Benefits of SWATH® Acquisition, a DIA Technique over Traditional Data Dependent Analysis for High Resolution Untargeted Metabolomics Applications

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ABSTRACT

A data independent acquisition (DIA) technique known as SWATH® acquisition enables the identification and quantification of a higher number of metabolites in untargeted metabolomics workflows compared to standard data dependent acquisition (DDA further as IDA) approaches thus enabling a deeper profile of the metabolome. In addition, SWATH® acquisition allows the collection of MS and MSMS data in a single injection and builds a digitized map of every detectable metabolite in your sample. This allows retrospective data mining meaning as your hypothesis changes there is no need to go back and re-run your sample but just to re-mine the data.

INTRODUCTION

SWATH® acquisition, a data independent acquisition (DIA) workflow is well adopted in quantitative discovery proteomics, but still not commonly used in discovery metabolomics. SWATH® acquisition combines the benefits of quantitation at the MS^2 level of targeted MRM-based workflows with the MS^2 level based untargeted identification capability for metabolite identification of information dependent acquisition (IDA) workflows and the comprehensive nature of the $MSMS^{all}$ workflow. Because of the comprehensive, non-stochastic nature of the fragmentation in SWATH® acquisition, more fragmentation and thus structural information of the analytes compared to the IDA approach is achievable. Because of the stochastic nature of IDA, reproducibility and coverage is lower compared to DIA workflows. Here we describe the improvements in metabolite coverage using SWATH® acquisition without sacrificing quantitation compared with the traditional IDA approach.

MATERIALS AND METHODS

Human urine and commercially available human plasma were processed according to standard extraction procedures. Urine was diluted with water at a ratio of 1:4 and centrifuged for prior analysis, while plasma was extracted 1:4 with ice-cold methanol for protein precipitation. Injection volume was 5 µl for both type of samples. Separation was performed on a Agilent Technologies 1290 Infinity II with a BEH C18 column (100nm x 2.1 mm ID, 1.7 µm) using a flow rate 200 µl/min. Linear gradient was build within 1-10 min from 2-98% of 0.1% formic acid in acetonitrile, total length of LC separation was 14 min and column oven was set to 40°C. The SWATH® acquisition and information dependent acquisition (IDA) experiments were acquired on a TripleTOF® 6600 System. Data was processed using MasterView™ software and the SCIEX Accurate Mass Metabolite Spectral Library using search settings accordingly: candidate search algorithm, results sorted by Purity (IDA) or Fit (SWATH®). Our MS workflow is summarized in the Table 1. For the IDA acquisition we set to selected different numbers of precursor ions for MS^2 and for the SWATH® acquisition we varied the window number and size to test which parameters resulted in the highest identification and coverage of metabolites.

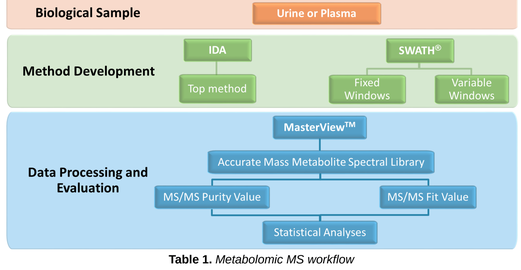


Table 1. *Metabolomic MS workflow*

In general, MS setting were for both modes as follow: Curtain Gas 35 psi, GS1 40 psi, GS2 40 psi, ISVF 5500 V, Source temp. 600°C, Declustering Potential 80 V and additional in MS^2 mode collision energy was 30 V with 15 V spread. Different MS settings were chosen as listed in Table 2 and 3. The Top20 IDA acquisition method was used to calculate variable windows (vw) using a SWATH Variable Window Assay calculator version 1.1 with minimum window size 3 Da. Examples of graph illustrating correlation of SWATH window sizes versus Precursor Ion Density are presented in Figure 1.

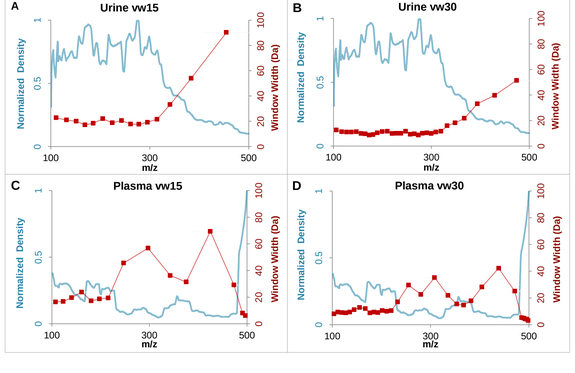
Table 2. *IDA acquisition MS method settings*

IDA aquisition	Top 5	Top 10	Top 15	Top 20
Nr. precussor ions	5	10	15	20
TOF MS range/Da	100-500	100-500	100-500	100-500
MS Acc. time/ms	50	50	50	50
TOF MSMS range/Da	50-500	50-500	50-500	50-500
MSMS Acc. time/ms	100	50	35	25
Cycle Time/ms	600	600	600	600

Table 3. SWATH® acquisition MS settings fixed windows (fw) and variable windows (vw).

SWATH® aquisition	fw15	fw20	fw30	vw15	vw20	vw30
Window size/Da	26	19	12	variable	variable	variable
TOF MS range/Da	100-500	100-500	100-500	100-500	100-500	100-500
MS Acc. time/ms	50	50	50	50	50	50
TOF MSMS range/Da	50-500	50-500	50-500	50-500	50-500	50-500
MSMS Acc. time/ms	30	25	18	30	25	18
Cycle Time/ms	550	600	657	550	600	640

Figure 1. *Variable window calculations. A) Urine sample with 15 variable windows (vw) and B) with vw30; C) Plasma sample with vw15 and D) with vw30.* — *Input Histogram* —■— *Variable Windows*



RESULTS AND DISCUSSION

In the first part of our study we optimized our IDA and SWATH® acquisition methods by varying the numbers of selected precursor ions for MS^2 by various fixed (fw) and variable window (vw) and we tested the performance of different methods by matching to the metabolite spectral library of 557 metabolites in human urine samples (Figure 2 and 3).

Figure 2. *Gain in metabolites library coverage with urine sample using various IDA acquisition methods*

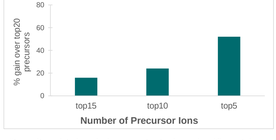
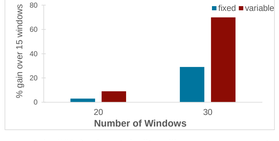


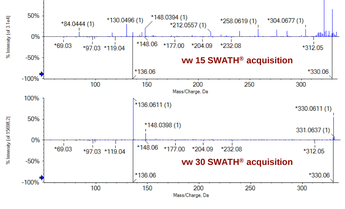
Figure 3. *Gain in metabolites library coverage with urine sample using various SWATH® acquisition approaches*



Results obtained demonstrated a significant improvement (~ 55%) of metabolites library coverage at the MS^2 level by using top5 compare to the top20 IDA method. This could be due to higher accumulation time that increase of spectra quality; e.g. ~80% of high quality of MS^2 spectra were measured with top5 whereas only ~50% with top20. Increasing number of windows selected for SWATH® acquisition resulted in ~ 30% raise in library coverage using fixed windows and ~70% raise using variable windows strategy.

Downsizing the windows size and also varying it depending of precursor ion mass density improves the overall ion selectivity as shown in Figure 4 for MS^2 of cyclic adenosine monophosphate.

Figure 4. *Cyclic adenosine monophosphate (cAMP) MS^2 spectrum comparison between SWATH® acquisition with 15 and 30 variable windows with cycle time ~0.6 s in urine. A blue spectrum represents measured all MS^2 ions and grey spectrum shows cAMP MS^2 fragmentation in the SCIEX Accurate Mass Metabolite Spectral Library*



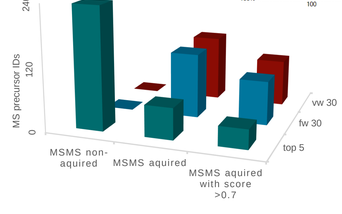


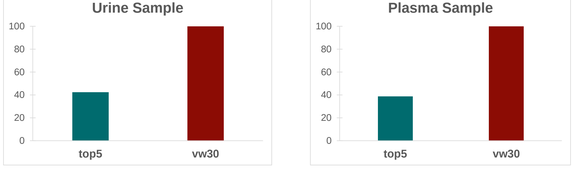
Figure 5. *Number of Metabolites IDs acquired on MS level with and without MS^2 confirmation using IDA and SWATH® acquisition. MasterView™ software with SCIEX Accurate Mass Metabolite Spectral Library were used to process data*

In the second step, we compared the ID rate from SWATH® acquisition to standard IDA acquisition in the Urine sample. Metabolite library coverage at the MS level was higher using the IDA acquisition, but number of confident identification on MS^2 level was much lower compare to different SWATH® acquisition approaches, shown in Figure 5. The opposite scenario is shown when SWATH® acquisition we measured very low number of metabolite identification without MS^2 confirmation and gain in number of identified metabolites (Figure 5).

Figure 6 illustrates that SWATH® acquisition using 30 variable windows is able to identify up to 60% more metabolites from the spectral library than by IDA top5 acquisition. More confident MS^2 based identifications then lead to more quantifiable metabolites in a metabolite expression experiment, which at the end allows better understanding of the biology.

In the third step, we compared the performance of the chosen methods when a different biological matrixes are used. As is shown in Figure 6, SWATH® acquisition with 30 variable windows overperform the top5 IDA in urine and also in plasma matrix.

Figure 6. *Gain (%) in metabolite coverage with top5 IDA and vw30 SWATH® acquisition in selected biological matrices; human urine and plasma. Graphs are normalized to the highest library coverage, which is in both cases vw30 SWATH® acquisition*



CONCLUSIONS

- **Elevated coverage of metabolite library and greater MS^2 level metabolite identification** agaisnt the SCIEX Accurate Mass Metabolite Spectral Library was obtain using the **SWATH® acquisition** compare to traditional IDA acquisition independent of measured sample (Figure 5 and 6)
- Using the **variable windows** instead of fixed windows will **significantly improve the metabolite library coverage** when SWATH® acquisition is used (Figure 3)
- **Increasing the number of windows** will refine the quality of the MS^2 spectra, thereby **increasing the slectivity**, as is shown in Figure 1 and 4. Setting up the correct window witdh of variable windows can be curial when different matrixes are measured (Figure 1)
- Nevertheless, the advantages of SWATH® acquisition are:
  - **No need for method development -** Use a generaic method setup
  - **No need re-run degradeted or descarted samples** - You can mine the digitized SWATH® map over and over

TRADEMARKS/LICENSING

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