Streamlined Unknown Screening for Postmortem Analysis

Using the SCIEX X500R QTOF System and SWATH® Acquisition in a Forensic Toxicology Laboratory

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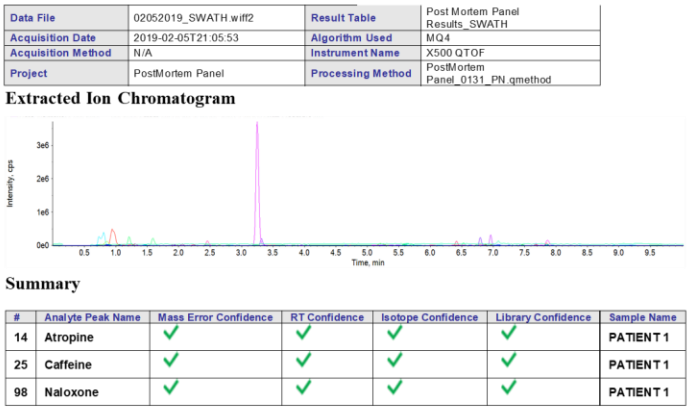
Gathering evidence to determine the cause of death is paramount in the service of the public interest and the judicial process. To this end, accurate identification of drugs present in postmortem samples is crucial for forensic toxicologists to successfully conduct case examinations as the findings of these examinations often raise important questions and provide immediate answers about cause of death and other related antemortem events.

The rapid emergence of novel psychoactive substances (NPS), designer drugs, and the abuse of prescribed drugs require fast and comprehensive drug screening approaches. Traditionally, postmortem drug screens are either performed by immunoassay or GC-MS. However, immunoassay techniques are often not conclusive enough (false positives) and lack sensitivity. GC-MS requires sample derivatization and lengthy chromatographic runs to accurately identify NPS and other drugs present in a biological postmortem sample. As a result, there is a need for rapid and robust screening methods that allow positive identification of NPS and other drugs with a high level of sensitivity and selectivity.



Data File	02052019_SWATH.wiff2	Result Table	Post Mortem Panel Results_SWATH
Acquisition Date	2019-02-05T21:05:53	Algorithm Used	MQ4
Acquisition Method	N/A	Instrument Name	X500 QTOF
Project	PostMortem Panel	Processing Method	PostMortem Panel_0131_PN.qmethod

#	Analyte Peak Name	Mass Error Confidence	RT Confidence	Isotope Confidence	Library Confidence	Sample Name
14	Atropine	✓	✓	✓	✓	PATIENT 1
25	Caffeine	✓	✓	✓	✓	PATIENT 1
98	Naloxone	✓	✓	✓	✓	PATIENT 1

Figure 1. Confidently Identify all Analytes Present Within an Unknown Postmortem Blood Sample. Obtain accurate mass data of all novel psychoactive substances and other drugs of interest present in a postmortem blood sample using SWATH® acquisition. Chromatogram and results table showing all positive target compounds identified in a blood sample based on the different acceptance criteria are easily generated using SCIEX OS Software.

High resolution mass spectrometers (HRMS) in the forensic laboratory allows toxicologists to rapidly obtain complete chemical profiles from biological samples. The acquisition of accurate mass, analyte specific MS/MS spectra often provides increased confidence in compound identification at low analyte concentrations.

In this technical note, a comprehensive drug screening workflow for the analysis of postmortem blood samples is described. The workflow was streamlined using a simplified sample preparation approach in combination with SWATH® Acquisition on the SCIEX X500R QTOF System.

Key Features of Postmortem Method

- Postmortem panel consisted of 151 drugs with limits of detection (LOD) down to the sub-ng/mL range
- Sample preparation was significantly simplified, using a protein precipitation with methanol and acetonitrile, followed by reconstitution with mobile phase
- Robust and reliable chromatographic separation was achieved using the vMethod™ Application for 664 forensic compounds[1] using the ExionLC™ AC HPLC system
- Analytes were monitored in positive ionization mode using SWATH® Acquisition on the SCIEX X500R QTOF with SCIEX OS Software
- The method allowed identification and quantification of nanogram (ng) detection limits of these drugs in a complex biological matrix

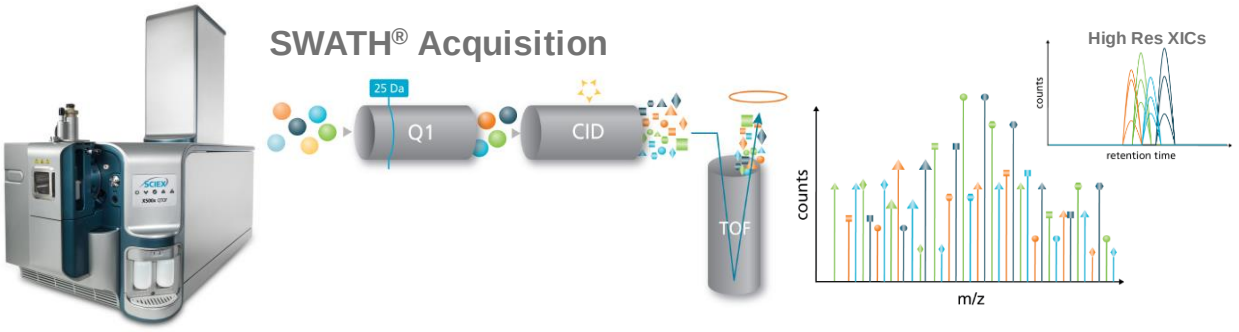


Figure 2. Avoid Missing Important Forensic Compounds with SCIEX X500R QTOF System and SWATH® Acquisition. In this workflow, instead of the quadrupole (Q1) transmitting a narrow mass range through to the collision cell, a wider window containing more analytes is passed. This produces a richer MS/MS spectrum 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 through extraction of the specific accurate mass fragment ions.

Experimental Details

Sample Preparation: Stock standard mixtures in neat solutions were prepared by diluting with methanol: water (20:80, v/v) to appropriate concentrations. These diluted standard mixtures were used to determine the retention times of the 151 targeted compounds. The full list of the forensic compounds used this method, including accurate mass information and limits of detection (LOD) is detailed in Table 1.

10 µL of the stock standard solution mixture containing the 151 different drugs were spiked into 90 µL of whole blood matrix for initial method development. Forensic case postmortem blood samples were extracted by using a protein precipitation procedure. In short, 900 µL of Methanol: MeCN (50:50, v/v) were added into the above mixture and vortexed for 1 min then followed by 3 min sonication and another 1 min vortex mixing. Then the samples were centrifuged for 5 min at 8,000 rpm. The supernatant was transferred out and completely dried down under nitrogen gas. The residues were reconstituted with 500 µL methanol: water (20:80, v/v). The protein precipitation procedure is shown in Figure 3.

Mix	•10 µL of std mixture with 90 µL of human whole blood
Load to tube	•900 µL of MeOH: MeCN (50:50, v/v)
Vortex	•1 min
Sonicate	•3 min
Vortex	•1 min
Centrifuge	•5 min at 8,000 rpm
Transfer	•Transfer supernatant to glass vial
Evaporate	•Evaporate to dryness under nitrogen
Reconstitute	•Add 500 µL of MeOH: water (20:80, v/v)

Figure 3. Protein Precipitation Procedure for Whole Blood Samples. A 9-step protein precipitation protocol was used for selectively extracting drugs from whole blood samples for analysis with the X500R QTOF System.

Liquid Chromatography: HPLC separation was performed on a Phenomenex Kinetex Phenyl-Hexyl column (50 × 2.1 mm, 2.6µm, 00B-4495-E0) on the SCIEX ExionLC™ AC system. Mobile phases used were water and methanol with appropriate additives. The injection volume was 5 µL and the total LC runtime was 8.5 minutes.

Mass Spectrometry: MS and MS/MS data were collected using SWATH® Acquisition on the SCIEX X500R QTOF System with SCIEX OS Software, each SWATH® Acquisition scan beginning with a TOF MS experiment.

Data Analysis: Targeted data processing was performed using SCIEX OS Software for positive analyte identification based on previously determined criteria. Four main confidence criteria were used including mass error (M), retention time (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, as shown in Figure 4.

Qualitative Rule	✓ Acceptable Difference		▲ Marginal Difference		● Unacceptable Difference		Combined Score Weight (%)
Mass Error (ppm)	<	5	<	10	>=	10	15
Fragment Mass Error (ppm)	<	5	<	10	>=	10	0
Error in Retention Time	<	5	<	10	>=	10	30
% Difference Isotope Ratio	<	20	<	40	>=	40	5
Library Hit Score	>	60	>	30	<=	30	50
Formula Finder Score	>	50	>	20	<=	20	20

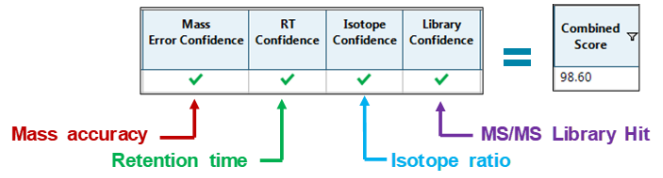


Figure 4. Confidence Criteria Used for Data Processing Using SCIEX OS Software. Mass Error (15%), Retention Time (30%), Isotope Ratio Difference (5%) and Library Score (50%) were used to generate a combined score.

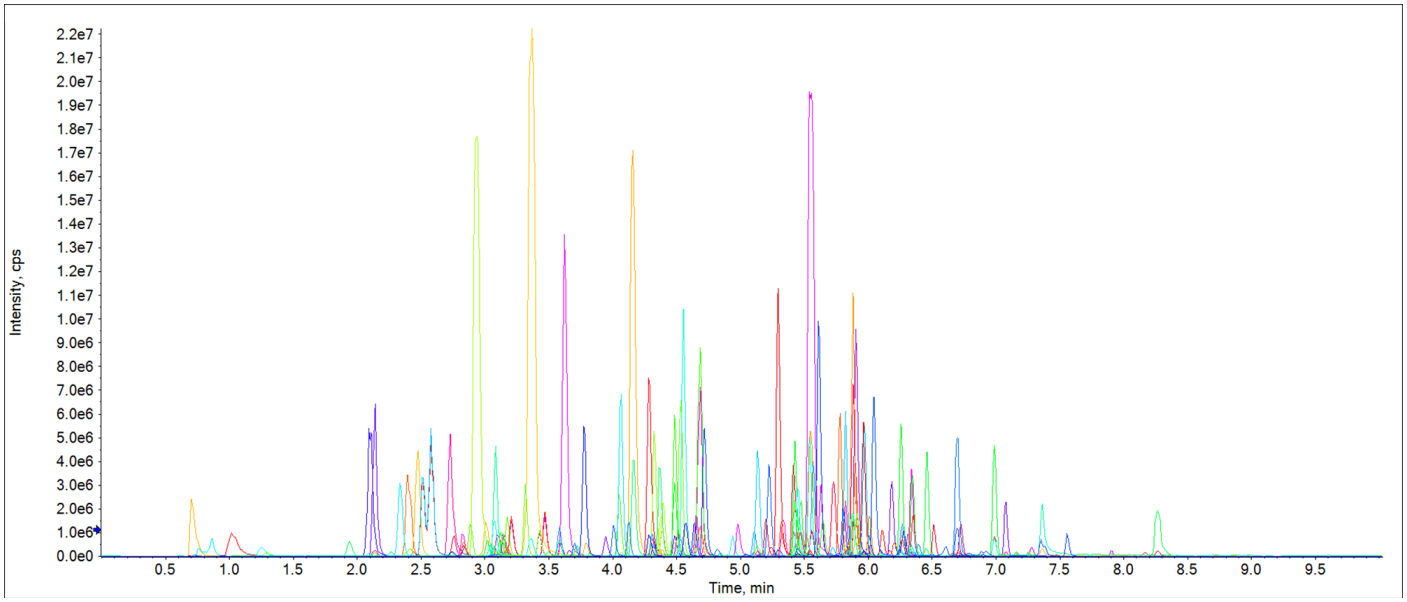


Figure 5. Obtain Fast and Confident Identification of Hundreds of NPS and Other Drugs of Interest in Biological Matrices. Extracted Ion Chromatogram (XIC) shows a rapid LC separation (8.5 min) and identification of 151 forensic compounds of interest spiked in whole blood using SWATH® acquisition.

vMethod Application for Comprehensive Screening of Postmortem Samples

Control whole blood samples spiked with all 151 forensic compounds of interest were prepared at various concentrations ranging from 2-12000 ng/mL. These standard solutions were extracted and injected to build a data analysis processing method.

The separation conditions for the vMethod Application for 664 forensic compounds[1] were initially used and further optimized to a final 8.5 min LC run time. Figure 5 shows the extracted ion chromatogram (XIC) for all 151 forensic compounds in a control whole blood sample using the optimized LC conditions.

Information-dependent acquisition (IDA) was initially applied to acquire and store MS/MS spectra for each target compound of interest. However, SWATH® Acquisition was chosen as the preferred data acquisition method as this strategy provides comprehensive fragment ion spectra generation over the whole run, minimizing the risk of missing potential forensic compounds present in postmortem blood samples in comparison to IDA[2,3].

Through the method development process, it was important to obtain the limit of detections (LOD) of all 151 compounds. Figure 6 shows the XIC, TOF-MS and MS/MS spectra of 3 representative forensic compounds spiked in whole blood matrix at different LOD concentrations. SWATH® Acquisition generated comprehensive and high-quality MS/MS spectra, enabling reliable compound fragmentation for spectral library database searching for the analytes.

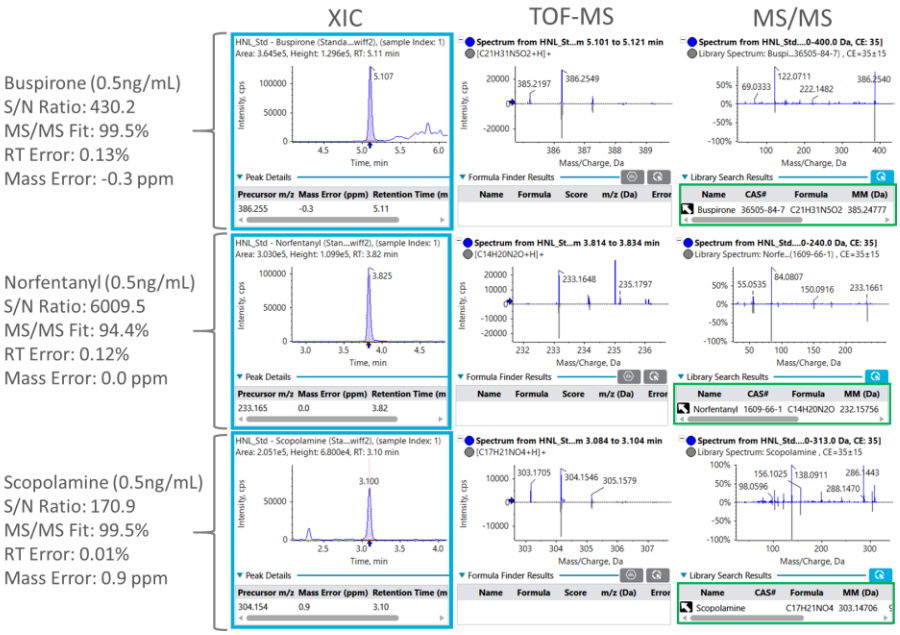


Figure 6. SWATH Acquisition Leads to Increased Compound Identification. XICs, TOF-MS and MS/MS spectra obtained showing confident and detailed identification of buspirone (top), norfentanyl (middle) and scopolamine (bottom) spiked in whole blood at low ng/mL concentrations.

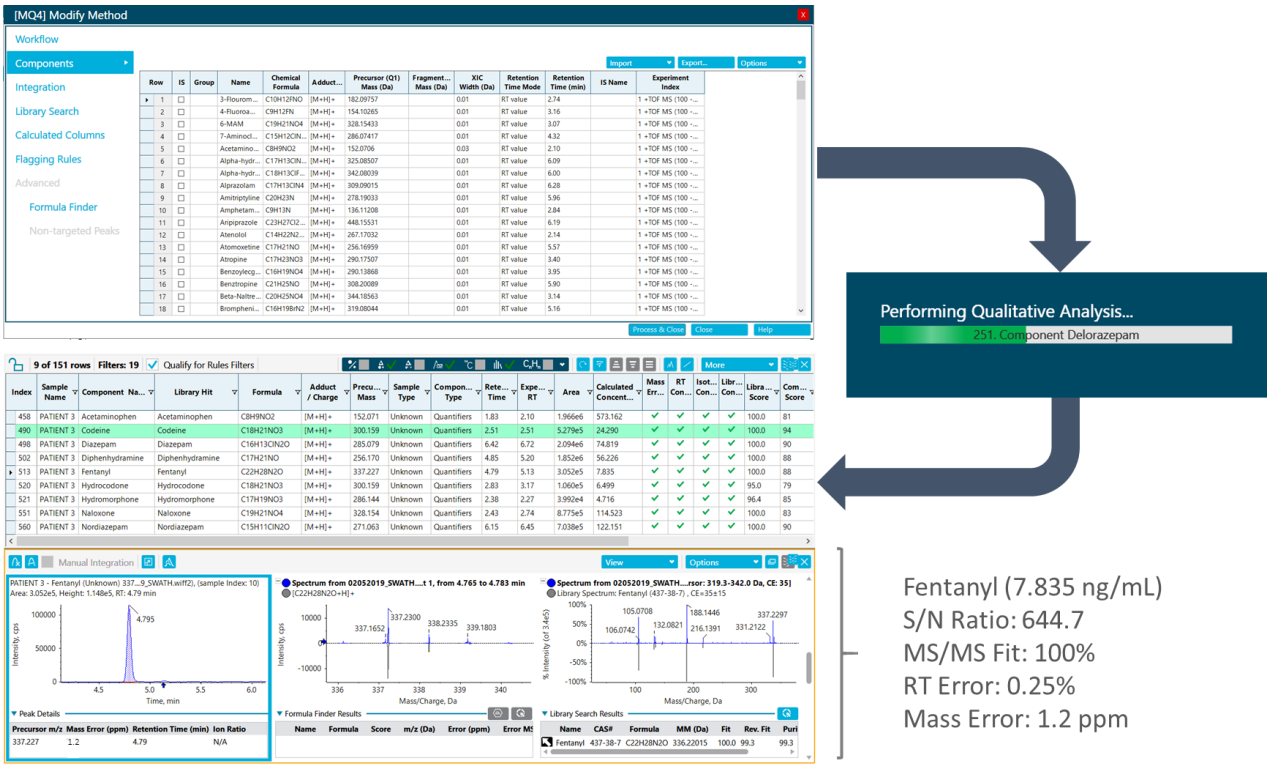


Figure 7. Re-Interrogate the Data Obtained from the Analysis of Postmortem Samples Using SCIEX OS Software. Data acquired from a postmortem blood sample, suspected of containing an NPS, was reprocessed after modifying the processing method window (top). The novel opioid Fentanyl (bottom) was retrospectively identified from the SWATH® Acquisition data.

Discovering Novel Psychoactive Substances Present in Postmortem Samples Through Retrospective Analysis

As a data independent acquisition strategy, SWATH® Acquisition allows the forensic toxicologists to collect MS and MS/MS information on every detectable peak within a sample, essentially creating a digital record of the sample. This allows the option to re-interrogate the sample data should new questions arise in the future.

Figure 7 displays a postmortem blood sample screened using SWATH® Acquisition, where 10 forensic compounds targeted in the data processing method were successfully identified and quantified above the LOQ in the first round of data processing. Next, the same sample was re-interrogated for the presence of a potential known NPS (i.e., Fentanyl), by extracting the compound's molecular formula ($C_{21}H_{26}N_2O$). Based on the confidence criteria set in SCIEX OS Software Fentanyl was detected with good confidence in the interrogated sample. This highlights the ability for users to retrospectively analyze previously acquired SWATH data sets and screen for new compounds without having to re-inject samples, when newly identified forensic targets are discovered.

Streamlined Sample Reporting for Efficient Forensic Case Turnaround Time

The data analysis component of SCIEX OS Software is designed to provide a centralized results grid for streamlined review and efficient sample report processing. Retention time, mass, isotope ratio error, and mass spectral library search score are calculated automatically and visualized using "traffic lights". Compounds identified with high confidence are indicated using green check symbols.

The results table can then be sorted by the 'traffic light' columns and/or filtered by 'identification criteria' for review and reporting of the positively identified compounds.

Figure 8 shows a customized report generated by SCIEX OS Software, after the processing of a postmortem blood sample, where 10 forensic compounds of interest were confidently identified based on the acceptance criteria. The sample report included XICs, TOF-MS and MS/MS spectra as well as the library matches. Table 2 summarizes the results of the postmortem blood sample and includes the list of compounds detected along with their concentration, library score and combined score.

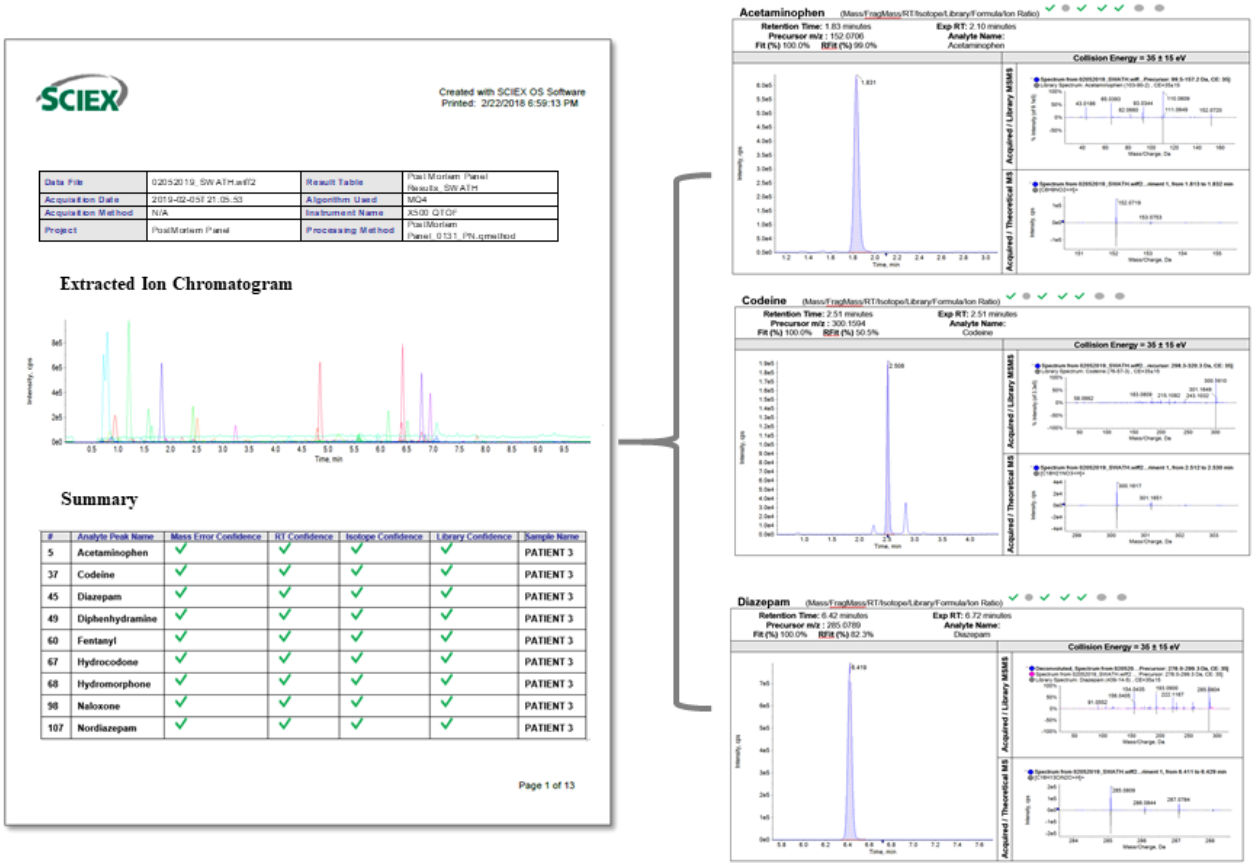


Figure 8. Streamlined Data Processing Through Customized Sample Reports Using SCIEX OS Software. A postmortem blood sample report was generated identifying all of the forensic compounds identified (left**)** and representative XICs, TOF-MS and MS/MS spectra (right).

Table 2. Summary Table for Postmortem Blood Sample for Case Sample #3. Inter-day average (n=3) for the detection of 10 compounds screened in a postmortem blood sample.

Drug Detected	Concentration (ng/mL)	Library Score (%)	Combined Score (%)
Acetaminophen	*773.162 ± 7.4*	*100*	*81.3 ± 0.2*
Codeine	*24.290 ± 2.1*	*95.7*	*89.5 ± 0.1*
Diazepam	*74.819 ± 2.4*	*98.3*	*94.6 ± 0.6*
Diphenhydramine	*56.226 ± 1.2*	*100*	*94.9 ± 1.1*
Fentanyl	*7.835 ± 0.3*	*100*	*90.3 ± 0.8*
Hydrocodone	*6.499 ± 0.4*	*95*	*79.3 ± 0.5*
Hydromorphone	*4.716 ± 0.1*	*96.4*	*85.4 ± 1.2*
Morphine	*29.732 ± 1.8*	*89.8*	*73.2 ± 0.5*
Naloxone	*114.523 ± 3.4*	*100*	*82.8 ± 1.2*
Nordiazepam	*122.151 ± 1.4*	*100*	*90.1 ± 0.4*

Conclusions

A comprehensive drug screening workflow for the analysis of postmortem blood samples was successfully developed using the SCIEX X500R QTOF System.

- The adaptation of the vMethod Application LC-MS conditions enabled the rapid implementation and optimization of the screening workflow for 151 forensic compounds of interest.
- SWATH® Acquisition generated comprehensive and high-quality MS/MS spectra, which enabled reliable compound fragmentation comparison to library spectra for confident drug identification. The data independent nature of SWATH® Acquisition allows for retrospective analysis to avoid missing potential NPS present in postmortem samples.
- The data analysis component of SCIEX OS Software provided a simplified interface for streamlined data review based a rigorous scoring system, a “traffic light” display, and an efficient sample report generation process.

Table 1. List of Compounds Used in the Postmortem Panel Along with Chemical Formula, Precursor Mass and Cutoff Concentration (LOD).

Component Name	Retention Time	Chemical Formula	Precursor (Q1) Mass (Da)	Adduct & Charge	LOD (ng/mL)
3-Fluoromethcathinone HCl	*2.74*	$C_{10}H_{12}FNO$	*182.09757*	*[M+H]+*	*5*
4-Fluoroamphetamine	*3.16*	$C_9H_{12}FN$	*154.10265*	*[M+H]+*	*5*
6MAM	*3.07*	$C_{19}H_{21}NO_4$	*328.15433*	*[M+H]+*	*2.5*
7-Aminoclonazepam	*4.32*	$C_{15}H_{12}ClN_3O$	*286.07417*	*[M+H]+*	*5*
Acetaminophen	*2.1*	$C_8H_9NO_2$	*152.0706*	*[M+H]+*	*250*
Alphahydroxyalprazolam	*6.09*	$C_{17}H_{13}ClN_4O$	*325.08507*	*[M+H]+*	*5*
Alphahydroxymidazolam	*6*	$C_{18}H_{13}ClFN_3O$	*342.08039*	*[M+H]+*	*5*
Alprazolam	*6.28*	$C_{17}H_{13}ClN_4$	*309.09015*	*[M+H]+*	*5*
Amitriptyline HCl	*5.96*	$C_{20}H_{23}N$	*278.19033*	*[M+H]+*	*12.5*
Amphetamine	*2.84*	$C_9H_{13}N$	*136.11208*	*[M+H]+*	*10*
Aripiprazole	*6.19*	$C_{23}H_{27}Cl_2N_3O_2$	*448.15531*	*[M+H]+*	*25*
Atenolol	*2.14*	$C_{14}H_{22}N_2O_3$	*267.17032*	*[M+H]+*	*50*
Atomoxatine HCL	*5.57*	$C_{17}H_{21}NO$	*256.16959*	*[M+H]+*	*5*
Atropine	*3.4*	$C_{17}H_{23}NO_3$	*290.17507*	*[M+H]+*	*1*
Benzoylecgonine	*3.95*	$C_{16}H_{19}NO_4$	*290.13868*	*[M+H]+*	*2.5*
Benztropine	*5.9*	$C_{21}H_{25}NO$	*308.20089*	*[M+H]+*	*25*
Beta-Naltrexol	*3.14*	$C_{20}H_{25}NO_4$	*344.18563*	*[M+H]+*	*5*
Brompheniramine maleate	*5.16*	$C_{16}H_{19}BrN2$	*319.08044*	*[M+H]+*	*1*
Buphedrone HCl	*3.43*	$C_{11}H_{15}NO$	*178.12264*	*[M+H]+*	*5*
Buprenorphine	*5.26*	$C_{29}H_{41}NO_4$	*468.31084*	*[M+H]+*	*0.5*
Bupropion HCl	*4.51*	$C_{13}H_{18}ClNO$	*240.11497*	*[M+H]+*	*12.5*
Buspirone	*5.1*	$C_{21}H_{31}N_5O_2$	*386.25505*	*[M+H]+*	*0.5*
Butorphanol tartrate	*4.66*	$C_{21}H_{29}NO_2$	*328.22711*	*[M+H]+*	*5*
Butylone HCl	*3.47*	$C_{12}H_{15}NO_3$	*222.11247*	*[M+H]+*	*0.5*
Caffeine	*3.25*	$C_8H_{10}N_4O_2$	*195.08765*	*[M+H]+*	*250*
Carbamazepine	*5.54*	$C_{15}H_{12}N_2O$	*237.10224*	*[M+H]+*	*2500*
Carboxyzolpidem	*3.59*	$C_{19}H_{19}N_3O_3$	*338.14992*	*[M+H]+*	*2.5*
Carisoprodol	*5.63*	$C_{12}H_{24}N2O_4$	*261.18088*	*[M+H]+*	*25*
Chlorpheniramine maleate	*4.98*	$C_{16}H_{19}ClN_2$	*275.13095*	*[M+H]+*	*5*
Chlorpromazine HCl	*6.31*	$C_{17}H_{19}ClN_2S$	*319.10302*	*[M+H]+*	*5*
Citalopram HBr	*5.33*	$C_{20}H_{21}FN_2O$	*325.17107*	*[M+H]+*	*5*
Clomipramine	*6.34*	$C_{19}H23ClN_2$	*315.16225*	*[M+H]+*	*12.5*

Component Name	Retention Time	Chemical Formula	Precursor (Q1) Mass (Da)	Adduct & Charge	LOD (ng/mL)
Clonidine	2.82	$C_9H_9Cl_2N_3$	230.02463	[M+H]+	5
Clozapine	5.42	$C_{18}H_{19}ClN_4$	327.1371	[M+H]+	5
Cocaethylene	4.72	$C_{18}H_{23}NO_4$	318.16998	[M+H]+	2.5
Cocaine	4.31	$C_{17}H_{21}NO_4$	304.15433	[M+H]+	2.5
Codeine	2.51	$C_{18}H_{21}NO_3$	300.15942	[M+H]+	2.5
Cotinine	2.73	$C_{10}H_{12}N_2O$	177.10224	[M+H]+	250
Cyclobenzaprine HCl	5.82	$C_{20}H_{21}N$	276.17468	[M+H]+	5
Delorazepam	6.29	$C_{15}H_{10}Cl_2N_2O$	305.02429	[M+H]+	0.5
Desalkylflurazepam	6.17	$C_{15}H_{10}ClFN_2O$	289.05385	[M+H]+	5
Desipramine HCl	5.88	$C_{18}H_{22}N_2$	267.18558	[M+H]+	25
Desomorphine	3.09	$C_{17}H_{21}NO_2$	272.16451	[M+H]+	5
Dextromethorphan	5.23	$C_{18}H_{25}NO$	272.20089	[M+H]+	1
Diazepam	6.72	$C_{16}H_{13}ClN_2O$	285.07892	[M+H]+	5
Diazepam D5	6.7	$C_{16}H_8[2H]_5ClN_2O$	290.1103	[M+H]+	5
Dihydrocodeine HCL	2.76	$C_{18}H_{23}NO_3$	302.17507	[M+H]+	5
Diltiazem HCL	5.73	$C_{22}H_{26}N_2O_4S$	415.1686	[M+H]+	12.5
Diphenhydramine HCl	5.2	$C_{17}H_{21}NO$	256.16959	[M+H]+	5
Diphenoxylate	6.51	$C_{30}H_{32}N_2O_2$	453.25365	[M+H]+	5
Doxepin HCl	5.42	$C_{19}H_{21}NO$	280.16959	[M+H]+	12.5
Doxylamine succinate	4.28	$C_{17}H_{22}N_2O$	271.18049	[M+H]+	25
Duloxetine HCL	5.84	$C_{18}H_{19}NOS$	298.12601	[M+H]+	12.5
EDDP perchlorate	5.29	$C_{20}H_{23}N$	278.19033	[M+H]+	25
Ephedrine HCl	2.52	$C_{10}H_{15}NO$	166.12264	[M+H]+	25
Estazolam	6.1	$C_{16}H_{11}ClN_4$	295.0745	[M+H]+	5
Ethylone HCl	3.47	$C_{12}H_{15}NO_3$	222.11247	[M+H]+	5
Etizolam	6.36	$C_{17}H_{15}ClN_4S$	343.07787	[M+H]+	5
Etomidate	6.27	$C_{14}H_{16}N_2O_2$	245.12845	[M+H]+	5
Fentanyl	5.13	$C_{22}H_{28}N_2O$	337.22744	[M+H]+	0.5
Flunitrazepam	6.21	$C_{16}H_{12}FN_3O_3$	314.09355	[M+H]+	5
Fluoxetine HCl	5.9	$C_{17}H18F_3NO$	310.14133	[M+H]+	12.5
Flurazepam	5.32	$C_{21}H_{23}ClFN_3O$	388.15864	[M+H]+	5
Fluvoxamine maleate	5.78	$C_{15}H_{21}F_3N_2O_2$	319.16279	[M+H]+	25

Component Name	Retention Time	Chemical Formula	Precursor (Q1) Mass (Da)	Adduct & Charge	LOD (ng/mL)
Gabapentin	2.39	$C_9H_{17}NO_2$	172.13321	[M+H]+	125
Haloperidol	5.54	$C_{21}H_{23}ClFNO_2$	376.14741	[M+H]+	1
Hydrocodone	3.17	$C_{18}H_{21}NO_3$	300.15942	[M+H]+	2.5
Hydromorphone	2.27	$C_{17}H_{19}NO_3$	286.14377	[M+H]+	2.5
Hydroxybupropion	4.39	$C_{13}H_{18}ClNO_2$	256.10988	[M+H]+	5
Hydroxyzine di-HCl	6.01	$C_{21}H_{27}ClN_2O_2$	375.18338	[M+H]+	5
Imipramine	5.88	$C_{19}H_{24}N_2$	281.20123	[M+H]+	25
Ketamine HCl	3.79	$C_{13}H_{16}ClNO$	238.09932	[M+H]+	2.5
Lamotrigine	4.16	$C_9H_7Cl_2N_5$	256.01513	[M+H]+	250
Levamisole	3	$C_{11}H_{12}N_2S$	205.0794	[M+H]+	5
Levetiracetam	2.48	$C_8H_{14}N_2O_2$	171.1128	[M+H]+	250
Lidocaine	3.37	$C_{14}H_{22}N_2O$	235.18049	[M+H]+	250
Loperamide	6.37	$C_{29}H_{33}ClN_2O_2$	477.23033	[M+H]+	1
Lorazepam	6	$C_{15}H_{10}Cl_2N_2O_2$	321.01921	[M+H]+	5
mCPP [1(3Chlorophenyl)piperazine HCL	4.38	$C_{10}H_{13}ClN_2$	197.084	[M+H]+	12.5
MDMA (methylenedioxymethamphetamine)	3.32	$C_{11}H_{15}NO_2$	194.11756	[M+H]+	10
MDPV HCL (3,4methylenedioxypyrovalerone)	4.39	$C_{16}H_{21}NO_3$	276.15942	[M+H]+	5
MEGX (monoethylglycycinexylidide)	2.94	$C_{12}H_{18}N_2O$	207.14919	[M+H]+	250
Meperidine	4.32	$C_{15}H_{21}NO_2$	248.16451	[M+H]+	12.5
Mephedrone HCL	3.43	$C_{11}H_{15}NO$	178.12264	[M+H]+	5
Meprobamate	4.53	$C_9H_{18}N_2O_4$	219.13393	[M+H]+	250
Methadone	5.91	$C_{21}H_{27}NO$	310.21654	[M+H]+	2.5
Methamphetamine	3.07	$C_{10}H_{15}N$	150.12773	[M+H]+	10
Methedrone HCl	3.18	$C_{11}H_{15}NO_2$	194.11756	[M+H]+	5
Methylone HCl	2.88	$C_{11}H_{13}NO_3$	208.09682	[M+H]+	5
Methylphenidate HCl	4.16	$C_{14}H_{19}NO_2$	234.14886	[M+H]+	12.5
Metoprolol TARTRATE	4.05	$C_{15}H_{25}NO_3$	268.19072	[M+H]+	5
MHC ((±)-10,11-Dihydro-10-Hydroxycarbamazepine)	4.69	$C_{15}H_{14}N_2O_2$	255.1128	[M+H]+	250
Midazolam	5.65	$C_{18}H_{13}ClFN_3$	326.08548	[M+H]+	5
Mirtazapine	4.48	$C_{17}H_{19}N_3$	266.16517	[M+H]+	12.5
Mitragynine	5.43	$C_{23}H_{30}N_2O_4$	399.22783	[M+H]+	1.25
Morphine	1.94	$C_{17}H_{19}NO_3$	286.14377	[M+H]+	5

Component Name	Retention Time	Chemical Formula	Precursor (Q1) Mass (Da)	Adduct & Charge	LOD (ng/mL)
Morphine-3-beta-glucuronide	*1.05*	$C_{23}H_{27}NO_9$	*462.17586*	*[M+H]+*	*24.7*
Naloxone	*2.74*	$C_{19}H_{21}NO_4$	*328.15433*	*[M+H]+*	*2.5*
Naltrexone	*3.02*	$C_{20}H_{23}NO_4$	*342.16998*	*[M+H]+*	*5*
Naphyrone HCl	*5.48*	$C_{19}H_{23}NO$	*282.18524*	*[M+H]+*	*5*
Naproxen	*6.26*	$C_{14}H_{14}O_3$	*231.10157*	*[M+H]+*	*250*
N-desmethylclomipramine HCL	*6.35*	$C_{18}H_{21}ClN_2$	*301.1466*	*[M+H]+*	*12.5*
N-Desmethyldoxepin	*5.43*	$C_{18}H_{19}NO$	*266.15394*	*[M+H]+*	*12.5*
Nefazodone HCl	*6.46*	$C_{25}H_{32}ClN5O_2$	*470.23173*	*[M+H]+*	*12.5*
Nitrazepam	*6.1*	$C_{15}H_{11}N_3O_3$	*282.08732*	*[M+H]+*	*50*
Norbuprenorphine	*4.84*	$C_{25}H_{35}NO_4$	*414.26389*	*[M+H]+*	*1.25*
Nordiazepam	*6.45*	$C_{15}H_{11}ClN_2O$	*271.06327*	*[M+H]+*	*5*
Norfentanyl oxalate	*3.82*	$C_{14}H_{20}N_2O$	*233.16484*	*[M+H]+*	*0.5*
Norhydrocodone HCL	*3.12*	$C_{17}H_{19}NO_3$	*286.14377*	*[M+H]+*	*12.5*
Norketamine HCl	*3.72*	$C_{12}H_{14}ClNO$	*224.08367*	*[M+H]+*	*2.5*
Normeperidine	*4.37*	$C_{14}H_{19}NO_2$	*234.14886*	*[M+H]+*	*12.5*
Noroxycodone HCl	*3.04*	$C_{17}H_{19}NO_4$	*302.13868*	*[M+H]+*	*5*
Nortriptyline HCl	*5.97*	$C_{19}H_{21}N$	*264.17468*	*[M+H]+*	*12.5*
O-Desmethyl tramadol HCL	*3.08*	$C_{15}H_{23}NO_2$	*250.18016*	*[M+H]+*	*12.5*
O-desmethyl venlafaxine	*3.7*	$C_{16}H_{25}NO_2$	*264.19581*	*[M+H]+*	*12.5*
Olanzapine	*3.5*	$C_{17}H_{20}N_4S$	*313.14814*	*[M+H]+*	*1*
Orphenadrine HCl	*5.54*	$C_{18}H_{23}NO$	*270.18524*	*[M+H]+*	*25*
Oxazepam	*6.13*	$C_{15}H_{11}ClN_2O_2$	*287.05818*	*[M+H]+*	*5*
Oxycodone	*3.07*	$C_{18}H_{21}NO_4$	*316.15433*	*[M+H]+*	*2.5*
Oxymorphone	*2.09*	$C_{17}H_{19}NO_4$	*302.13868*	*[M+H]+*	*2.5*
Paroxetine Maleate	*5.88*	$C_{19}H_{20}FNO_3$	*330.15*	*[M+H]+*	*12.5*
Pentazocine HCL	*4.55*	$C_{19}H_{27}NO$	*286.21654*	*[M+H]+*	*25*
Phenazepam	*6.39*	$C_{15}H_{10}N_2OBrCl$	*348.97378*	*[M+H]+*	*5*
Phencyclidine	*4.94*	$C_{17}H_{25}N$	*244.20598*	*[M+H]+*	*2.5*
Phentermine	*3.36*	$C_{10}H_{15}N$	*150.12773*	*[M+H]+*	*50*
Phenytoin	*5.45*	$C_{15}H_{12}N_2O_2$	*253.09715*	*[M+H]+*	*250*
Pregabalin	*2.33*	$C_8H_{17}NO_2$	*160.13321*	*[M+H]+*	*125*
Primidone	*4.07*	$C_{12}H_{14}N_2O_2$	*219.1128*	*[M+H]+*	*250*

Component Name	Retention Time	Chemical Formula	Precursor (Q1) Mass (Da)	Adduct & Charge	LOD (ng/mL)
Promethazine HCl	*5.72*	$C_{17}H_{20}N_2S$	*285.142*	*[M+H]+*	*1*
Propranolol HCL	*5.13*	$C_{16}H_{21}NO_2$	*260.16451*	*[M+H]+*	*10*
Protriptyline HCl	*5.82*	$C_{19}H_{21}N$	*264.17468*	*[M+H]+*	*12.5*
Pseudoephedrine	*2.58*	$C_{10}H_{15}NO$	*166.12264*	*[M+H]+*	*500*
Quetiapine fumarate	*5.57*	$C_{21}H_{25}N_3O_2S$	*384.17402*	*[M+H]+*	*12.5*
Risperidone	*5.11*	$C_{23}H_{27}FN_4O_2$	*411.21908*	*[M+H]+*	*2.5*
Ritalinic Acid	*3.58*	$C_{13}H_{17}NO_2$	*220.13321*	*[M+H]+*	*12.5*
Scopolamine HBr	*3.1*	$C_{17}H_{21}NO_4$	*304.15433*	*[M+H]+*	*0.5*
Sertraline HCl	*6.27*	$C_{17}H_{17}Cl_2N$	*306.08108*	*[M+H]+*	*12.5*
Tapentadol HCL	*4.13*	$C_{14}H_{23}NO$	*222.18524*	*[M+H]+*	*2.5*
Temazepam	*6.39*	$C_{16}H_{13}ClN_2O_2$	*301.07383*	*[M+H]+*	*5*
Thioridazine	*6.7*	$C_{21}H_{26}N_2S_2$	*371.16102*	*[M+H]+*	*25*
Topiramate	*4.82*	$C_{12}H_{21}NO_8S$	*340.10606*	*[M+H]+*	*250*
Tramadol HCl	*4.01*	$C_{16}H_{25}NO_2$	*264.19581*	*[M+H]+*	*2.5*
Trazodone HCl	*5.22*	$C_{19}H_{22}ClN_5O$	*372.15856*	*[M+H]+*	*12.5*
Trimipramine	*6.04*	$C_{20}H_{26}N_2$	*295.21688*	*[M+H]+*	*12.5*
Venlafaxine HCl	*4.72*	$C_{17}H_{27}NO_2$	*278.21146*	*[M+H]+*	*12.5*
Verapamil HCl	*5.61*	$C_{27}H_{38}N_2O_4$	*455.29043*	*[M+H]+*	*25*
Zaleplon	*5.8*	$C_{17}H_{15}N_5O$	*306.13494*	*[M+H]+*	*10*
Ziprasidone	*5.45*	$C_{21}H_{21}ClN_4OS$	*413.11974*	*[M+H]+*	*5*
Zolpidem	*4.63*	$C_{19}H_{21}N_3O$	*308.17574*	*[M+H]+*	*2.5*
Zonisamide	*3.77*	$C_8H_8N_2O_3S$	*213.03284*	*[M+H]+*	*25*
Zopiclone	*4.57*	$C_{17}H_{17}ClN_6O_3$	*389.11234*	*[M+H]+*	*25*

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