SWATH® Acquisition Enables the Ultra-Fast and Accurate Determination of Novel Synthetic Opioids

Data Independent Acquisition on TripleTOF® and X-Series QTOF Systems for Seized Drug Analysis

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Novel synthetic opioids continue to cause widespread intoxications resulting in fatalities worldwide. As these compounds continue to surge within society, their timely and accurate detection is crucial to forensic investigators. Analysis of seized powders is highly contingent on specific and sensitive screening applications.

The combination of LC separations with SWATH Acquisition, on a TripleTOF or X500R system, gives forensic investigators a higher level of confidence in modern, synthetic opioid characterization by reliably obtaining comprehensive MS/MS spectral fragment information on every detectable component in the sample. Furthermore, it permits high throughput sample analysis, which can help reduce case backlog.

In this technical note, the use of SWATH Acquisition for the ultra-fast and accurate identification of novel synthetic opioids present in different seized drug samples was evaluated, and compared to typical GC/MS analysis.

Features of SWATH Acquisition for Forensic Drug Chemistry Analysis

- SWATH Acquisition helps the forensic investigator to quickly identify every component present in a seized illicit substance, whether at low or high concentration.
- As a data independent acquisition strategy, the forensic investigator can collect MS and MS/MS information on every detectable peak within the acquired mass range, giving the option to comprehensively re-interrogate the sample data should new questions arise in the future.
- SWATH Acquisition utilizes universal MS acquisition settings, requiring little to no method development to identify numerous compounds present in a seized sample in a single run.

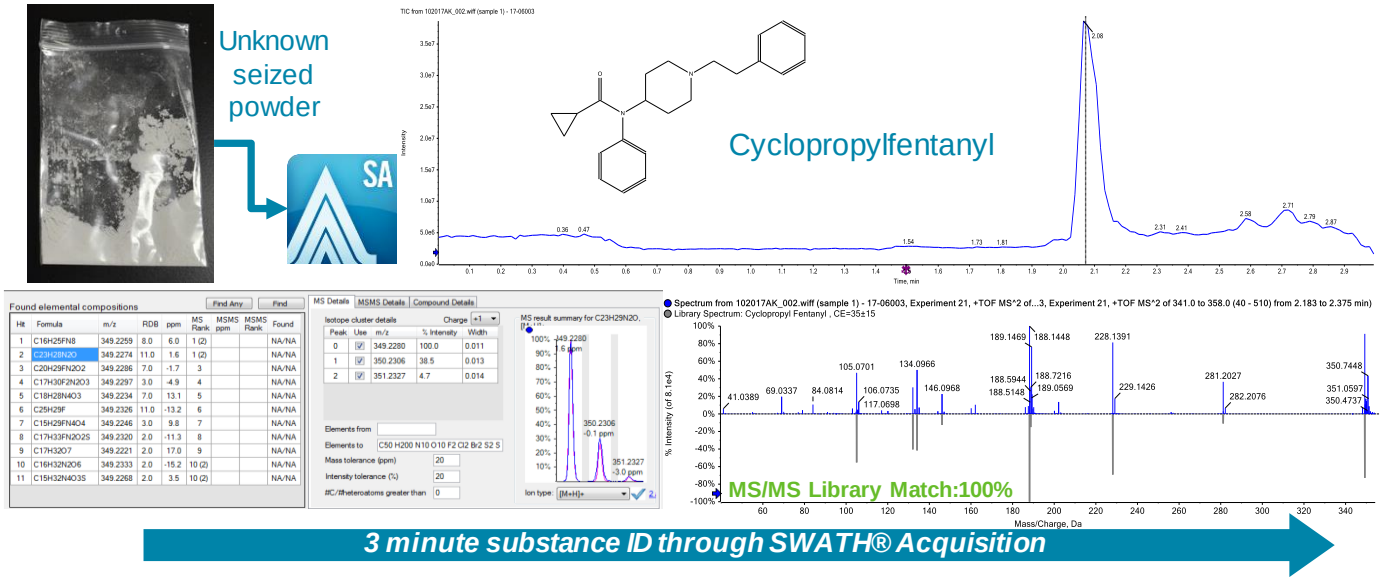

Figure 1. Obtain High Quality MS/MS Spectra with Confident Identification of Synthetic Drugs within a Single Workflow. Extracted ion chromatogram (XIC) (top) of an unknown seized powder was obtained using a 3 minute LC-MS method using SWATH Acquisition. Using the formula finder and MS/MS spectral library search, the novel opioid cyclopropylfentanyl was accurately identified.

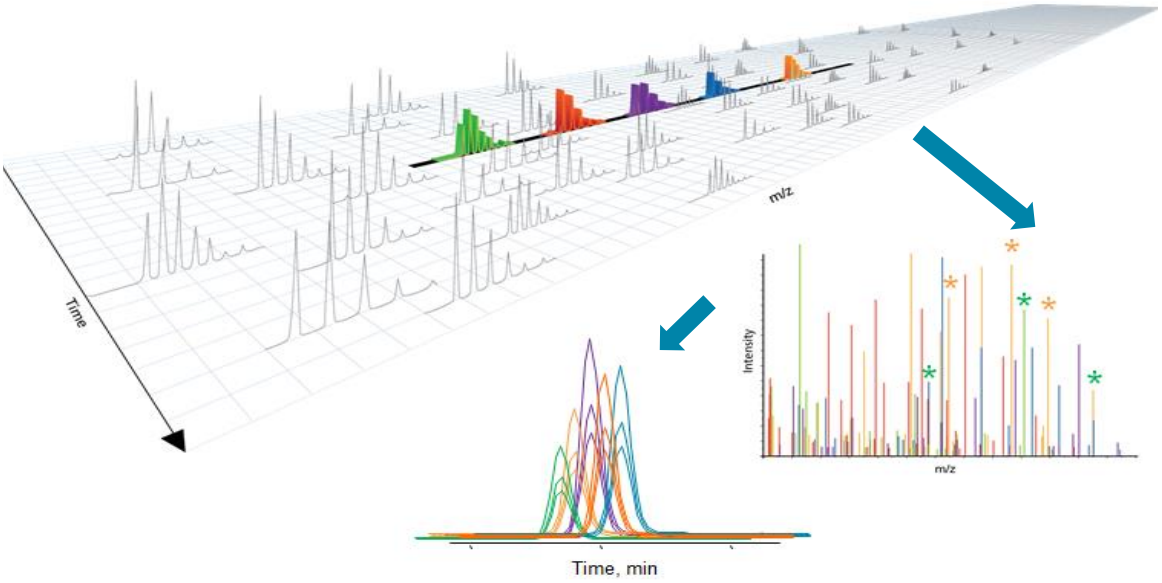

Figure 2. SWATH Acquisition Workflow. In this workflow, instead of the quadrupole (Q1) transmitting a narrow mass range through to the collision cell, a wider window containing more analytes is passed. This produces a richer MS/MS spectrum which is a composite of all the analytes within that Q1 m/z window. This MS/MS is performed across the full m/z range of target compounds, ensuring MS/MS is collected on every detectable compound. Because the fragment ions are generated using high resolution acquisition, detected compounds can be accurately identified.

Experimental Details

Sample Preparation: Powder drug samples, seized as part of forensically relevant investigations, were aliquoted into standard autosampler vials and diluted with 1 mL of methanol, followed by vortex mixing to ensure homogeneity. Subsequently, these samples were diluted 1:100 in LC mobile phase prior to LC/QTOF analysis.

Liquid Chromatography: An HPLC gradient separation was performed at 30 °C on a Phenomenex Kinetex C18 column (50 × 3 mm, 2.6µm). Ammonium formate in water (10 mM, pH 3) and methanol/acetonitrile (50:50) with 0.1% formic acid were used as mobile phase. The LC flow rate was 0.5 mL/min and the LC runtime was 3 minutes. Injection volume was 10 µL.

Mass Spectrometry: MS and MS/MS data were collected using SWATH Acquisition on the SCIEX TripleTOF 5600+ System with Analyst® TF software 1.6, each SWATH Acquisition scan beginning with a TOF MS experiment. Acquisition parameters are detailed in Table 1.

Data Analysis: Targeted data processing was performed using MasterView™ Software 1.1 and PeakView® Software 2.2 for positive analyte identification based on previously determined criteria. Four main confidence criteria were used including mass error (M), retention time shift (R), isotope ratio difference (I), and library score (L).

Table 1: Data Acquisition Parameters Used for the Analysis of Seized Drugs Samples.

Parameter	SWATH Acquisition Set Point
Electrospray Ionization	Positive
Precursor Ion Acquisition	TOF MS
TOF MS Scan Range	100-510 Da
Candidate Ions per Cycle	All
Collision Energy	35±15 eV
Product Ion Acquisition	SWATH Acquisition
Q1 Isolation Windows	Variable: 6-34 Da
Fragment Scan Range	40-510 Da

Subsequently, a combined score (C) was computed based on these four confidence categories (MRIL) with custom weightings. Finally, when there was no comparison sample (blank sample or sample spiked with drugs at reference level), the absolute peak intensity was used as additional criteria to help reduce false positive results.

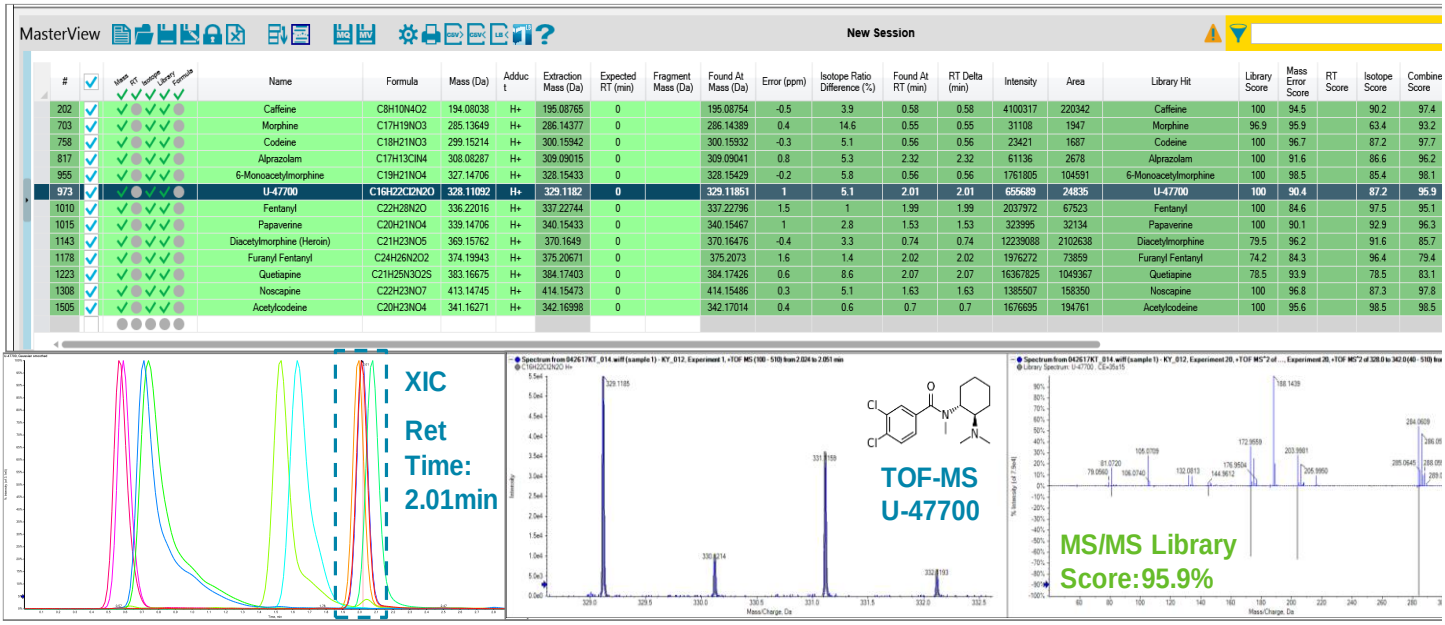

Figure 3. Quickly Retrieve Analyte Specific Information Present in an Unknown Powder. View of all novel psychoactive substances and other adulterants present in a powder sample by MasterView™ Software (Top). XICs of all compounds identified in the sample, showcasing TOF MS and MS/MS spectrum identification of the novel opioid U-47700 using spectral library search (Bottom).

Fast Identification of Multiple Compounds Present in Seized Drug Samples

Using SWATH Acquisition for analysis of dilute-and-shoot seized drug samples, several novel synthetic opioids, as well as other drugs of abuse, were successfully identified.

Figure 3 highlights a sample positive for U-47700, furanylfentanyl, and fentanyl, as well as heroin drug constituents, alprazolam, and other adulterating agents. Despite the ultra-fast sample acquisition run times, the MS/MS collected using variable window SWATH Acquisition had high resolution and mass accuracy, enabling the accurate identification of all components present in the seized drug sample.

U-47700 and fentanyl, in particular, were accurately identified, within the same SWATH Acquisition Q1 isolation window, even though these components share similar retention times and have closely related precursor ions (329 and 337 Da, respectively). High quality of MS/MS spectra was generated, and the library score of 95.9% was obtained for U-4700 and 95.1% for fentanyl.

High Quality MS/MS Leads to Accurate Compound Characterization

SWATH Acquisition generates comprehensive and high-quality MS/MS spectra, which enables reliable compound fragmentation for easy spectral library database searching.

Figure 4 shows the XIC of a seized drug sample indicating the presence of an illicit compound at 0.56 min. The MS/MS spectrum at that retention time generated two potential candidates for positive identification: 6-monoacetylmorphine (6-MAM) and naloxone. These two analytes are isobaric species with high incidences of presence in seized drug powders and toxicological casework samples.

The high-quality MS/MS fragmentation generated from SWATH Acquisition enabled the positive identification of 6-MAM (100% library score) instead of Naloxone (78.9% library Score). This principle of identification of co-eluting species based on high resolution accurate fragment ion masses would not be feasible using less specific analytical approaches like unit resolution mass spectrometry or molecular spectroscopy.

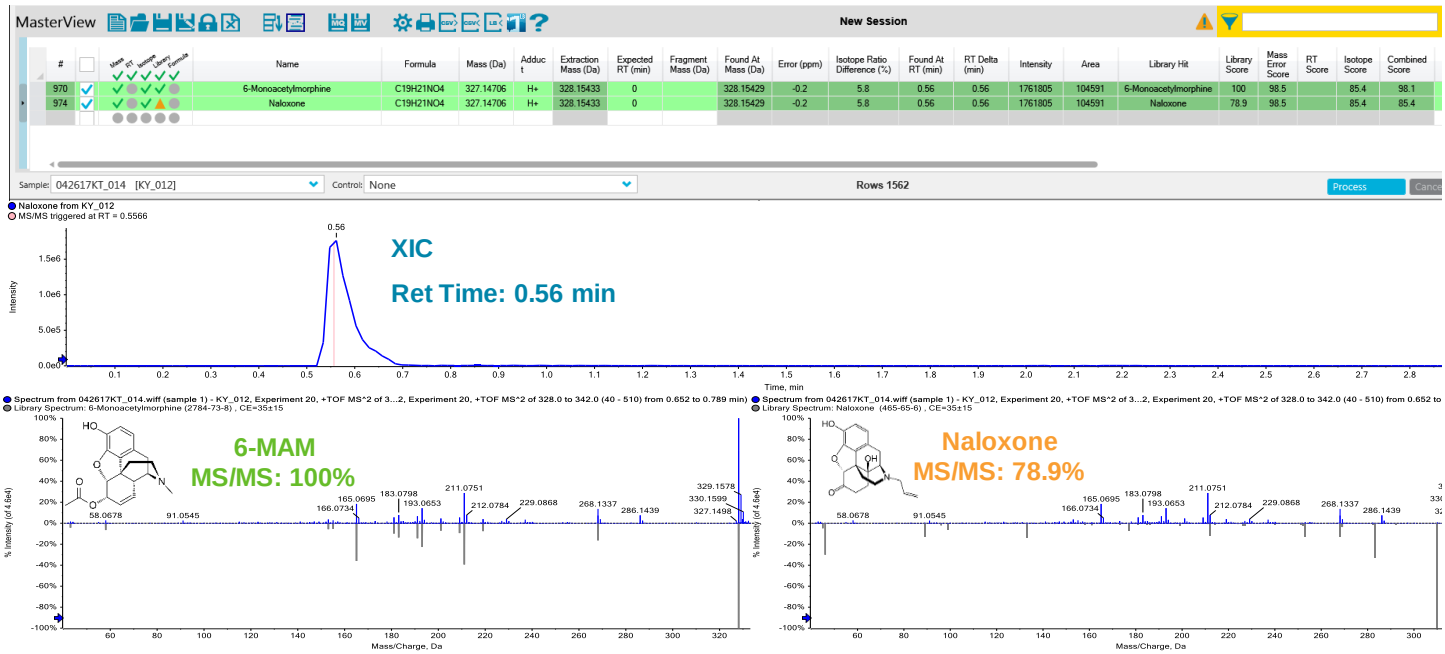

Figure 4. Minimizing False Positives by Obtaining High Quality MS/MS Spectra. MasterView™ software lists isobaric compounds identified in a powder sample (top), for which the XIC is displayed (middle). MS/MS spectra comparison between 6-MAM (bottom left) and Naloxone (bottom right) indicates significant fragmentation differences that enables the positive identification of 6-MAM through the confirmation using spectral libraries.

Enhanced Synthetic Opioid Detection with SWATH Acquisition in Comparison to Other Analytical Approaches

As part of this comprehensive study, more than 500 seized drug samples, prepared via dilute-and-shoot, were analyzed and compared using SWATH Acquisition and GC/MS acquired by full scan mode. Analytes identified by each method were compared side by side, and the unidentified compounds by a specific instrument were tallied.

A subset of seized drug samples obtained within a geographical region was further analyzed in this comparative study. It was determined that more than 10% of the overall targets were missed by GC/MS analysis, but positively identified by SWATH Acquisition. When further evaluating this gap, it was found that in comparison of novel opioid identification, more than 34% of novel opioids were missed by GC/MS (e.g. furanylfentanyl) (Figure 5).

A chart displaying an overall list of the different opioids found in this comprehensive study is also detailed in Figure 5. Other analytes missed by GC/MS but positively identified by SWATH Acquisition included novel stimulants and other psychoactive substances.

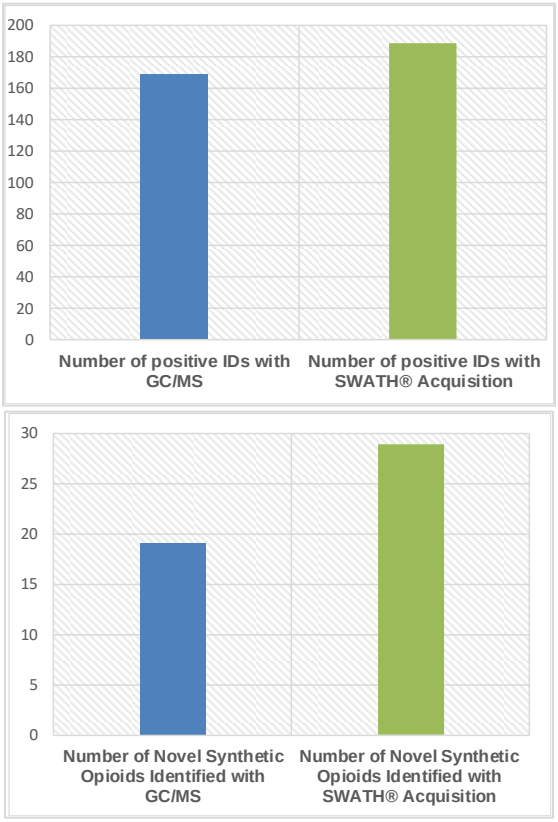

Figure 5. SWATH Acquisition Leads to Increased Compound Identification. (Top) Comparison chart shows a higher number of positively identified substances with SWATH Acquisition (green) in comparison to GC/MS (blue). Nine opioids were found in the SWATH Acquisition data but missed by GC/MS (Bottom).

Conclusions

The combination of ultra-fast LC separations with SWATH Acquisition provides forensic investigators quick and reliable identification of novel synthetic opioids present in seized drug samples.

- Using data independent acquisition strategies, several novel synthetic opioids, as well as other drugs of abuse, were successfully characterized.
- SWATH Acquisition generated comprehensive and high-quality MS/MS spectra, enabling reliable compound fragmentation comparison to library spectra for confident drug identification.
- Due to the comprehensive nature of SWATH Acquisition, improved novel synthetic opioid detection was achieved in comparison to GC/MS.

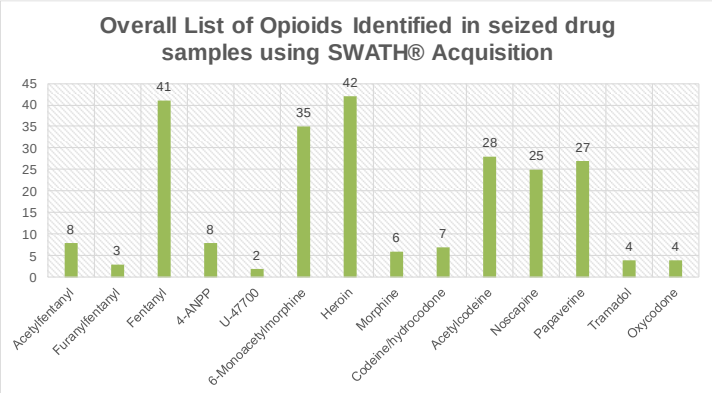

Figure 6. Overall List of Opioids Identified in a Larger Sample Cohort. More than 500 seized samples were analyzed with this dilute-and-shoot SWATH acquisition strategy. All of these opioids were accurately identified using the SWATH Acquisition workflow across the comprehensive study.

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