Impact of novel MS/MS^{ALL} acquisition and processing techniques on forensic toxicological screening

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INTRODUCTION

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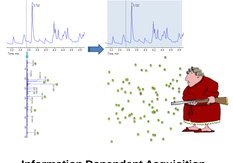
Rapid forensic toxicology screening by high resolution mass spectrometry is a powerful technique. However, some compounds cannot be unambiguously identified with high resolution MS1 measurements alone. MS/MS fragmentation yields confident identifications of these compounds, but how to ensure quality 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. Data-independent techniques, such as SWATH™ acquisition (the MS/MS of all possible candidates), improve identifications significantly and enable retrospective analysis of the data. The impact of improvements to SWATH acquisition, including variable precursor window sizes, overlapping windows, and data processing were evaluated.

- **Investigate variations of SWATH acquisition (MS/MS^{ALL})**
- **Compare identification results to best practice IDA methods**
- **Deconvolution of MS/MS from multiple precursors**

MATERIALS AND METHODS

Urine was spiked with over 120 drugs and compounds often found in forensics screening panels. The data was collected on a TripleTOF® 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 2.0, using a research prototype of MasterView™ software.



Information Dependent Acquisition

At any time point during an LC run, unit resolution MS/MS (0.7 Da wide precursor isolation window) are acquired for the top candidates. Good method settings ensure that almost all potential candidates are acquired at least once during the run. However, inevitably, there are some gaps in the coverage, and methods must be modified depending on LC peak widths and sample complexity to achieve optimal coverage.

SWATH Acquisition

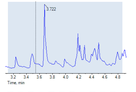
At every time point during an LC run, an MS/MS for a window of precursor ions is acquired. MS/MS for all possible candidates are acquired at every point in the LC run. Method settings are more generic. Individual MS/MS can be convolved with multiple precursors.

Available since Analyst TF 1.6.

Variable Window SWATH Acquisition

Identical to SWATH acquisition, except the width of the isolation window is varied. MS/MS for all possible candidates are acquired at every point in the LC run. Convolution due to multiple precursors is reduced.

Available in Analyst TF 1.7.



Overlap SWATH Acquisition

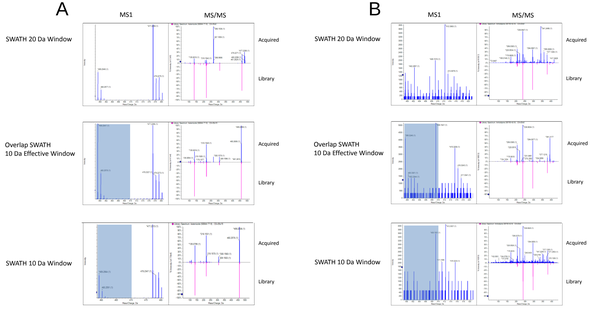
Identical to SWATH acquisition, except in each cycle, the beginning and end of each window are shifted. MS/MS for all possible candidates are acquired at every point in the LC run. Convolution due to multiple precursors is different from cycle to cycle, due to the shifted windows.

The differences between shifted windows enable the calculation of an MS/MS that would have resulted from a narrower window.

The data was acquired using prototype software, and the results were demultiplexed using custom software running on an NVDIA 660 graphics card.

Research prototype software.

RESULTS

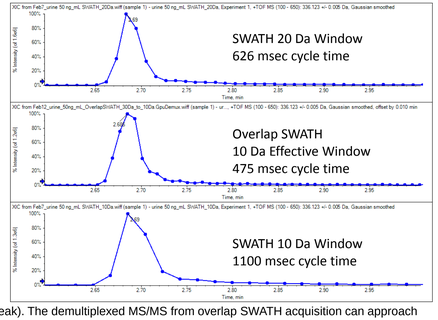


Advantages of narrower isolation windows

Two examples demonstrating the advantage of having narrower isolation windows.
A) Astemizole, a compound with a high intensity signal but with a significant interference within the same 20 Da SWATH window. Despite using background subtraction to obtain the MS/MS, there are significant interferences, resulting in a poor library match purity score of 2.2%.
B) Amlodipine, a compound with low intensity signal and numerous interferences within the 20 Da SWATH window. The MS/MS is clearly a mixture of several compounds. Purity score of 38.8%
In both examples, the overlap SWATH acquisition (after demultiplexing was performed) resulted in MS/MS that were significantly reduced in interferences. The library match purity scores were improved to 97.5% and 92.7% respectively. The spectral quality of the demultiplexed spectra were equivalent to spectra acquired from SWATH windows that were actually set to 10 Da wide.

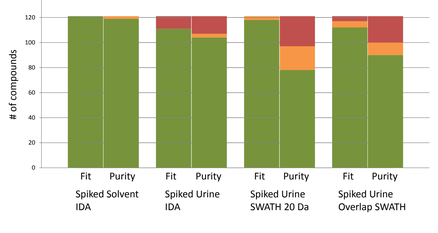
Improved cycle times

Extractions of berberine from the MS1 experiment of either a 20 Da window SWATH acquisition, an overlap SWATH acquisition (30 Da windows with a 10 Da overlap between cycles) or a 10 Da window SWATH acquisition. The accumulation time for MS/MS experiments was held constant at 25 msec. 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). The demultiplexed MS/MS from overlap SWATH acquisition can approach the quality of a true 10 Da SWATH acquisition MS/MS, while having an improved cycle time.



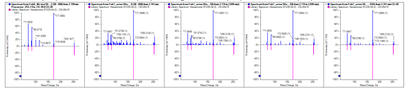
Screening using various acquisition modes

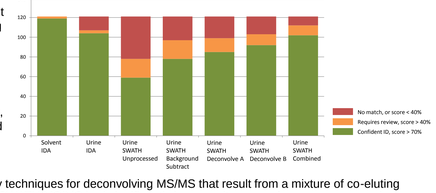
Various methods were used to screen compounds spiked into either solvent or urine. Identifications were made using mass accuracy, isotope patterns, retention time accuracy, and library scores. For this comparison, only library scores were plotted. Fit: A measure of how well a library spectrum matches the unknown spectrum. Purity: A measure of how well the unknown spectrum matches the library spectrum. Lower values indicate that either the match is less certain or that additional fragment ion peaks from another compound are present in the unknown spectrum.
In a spiked clean solvent sample, using IDA acquisition, both fit and purity scores were good for all screened compounds.
Spiked urine resulted in some ion suppression and interferences. A few compounds did not trigger MS/MS, resulting on no score. For the majority of compounds, an MS/MS was triggered, and the unit resolution isolation resulted in good purity scores.
With SWATH, since all possible MS/MS are acquired, no compounds were missed, resulting in acceptable fit scores for all compounds. However, purity scores were lower due to the wider 20 Da isolation windows.
With overlap SWATH, the effective 10 Da windows gave slightly improved purity scores compared to 20 Da windows.



Deconvolution of SWATH MS/MS improves library match purity scores

The wider windows of SWATH acquisition often lead to convolved spectra, resulting in lower purity scores. Compounds were still confidently identified using mass accuracy, isotope patterns, retention time accuracy, and library fit scores. However, achieving consistently high purity scores is important, particularly when an unknown compound is not in a library and must be identified by other methods, such as formula finding and ChemSpider. These methods work best with reasonably pure MS/MS.





Fortunately, there are many techniques for deconvolving MS/MS that result from a mixture of co-eluting compounds. 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 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.

CONCLUSIONS

SWATH acquisition methods:

- **Acquire MS/MS for all compounds, at every time point**
- **Achieve identification results comparable to unit resolution IDA methods**
- **Overlap SWATH acquisition can improve cycle times and improve identification results**

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TRADEMARKS/LICENSING