K24 Impact of Novel Accurate Mass MS/MSALL Acquisition and Processing Techniques on Forensic Toxicological Screening

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After attending this presentation, attendees will be better informed about a novel data-independent mass spectrometric technique that allows Tandem Mass Spectrometry (MS/MS) of All possible candidates (MS/MSALL) and provides significant improvements in identification. Attendees will be aware of the ability to retrospectively analyze the data for compounds that were not originally targeted. This presentation will describe how powerful a technique rapid forensic toxicology screening by high-resolution mass spectrometry is, while showing that some compounds cannot be unambiguously identified with high-resolution MS measurements alone.

This presentation will impact the forensic science community by illustrating that data-independent techniques, such as SWATH™ acquisition (the MS/MS of all possible candidates), significantly improve identifications and enable retrospective analysis of the data.

Introduction: MS/MS fragmentation yields confident identifications of these compounds, but how to ensure quality MS/MS of these compounds? Data dependent techniques, although very powerful, cannot guarantee the measurement of all possible MS/MS candidates. Targeted MS/MS ensures acquisition of the target compounds, but limits the number of compounds.

Objectives: To evaluate the impact of improvements to SWATH™ acquisition, including variable precursor window sizes, overlapping windows, and deconvolution of MS/MS from multiple precursors.

Methods: Urine was spiked with more than 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) a TOF/MS survey scan with Information Dependent Acquisition (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 2.0, using a research prototype of MasterView™ software.

Results: Astemizole and amilodipine both demonstrated the advantage of having narrower SWATH™ isolation windows. The overlap 20 Da SWATH™ window acquisition (after demultiplexing was performed) resulted in MS/MS that were significantly reduced in interferences. The library match purity scores were improved from 2.2% to 97.5% and 38.8% to 92.7%, respectively. Having narrower isolation windows improves the specificity of MS/MS data, but at a cost. Either accumulation times must be decreased (which would make signal to noise worse) or cycle times will get longer (reducing the number of points across a peak). It is shown with the example of berberine that using overlap SWATH™ acquisition that the demultiplexed MS/MS can approach the quality of a true 10 Da SWATH™ acquisition MS/MS, while having an improved cycle time. Deconvolution of SWATH™ MS/MS was also shown to improve library match purity scores. Unprocessed SWATH™ MS/MS had significantly lower purity scores for many compounds. Simple background subtraction resulted in MS/MS of much better quality. Two other deconvolution techniques were tried. Method A was similar to techniques used for deconvolving Gas Chromatography/Mass Spectrometry (GC/MS) signals, and was implemented to run on an NVIDIA 660 graphics card. Method B is a novel technique making use of Principal Components Variable Grouping (PCVG) to obtain a SWATH™ MS/MS. When the techniques were combined, results were equivalent to those achieved using unit resolution IDA. For a few compounds, IDA was not triggered, resulting in no identification. While the SWATH™ acquisition was able to confidently identify these compounds with good purity scores.

Conclusion: SWATH™ acquisition methods acquire MS/MS for all compounds, at every time point, achieve identification results comparable to unit resolution IDA methods and overlap SWATH™ acquisition can improve cycle times and improve identification results.

Unknown Screening, Data Independent Acquisition, Comprehensive Analyte Coverage