In proteomics, and many other sample types, the complexity and dynamic range of compounds are very large. This poses challenges for the traditional data dependent workflows, requiring very high speed MS/MS acquisition to deeply interrogate the sample in order to both identify and quantify a broad range of analytes. Data independent acquisition strategies have been used to increase the reproducibility and comprehensiveness of data collection. However, mass spectrometers have been limited in the speed and quality of the data that they acquire. With the TripleTOF® Systems, it is now possible to perform a data-independent workflow with high speed and high resolution in both MS and MS/MS modes.

Targeted quantification is often desired to obtain accurate quantification in complex mixtures. Multiple Reaction Monitoring (MRM) is the most dominant workflow for performing this type of analysis, but the nominal mass selection of triple quads can allow mass interferences. There have been a number of workflow extensions that provide higher specificity to targeted quantification. On the TripleTOF systems using the MRM® workflow, the instrument is set up to acquire full scan MS/MS data on a fixed precursor, over and over again across the LC-MS run (Figure 1). The Q1 is fixed, the peptide is fragmented in the collision cell and the full scan TOF MS/MS is acquired. After data acquisition, extracted ion chromatograms on sequence specific ions are generated.

MS/MSALL with SWATH® Acquisition brings together data independent acquisition for comprehensive acquisition and targeted data processing for the highest quality data.¹

Figure 1. MRM® Workflow Using the TripleTOF® Systems. In the MRM® workflow, a fixed number of analytes are targeted and high resolution MS/MS spectra are collected across an LC run. Precursor masses are selected at narrow resolution such that mainly the target compound is passed into the collision cell (top). This produces a full scan MS/MS spectrum enriched for the analyte of interest (right). Then, any number of fragment ions can be extracted at high resolution post-acquisition to generate MRM-like data (bottom).
In **MS/MS**ALL with SWATH® Acquisition, the Q1 quadrupole is typically stepped at 25 amu increments across the mass range of interest, passing a 25 amu window through to the collision cell. The transmitted ions are fragmented and the resulting fragments are analyzed in the TOF MS Analyzer at high resolution. This sequential windowed acquisition is termed SWATH Acquisition (Figure 2). A large mass range can be interrogated in an LC-MS time frame because of the larger mass steps. This yields a more complicated MS/MS spectrum at each step; however the high resolution MS/MS enables tighter extraction windows to maintain high specificity.

A three dimensional dataset is acquired which is then interrogated after acquisition (Figure 4). After acquisition, the data files are interrogated for proteins and peptides of interest, using previously determined fragmentation patterns or spectral libraries. Using narrow extraction widths on the target fragment ions, high specificity XICs are generated post-acquisition, and integration provides accurate quantitation.

The next acquisition innovation was the ability to use variable sized Q1 windows to optimize based on precursor density. Please see the variable window technical note for more information.2

Figure 2. **MS/MS**ALL with SWATH® Acquisition. In this workflow, instead of the Q1 quadrupole transmitting a narrow mass range through to the collision cell, a wider window containing more analytes is passed. This produces a more complex MS/MS spectrum which is a composite of all the analytes within that Q1 m/z window. Because the fragment ions are high resolution, high quality XICs can be generated post-acquisition to produce the MRM-like data. This Q1 window can be stepped across the mass range, collecting full scan composite MS/MS spectra at each step, with an LC compatible cycle time. This enables a data-independent LC workflow. After data acquisition, the data is interrogated by generating extracted ion chromatograms (XICs) from the high resolution MS/MS for specific peptide fragment ions to generate MRM-like data to integrate for quantitation.
The advantages of this SWATH® Acquisition for targeted quantification are numerous. No upfront assay development is required on specific targets, all data is acquired and targets are mined post-acquisition. Setting up acquisition methods is straightforward (Figure 3) and once established, the same method can be used on all proteomic samples.

Data collection is comprehensive so quantitative data on additional compounds can be mined retrospectively. Additionally, in the event of overlapping fragment ions, the quantification of any peptide or protein can be "rescued" by re-assigning quantification to a different fragment ion or peptide. In similar analyses using data-dependent or targeted MRMs, the offending peptide or protein would have to be excluded from the results (illustrated in Figure 4). Finally, the limits of detection and quantification rival those of the leading triple quads available today.

Figure 3. Easy Creation of SWATH® Acquisition Methods. User can design an optimal acquisition method based on the chromatography used and the complexity of the sample to be analyzed, by controlling Q1 isolation windows, accumulation time and cycle time. Once the method is established, the same method can be used over and over again to analyze many different proteomic samples.

Figure 4. Comprehensive Data Acquisition for High Quality Quantitation with Highest Multiplexing. The LC-MS contour plot of a single SWATH acquisition window illustrates the number of peptide precursors that are transmitted through the Q1 window, which is set to transmit 850 to 875 m/z (Top). The horizontal black box highlights all the un-fragmented precursors that have transferred through Q1. The vertical strips of data are the MS/MS fragment ions of the peptides with precursor masses between 850-875 m/z that have eluted at that point in the LC run. A peptide is highlighted at 21 min and the spectrum shown (Bottom left). The MS/MS is actually two closely eluting phosphopeptides with the same sequence but the phosphate located on two different serines. Extraction of the site-specific fragment ions enables quantitation of the two different peptide forms (bottom right).
In one example of the workflow, a digest of a yeast cell lysate was analyzed using MS/MS\textsuperscript{ALL} with SWATH Acquisition and interrogated for a large number of proteins. In a single injection, MS/MS quantification is obtained on proteins across a broad dynamic range, down to 100s of copies per cell (Figure 5, left). Typically, better quantitation is achieved using SWATH Acquisition than when using MS based quantitation (Figure 5, right) because of the specificity afforded by quantifying at the MS/MS level.

Conclusions

- Comprehensive quantitative analysis with qualitative confirmation
- Quantification with no method development for all species in a single analysis
- Archive of all analytes enables retrospective in silico interrogation
- High resolution quantification reduces potential for interferences
- Quantitative performance comparable to leading triple quadrupole instruments

References

2. Improved Data Quality Using Variable Q1 Window Widths in SWATH\textsuperscript{®} Acquisition, SCIEX technical note RUO-MKT-02-2879-A.