K23 SWATH™ Acquisition vs. Information-Dependent Data Acquisition (IDA) With Application to Broad-Based Drug Screening

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After attending this presentation, attendees will understand the use of two powerful data acquisition modes as they pertain to laboratory screening for drugs of abuse by Liquid Chromatography/Time-Of-Flight/Mass Spectrometry (LC/TOF/MS). Attendees will be able to evaluate the capabilities and limitations of each and determine which mode is best suited to specific laboratory applications.

This presentation will impact the forensic science community by providing an assessment of data-rich LC/TOF acquisition modes and their effectiveness in solving complex drug-screening problems.

LC/TOF mass spectrometry is increasingly being utilized in laboratories across the country due to the ability to perform sensitive, highly specific, rapid screening for large numbers of compounds in short run times. The fast electronics of these systems allow the use of various data acquisition modes that trade-off file size and data complexity with sensitivity and various degrees of analytical specificity. We describe a comparison of two such data acquisition modes for an application focused on analysis of human blood samples for recreational and novel psychoactive drugs.

SWATH™ acquisition is a data-independent acquisition mode for use in broad-based drug screening that collects high resolution accurate mass data on all fragments of all parent masses throughout the chromatographic run. Complimentary to this method, the system offers a data-dependent acquisition mode, known as information-dependent data acquisition (IDA) that collects data only when triggered by criteria set within the analytical method. Both acquisition modes generate accurate mass data that can be searched against accurate mass extracted ion chromatogram (XIC) lists and accurate mass spectral library databases. This study employed the use of SWATH™ acquisition and IDA to blood samples injected in duplicate on opposing methods.

As part of a larger Institutional Review Board approved study, blood samples were collected from participants at an electronic dance music festival in Miami, FL. Blood samples were collected by a trained phlebotomist following explanation of study design and completion of informed consent. In total, 139 blood samples were collected over a three-year period, of which 40 samples were chosen at random for analysis in this study.

Screening analysis was performed at the Center for Forensic Science Research and Education (Willow Grove, PA). Blood samples (0.5mL) were extracted using Borax buffer (0.1M, pH 10.4) and n-butyl chloride/ethyl acetate (70:30). Instrumental analysis was performed using a TripleTOF™ 5600^{+} mass spectrometer (Sciex, Ontario, Canada) coupled with a Shimadzu Nexera XR ultra high performance liquid chromatograph (Shimadzu, Kyoto, Japan). A reverse phase gradient of ammonium formate (10mM, pH 3) and methanol/acetonitrile (50:50) was used to create chromatographic separation on a Phenomenex® Kinetex C18 analytical column (50mm x 3.0mm, 2.6µm) at a flow rate of 0.4mL min^{-1}. Following positive electrospray ionization, precursor ions were acquired by TOF MS scan and isolated based on overlapping mass range windows (SWATH™ acquisition) or traditional unit mass isolation (IDA). Fragmentation was achieved using a rolling collision energy of 35±15eV. Data processing was

performed using PeakView Software (Version 2.2) under identical criteria as previously determined and verified through method development and validation.

Of the 40 blood samples chosen at random, 30 (75%) screened negative by both SWATH™ acquisition and IDA. The remaining 10 samples were sent to NMS Labs (Willow Grove, PA) for appropriate confirmatory testing based on the screening results from both acquisition modes. Of these 10 samples, SWATH™ acquisition positively identified all compounds present in 9 (90%) samples, with 1 (10%) false positive. IDA positively identified all compounds present in 6 (60%) samples, with 1 (10%) false positive. Three (30%) false negative samples occurred during IDA screening for the following analytes: MDMA, oxymorphone, and levamisole. All three of the false negatives for IDA were attributed to failed library criteria.

In conclusion, SWATH™ acquisition and IDA were successfully used for the acquisition of accurate mass data from blood samples taken from human subjects. Both acquisition modes were able to identify compounds present in the blood samples, but SWATH™ acquisition more accurately identified compounds in relation to confirmatory testing. SWATH™ acquisition and IDA have distinct features and characteristics, but the data acquired in this study shows that SWATH™ acquisition could be preferred over IDA based on more reliable positive screening results.

LC/qTOF, SWATH™, SCIEX™