# A Review of the Advances in High Resolution Accurate Mass Spectrometry for Industrialising Omics

*by Arianna Jones, Proteomics Manager, SCIEX, Framingham, Massachusetts, USA arianna.jones@sciex.com*

*Arianna Jones is the Global Market Manager for the Proteomics Business at SCIEX, where she evaluates industry trends and addresses customer's critical challenges with the product development team. Additionally, Arianna is part of the open innovation team, which looks at new opportunities to solve unmet needs in adjacent markets. She received her bachelor's degree in Animal Physiology and Neuroscience at the University of California, San Diego.*

**As mass spectrometry (MS) users' needs grow, particularly in omics research and the pharmaceutical and biopharmaceutical (bio/pharma) industries, accurate MS instruments and tools need to keep pace to meet these needs. The constant development of MS analytical science has pushed boundaries in the past 20 years. The high throughput required by the bio/pharma industries means that we need not only ever increasingly precise and sensitive systems for quantitative and qualitative analyses, but also speed in acquiring the data. The data also needs to be as comprehensive as possible, providing meaningful information that can be linked with physiology and disease mechanisms. This is imperative, not only for drug discovery in bio/pharma, but also in basic life sciences research, where investigating disease biomarkers and treatment targets is fundamental. It is these novel discoveries and ongoing research and development that is facilitating the realisation of precision and personalised medicine today and in the near future [1].**

To meet these growing needs and demands from biomedical research scientists and the bio/pharma industry, we have been working to progress and push the boundaries of what is possible in high-resolution accurate mass spectrometry (HRMS). Much of discovery research now employs a multi-omics approach, looking at the genomics, transcriptomics, proteomics, metabolomics, and other -omics of human health and disease, using complex biological samples from humans as well as model systems, available in varying amounts. This means that mass spectrometry using electrospray ionisation needs to be performed at different liquid chromatographic (LC) flow rates, such as nanoflow for proteomics, where sample amounts tend to be especially limited, and microflow for metabolomics, where sample amounts are frequently more abundant. Switching LC systems, sources, solvent set-ups, columns, and associated chemistries to accommodate different flow rates is time- and resource-consuming.

Many laboratory managers minimise this downtime by batching tasks, so that the LC-MS instrument is block-booked for various purposes weeks at a time, e.g. 3 weeks for proteomics analyses, then 3 weeks for metabolomics runs. However, having to wait up to almost a month (or in some cases, longer) to analyse one's samples is not an ideal situation for any scientist. As a scientist myself, I understand the frustration and consequences this can cause, especially with stringent timeframes and deadlines that need to be met, both in academic and industry-led research. Therefore, the team at SCIEX has been working to develop a technical solution to help resolve this issue. We thought, wouldn't it be amazing if we could switch flow rate easily, so the lab could run at optimal efficiency?

What we came up with was the OptiFlow® Source, which is a single ionisation source that can cover the full spectrum of flow rates from nanoflow to microflow, for high-sensitivity LC-MS analyses (see Figure 1). This low flow source allows users to be always working in the 'sweet spot' – retaining the sensitivity of low flow, without compromising on robustness and ease of use more usually associated with higher flow rates. Upon testing the new ion source, we realised that many of the user-adjustable source settings could actually be predetermined and locked-down. So, we developed a range of SteadySpray


*Figure 1: SCIEX OptiFlow Turbo V Ion Source. 1) Based on the trusted Turbo V source design, and with finger-tight fittings for tool-free setup. 2) Supports a wide flow rate range and intelligent probe recognition to eliminate all manual source adjustments. Micro: 1–10, 10–50, 50–200 µL/min. Nano: 100–1000 nL/min. 3) Integrated column heater with expanded range up to 90°C for retention time consistency. 4) New SteadySpray probes ensure consistent droplet formation for high quality spray [2].*

Electrodes and Probes that are fitted to produce the correct spray tip protrusion and electrode position for optimal nebulisation and signal for the user's chosen flow regime. It takes mere minutes to switch the MS instrument between nanoflow (100–1000 nL/min) and microflow (1–200 µL/min) regimes, thus removing a critical barrier to switching flow rates as-you-go. This enables users to simply choose and use the flow rate that is right for their experiment, without having to book weeks in advance or wait. And if combined with the nanoLC™ 425 System, the same LC can be used to cover the nanoflow to microflow regime, further simplifying and reducing the time to switch modes.

Once the OptiFlow Source was developed, we went back to the drawing board to see how else we could optimise uptime and advance additional HRMS capabilities. Instrument scan speed and throughput were the classic paradigms that we kept coming across. Like longer battery life per charge for mobile phones, these are the basic functionalities that users constantly want improved for their LC-MS experiments. We set about increasing the scan speed and throughput capacity for HRMS, resulting in the TripleTOF® 6600+ which is able to reach tandem MS scan speeds of 100 Hz for both quantitative and qualitative analyses. This is possible regardless of whether the experiment is a targeted high-resolution quantification run, e.g. MRMHR workflow, or an untargeted workflow using either data-dependent or data-independent SWATH® acquisition.

By increasing scan speed while retaining sensitivity, precision and robustness, the capacity is thus increased for higher throughput analysis of samples. Additionally, while looking to maximise uptime, we also made multiple software improvements, including programming the Analyst® TF Software (version 1.8) to eliminate the acquisition of unwanted data, and introducing Scheduled Ionization that allows users temporal control over the number of ions entering the system.

In order to preserve the most information from precious biological samples and handle the higher throughput, variable window SWATH acquisition was developed in 2014 (see Figure 2). This technology was built on the original SWATH acquisition method, and allows the Q1 window to be variable in size. What this means is that size of the Q1 windows can be adjusted depending on the m/z regions being analysed. For example, regions that are m/z dense require

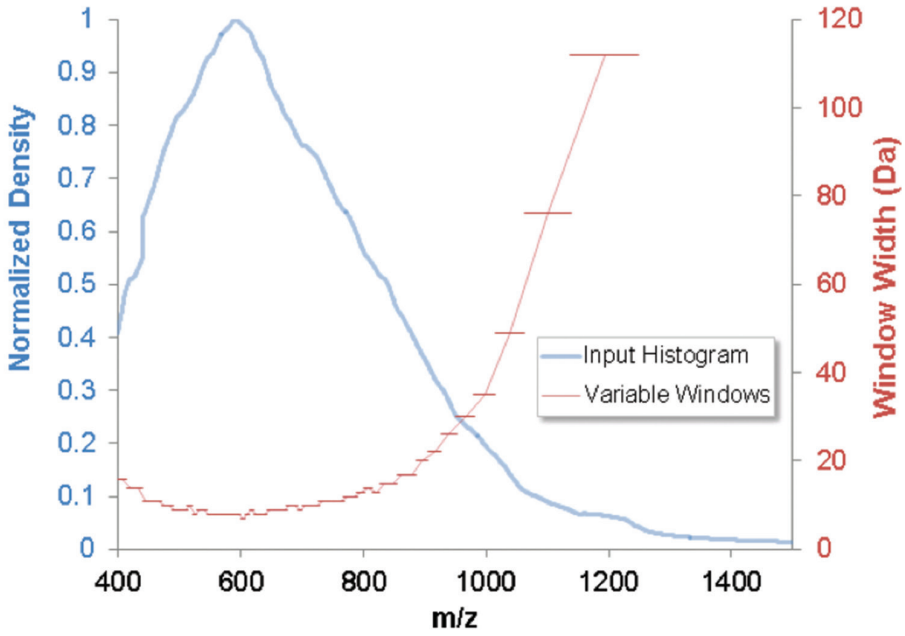


*Figure 2: Investigating variable Q1 window widths for SWATH™ acquisition. To achieve better specificity in complex matrices, smaller Q1 windows are desirable especially in the m/z dense regions where many peptide precursors are measured. The m/z density histograms constructed from the time-of-flight (TOF) MS data for the proteome of interest (blue line) can be used to construct variable sized windows (red line), where the density of precursors in each of the isolation windows is equalised across the m/z range [3].*

the higher specificity offered by smaller Q1 windows to distinguish the different spectra where many peptide precursors are measured, while wider Q1 windows can be used for areas where there are fewer precursors to measure. In this way, the entire mass range is interrogated thoroughly, providing more detail than before, and in an efficient and timely manner. The scientist can be assured that all detectable analytes are captured and that all species are accurately quantified and qualitatively characterised in full. The data is stored digitally, forming an immortalised record of the sample, which can be accessed and referred to time and time again.

In the latest generation of data-independent acquisition, Scanning SWATH acquisition has been introduced on the TripleTOF 6600+ system. Here, the Q1 window is actually sliding across the mass range, which in turn provides an additional dimension in the quantitative data. With this approach, the correlation between fragment and precursor can be determined, which can provide greater confidence in identification and improved data deconvolution.

An example of how these technologies have been used all together is in the Ralser lab, where the research focus is on the elucidation of metabolic network functionality at the genomic scale. The Ralser lab operates several mass spectrometers that record thousands of metabolomes, proteomes, and ionomes, which are then combined by using methods employing artificial intelligence. This data is used to identify key regulatory mechanisms that coordinate metabolic functionality in cells, particularly those involved in the metabolic cooperation that results from the exchange of metabolites between single cells. "The TripleTOF® 6600+ with Scanning SWATH® retains the sensitivity and data quality we have come to expect but adds the fast scanning capability that is critical for the next generation of proteomic applications, that we have increasingly become dependent on," says Dr Markus Ralser, PhD, the Senior Group Leader of the Molecular Biology of Metabolism Laboratory at The Francis Crick Institute, London, and Director of Biochemistry at the Charité, Berlin.

Collectively, the advances we have made in our MS instrumentation and acquisition mean that scientists can now more easily analyse large numbers of samples from diverse cohorts for large-scale multi-omics projects. Through our focus on industrialising omics, we have aimed at providing the innovative scientific pioneers, working at the intersection of precision health and personalised medicine, with the means they need to better investigate and understand the complex mechanisms at play in human health and disease.

## References

1. Sleno L. Foreword – Applications of high-resolution mass spectrometry in drug discovery and development. In: Sleno, L

(Editor). Applications of high-resolution mass spectrometry in drug discovery and development. Future Science Ltd., London, UK. 2014.

2. SCIEX. OptiFlow® Turbo V Ion Source. A Revolution in Low Flow Separations to Maintain the Perfect Balance of Robustness and Sensitivity. https://sciex.com/products/ion-sources/optiflow-turbo-v (October 2019).

3. SCIEX. Biomarkers and omics – Improved data quality using variable Q1 window widths in SWATH® acquisition. 2017. https://sciex.com/Documents/tech%20notes/Variable_Window_SWATH_Acquisition_TripleTOF_RUO-MKT-02-2879-B.pdf (October 2019).