Comparison of accurate mass MS/MS acquisition and processing techniques on forensic toxicological screening

David M. Cox¹, Michael Jarvis¹, Xiang He², Evelyn McClure¹, Adrian M. Taylor¹  
¹ AB SCIEX, Concord, Ontario, Canada  
² AB SCIEX, Redwood City, CA, USA

Introduction:

Desirable attributes of a screening method include: rapid, unambiguous identification, with little or no method development and the ability to review past results without having to re-inject a sample. High resolution mass spectrometry (TOF or ion trap) yields generic methods that identify some compounds. However, many compounds cannot be unambiguously identified with MS1 evidence alone. MS/MS fragmentation yields rich, complimentary data to assist in identifying compounds. Obtaining MS/MS for every possible compound in a sample can be challenging. Data dependent techniques often miss candidates, and, due to their stochastic nature of candidate sampling, can give different results for injections of the same sample. Targeted techniques are limited in the number compounds that can be monitored, and require significant maintenance of known retention times. Data-independent techniques, such as SWATH™ acquisition, are capable of capturing MS1 and MS/MS evidence for all possible candidates. Such a generic and rapid method has the potential to identify more compounds with less method development, and enable retrospective data analysis. We compared several acquisition techniques, and several data processing techniques, in a toxicological screening scenario.

Methods:

Urine was spiked with over 120 drugs and compounds often found in forensics screening panels. The data was collected on a Triple TOF® 5600 system using one of the following methods 1) using a TOF-MS survey scan with IDA-triggering of up to 20 product ion scans or 2) SWATH acquisition. For SWATH acquisition, the precursor isolation window width was varied for each MS/MS experiment, or the windows were overlapped between each cycle. Data was processed in PeakView® software 2, using a research prototype of MasterView™ software.

Results:

To simulate identifying an unknown compound, clenbuterol was selected. Searching ChemSpider using MS1 precursor mass accuracy only, over 300 compounds were found even when using 1 ppm mass tolerance. Including isotopic information reduced the number of possible compounds to 76, regardless of typical mass tolerances. All of these possible structures shared the exact same molecular formula, therefore no information from MS1 could possibly differentiate them. Using MS/MS information and a structural
Comparison of accurate mass MS/MS acquisition and processing techniques on forensic toxicological screening

David M. Cox¹, Michael Jarvis¹, Xiang He², Evelyn McClure¹, Adrian M. Taylor¹
¹ AB SCIEX, Concord, Ontario, Canada
² AB SCIEX, Redwood City, CA, USA

fragmentation prediction tool, it was possible to reduce the list of possible structures to just clenbuterol (and its enantiomers), hydroxyl and amine positional isomers of clenbuterol, and one other compound.

Clenbuterol

To obtain MS/MS data for as many compounds as possible, either information dependent acquisition (IDA) or SWATH acquisition (data independent) was used. Library match purity scores were used as a surrogate indicator of how much effort would be required to identify an unknown compound. IDA failed to trigger on several compounds in spiked urine, leaving no MSMS data for interpretation. Unprocessed SWATH spectra had interferences from multiple
Comparison of accurate mass MS/MS acquisition and processing techniques on forensic toxicological screening

David M. Cox¹, Michael Jarvis¹, Xiang He², Evelyn McClure¹, Adrian M. Taylor¹
¹ AB SCIEX, Concord, Ontario, Canada
² AB SCIEX, Redwood City, CA, USA

compounds within the same spectrum. However, simple background subtraction was often enough to improve these spectra to something that could be interpreted easily. More sophisticated deconvolution techniques were able to significantly improve on this.

In addition to data processing techniques to reduce convolution, different acquisition techniques were compared. One of the simplest, yet most effective, changes was to vary the width of the SWATH window based on expected MS1 complexity. Regions with lots of precursors were given narrow SWATH windows, while regions with very few candidates were given large windows. Variable window acquisition also assists in separating the precursors that generate the same MSMS fragments (e.g. drugs and their metabolites or analogs) for higher data quality.

The decrease in isolation window size reduced the amount of convolved MSMS significantly. No changes to processing techniques were required to analyze this format of data compared to regular SWATH acquisition data.

Another SWATH acquisition technique used to reduce convolution was overlapping of SWATH acquisition windows between cycles. In this technique, alternating cycles had identical SWATH windows, however the start and end of these windows was shifted so as to introduce an overlap of windows between cycles. Thus precursors might be present in one window in odd numbered cycles but in a shifted window during even cycles. While interferences might be present in odd cycles, but absent in even cycles. The algorithm to demultiplex these signals was implemented to run on an NVIDIA 660 graphics card. The demultiplexed data was then processed using normal SWATH acquisition data processing tools.
Comparison of accurate mass MS/MS acquisition and processing techniques on forensic toxicological screening

David M. Cox¹, Michael Jarvis¹, Xiang He², Evelyn McClure¹, Adrian M. Taylor¹
¹ AB SCIEX, Concord, Ontario, Canada
² AB SCIEX, Redwood City, CA, USA

Conclusion:

SWATH acquisition methods acquire MS/MS for all compounds, at every time point, achieve identification results comparable to unit resolution IDA methods. Convolution of signals can be reduced by background subtraction, deconvolution techniques, or alternative SWATH acquisition strategies such as variable windows or overlapping windows.