

Advancing Forensic DUID Screening with Mass Spectrometry

Optimized Evolution of a Toxicology Laboratory from Immunoassay to the SCIEX X500R QTOF System

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Over the past decade, the National Safety Council's Alcohol, Drugs and Impairment Division (NSC-ADID) started an initiative to standardize forensic toxicology laboratory testing for cases involving driving under the influence of drugs (DUID).

Target forensic compounds of interest were divided into two tiers: Tier I drugs include the most frequently encountered drugs found in DUID casework, and those which could be screened and confirmed with commercially available immunoassay and GC-MS instrumentation. Tier II analytes were those that had limited occurrence or required more advanced instrumentation such as LC-MS/MS, which is typically not readily available in every forensic laboratory.



Figure 1. Confidently Identify All Analytes Present Within a Forensic DUID Case Sample. Obtain a simplified sample report showing all positively identified compounds present in a case sample. (Top) Chromatogram and results table showing all target compounds identified in the blood sample based on difference acceptance criteria. (Right) Detailed XIC, TOF MS and MS/MS spectral library identification of fentanyl and codeine present in the screened sample.



More recently, the NSC-ADID made further changes on the list of target analytes for impaired driving and motor vehicle fatality forensic testing, due to recent advances in analytical technology and rapidly growing of novel psychoactive substances (NPS), like synthetic cannabinoids, bath salts and novel opioid analogs.¹

In this technical note, a comprehensive drug screening workflow for the analysis of forensic DUID blood samples is described. The methodology was developed using a simplified sample preparation approach in combination with the SCIEX X500R QTOF System following the new NSC-ADID recommendations for forensic testing in DUID and motor vehicle fatality cases.





Experimental Details

Sample Preparation: Control whole blood samples were spiked with a stock standard solution mixture containing all the different drugs for initial method development. A detailed list of the forensic compounds targeted, including accurate mass information and limits of detection (LOD) used for this screening are detailed on Supplement A of this technical document. Forensic DUID case samples and controls were extracted for LC-MS screening using the protocol in Figure 2.²



Figure 2. Sample Preparation Protocol.

Liquid Chromatography: HPLC separation was performed at 30 °C on a Phenomenex Kinetex Phenyl-Hexyl column (50×2.1 mm, 2.6μ m) on the SCIEX ExionLCTM AC system using the following conditions: Mobile Phase A: 10 mM Ammonium Acetate in H₂O:ACN (90:10). Mobile Phase B: 10 mM Ammonium Acetate in ACN:H₂O (90:10) plus 0.1% Formic Acid. LC separation conditions are detailed in Figure 3.





ESI- Screen: LC Runtime: 5.1 min, Flow Rate: 0.5 mL/min



Figure 3. Chromatography Conditions.

Mass Spectrometry and Data Analysis: MS and MS/MS data were collected using the SCIEX X500R QTOF System. For all positive ionizable compounds, an Information Dependent Acquisition (IDA) approach was used. For the negative ionizable target compounds, the MRM^{HR} workflow with the *Apply TOF start/stop* mass feature was used. Both screening strategies included a TOF MS experiment in each cycle. Detailed acquisition parameters are shown in Figure 4.

ESI	+ Scr	een:	Information	Depen	de	nt A	cquisition ((IDA)		
Method duration	7.5	🗘 min	Total scan time:	0.523599	iec					
Estimated cycles:	859									
Source and Gas Paran	neters -									
Ion source gas 1	60	🗘 psi	Curtain gas	30	:		Temperature	600	\$	ŀ
Ion source gas 2	60	🗘 psi	CAD gas	7	\$					
Experiment IDA	• -									
Polarity	Positive	v v	Spray voltage	2500	\$	v				
TOF MS										
TOF start mass	100	🗘 Da	Declustering potential	50	:	v	Collision energy	10	۵	ŀ
TOF stop mass	650	🗘 Da	DP spread	0	:	v	CE spread	0	\$	h
Accumulation time	0.1	sec								
IDA Criteria Small mole	cule 👻									
Maximum candidate ions	14	0	Dynamic backgroun	d subtraction						
Intensity threshold exceeds	10	Cps	Exclude former cand	sidate ions						
			For 6	Ç sec						
			After 1	occurrence	xes					
Advanced Criteria										
TOF MSMS										
Precursor ion	830	0 Da	Declustering potential	50	:	V	Collision energy	35	۰	ŀ
TOF start mass	25	🗘 Da	DP spread	0	:	v	CE spread	15	\$	ŀ
TOF stop mass	650	🗘 Da	Accumulation time	0.025	:	sec				

ESI- Screen: MRM^{HR} Workflow (High Resolution MRM)

Metho	d duration	5.1	\$	min	Total scan time:	0.241059 sec					
Entime	ated cycles: 1269										
Source	e and Gas Par	ameters									
lon so	utor gas 1	60	\$	pei	Curtain gas	30	t Temp	erature 800	÷ <		
101 101	urce get 2	80	:	pai	CAD gas	7	•				
Experi	ment MRM H	. v -									
Polarit	¥	Negative	۷		Spray voltage	-4500	• v				
TOF MS											
TOF st	ant mass	300	:	Da	Declustering potential	-60	🗘 V Collisi	on energy -10	• v		
10F 18	top mass	1000	:	Da	OP spread	0	t V CE spr	a bas	\$ v		
Accum	sulation time	0.1	:	161							
TOF MS	IMS ce dynamic range										
Mass	Table O	Apply fragmen	t ion n	ness 💌 App	ly TOF start/stop mass	Apply scan	schedule Inportanta	staff. Sort by processor is			
	Compound 10	Group name	Prec	ersor ion (Da)	TOF start mass (Da)	TOF stop mass (Da)	Accumulation time (sec	Deckastering potential (V	Collision energy (V)	CE spread (V)	Retention
1	Secolarbital	Group 1	257.	12	35.00000	300.00000	6.0100	-60	-35	15	2.20
2	Butabital	Group 1	225.	11	21.00000	300.00000	0.0100	-60	-35	15	1.92
	East-backet d	Group 1	225.3	12	15.00900	300.00000	0.0100	-60	-35	25	2.10
3											
3	Phenobarbital	Group 1	231.0	28	35.00000	300.00000	6.0100	-60	-35	13	1.80
3 4 5	Phenobarbital THCC	Group 1 Group 2	231/	26 29	35.00000	300.00000 350.00000	0.0100	-60	-35	15	1.80
3 4 5 6	Phenobarbital THCC Butalbital DS	Group 1 Group 2 Group 1	231.0 343.1 228.1	20 29 14	35.00800 35.00800 35.00800	306.00000 356.00000 306.00000	6.0100 6.0100 6.0100	-60 -60 -60	-35 -35 -35	15 15 15	1.80 3.20 1.92

Figure 4. MS Conditions.

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, retention time, isotope ratio difference, and library score. Subsequently, a combined score was computed based on these four confidence categories with custom weightings.



Table 1. Inter-Day Sverage Combined Scores (n=9) for 60 Compounds Screened in Forensic DUID Samples at the LOD using the SCIEX X500R QTOF System.

			Mass Error Confi	dence	RT Confidence	Isotope Confidence	Library Confidence	Combined Score 98.60	V		
	Μ	ass accurac Re	tention	time		Ĺ	sotope ra	MS/MS Library atio	Hit		
6-MAM	90.0 %	Cotinine	89.1 %	Metha	adone		96.4 %	Noroxycodone	95.8 %	Secobarbital	97.1 %
7-Aminoclonazepam	90.6 %	Diazepam	97.6 %	Metha	amphetamine	e	96.7 %	O-Desmethyl tramadol	97.4 %	Butalbital	97.2 %
Alphahydroxyalprazolam	87.1 %	EDDP	97.2 %	Methy	/lphenidate		97.2 %	Oxazepam	97.2 %	Pentobarbital	96.9 %
Alphahydroxymidazolam	92.2 %	Etizolam	95.1 %	Midaz	zolam		96.4 %	Oxycodone	94.6 %	Phenobarbital	96.9 %
Alprazolam	97.7 %	Fentanyl	97.2 %	Mitrag	gynine		95.8 %	Oxymorphone	90.3 %	тнс-соон	70.1 %
Amphetamine	95.9 %	Gabapentin	96.8 %	Morph	hine		93.6 %	Phenazepam	94.3 %		
Benzoylecgonine	95.2 %	Hydrocodone	95.5 %	Morph	hine-3-beta-g	glucuronide	93.5 %	Phencyclidine	98.1 %		
Beta-Naltrexol	95.2 %	Hydromorphone	94.8 %	Naltre	exone		94.0 %	Pregabalin	84.5 %		
Buprenorphine	97.0 %	Ketamine	97.7 %	Norbı	ıprenorphine	•	95.7 %	Ritalinic Acid	97.8 %		
Carboxyzolpidem	97.5 %	Lorazepam	96.4 %	Nordia	azepam		96.0 %	Tapentadol	98.4 %		
Carisoprodol	97.7 %	MDA	96.4 %	Norfe	ntanyl		81.8 %	Temazepam	97.0 %		
Cocaethylene	97.9 %	MDMA	97.4 %	Norhy	drocodone		97.1 %	Tramadol	98.4 %		
Cocaine	96.0 %	Meperidine	96.8 %	Norke	tamine		96.4 %	Zolpidem	98.5 %		
Codeine	96.8 %	Meprobamate	97.7 %	Norm	eperidine		95.9 %				

Using a vMethod[™] to Develop a Comprehensive Screening Workflow Applied to Forensic DUID Blood Samples

The vMethod[™] Application for 664 forensic compounds³ was initially used to obtain retention times and MS/MS spectra quality to build a data analysis processing method for the 60 target forensic compounds of interest. Two different acquisition strategies were utilized to streamline the screening workflow. For all positive ionizable compounds IDA was chosen as the acquisition mode, as it enabled the acquisition of MS/MS spectra on many precursors, in an intensity dependent manner. Subsequently, resulting MS/MS spectra is the used to match to potential analytes using MS/MS library spectral matching.

For the 5 target compounds (barbiturates and THC-COOH) that favor negative electrospray ionization, MRM^{HR} workflow was used as targeted acquisition strategy. MRM^{HR} workflow was performed using full scan MS/MS acquisition; by



Figure 5. Obtaining Fast and Confident Identification of Forensic Compounds of Interest in Biological Matrices. (Left) Extracted Ion Chromatogram shows a rapid LC separation (6 min) and identification of 55 forensic compounds of interest spiked in whole blood at LOD concentrations using IDA-MS/MS.(**Right**) Extracted Ion Chromatogram shows the rapid identification of barbiturates and THC-COOH spiked in whole blood at LOD concentrations using MRM^{HR} workflow.



defining the m/z range desired using the *Apply TOF start/stop* mass feature. This mode was beneficial as it enhanced compound identification at the LOD when performing MS/MS spectral library matching.

Figure 5 displays XIC chromatograms showing the detection of all target compounds analyzed with both positive and negative electrospray ionization modes in control blood samples spiked at the LODs, based on the latest NSC-ADID recommendations.¹

Throughout the method development process, it was important to obtain high combined scores for all compounds based on the four main confidence criteria defined in the processing method. Additional qualification criteria were implemented by setting an analyte concentration threshold based on the LODs to minimize false positives and/or false negative hits. Figure 6 shows the successful detection of 6-MAM and Fentanyl at their corresponding LODs, with mass errors less than 2ppm and MS/MS scores over 90%. Table 1 shows the average (n=9) combined scores obtained for all 55 target compounds, in control blood samples spiked at the LOD analyzed over the course of 3 days. Inter-day reproducibility resulted in %RSDs ranging between 1-10% for the target analytes.

It was found that THC-COOH had sufficient S/N ratios (> 200) and mass error less than 1 ppm at the LOD (10 ng/mL) for positive identification. However, low-abundance MS/MS spectra were obtained at that concentration level, subsequently resulting in an average combined score of 70%. Further optimization on the sample extraction protocol is recommended to enhance THC-COOH sensitivity and MS/MS fragmentation.



Figure 6. Successful Application of the SCIEX X500R QTOF System for Enhanced Compound Identification at Trace Concentration Levels. XICs, TOF MS and MS/MS spectra obtained showing confident and detailed identification of 6-MAM (Top) and fentanyl (bottom) spiked in whole blood at low ng/mL levels.





Component 🛛	Actual Concentr	RT RT	Area 🗤	Area R ⊽	Retent Time	Retenti ⊽ Time D	U	Calculated Concentrat ⊽	Adduct / C 🛛	Formula 🖓	Precursor Mass	Ac ⊽	Error	Confi	Confi	Confi	Found At Mass	, Mass Error (⊽	Library Hit ⊽	Library Score
Cocaethylene	N/A	4.14	5.586e4	0.110	4.14	0.00	V	0.747	[M+H]+	C18H23N	318.170		 Image: A set of the set of the	 Image: A set of the set of the	 Image: A second s	~	318.1704	1.4	Cocaethylene	97.7
Cocaine	N/A	3.93	3.241e4	0.064	3.94	0.01	V	0.430	[M+H]+	C17H21N	304.154		 Image: A set of the set of the	 Image: A set of the set of the		×	304.1544	0.1	Cocaine	86.7
Codeine	N/A	3.22	1.753e4	0.035	3.30	0.08	V	0.759	[M+H]+	C18H21N	300.159		 Image: A set of the set of the	 Image: A set of the set of the		×	300.1603	3.0	Codeine	80.6
Cotinine	N/A	2.04	8.459e6	16.675	1.96	0.08	V	482.317	[M+H]+	C10H12N	177.102	1	 Image: A set of the set of the	 Image: A second s	 Image: A set of the set of the	×	177.1021	-0.9	Cotinine	100.0
Diazepam D5	1.00	5.51	5.073e5	N/A	5.51	0.00		N/A	[M+H]+	C16H8[2H	290.110		 Image: A second s	 Image: A second s	 Image: A second s	 Image: A second s	290.1106	0.9	Diazepam D5	96.8
Fentanyl	N/A	4.32	1.824e5	0.360	4.33	0.01		2.093	[M+H]+	C22H28N	337.227	!	~	 Image: A second s	 Image: A start of the start of	 	337.2276	0.3	Fentanyl	98.3
Norfentanyl	N/A	3.52	3.102e4	0.061	3.52	0.00	V	1.324	[M+H]+	C14H20N	233.165	1	×	~			233.1651	1.0	Norfentanyl	53.9
Component v	, Actual Concentr ⊽	, Expected RT	Area ⊽	Area R V	Retent _V Time	Retenti ⊽ Time D	U 7	Calculated Concentrat ⊽	Adduct / C ⊽	Formula ♥	Precursor Mass	Co Ac T	Mass Error	RT Confi	Isotope Confi	Library Confi	Found At Mass	, Mass Error (⊽	. Library Hit ⊽	Library Score
тнс-соон	N/A	3.19	3.637e4	0.372	3.16	0.03	V	92.518	[M-H]-	C21H28O4	343.191	1	~	 	 	 	343.1918	0.8	THC-COOH Negative	97.0
1000000 000000 000000 000000 005 110 ▼ Pesk Details Procursor m/x Mass En	1 15 20 25 30 Time, min 1.96	3.5 4.0 Time (min)	200000 150000 50000 50000 717 Formula Finder R Name Fo	177.1021 177.1021 178. 6 177 17 Mass/Cr esuits rmula Score	1056 8 179 18 harpe, Da m/z (Da) Erri		0% 0% 0% 0% 0% 0% 58earch Resi 10 10 10 10 10 10 10 10 10 10 10 10 10	0 100 0495 98,0602 118.06 0 100 Mass/Charge, D Mass/Charge, D 6 C101121202	155 177 1023 5 005 1 150 3 G M (Da) Fit (0.9497 7 100.0	Pred: 1.52460, Hei 70000 50000 50000 20000 10000 0 40000 10000 0 41 ▼ Peak Details ■ Precursor m/z M 337.227 0	4.2 4.3 Time ass Error (ppm) Re 3 4.2	4.4 4.4 tention Time	45 (min) ion N/A	© [C.22H2 00 00 00 00 00 00 00 00 00 0	337.147 336 3 inder Results	37,2276 338,2 1 338,2 1 338,2 1 338,2 1 338,2 1 338,2 1 338,2 1 338,2 1 338,2 1 338,2 1 338,2 1 338,2 1 338,2 1 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	339 340 srge. Da m/z (Da) Error	Generation of the second secon	y several restrictly (42:/38-7) (2) 122.0807 188-143 123.0807 188-143 125.0607 188-143 126.132.0807 188-143 127.132.0807 188-143 127.132.0807 188-143 100 200 100 200	337.2273 58 9.0902 300 500 500 500 500 500 500 500
UI 4 - Norfentaryl (Unkn rea: 3.10244, Height: 1.65 20000 1 15000 100000 5000	nyl 100vn) OS.wiff2), (sam 2264. RT: 3.52 min 3.524	ple Index: 18) -	Spectrum fre C14H20N2O+ 3000 - 2500 - 2000 - 1500 - 2	m 04-24-18 D Hj+ 233.1651 33.0788	from 3.523 to 3.54	3 min Spec Ubra 100 Igp 9 6 pp / Aprix	55.0 0%	04-24-18 Decursor In: Norfentanyl (1609-66-1 84.0807 96.0324	2332 Da, CE: 35). CE=35215	THC-CO	(Unknown) 34. NEG E 1.786e4, RT: 3.16 m	wiff2), (sampi sin 3.157	e Index: 18)	Spectr © [C21142 6000 5000	um from 04-3 804-H]-) -) -	24-18 DUI) 343.1918 	t 1, from 3.155 to 3	161 min ⊡ ● Spe ● Lib Igree to p /de	ectrum from 04-24-18 DUI recu ary Spectrum: THC-COOHve (642) 50%	sor: 343.2 Da, CE 0-14-4) , CE=35: 1557 299.20 299.18

-

14 DT 1 17

Figure 7. Minimize False Positives/Negatives by Streamlining Accurate Mass Data Processing of all Compounds of Interest Present in a Forensic DUID Case Sample using SCIEX OS Software. Using multiple acceptance criteria enabled the accurate identification of target analytes present in a forensic blood sample. (Top) Sample list of all compounds passing the acceptance criteria (green traffic lights) and concentration thresholds (cells highlighted in red) set within the processing method. (Bottom) XICs of all compounds identified in the sample, showcasing TOF MS and MS/MS spectral library identification details.

Enhanced Forensic Compound Identification using the SCIEX X500R QTOF System

One of the principal goals of developing this comprehensive analysis workflow was to successfully migrate the current immunoassay approach to the SCIEX X500R QTOF System. The current immunoassay sample preparation and analysis workflow utilizes 1mL of forensic blood sample and 2mL of acetonitrile for extraction, whereas with the QTOF MS strategy the laboratory was able to reduce the sample size to 100 μ L while still meeting the NSC-ADID recommended cutoffs.

The ability of meeting these cutoffs with minimal sample is ideal, as often forensic case samples are limited in volume. Additionally, it eliminates the laboratory's need for using multiple reagent kits (9 kits currently utilized) as the QTOF MS approach provides the enhanced selectivity and sensitivity to streamline the detection of Tier I and Tier II compounds. As part of the implementation plan, 30 forensic DUID case samples were screened with both immunoassay and QTOF MS for results comparison.

Table 2 shows all compounds detected in the 30 forensic DUID samples examined with both immunoassay analyzer and the SCIEX X500R QTOF system. Compounds highlighted in green were specifically detected using QTOF MS but missed or classified as a single compound class (e.g., *OPI* for Opiates and metabolites) by the immunoassay approach.

Figures 1 and 7 show the detailed analysis of two different DUID samples in the study. In reference to the sample displayed on Figure 7, the immunoassay analyzer detected THC-COOH exclusively.



Table 2. List of Compounds Identified in Forensic DUID Samples using Immunoassay Analyzer and the SCIEX X500R QTOF System.

Immu	noAssa	y Results				Mas	s Spectrometry I	Results			
DUI 1	COKE		Benzoylecgonine	Cocaethylene	Cocaine	Cotinine	Nordiazepam				
DUI 2	THC		Cotinine	тнс-соон							
DUI 3		NEG					NEG				
DUI 4	THC		Cotinine	Fentanyl	Norfentany	ТНС-СООН					
DUI 5	THC	COKE	Benzoylecgonine	Cocaine	Cotinine	тнс-соон					
DUI 6	THC		Cotinine	тнс-соон							
DUI 7	AMPH		Amphetamine	Cotinine	Metham	phetamine					
DUI 8	NEG		Cotinine	Lorazepam							
DUI 9	PCP	THC	Cotinine	PCP	THC	-СООН					
DUI 10	AMPH		Amphetamine	Cotinine	Ritali	nic Acid	Methamphetamine				
DUI 11	THC	BENZO	Cotinine	Diazepam	THC	-СООН	Nordiazepam				
DUI 12	THC		Fentanyl	Norfentanyl	THC	-СООН					
DUI 13	THC		Cotinine	тнс-соон							
DUI 14	THC		Cotinine	тнс-соон							
DUI 15	THC		Cotinine	тнс-соон							
DUI 16	THC		Cotinine	тнс-соон							
DUI 17	THC		Cotinine	тнс-соон							
DUI 18	THC		Cotinine	тнс-соон							
DUI 19	THC		Cotinine	THC-COOH							
DUI 20	THC		Cotinine	ТНС-СООН							
DUI 21	THC		Cotinine	ТНС-СООН							
DUI 22	THC		Cotinine	ТНС-СООН							
DUI 23	THC		Cotinine	THC-COOH							
DUI 24	COKE	OPI	Benzoylecgonine	Buprenorphine	Cocaine	Codeine	Cotinine	Hydromorphone	Morphine	Metamphetamine	Morphine-3- Glucuronide
DUI 25	COKE		Benzoylecgonine	Cocaethylene	Cocaine	Cotinine					
DUI 26	OPI		Codeine	Cotinine	Fentanyl	Morphine	Morphine-3- Glucuronide	Hydromorphone			
DUI 27	AMPH	COKE OPI	Amphetamine	Benzoylecognine	Cotinine	Fentanyl	Hydromorphone	Metamphetamine	Morphine	Morphine-3- Glucuronide	Norfentanyl
DUI 28	OPI		Benzoylecgonine	Codeine	Cotinine	Fentanyl	Hydromorphone	Morphine	Morphine-3- Glucuronide		
DUI 29	AMPH		Amphetamine	Cotinine	Fentanyl	Norfentanyl	Metamphetamine				
DUI 30	THC		Cotinine	тнс-соон							

Compounds highlighted in GREEN are not screened for using the immunoassay analyzer operating in the laboratory but were detected in the MS assay.



However, when analyzed with the SCIEX X500R QTOF System, the same compound was identified but also three compounds of interest, which were not tested by immunoassay were detected:

- Cotinine (~ 482.32 ng/mL) Combined Score 100%
- Fentanyl (~2.1 ng/mL) Combined Score 98.3%
- Norfentanyl (~1.32 ng/mL) Combined Score 53.9%
- THC-COOH (~92.52 ng/mL) Combined Score: 97%

It is important to highlight that norfentanyl was considered a positive hit although obtaining a combined score of 53.9%. Analyte review based on the acceptance criteria like retention time, mass error on the TOF MS scan, concentration threshold (> 1 ng/mL) as well as parent drug metabolism pathway knowledge, were supporting evidence of compound presence in the forensic DUID sample.

Conclusions

A comprehensive drug screening workflow for the analysis of forensic DUID blood samples has been successfully developed using the SCIEX X500R QTOF System based on the new NSC-ADID recommendations.

- The vMethod[™] Application for forensic compound screening was successfully used to obtain retention times and MS/MS spectra necessary to build a targeted analysis workflow for the 60 forensic compounds of interest in DUID case samples.
- Average combined scores based on multiple acceptance criteria (Ret. Time, Mass error, Isotope ratio, MS/MS library hit and concentration) ranged between 70-98% for all target analytes, resulting in successful compound identification.
- The developed QTOF MS screening approach enabled the identification of multiple number of the targeted compounds present in authentic forensic DUID case samples in comparison to immunoassay based screening.
- The adaptation of QTOF MS technology enabled the use of microliter volumes of forensic blood samples, while meeting NSC-ADID cutoff recommendations. Thus, eliminating the use of multiple immunoassay reagent kits used for screening.

References

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- Desharnais B. et al., Protein precipitation of whole blood for quantification of 58 different analytes by LC-MS/MS: method development challenges, https://goo.gl/2Ma9RU
- vMethod[™] Application Single-Injection Screening of 664 Forensic Toxicology Compounds on a SCIEX X500R QTOF System.



Supplement A. List of Target Forensic DUID Compounds.

Component Name	Retention Time	Formula	Precursor (Q1) Mass (Da)	Adduct & Charge	LOD (ng/mL)
6-Monoacetylmorphine	3.32	$C_{19}H_{21}NO_4$	328.1543	[M+H]+	5
7-Aminoclonazepam	3.97	$C_{15}H_{12}CIN_3O$	286.0742	[M+H]+	10
Alpha-hydroxyalprazolam	4.71	$C_{17}H_{13}CIN_4O$	325.0851	[M+H]+	10
Alpha-hydroxymidazolam	4.36	$C_{18}H_{13}CIFN_3O$	342.0804	[M+H]+	10
Alprazolam	4.95	$C_{17}H_{13}CIN_4$	309.0902	[M+H]+	10
Amphetamine	3.24	$C_9H_{13}N$	136.1121	[M+H]+	20
Benzoylecgonine	3.59	$C_{16}H_{19}NO_4$	290.1387	[M+H]+	5
6-Beta-Naltrexol	3.28	$C_{20}H_{25}NO_4$	344.1856	[M+H]+	10
Buprenorphine	4.39	$C_{29}H_{41}NO_4$	468.3108	[M+H]+	1
Zolpidem Phenyl-4-carboxylic acid	3.5	C ₁₉ H ₁₉ N3O3	338.1499	[M+H]+	5
Carisoprodol	4.86	$C_{12}H_{24}N2O_4$	261.1809	[M+H]+	50
Cocaethylene	4.14	$C_{18}H_{23}NO_4$	318.1700	[M+H]+	5
Cocaine	3.93	$C_{17}H_{21}NO_4$	304.1543	[M+H]+	5
Codeine	3.22	$C_{18}H_{21}NO_3$	300.1594	[M+H]+	5
Cotinine	2.04	$C_{10}H_{12}N_2O$	177.1022	[M+H]+	5
Delorazepam	5.2	$C_{15}H_{10}CI_2N_2O$	305.0243	[M+H]+	10
Diazepam	5.53	$C_{16}H_{13}CIN_2O$	285.0789	[M+H]+	10
EDDP	4.59	$C_{20}H_{23}N$	278.1903	[M+H]+	50
Etizolam	5.12	$C_{17}H_{15}CIN_4S$	343.0779	[M+H]+	10
Fentanyl	4.32	$C_{22}H_{28}N_2O$	337.2274	[M+H]+	1
Gabapentin	3.12	$C_9H_{17}NO_2$	172.1332	[M+H]+	250
Hydrocodone	3.41	$C_{18}H_{21}NO_3$	300.1594	[M+H]+	5
Hydromorphone	3.05	$C_{17}H_{19}NO_3$	286.1438	[M+H]+	5
Ketamine	3.55	C ₁₃ H ₁₆ CINO	238.0993	[M+H]+	5
Lorazepam	4.9	$C_{15}H_{10}CI_2N_2O_2$	321.0192	[M+H]+	10
MDA	3.3	$C_{10}H_{13}NO_2$	180.1019	[M+H]+	20
MDMA	3.4	$C_{11}H_{15}NO_2$	194.1176	[M+H]+	20
Meperidine	3.89	$C_{15}H_{21}NO_2$	248.1645	[M+H]+	25
Meprobamate	4.11	$C_9H_{18}N_2O_4$	219.1339	[M+H]+	500
Methadone	4.71	C ₂₁ H ₂₇ NO	310.2165	[M+H]+	5
Methamphetamine	3.35	C ₁₀ H ₁₅ N	150.1277	[M+H]+	20



Component Name	Retention Time	Formula	Precursor (Q1) Mass (Da)	Adduct & Charge	LOD (ng/mL)
Methylphenidate	3.77	$C_{14}H_{19}NO_2$	234.1489	[M+H]+	25
Midazolam	4.3	$C_{18}H_{13}CIFN_3$	326.0855	[M+H]+	10
Mitragynine	4.59	$C_{23}H_{30}N_2O_4$	399.2278	[M+H]+	2.5
Morphine	2.97	$C_{17}H_{19}NO_3$	286.1438	[M+H]+	10
Morphine-3-glucuronide	1.94	C ₂₃ H ₂₇ NO ₉	462.1759	[M+H]+	49.4
Naltrexone	3.32	$C_{20}H_{23}NO_4$	342.1700	[M+H]+	10
Norbuprenorphine	3.95	$C_{25}H_{35}NO_4$	414.2639	[M+H]+	2.5
Nordiazepam	5.12	$C_{15}H_{11}CIN_2O$	271.0633	[M+H]+	10
Norfentanyl	3.52	$C_{14}H_{20}N_2O$	233.1648	[M+H]+	1
Norhydrocodone	3.34	$C_{17}H_{19}NO_3$	286.1438	[M+H]+	25
Norketamine	3.46	C ₁₂ H ₁₄ CINO	224.0837	[M+H]+	5
Normeperidine	3.85	$C_{14}H_{19}NO_2$	234.1489	[M+H]+	25
Noroxycodone	3.28	$C_{17}H_{19}NO_4$	302.1387	[M+H]+	10
O-Desmethyl-cis-tramadol	3.31	$C_{15}H_{23}NO_2$	250.1802	[M+H]+	25
Oxazepam	4.84	$C_{15}H_{11}CIN_2O_2$	287.0582	[M+H]+	10
Oxycodone	3.34	$C_{18}H_{21}NO_4$	316.1543	[M+H]+	5
Oxymorphone	3	$C_{17}H_{19}NO_4$	302.1387	[M+H]+	5
Phenazepam	5.28	C ₁₅ H ₁₀ N ₂ OBrCl	348.9738	[M+H]+	10
Phencyclidine	4.25	C ₁₇ H ₂₅ N	244.2060	[M+H]+	5
Pregabalin	3.11	$C_8H_{17}NO_2$	160.1332	[M+H]+	250
Ritalinic acid	3.46	$C_{13}H_{17}NO_2$	220.1332	[M+H]+	25
Tapentadol	3.74	$C_{14}H_{23}NO$	222.1852	[M+H]+	5
Temazepam	5.21	$C_{16}H_{13}CIN_2O_2$	301.0738	[M+H]+	10
Tramadol	3.74	$C_{16}H25NO_2$	264.1958	[M+H]+	5
Zolpidem	4	$C_{19}H_{21}N_3O$	308.1757	[M+H]+	5
Secobarbital	2.19	$C_{12}H_{18}N_2O_3$	237.1245	[M-H]-	250
Butalbital	1.9	$C_{11}H_{16}N_2O_3$	223.1088	[M-H]-	250
Pentobarbital	2.05	$C_{11}H_{18}N_2O_3$	225.1245	[M-H]-	250
Phenobarbital	1.76	$C_{12}H_{12}N_2O_3$	231.0775	[M-H]-	250
THC-COOH	3.19	$C_{21}H_{28}O_4$	343.1915	[M-H]-	10

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