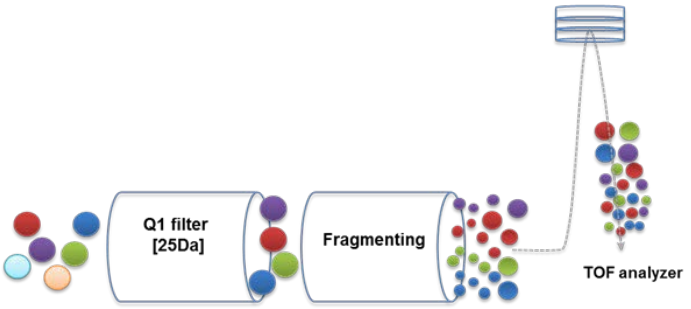
Using MS/MS^{All} with SWATH® Acquisition for Forensic Drugs Screening with SCIEX TripleTOF® 4600/5600+ LC-MS/MS System

Xiang He,[1] Adrian Taylor,[2] Michael Jarvis,[2] David Cox[2] and Alexandre Wang[2].
[1]SCIEX, 1201 Radio Rd, Redwood City, CA 94065, USA; [2]SCIEX, 71 Four Valley Drive, Concord, Ontario, L4K 4V8 Canada.

Overview

In this technical note, we investigated the use of MS/MS^{All} with SWATH® acquisition for screening applications in a forensic toxicological setting. We demonstrated that SWATH® acquisition is a viable tool for screening applications in biological samples with excellent positive detection rate. A novel SWATH® (variable window) data acquisition was tested and showed better results than the fixed window SWATH® acquisition method. Furthermore, SWATH® acquisition enabled more sensitive quantitation of lower concentration species in complex matrices utilizing the more selective MS/MS information.



MS/MS^{All} with SWATH® Acquisition

Introduction

Many techniques have been used for forensic drugs screening including immunoassay, gas chromatography mass spectrometry (GC-MS), and liquid chromatography tandem mass spectrometry (LC-MS/MS). Limitations in current technologies include insufficient selectivity and sensitivity, cross-reactivity, difficulty in adding new compounds, and lack of retrospectively analytical capability. Single stage high resolution accurate mass platforms (e.g. Time-of-flight or TOF) solved some of these challenges but have not been able to provide clean and characteristic MS/MS information for high-confidence structural confirmation. Quadrupole TOF (QTOF) mass spectrometers combine quadrupole with TOF analyzer and enable selection of precursor ions within narrow *m/z* window (< 1 a.m.u.) before fragmentation in Q2, thereby provide high-quality MS/MS information for data analysis.

For screening applications, users often have no prior knowledge about the number and the identities of the drugs in the samples, but need to report possibly all correct identifications (true positives) and not to report erroneous compounds (false positives) or miss correct compounds (false negatives). Therefore, the collected screening data should contain the necessary information for confident identification of any drug in the sample, dictating a non-targeted data acquisition approach for both MS and MS/MS levels.

Traditionally, the information dependent acquisition (IDA)-MS/MS has been used as the main data acquisition approach for forensic drug screening. The typical steps in each data acquisition cycle include: (1) TOF-MS scan in full mass range; (2) MS/MS scans of the top "n" ions primarily based on their intensity ranking. Information from TOF-MS scan, such as the mass to charge ratios, isotope patterns, LC retention times, were evaluated together with MS/MS information for the confident identification of any drug in the sample. Due to the high scanning speed (up to 100 Hz for single collision energy) on TripleTOF® 4600 and 5600+ systems, almost all potential drug targets in the samples can be confirmed with confident MS/MS library matching.

IDA-MS/MS is a non-targeted data acquisition method and the user needs to define the maximum number of candidates in each data cycle. More intense ions take higher priority within any data cycle, so for less abundant species especially in complex sample matrices, the associated MS/MS information might be missed. Therefore, an unbiased MS/MS data acquisition approach that collects MS/MS information for everything at all times (MS/MS^{All}) will solve this potential concern. Most of the existing MS/MS^{All} techniques collect MS and MS/MS information for all ions in an alternating fashion, i.e. MS scan of all precursor ions, followed by MS/MS scan of the fragments of all precursor ions. Without precursor ion selection, such approaches suffer from insufficient sensitivity, selectivity and narrower linear range compared to IDA-MS/MS.

MS/MS^{All} with SWATH® acquisition is a novel MS/MS^{All} technique. In every data cycle, the instrument acquires TOF-MS information; then it sequentially acquires MS/MS information of all precursor ions across a specified mass range in pre-divided mass windows. SWATH® acquisition is a unique MS/MS^{All} technique that records MS/MS information of everything at all times, and it significantly improves the MS/MS data quality by allowing sequentially programed MS/MS experiments therefore more selective MS/MS data collection (Figure 1).

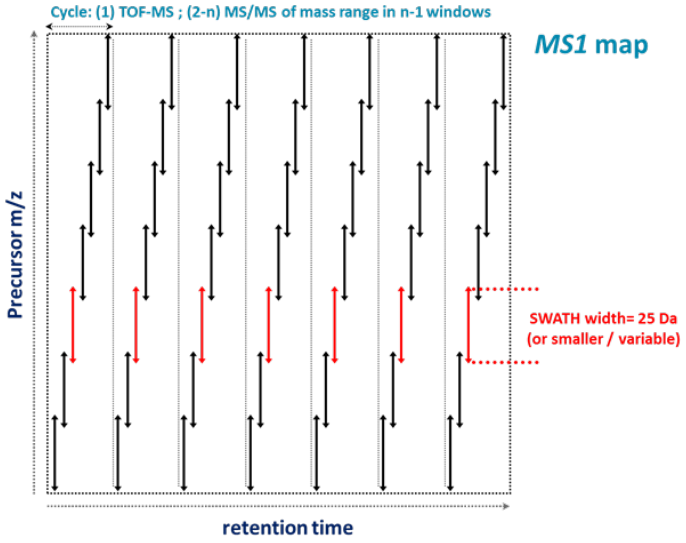


Figure 1: Principle of SWATH® acquisition in fixed window size.

In this technical note, we investigated the use of SWATH® acquisition for two sets of forensic drug containing samples; in human urine and blood matrices. We evaluated the true-positive detection rate in these two sets of samples for screening purposes, as well as the improvement on detection sensitivity with MS/MS information for quantitation purposes.

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Experimental

Sample Preparation

SPE (urine). Fourteen authentic human urine samples were extracted with SPE and dried down, before being reconstituted with 5% acetonitrile in water with a final dilution factor of 2. Injection volume was 2 µL.

Dilute and shoot (urine). Urine samples were diluted 10-fold with 10% methanol in water followed by ultra-centrifugation. Injection volume was 10 µL.

Protein precipitation (blood). 1 mL Blood sample was mixed with cold acetonitrile (1:2 v/v) and centrifuged. The supernatant was dried and reconstituted with 1 mL 20% methanol in water. Ten spiked blood samples and a blank were processed. Injection volume was 10 µL.

Liquid Chromatography

HPLC separation was performed at 30 °C on a reversed-phase HPLC column (50 × 2.1 mm). Mobile phases used were water and methanol with appropriate additives. The LC flow rate was 1.5 mL/min and the LC runtime was 6.5 min.

MS and MS/MS condition

MS and MS/MS Data was collected on both TripleTOF® 4600 and 5600+ mass spectrometers with Analyst® TF 1.7 software. Table 1 listed the data acquisition methods and source conditions for urine samples. For blood samples, only SWATH® acquisition with fixed window was used. For urine samples, both fixed and variable SWATH® acquisition windows were tested for the same mass range.

Table 1: Data acquisition methods and source parameters analyzing blood and urine samples

	SWATH®; fixed (blood)	SWATH®; variable (urine)
TOF-MS	100 to 1000 *m/z*, 0.1 sec	100 to 1000 *m/z*, 0.1 sec
Precursors of MS/MS	100 to 750 *m/z*, in 17 windows (40 m/z ea.)	120 to 475 *m/z*, in 15 windows (variable size)
MS/MS	40 to 1000 *m/z*, 25 ms x 17	40 to 1000 *m/z* x 15
Collision energy ramp	20 to 50 V	20 to 50 V
Total cycle time	**0.575 sec**	**0.525 sec**

List of Target Compounds

For blood samples, there were more than 450 drugs that were monitored. For urine samples, there were 67 drugs on the target list. The frequently detected compounds in human urine, such as acetaminophen, caffeine and its metabolites, were not included for urine samples.

Data analysis: Confidence settings and screening criteria

Data was processed in MasterView™ software 1.1. Reporting was performed also in MasterView™ software with customized report template.

Table 2: List of drug targets for urinary screening

Drugs analyzed in positive mode		
1-(3-chlorophenyl)piperazine	*Doxepin*	*Norbuprenorphine*
6-Monoacetylmorphine	*Doxylamine*	*Nordiazepam*
7-Aminoclonazepam	*Duloxetine*	*Nordoxepin*
Alpha-Hydroxyalprazolam	*EDDP*	*Norfentanyl*
Alprazolam	*Fentanyl*	*Norhydrocodone*
Amphetamine	*Fluconazole*	*Noroxycodone*
Atenolol	*Fluoxetine*	*Norpropoxyphene*
Baclofen	*Gabapentin*	*Nortriptyline*
Benzoylecgonine	*Glipizide*	*Oxazepam*
Buprenorphine	*Hydrocodone*	*Oxycodone*
Ceterizine	*Hydromorphone*	*Oxymorphone*
Chlorpheniramine	*Hydroxyzine*	*PCP*
Citalopram	*Lidocaine*	*Phentermine*
Clindamycin	*Lorazepam*	*Propoxyphene*
Codeine	*Meprobamate*	*Quinine*
Cotinine	*Methadone*	*Sertraline*
Cyclobenzaprine	*Methamphetamine*	*Tapentadol*
Desipramine	*Metoclopramide*	*Temazepam*
Dextromethorphan	*Mirtazapine*	*THC-COOH*
Diazepam	*Morphine*	*Tramadol*
Dihydrocodeine	*Naloxone*	*Tranylcypromine*
Diphenhydramine	*Naproxen*	*Trazodone*
Donepezil		

Figure 2 is an exemplary confidence setting used for screening. Four main confidence criteria were used for positive identification determination, which were mass error (M), RT error (R), isotope ratio difference (I), and library score (L). 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 an additional criteria to help reduce false positive rate.

Figure 2. Confidence settings in MasterView™ 1.1 Software

Results and Discussion

How to determine a positive identification

Although the screening data were acquired in non-targeted fashion, they were analyzed in a targeted way, which means a compound list was pre-assembled for analysis. This list contained the chemical formulas (for extracted ion mass calculation), mass extraction window, retention times and retention time window.

Table 4 showed the four hypothetical scenarios of screening outcome for a sample that contained 100 drugs. The ideal screening outcome was to report the correct 100 drugs. Such report required a (universally) **perfect criteria** which might work for the said sample but unlikely for all situations (Table 4). Very often, a loose criteria was used and a large number of positives were reported (**Criteria too loose**, Table 4), but there were still a handful true positives missed. Applying a more strict criteria would improve true positive rate (**Criteria too strict**, Table 3), but in the mean time generated more false negatives. It was essential to find good combinations of criteria for determining positive identifications (**Good criteria**).

Table 3: Partial list of drug targets (total of >450 drugs) for blood samples

Drugs analyzed in positive mode

2-Hydroxyethylflurazepam	*Benzoylecgonine*	*Cocaine*	*Fluoxetine*	*Mephedrone*	*Nicotine*	*Oxycodone*	*Sertraline*
6-Monoacetylmorphine	*Buprenorphine*	*Codeine*	*Gabapentin*	*Meprobamate*	*Nitrazepam*	*Oxymorphone*	*Temazepam*
7-Aminoclonazepam	*Bupropion*	*Cyclobenzaprine*	*Hydrocodone*	*Metaxalone*	*Norbuprenorphine*	*Paroxetine*	*THC*
7-Aminoflunitrazepam	*BZP*	*Demoxepam*	*Hydromorphone*	*Methadone*	*Norcitalopram*	*Phenazepam*	*THC-COOH*
Acetaminophen	*Carbamazepine*	*Dextromethorphan*	*Levetiracetam*	*Methamphetamine*	*Nordiazepam*	*Phentermine*	*THC-OH*
Alpha-hydroxyalprazolam	*Carisoprodol*	*Diazepam*	*Lidocaine*	*Midazolam*	*Norsertraline*	*Phenytoin*	*Tramadol*
Alpha-Hydroxymidazolam	*Chlordiazepoxide*	*Doxylamine*	*Lorazepam*	*Mirtazapine*	*Nortriptyline*	*Prazepam*	*Trazodone*
Alpha-hydroxytriazolam	*Chlorpheniramine*	*EDDP*	*MDA*	*Morphine*	*O-Desmethylvenlafaxine*	*Primidone*	*Triazolam*
Alprazolam	*Citalopram*	*Estazolam*	*MDMA*	*Naloxone*	*Olanzapine*	*Promethazine*	*Venlafaxine*
Amitriptyline	*Clobazam*	*Fentanyl*	*MDPV*	*N-Ethylcathinone*	*Oxazepam*	*Quetiapine*	*Zolpidem*
Amphetamine	*Clonazepam*	*Flunitrazepam*	*Meperidine*				

Table 4. Different hypothetical scenarios in determining positive identifications

	Perfect criteria	Criteria too loose	Criteria too strict	Good Criteria
Expected drugs [A]	100	100	100	100
Reported positives [B]	100	125	80	98
True positives [C]	100	85	75	95
False positives [B-C]	0	40	5	3
False negatives [A-C]	0	15	25	5

MS/MS^{All} with SWATH® acquisition Quantitation:

Quantitation can be done with MS information (TOF-MS full scan) or MS/MS information (MRM^{HR} and SWATH® acquisition).

1. Protein-precipitated blood samples.

For protein-precipitated blood samples, we evaluated the quantitative performances with MS information using TOF-MS full scan and MS/MS information with SWATH® acquisition. Due to added selectivity with MS/MS information, for several compounds, it was observed that quantitation with MS/MS had superior performance over single MS.

In Figure 3 and 4, quantitation of temazepam and clobazam with both MS and MS/MS was shown. Temazepam and clobazam are isobaric compounds. They have the same molecular formula of C16H13ClN2O2 and their retention times are similar. As a result, a reasonable LC separation was needed for accurate quantitation of both compounds with MS information (Figure 3-A and 4-A). However, temazepam and clobazam have their different fragment ions at 255.069 and 259.066 Th, respectively. Quantitation with these specific fragment ions significantly clarified the situation (Figure 3-B and 4-B).

Lower background signals were also observed with MS/MS quantitation. For blood sample #4, the background signal for bupropion precursor ion (Figure 5-A) was higher than that for its major fragment ion (Figure 5-B).

2. Diluted urine samples.

Figure 6 shows the extracted ion chromatograms of methadone, naloxone and sufentanil with both MS (top row) and MS/MS (acquired with SWATH® acquisition, bottom row). Overall, we observed cleaner LC chromatograms and/or lower baseline in MS/MS mode.

In the case of a SWATH® acquisition scan where ions are selected with a wide *m/z* window (i.e. 25 Th), to enter Q2 for fragmentation, there is a chance that compounds that fragmented at the same time (in same SWATH® acquisition mass window) will produce similar fragment ions. This can result in interference between compounds if they are not chromatographically resolved and the same fragment ion is

extracted for both compounds. Selectivity is lost if other fragment ions cannot be found that are diagnostic of each compound.

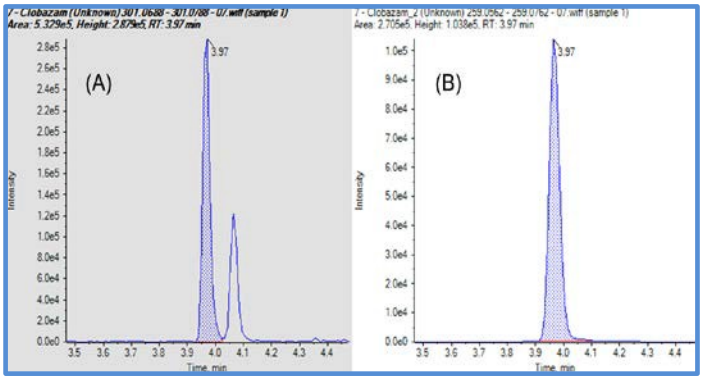


Figure 3. Extracted ion chromatograms of (A) clobazam precursor (3.97 min): 301.074±0.005 Th; and (B) clobazam fragment (3.97 min): 301.1→ 259.066 ±0.01 Th from blood sample #7

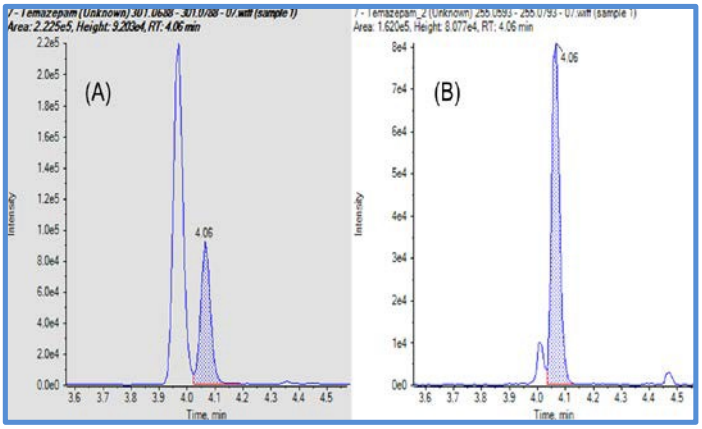


Figure 4: Extracted ion chromatograms of (A) temazepam precursor (4.06 min): 301.074±0.005 Th; and (B) temazepam fragment (4.06 min): 301.1→255.069±0.01 Th. This is blood sample #7

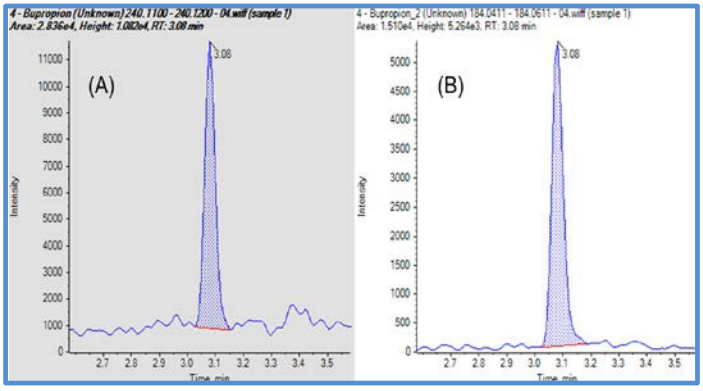


Figure 5: Extraction ion chromatograms of (A) bupropion precursor (3.08 min): 240.115±0.005 Th; and (B) bupropion fragment (3.08 min): 240.1→184.051±0.01 Th. This is blood sample #4.

We tested diluted urine samples spiked with multiple drugs and identified the following pairs of compounds that showed the above described issue: (1) amphetamine and methamphetamine, (2) MDA and MDMA, and (3) desmethyltapentadol and tapentadol. These compounds (in their respective groupings) differed only by one methylene group and generated the same major fragment ion in each group. For instance, amphetamine and methamphetamine have *m/z* of 136 and 150 Th and they were fragmented simultaneously (124 to 150 Th) in the method with fixed window. Both generated major fragment ion at 91.0553 Th. Extraction of ion 91.0553±0.005 Th from the MS/MS scan of precursor ions from 124 to 150 Th yielded two peaks at 1.96 and 2.25 min for amphetamine and methamphetamine, respectively. Same situations were observed for MDA and MDMA, and desmethyltapentadol and tapentadol (Figure 7).

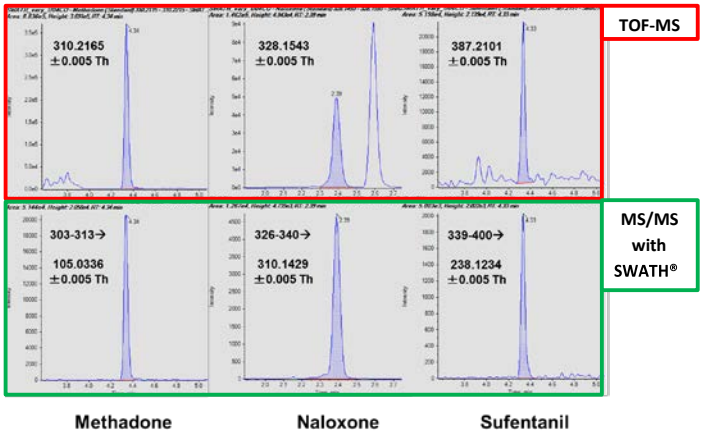


Figure 6. Quantitation in MS and MS/MS level with SWATH® acquisition for methadone, naloxone, sufentanil in 10-fold diluted urine at 40% cutoff concentrations.

A new feature in the latest version of Analyst® software (Analyst® TF 1.7) allows setting the SWATH® acquisition windows to variable sizes to further improve the selectivity of MS/MS acquisition. With this, SWATH® acquisition windows could be redesigned for the pairs discussed previously (amphetamine/methamphetamine, MDA/MDMA, and desmethyltapentadol/tapentadol). Figure 8 is an example of a SWATH® acquisition for ions from 120 to 500 Th with 16 SWATH® acquisition windows of variable size. All the compounds in adjacent cells with same colors were purposely distributed into different SWATH® acquisition windows for more selective MS/MS data acquisition.

With variable SWATH® acquisition window acquisition, the source of ambiguity was removed. We observed a clean peak for 120-140 → 91.0533±0.005 Th at 1.96 min (amphetamine), 164-190 → 105.0694 ±0.005 Th at 2.30 min (MDA), and 217-240 → 107.0512 ±0.005 Th at 2.27 min (tapentadol) (Figure 9).

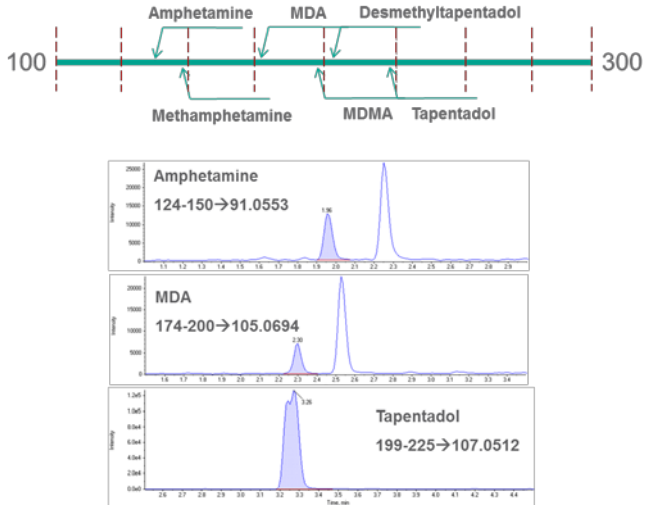


Figure 7. Data examples of SWATH® acquisition with fixed window size.

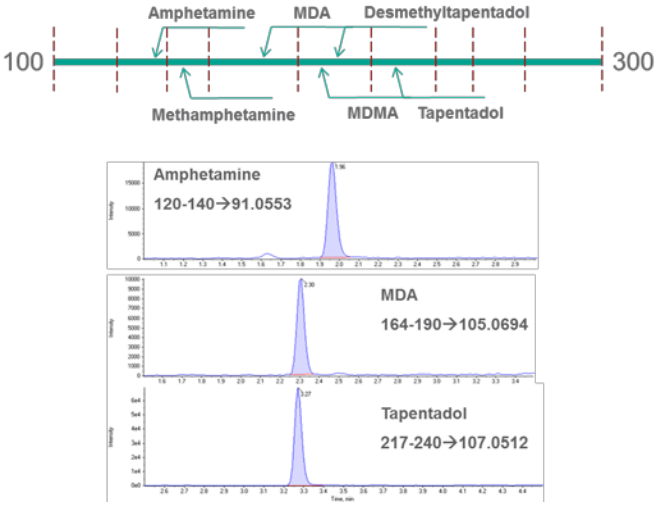


Figure 9. Data examples of SWATH® acquisition with variable window size.

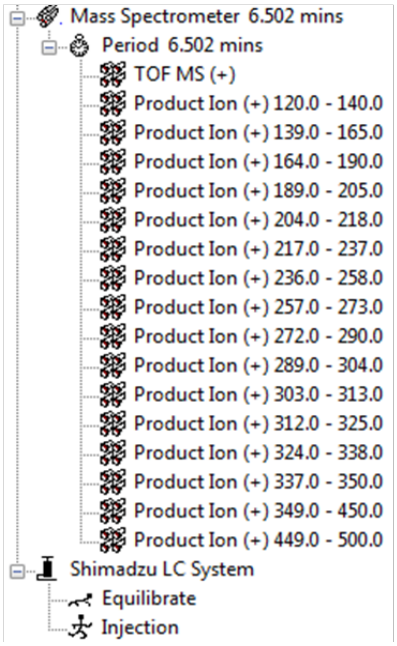
We tested >50 common forensic drugs in diluted urine samples. Figure 10 shows the calibration curves and extracted ion chromatograms (XIC) of two exemplary drugs, imipramine and sufentanil, at 40%, 100% and 300% cutoff concentrations.

The number of compounds that can be quantitated with SWATH® acquisition can be infinite. However, to have the best selectivity, users need (1) a well-designed data acquisition method, (2) appropriately selected internal standards, and (3) information of the unique and available fragments from target compound.

Screening:

Blood samples (protein precipitation)

SWATH acquisition with variable window



Pairs to separate (*not a complete list*)				
Amphetamine (136)				
Methamphetamine (150)				
MDA (180)				
MDMA (194)	Nortapentadol (204)			
	Tapentadol (222)			
Norketamine (224)				
Ketamine (238)	Nortramadol (250)			
	Tramadol (264)	Norvenlafaxine (264)	Nordoxepin (266)	Nordiazepam (271)
Morphine (286)	Norchlordiazepoxide (286)	Venlafaxine (278)	Doxepin (280)	Diazepam (285)
Codeine (300)	Chlordiazepoxide (300)	Norsertraline (292)	Norfluoxetine (296)	
Alprazolam (309)	Norcitalopram (311)	Sertraline (306)	Fuoxetine (310)	
Hydroxyalprazolam (325)	Citalopram (325)	Norclozapine (313)	THC (315)	
Norpropoxyphene (326)		Clozapine (327)	THC-OH (331)	
Propoxyphene (340)	Triazolam (343)			
Norbuprenorphine (414)	Hydrotriazolam (359)	Norverapamil (441)		
Buprenorphine (468)		Verapamil (455)		

Figure 8. MS/MS^{All} with SWATH® acquisition method using windows of variable sizes.

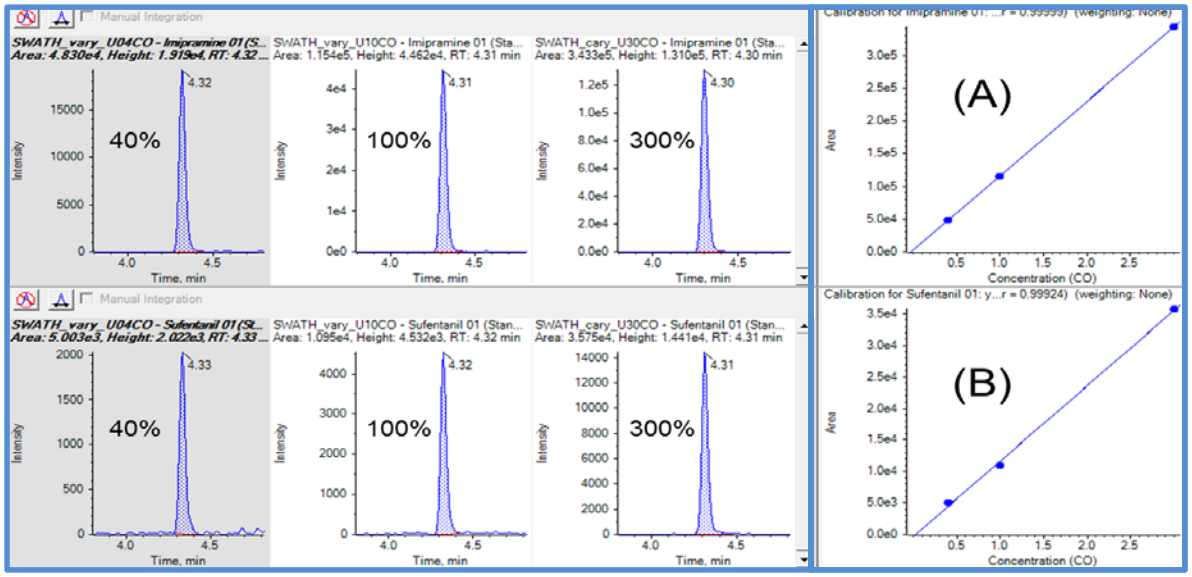


Figure 10. XICs and calibration curves of (A) imipramine, 272-290 → 86.098±0.005 Th; and (B) sufentanil, 339-400 → 238.1334±0.005 Th in diluted urine at 40, 100 and 300% of cutoff concentrations. Linear fit regression was used with r value at 0.99999 and 0.99924.

Ten protein-precipitated spiked blood samples and one blank blood sample were analyzed. Table 5 lists the criteria for determining positive identifications for both blood and urine samples. In blood, to be qualified as a positive identification, the entry needed to have absolute mass error less than 5 ppm, RT error less than 3%, library 'Fit' score better than 30%, combined score better than 70%, and an absolute intensity higher than 5000 cps. Because a matrix blank was used, a sample to control comparison processing was performed during data analysis by MasterView™ software automatically. Therefore, the last rule was that any detected peak needed to be 200% stronger than the same peak in the blank sample. Any positive identification needed to meet all the criteria. The operator also reviewed the data before the final report, especially for entries with marginal performances.

There were a total of 101 drugs that were expected in these 10 samples based on past tests using immunoassay, GC, and LC-UV with corresponding sample preparations. In this study, only protein precipitation was performed to simplify the preparation. Table 6 listed all the drugs that were detected by LC-MS/MS with SWATH® acquisition. A total of 91 drugs were reported as positives (90.1% detection rate) and no false positive was detected.

Among the missed detections (false negatives), firstly, some were simply not observed, such as norsertraline in #3, meperidine and lidocaine in #5, and THC and THC-COOH in #9. Majority of these missed detections were in low abundance.

Table 5. Criteria for positive identification of compounds from blood and urine samples

	Blood	Urine
Mass error (±ppm)	5	5
RT error (±%)	3	3
Isotope ratio diff. (%)	Not used	Not used
Library score (%)	30 (fit)	30 (fit)
Combined score (%)	70	55
Intensity (cps)	5000	2600

It is worth noting that the final sample reconstitution solution was 20% methanol in water, under which condition the recovery of THC and its metabolites was not ideal. Secondly, there were a few drugs that were detected with unmatched MS/MS confirmation, such as THC and THC-OH in #1, nitrazepam, clonazepam and lorazepam in #7, and olanzapine in #10. The reason for this was either low abundance of the precursor ions leading to weak MS/MS spectra, or the extra fragment ions in the MS/MS spectra from other precursors within the same SWATH® acquisition window of 40 Th. This was a drawback for SWATH® acquisition with fixed SWATH® acquisition window and can be addressed with variable SWATH® acquisition window, in which the precursor ion window can be adjusted to remove interferences. Overall, the detection rate was >90% and in line with IDA-MS/MS workflow (93.1% detection rate). The additional three positive identifications by IDA-MS/MS were nitrazepam, clonazepam and lorazepam in sample #7, data not shown).

Figure 11 showed the MS/MS matching for amitriptyline in sample 4. The blue trace was the observed MS/MS data for precursor ions from 259 to 300 Th in the vicinity of 3.95 min, where amitriptyline eluted. Background subtraction was performed to achieve cleaner MS/MS spectrum automatically by MasterView™ Software. Since 'Fit' score was used, no penalty was given for the additional ions (mostly the C13 isotope peak of amitriptyline precursor) shown in the MS/MS spectrum.

1	2	3	4	5
THC-COOH	Primidone	Venlafaxine	Trazodone	Carisoprodol
Nicotine	Phenytoin	O-Desmethylvenlafaxine	Tramadol	MDPV
11-OH-THC[1]		Doxylamine	Citalopram	Cocaine
	7	Chlorpheniramine	Amitriptyline	Zolpidem
6	Zolpidem	Cyclobenzaprine	Mirtazapine	Methadone
Hydrocodone	Alprazolam	Fluoxetine	MDMA	Diazepam
Codeine	Diazepam	Sertraline	Desmethylcitalopram	Meprobamate
Oxycodone	Midazolam	Amphetamine	Nortriptyline	Alprazolam
Morphine	Triazolam	Norsertraline[2]	Dextromethorphan	Nordiazepam
Hydromorphone	Estazolam		Bupropion	Mephedrone
6-MAM	Clobazam	8	MDA	Benzoylecgonine
Oxymorphone	Flunitrazepam	Naloxone	Paroxetine	N-Ethylcathinone
Acetaminophen	Alpha-hydroxymidazolam	Buprenorphine	Phentermine	Acetaminophen
	Prazepam	Acetaminophen		Methamphetamine
	2-Hydroxyethylflurazepam	Levetiracetam	9	EDDP
	Chlordiazepoxide	Norbuprenorphine	Trazodone	BZP
	7-Aminoflunitrazepam	Nicotine	Quetiapine	Meperidine[2]
	Temazepam		Alprazolam	Lidocaine[2]
	Alpha-Hydroxyalprazolam		Gabapentin	
	Alpha-Hydroxytriazolam		Morphine	10
	Nordiazepam		Morphine	Carbamazepine
	Nitrazepam[1]		Acetominophen	Zolpidem
	7-Aminoclonazepam		THC[2]	Carisoprodol
	Clonazepam[1]		THC-COOH[2]	Chlordiazepoxide
	Phenazepam			Fentanyl
	Oxazepam			Metaxalone
	Lorazepam[1]			Meprobamate
				Demoxepam
				Promethazine
				Olanzapine[1]

Table 6. Detected drugs in blood samples. Cells with red background were false negatives. (1)False negatives unmatched MS/MS information. (2)Not detected at all.

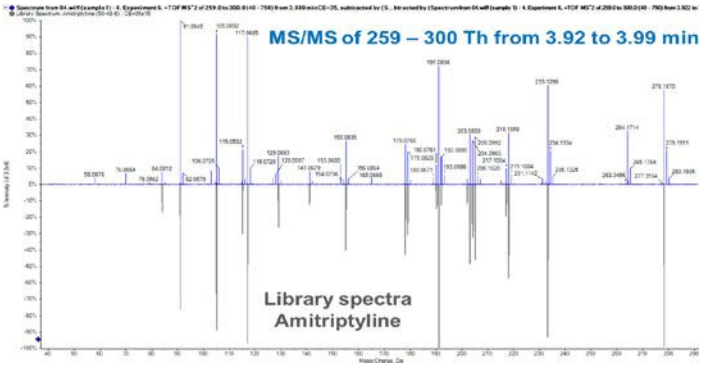


Figure 11: Library matching ('Fit') of amitriptyline in sample #4. This corresponded to 100% 'Fit' score despite the presence of several isotopic ions in observed MS/MS spectrum.

Urine samples (SPE)

Here we tested SWATH® acquisition with windows of variable size (SWV) for the 14 SPE-processed authentic urine samples. Table 5 listed the criteria for determining positive identifications in urine samples. To be qualified as a positive identification, the entry needed to have absolute mass error less than 5 ppm, RT error less than 3%, library 'Fit' score better than 30%, combined score better than 55%, and finally an absolute intensity higher than 2600 cps. The positive identification needed to meet all the criteria. Operator also reviewed the data before the final report, especially for entries that had a few categories that had marginal performances.

For any sample, the detected positive hits (DP) were compared to the expected list of drugs (TP) and the corresponding detection rate (DR) was calculated. Also, the calculated 'Fit' scores for all the positive identifications were averaged (AF) to reflect the average quality of library matching in each sample. Finally, the overall DR and AF were calculated for this method (Table 7).

Same as blood samples, there were no false positives reported based on the listed criteria. There were 10 false negatives due to unmatched MS/MS information (detection rate of 94% with average 'Fit' score of 95.2%). Compared to data using SWATH® acquisition with fixed window (90% detection; data not shown), the detection rate using SWATH® acquisition with variable window was significantly improved, proving the importance of MS/MS selectivity information.

Table 7. Detection rate and average 'Fit' scores in the 14 tested samples with SWV

SWV	Detected positives (DP)	Total positives (TP)	Detection rate (DR, %)	Average fit (AF,%)
S01	9	9	*100.0*	*95.3*
S03	9	*9*	*100.0*	*96.7*
S04	6	*6*	*100.0*	*99.8*
S05	15	*16*	*93.8*	*86.6*
S06	6	*6*	*100.0*	*89.0*
S07	5	*6*	*83.3*	*98.7*
S08	11	*13*	*84.6*	*99.3*
S09	24	*25*	*96.0*	*94.5*
S13	14	*14*	*100.0*	*99.3*
S14	8	*9*	*88.9*	*88.7*
S15	8	*8*	*100.0*	*87.5*
S16	5	*5*	*100.0*	*99.9*
S18	17	*18*	*94.4*	*99.2*
S20	*21*	*24*	*87.5*	*97.8*
ALL	***158***	***168***	***94.0***	***95.2***

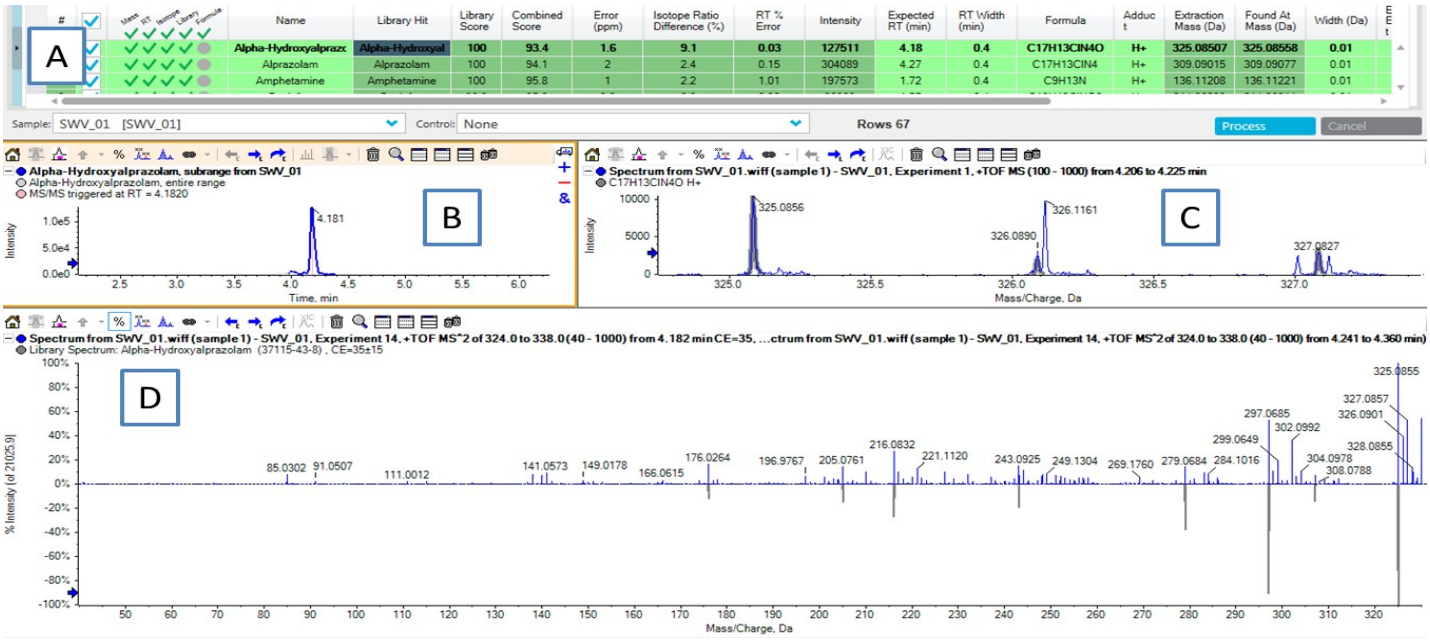


Figure 12. Identification of α-hydroxyalprazolam in sample 1. (A) Result summary showing mass error, RT error, isotope ratio difference, library score, combined score and intensity. (B) Extracted ion chromatograms of α-hydroxyalprazolam. (C) MS spectrum of α-hydroxyalprazolam at 4.21 min. (D) Library matching with 'Fit' score: observed MS/MS spectrum (blue) and library spectrum (grey).

Figure 13. Identification of metoclopramide in sample 4. (A) Result summary. (B) Extracted ion chromatograms of metoclopramide. (C) MS spectrum of metoclopramide at 2.81 min. (D) Library matching with 'Fit' score: observed MS/MS spectrum (blue) and library spectrum (grey).

Figure 12 and 13 shows two examples of positive identifications. Both cases showed excellent 'Fit' scores for MS/MS matching.

Conclusion

In this technical note, we described the use of MS/MS^{All} with SWATH® acquisition for forensic drug screening with TripleTOF® LC-MS/MS systems. We demonstrated that SWATH® acquisition is a viable tool for screening applications in both human urine and blood samples. We achieved >90% positive detection rate in blood samples with SWATH® acquisition (fixed window) and saw 95% detection rate in urine samples using a novel SWATH® (variable window) data acquisition.

With traditional TOF-MS-IDA-MS/MS, quantitation can only be performed from TOF-MS mode but not from the in situ sporadic TOF-MS/MS data points. It was shown in this study that, due to the continual and looped MS/MS scan function and better selectivity with the fragment ion information, SWATH® acquisition enabled more sensitive detection in MS/MS mode of lower concentration species in complex matrices.

Headquarters
500 Old Connecticut Path | Framingham, MA 01701 USA
Phone 508-383-7700
www.sciex.com

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