

SWATH[®] Acquisition vs. Information-Dependent Acquisition (IDA)

with Application to Broad-Based Drug Screening

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Introduction

SWATH[®] Acquisition is a data-independent acquisition mode that allows for broad-based drug screening and the acquisition of accurate mass MS/MS data for all possible candidates. SWATH[®] Acquisition employs Q1 isolation windows designed to pass only specific precursor ions in a given range through the quadrupole (Figure 1), allowing for more specific identification of fragment ions produced in the collision cell. This added level of specificity results in the ability to search the acquired accurate mass and MS/MS data against extensive databases and libraries. Information-Dependent Acquisition (IDA) employs unit Q1 isolation specific to one precursor ion, resulting in higher specificity of fragment ions produced.

While IDA can be analytically favorable over SWATH[®] Acquisition, MS/MS spectra are generated based on defined method criteria, adding potential uncertainty during acquisition.

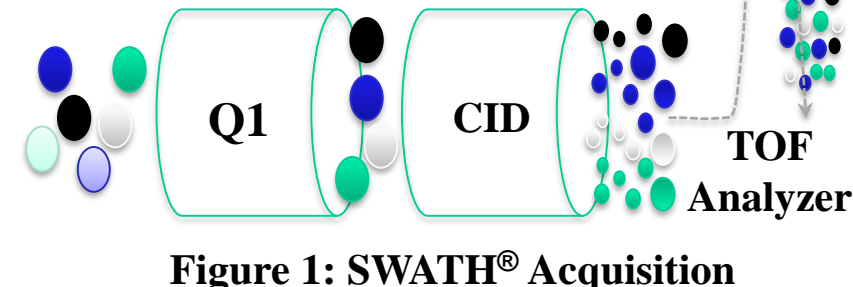


Figure 1: SWATH[®] Acquisition

Objective

To assess the performance of SWATH[®] Acquisition and IDA for broad-based drug screening, forty (40) blood samples, collected as part of a larger IRB-approved study, were chosen at random and screened using both acquisition modes. All samples containing analytes identified during the screening process were sent to NMS Labs for additional confirmation testing. Acquisition mode performance was determined based on agreement between screening and confirmation results. True positive (TP) identifications were defined as a positive screen and confirmation result, true negative (TN) identifications were defined as a negative screen and confirmation result, false positive (FP) identifications were defined as a positive screen but negative confirmation result, and false negative (FN) identifications were defined as a negative screen but positive confirmation result.

Methods

Sample Preparation

- Blood samples (0.5mL) were aliquoted to 13x100 test tubes
 - Blood controls were prepared by spiking control mixes to blank blood
 - 15 analytes across a variety of drug classes, including metabolites
 - High, mid, and low concentrations (ranging 0.05-75ng/mL)
- Internal standard (50µL, 0.1ng/µL) was added containing the compounds listed below:
 - Methylone-D3, MDMA-D5, Morphine-D3, and Alprazolam-D5

Sample Extraction

- Borax buffer (1mL, 0.1M, pH 10.4) was added and samples were vortexed
- Extraction solvent (3mL, 70:30 n-butyl chloride and ethyl acetate) was added
- Samples were capped and rotated for 10 minutes
- Samples were centrifuged at 4600 RPM for 15 minutes
- Supernatant was transferred using freeze/pour separation technique
- 10% HCl in methanol (100µL) was added
- Supernatant was evaporated to dryness at 35°C under 10 psi of nitrogen for 30 minutes
- Samples were reconstituted (100µL) in LC initial conditions

Instrumentation

Analysis by LC-QTOF

LC Instrumentation: Shimadzu Nexera XR Ultra High Performance Liquid Chromatograph

Column: Phenomenex[®] Kinetex C18 (2.6µm, 3.0 x 50mm)

Mobile Phase A (MPA): 10mM Ammonium Formate in Water, pH 3 with Formic Acid

Mobile Phase B (MPB): 0.1% Formic Acid in Acetonitrile and Methanol (50:50)

Table 1: LC Parameters	
Parameter	Set Point
Injection Volume	10µL
Flow Rate	0.4mL/min
Run Time	15.5 min

Table 2: LC Gradient		
Time (min)	% MPA	% MPB
0	95	5
1	95	5
10	5	95
13	5	95
13.5	95	5
15.5	95	5

MS Instrumentation: SCIEX TripleTOF[®] 5600+ Mass Spectrometer

Table 3: SWATH [®] and IDA Acquisition Parameters		
Parameter	SWATH [®] Set Point	IDA Set Point
Electrospray Ionization	Positive	Positive
Precursor Ion Acquisition	TOF MS	TOF MS
TOF MS Scan Range	100-510Da	100-1000Da
Candidate Ions per Cycle	All	16*
Collision Energy	35±15eV	35±15eV
Product Ion Acquisition	SWATH [®]	IDA
Q1 Isolation Windows	Variable: 6-34Da	Unit
Fragment Scan Range	40-510Da	40-1000Da
Cycle Time	0.77 sec	Variable

*Most intense (>100 cps)

Software

Data Processing

Software: PeakView[®] (Version 2.2) and MasterView[™] (Version 1.1)

An extracted ion chromatogram (XIC) list was generated containing 403 compounds with precursor accurate mass, fragment accurate mass (SWATH[®] acquisition only), and retention time data. Acquired data was processed based on pre-established criteria (Table 4) with acquired MS/MS data comparison to an accurate mass library database.

Table 4: Processing Criteria			
Criteria	Pass	Review*	Fail
Mass Error (ppm)	<5	<10	>10
Retention Time Error (min)	<0.25	<0.35	>0.35
Isotope Ratio (% Difference)	<30	<50	>50
Library Score	>90	>70	<70
Signal-to-Noise Ratio	>10	-	<10
Peak Intensity (counts)	>800	-	<800

*Additional criteria for analyst review included acceptable chromatography, acceptable chromatographic and mass spectral peak shape, acceptable library spectra, and control comparison.

Results

Table 5: Blood Results by Method (n=40)

Criteria	SWATH [®]	IDA
True Positive	9	6
True Negative	30	30
False Positive	1	1
False Negative	0	3

Both screening methods (SWATH[®] and IDA) produced one false positive, for different analytes in different blood samples. In total, 22 analytes were identified by SWATH[®] acquisition in 9 samples, all of which were confirmed. IDA only identified 19 of the 22 analytes, resulting in the three false negatives. All three analytes not positively identified during IDA screening were due to library spectra not being generated (Figure 2).

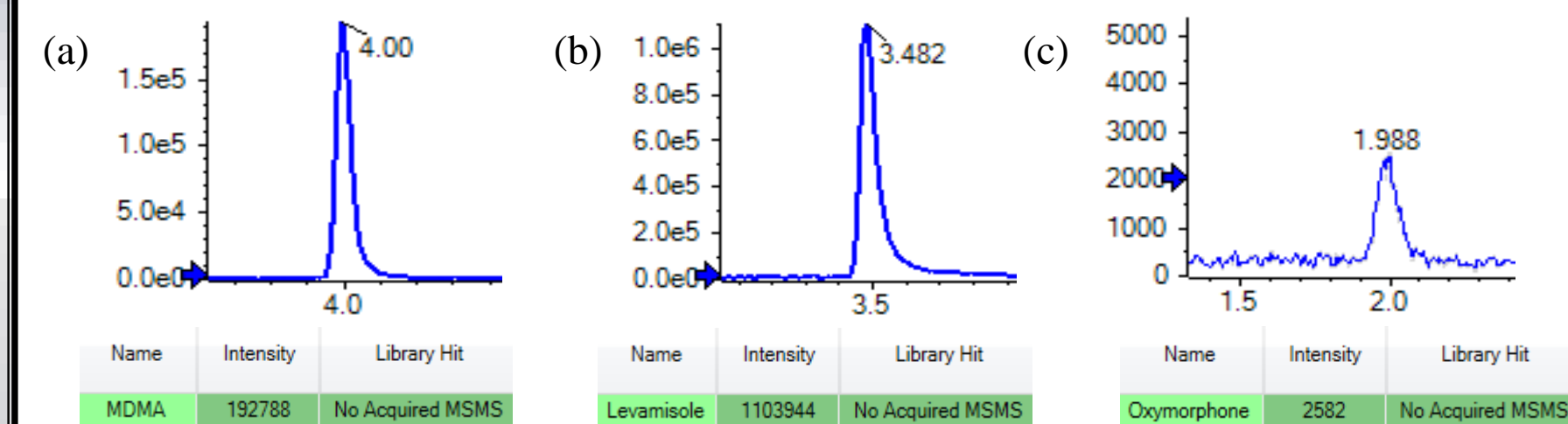


Figure 2: False Negatives by IDA Screen: Extracted Ion Chromatograms (TOF MS) and XIC List processed data for (a) MDMA, (b) Levamisole, and (c) Oxycodone

Conclusion

The applications of SWATH[®] Acquisition and IDA for broad-based drug screening were successful for the analysis of 40 blood samples collected in the field from living subjects, although SWATH[®] outperforming IDA overall. Accurate mass data, retention time data, and accurate mass library spectra were acquired via both acquisition modes and used during data processing for positive analyte identification. Accurate mass, retention time, and library score reliably produced positive identifications during processing of SWATH[®] acquired data, with no false negatives identified. While accurate mass and retention time were acceptable criteria for processing of IDA acquired data, library spectra generation was missed for certain compounds leading to misidentifications during data processing. Since library score is a criteria for positive analyte identification, absence of a library spectra resulted in failed library criteria and characterization as a false negative (n=3). This analysis and processing have proven SWATH[®] Acquisition to be a preferred means for broad-based drug screening on SCIEX QTOF systems, as SWATH[®] generated more acceptable and reliable results over IDA for analytes toxicologically relevant to forensic investigations.

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