data processing times, will bring greater efficiencies to users of the Peptide MAM analysis workflow. The optimum level of IDC 15 acquisition generates comparable %modification results to IDC Off data, with no effect on sensitivity or relative profiling of targeted attributes. Further, IDC 15 acquisition minimized the number of false positive new peaks in NPD, while identifying all expected new peaks during a spiked mAb sample MAM analysis. Taken together, these results clearly demonstrate that MAM workflow users on the Xevo G2-XS Platform can derive the full benefits of IDC acquired data, therefore realizing abetter and faster generation of accurate and meaningful Peptide MAM results.

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 < https://www.waters.com/webassets/cms/library/docs/720006567en.pdf> (2019).
2. Ippoliti, S.; Yu, Y., Q.; Mortishire-Smith, R. Peptide Mapping Using Intelligent Data Capture on Vion IMS QTof. Waters Application Brief, 720006636EN. (2019).

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waters_connect <https://www.waters.com/waters/nav.htm?cid=135040165>

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

Biopharmaceutical Platform Solution with UNIFI <https://www.waters.com/waters/10195515>

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