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The use of smaller Q1 isolation windows improves reproducibility in SWATH® based protein quantification even at higher spectral acquisition rate

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ABSTRACT

The aim of quantitative proteomics is to both identify and quantify proteins of wide dynamic range. Data independent acquisition (DIA) strategies have emerged as a key workflow to increase the reproducibility, comprehensiveness of data collection and improve biological conclusions, which is correlated with improvement in MS/MS speed, sensitivity and resolution in today's accurate mass LC/MS systems. The most widely used of these techniques is MS/MS^{ALL} with SWATH® Acquisition on the TripleTOF® Systems. In SWATH Acquisition, Q1 isolation windows are stepped across the mass range in an LC timescale, transmitting populations of peptides for fragmentation, and high resolution composite MS/MS spectra are acquired at each step. The complexity of the MS/MS spectra and the resulting specificity depends on the number of peptides eluting off the column at the same time within the same m/z isolation window. Here, the impact of varied Q1 window widths on quantitative data quality was assessed.

INTRODUCTION

A large scale identification and quantitation of proteins in complex mixtures is "bottom-up" proteomics, where proteins are digested into peptides and separated by reversed-phase chromatography prior to mass analysis by electrospray ionization mass spectrometry. Low MS/MS identification rate results in poor reproducibility of proteins present in complex samples, thus remain a major challenge with the traditional data dependent workflows. This poses challenges for such samples requiring very high speed MS/MS acquisition to deeply interrogate the sample in order to both identify and quantify a broad range of proteins. To get maximum information from the complex mixture data Independent acquisition strategies have been used to identify the proteins and the same has been used to quantify using workflow namely SWATH acquisition. TripleTOF 5600 System enabled with SWATH acquisition will ease the process. It is now possible to perform a data-independent workflow with high speed and high resolution in both MS and MS/MS modes (1, 2). In the current workflow we have focused on the label free quantitation using the SWATH with variable Q1 windows to get maximum reproducibility with accuracy. SWATH 2.0 data independent acquisition with smaller Q1 isolation window maximized the accuracy of quantitation for each of the identified proteins.

MATERIALS AND METHODS

Sample Preparation:

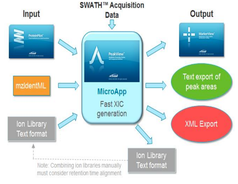
Human cell digest were purchased from Promega (V6951).
Nano LC-MS/MS condition:

In order to generate spectral library protein identification experiment was performed by information dependent acquisition mode after injecting tryptic digest of 500ng on column Human Cell Lysate using Eksigent nanoLC-Ultra™ 2D plus system coupled with SCIEX TripleTOF® 5600 system fitted with nano spray III source. The samples were loaded on the trap (Eksigent ChromXP™ 350 µm x 0.5 mm, 3 µm 120Å) and washed for 30 minutes at 3 µL/min. A 100 min gradient in multiple steps ranging from 5-50% Acetonitrile in water containing 0.1% formic acid was set up to elute the peptides from the ChromXP 3-C18, 0.075 x 150 mm, 3 um 120Å analytical column.

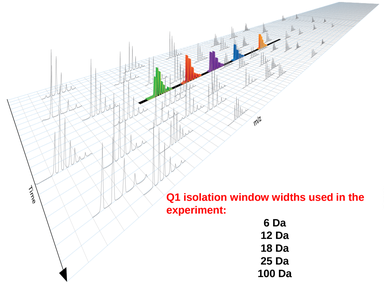
In DIA ion library generation method an Information Dependent Acquisition (IDA) method was used where maximum 20 most intense multiple charged ions per MS cycle were selected to perform MS/MS fragmentation. A dynamic exclusion criteria was applied to each of the ions for 10 seconds. The accumulation time for each MS/MS experiment was set to 70 ms.

In SWATH acquisition method Q1 transmission window was set to 6, 12, 18, 25, 100 Da and data was acquired independently with accumulation time of 49 msec, 98 msec, 145 msec, 204 msec, 833 msec respectively with three technical replicates for each of the sets. Total cycle time was kept constant at 5 sec. To generate spectral library ProteinPilot™ v. 5.0 was used. All SWATH Acquisition data were processed using the SWATH Acquisition MicroApp 2.0 in PeakView® Software. Peak groups were detected where the data was filtered by 1% FDR and replicates were analysed in MarkerViewTM v. 1.2.1 software.

Benefits for MS/MS^{ALL} with SWATH acquisition

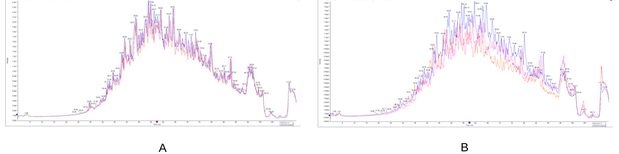


Triple TOF® 5600+ system



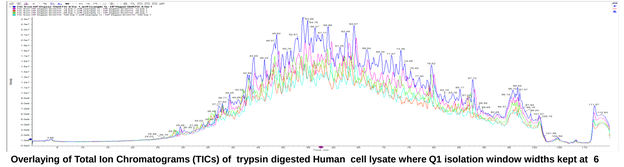
Q1 isolation window widths used in the experiment:

6 Da
12 Da
18 Da
25 Da
100 Da

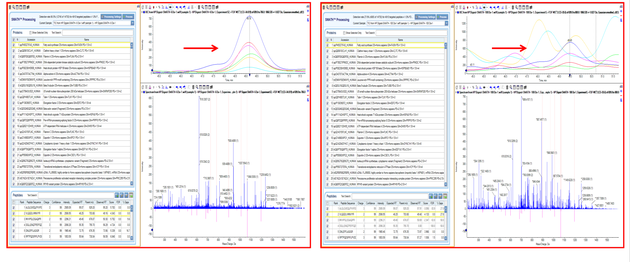


A B

Overlaying of Total Ion Chromatograms (TICs) of replicates of trypsin digested Human cell lysate where 6 Da Q1 (A) and 100 Da (B) isolation window widths were used respectively.

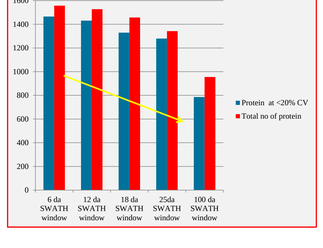


Overlaying of Total Ion Chromatograms (TICs) of trypsin digested Human cell lysate where Q1 isolation window widths kept at 6 Da, 12 da, 18 da, 25 da and 100 da.

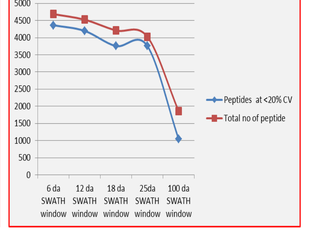


A B

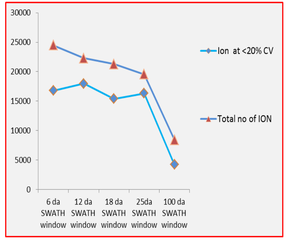
Comparative extracted ion chromatograms (XICs) of fragment ions of the peptide data acquired using 6 Da (A) and 100 Da (B) Q1 isolation window widths respectively



Number of proteins quantified at different Q1 isolation width setting



Number of peptides quantified across various Q1 selection windows



Total number of ions quantified within the <20% CV across various Q1 selection windows

RESULTS

Upon column injection of 500 ng of human cell digest a total of 1736 proteins were identified with 1% FDR. It was observed that when Q1 isolation width narrowed down from 100 Da to 6 Da, a significant improvement (ca. 180%) occurred in terms of percentage of quantifiable proteins, with %CV less than 20% among technical replicates. Further improvement (ca. 88% and 92%) was observed when Q1 isolation window width was reduced to 12 Da and 6 Da. This is mostly likely due to an improvement in specificity obtained from the reduced complexity of the MS/MS data across the whole mass range. The same trend was evident from the increase in the numbers of interfering transitions when Q1 isolation window was expanded from 6 Da to 100 Da. However there is a slight decrease (ca. 4.5%) in percentage of quantifiable proteins observed when the data was acquired keeping the Q1 isolation window width to 18 Da over 25 Da. In this study, MS/MS acquisition rate were kept 20.4 Hz, 10.2 Hz, 6.9 Hz, 4.9 Hz, 1.2 Hz while data was acquired keeping the Q1 isolation window widths 6 Da, 12 Da, 18 Da, 25 Da, 100 Da respectively. This suggests it is possible to achieve robust quantitation result in SWATH acquisition even at higher acquisition rate, where Q1 isolation window width plays key role. The higher specificity is advantageous to a number of aspects of the peak group scoring process.

CONCLUSIONS

1. **Keeping Q1 isolation window width at 6 Da in SWATH based data acquisition 84% of the total identified proteins (1736 at 1% FDR) showed <20% CV among three technical replicates.**
2. **There is a significant (180%) decrease in quantifiable proteins from 6da to 100 da Q1 isolation window in SWATH acquisition.**
3. **Both peptides and fragment ions numbers show exponential decrease of 418% and 397% respectively while SWATH data was acquired keeping variable Q1 windows from 6da to 100da.**
4. **MS/MS acquisition rate were kept 20.4 Hz, 10.2 Hz, 6.9 Hz, 4.9 Hz, 1.2 Hz while data was acquired keeping the Q1 isolation window widths 6 Da, 12 Da, 18 Da, 25 Da, 100 Da respectively suggest Q1 isolation width plays more significant role irrespective of data acquisition speed.**
5. **Lower Q1 SWATH window exhibits excellent reproducibility which may further help to quantify low abundant proteins in complex mixture without compromising data quality at very high acquisition rate.**

REFERENCES

1.Gillet LC *et al (2012) Mol. Cell. Prot. E-pub.*
2.Liu Y et al (2012) J Proteomics *75 (13)*, **3877-3855.**

TRADEMARKS/LICENSING

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