# Single-Injection Screening of 664 Forensic Toxicology Compounds Using an Innovative Benchtop High Resolution Mass Spectrometer



**The Power of Precision** 

Oscar G. Cabrices<sup>1</sup>, Alexandre Wang<sup>1</sup>, Xiang He<sup>1</sup>, Holly McCall<sup>1</sup>, Laura Baker<sup>1</sup>, Adrian Taylor<sup>2</sup>.

<sup>1</sup>SCIEX, 1201 Radio Rd, Redwood City, CA USA 94065. <sup>2</sup>SCIEX, 71 Four Valley Drive, Concord, Ontario, Canada L4K 4V8.

# INTRODUCTION

Quadrupole Time-of-flight mass spectrometry (QTOF-MS) provides high-resolution, accurate-mass data for full-scan information of both precursor ion and all product ions. This is an ideal approach for forensic toxicology screening where unknown compounds in complex samples must be identified from information-rich data sets.

Designed for routine use, the benchtop SCIEX X500R QTOF system could also be used for high-specificity, targeted quantitation as well as for non-targeted screening from single sample sets in a routine testing laboratory environment.

Herein, we present a single-injection method for screening 664 most up-to-date forensic compounds using the SCIEX X500R QTOF system and SCIEX OS Software The obtained data provides both structural information and retention times to enhance identification accuracy, especially for structurally similar isomers. Sample preparation procedures for urine and whole blood.



## Reproducibility of Retention Time Measurements

The reproducibility tests indicate that the RTs generated from our optimized LC conditions are consistent and reproducible. RTs measured on three separated analytical columns all have %CVs of less than 5% for each of the 664 compounds. RT inter-day reproducibility (tested on 80 compounds) resulted in %CVs less than 5% over 3 days.

Lastly, RT variability in human whole blood and urine samples (tested on 80 compounds) indicated that the %CV for 3 individual lots is less than 5%. In addition, the RT difference between neat solutions and matrix is less than 5% for all tested compounds. Results are shown for 80 out the 664 compounds the table below:

#### Retention time reproducibility for forensic compounds (partial list)



## **EXPERIMENTAL**

#### **Sample Preparation for Urine Matrix**

The stock standard mixtures in neat solutions were diluted with methanol: water (20:80, v/v) to appropriate concentrations. These diluted solutions were used to determine the retention time of the 664 compounds. Subsequently, the urine and whole blood samples were prepared to confirm the retention times in matrix. For urine samples, stock standards solutions (10.0  $\mu$ L) were added into human urine matrix (90.0  $\mu$ L) and then diluted 10folds with methanol:water (20:80, v/v). After centrifuged at 8,000 rpm for 5 min, the supernatant was used for LC-MS

Index	Name	Туре	Name	Used	Error Confidence	Confidence	Confidence	Confidence Mass Error (ppm)		Library Hit	Score	Isotope Ratio Difference
28	1	Unkno	Nitenpyram		✓	<ul> <li></li> </ul>	✓	✓	0.7	Nitenpyr	87.9	1.6
34	2	Unkno	Nitenpyram	<b>v</b>	✓	<ul> <li></li> </ul>	✓	✓	0.4	Nitenpyr	91.9	5.5
58	20	Unkno	Nitenpyram	<b>v</b>	✓	<ul> <li></li> </ul>	✓	<ul> <li></li> </ul>	0.5	Nitenpyr	92.8	2.5
64	50	Unkno	Nitenpyram		✓	<ul> <li></li> </ul>	✓	<ul> <li>Image: A set of the set of the</li></ul>	-0.7	Nitenpyr	95.1	5.7
70	100	Unkno	Nitenpyram		✓	~	✓	~	0.0	Nitenpyr	94.8	3.2



## **RESULTS AND DISCUSSION**

**Optimizing the Processing Method** To identify compounds in the analyzed samples, a targeted screening approach was employed using SCIEX OS software.

Samples were evaluated against a list of parameters containing the names, molecular formulas and retention times (RTs) for all targeted compounds. Appropriate integration parameters were defined for each component. For example, the compound, hydromorphone, was defined as the peak at 2.35 min with a 30 second half time window. An MS/MS library was used for MS/MS library matching.

The confidence criteria used for screening were mass error, RT error, isotope ratio difference, and library score. A traffic light system where different colors were assigned to different performance levels provided a way to assess the quality of the match. For example, in the case of mass error, green represented mass errors less than 5 ppm; orange, mass errors between 5 and 10 ppm; and red, mass errors larger than 10 ppm. Color representation for all the four criteria are shown in the diagram:

orkflow	Sele	ect or	verify	the a	nalyte and internal sta	ndard names ar	nd masses.				
omponents •										Experiment	Type 💙 🛛 In
tegration	Row IS Gro		Name	Chemical Formula	Adduct/Ch	Precursor Ma	Fra Ma	XIC Width (Da)	Retention Time (min)		
brary Search	•	1			2-OH-Ethylflurazepam	C17H14CIFN2O2	[M+H]+	333.08006		0.01	3.76
		2			6-MAM	C19H21NO4	[M+H]+	328.15433		0.01	1.90
cceptance Criteria		3			7-Aminoclonazepam	C15H12CIN3O	[M+H]+	286.07417		0.01	2.39
0.1		4			7-Aminoflunitrazepam	C16H14FN3O	[M+H]+	284.11937		0.01	2.57
onfidence Limits		5			Alpha-Hydroxyalprazolam	C17H13CIN4O	[M+H]+	325.08507		0.01	3.68
Qualitativa Pulac		6			Alpha-OH-Triazolam	C17H12Cl2N4O	[M+H]+	359.04609		0.01	3.47
Qualitative Rules		7			Alprazolam	C17H13CIN4	[M+H]+	309.09015		0.01	3.91



		between neat and matrix					
Component name	RT (min)	Column (n=3)	Inter-day (n=3)	Whole blood (n=3)	Urine (n=3)	Whole blood	Urine
6-MAM	3.05	1.5	0.3	0.0	0.2	1.0	0.8
7-Aminoclonazepam	4.35	0.6	0.4	0.0	0.1	0.2	0.2
7-Hydroxymitragyline	4.50	1.5	0.6	0.1	0.2	1.7	1.5
Acetyl Fentanyl	4.63	1.1	0.3	0.0	0.2	0.6	0.4
Alpha-Hydroxyalprazolam	6.09	0.3	0.1	0.0	0.2	0.0	0.0
Alpha-hydroxymidazolam	6.11	0.7	0.5	0.0	0.1	0.6	0.5
Alpha-hydroxytriazolam	5.87	0.2	0.2	0.1	0.1	-0.1	-0.1
Alpha-PPP	3.11	1.9	0.5	0.0	0.2	1.0	0.7
Alpha-PVP	4.05	1.5	0.4	0.0	0.1	0.5	0.3
Alprazolam	6.26	0.2	0.1	0.1	0.2	0.1	0.0
Amitriptyline	5.87	1.0	0.3	0.1	0.1	0.4	0.2
Amphetamine	2.79	2.1	0.5	0.0	0.2	0.7	0.8
Benzoylecgonine	3.95	0.3	0.1	0.0	0.1	0.3	0.1
Buphedrone	3.10	1.6	0.5	4.8	0.2	3.8	9.3
Buprenorphine	5.24	1.1	0.5	0.1	0.2	1.4	1.1
Carisoprodol	5.62	0.2	0.1	0.1	0.2	0.1	0.0
Clomipramine	6.24	1.1	0.3	0.1	0.2	0.4	0.3
Codeine	2.81	1.4	0.4	0.2	0.2	0.9	0.8
Cotinine	2.89	2.1	1.6	0.2	0.2	2.8	2.6
Cyclobenzaprine	5.73	1.0	0.3	0.0	0.2	0.5	0.3
Desalkvlflurazepam	6.16	0.2	0.2	0.0	0.1	0.0	-0.1
Desipramine	5.78	1.1	0.3	0.1	0.2	0.5	0.3
Desmethyldoxenin	5.34	11	0.3	0.1	0.2	0.5	0.4
Dextromethorphan	5 16	12	0.3	0.0	0.1	0.6	0.1
Diazenam	6.72	0.2	0.0	0.0	0.1	0.0	-0.1
Dibydrocodeine	2.73	1.6	0.1	0.0	0.1	0.0	0.1
Davenin	5.34	1.0	0.0	0.2	0.4	0.0	0.7
EDDR	5.20	1.1	0.4	0.1	0.2	0.5	0.4
	3.07	1.1	0.5	0.1	0.2	0.5	0.4
	3.07	1.5	0.0	0.2	0.2	0.5	0.4
	3.50	1.0	0.4	0.0	0.2	0.0	0.4
	3.27	1.7	0.5	0.2	0.2	0.5	0.4
Manaridina	4.32	1.3	0.3	0.1	0.2	0.6	0.5
Meperiaine	4.26	1.3	0.2	0.0	0.1	0.5	0.3
Mennearone	3.37	1./	0.4	0.0	0.2	0.6	0.4
	4.53	0.3	0.1	0.0	0.1	0.2	0.1
Methadone	5.80	1.1	0.3	0.0	0.1	0.3	0.2
	3.03	1.9	0.5	0.2	0.2	0.9	0.8
Methedrone	3.27	1.1	0.5	2.5	2.7	2.4	2.2
Methylone	2.85	1.7	0.5	0.0	0.3	0.7	0.7
Methylphenidate	4.09	1.3	0.4	0.0	0.1	0.5	0.3
Midazolam	5.84	1.8	1.3	0.1	0.2	2.6	2.5
Nortriptyline	5.87	1.1	0.3	0.0	0.1	0.3	0.2
O-Desmethyltramadol	3.02	1.8	0.3	0.0	0.2	0.6	0.4
Oxazepam	6.12	0.3	0.1	0.0	0.1	0.2	0.1
Oxycodone	3.03	1.5	0.4	0.0	0.2	0.6	0.4
Oxymorphone	2.07	1.9	0.6	0.0	0.5	1.0	1.3
Pregablin	2.20	2.0	1.4	0.3	0.8	-2.4	-2.3
Propoxyphene	5.58	1.1	0.3	0.0	0.2	0.4	0.2
Protriptyline	5.87	0.5	0.3	0.0	0.1	0.3	0.2
Ritalinic acid	3.58	0.5	0.2	0.0	0.2	0.0	-0.2
Sufentanil	5.55	0.9	0.3	0.1	0.1	0.8	0.6
Tapentadol	4.05	1.3	0.2	0.0	0.1	0.5	0.3
Temazepam	6.39	0.2	0.1	0.1	0.2	0.1	-0.1
Tramadol	3.93	1.5	0.2	0.0	0.1	0.5	0.3
Zolpidem	4.64	1.6	0.7	0.1	0.1	2.0	1.8

analysis.

#### **Sample Preparation for Blood Matrix**

For whole blood samples, 10.0  $\mu$ L of stock standard solutions were spiked into 90.0  $\mu$ L of human whole blood matrix. The samples were extracted by using a protein precipitation procedure. Basically, 900  $\mu$ L of Methanol: MeCN (50:50, v/v) were added into the above mixture and vortexed for 1 min then follow by 3 min sonication and another 1 min vortex. 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  $\mu$ L methanol: water (20:80, v/v).

A.Conc B.Conc B.Curve

7.60

9.50 min

3.80 min 5.70

## **LC Conditions**

Analytes (10 µL sample injection volume) were chromatographically separated using a Phenomenex Kinetex® 2.6 µm phenyl-hexyl (50 x 4.6 mm) column. 10 mM ammonium formate in water was used as mobile phase A and 0.05% formic acid in methanol was employed as mobile phase B.

### **MS/MS** Conditions

system.

Source conditions and the method settings for non-targeted, IDA-MS/MS acquisition methods are listed below. Those settings allow screening for the 664 targeted, as well as the additional non-targeted compounds.

Stop time:

B.Conc A.Conc

#### **Optimization of LC Conditions**

The performance of separation was evaluated with different mobile phases (acidic and neutral), gradient conditions, and column types. Results indicate that a majority of the isomeric compounds was fully resolved with neutral Buffer A and a 10 min linear gradient using a Phenomenex phenyl-hexyl column (Part Number: 00B-4495-E0).

Full chromatographic separation for 4 isomers, including Morphine, Hydromorphone, Norcodeine and Norhydrocodone, with the optimized LC condition.

![](_page_0_Figure_38.jpeg)

For example, the Noroxycodone (Top) and Oxymorphone (bottom) have exactly same precursor ion and very similar MS/MS spectra. However, these two compounds were fully resolved by using the LC condition in this method. The retention time is 3.05 min for Noroxycodone and 2.10 min for Oxymorphone.

Therefore, it is easier and more accurate to distinguish these two compounds by using retention time combined with MS and MS/MS information.

For a complete list of compounds, please refer to the SCIEX vMethod<sup>™</sup> application [1].

# CONCLUSIONS

- We have developed an LC-MS/MS-based toxicological screening method that includes the Retention Times for 664 forensic compounds.
- When combined with high-resolution mass spectrometry (HRMS) and HR-MS/MS information [2], the retention time identified herein enable more accurate compound identification. Overall, the ability to identify structural similar isomers was largely enhanced.
- In addition, because the data was acquired in a nontargeted approach the processing method designed here for screening targeted compounds can be quickly adjusted and used for unknown compound identification using a non-targeted data processing.

![](_page_0_Figure_46.jpeg)

Time -	Method duration Estimated cycles:	9.5 🗘 min 1088	Total scan time:	0.523599 sec		
Source	<ul> <li>Source and Gas Para Ion source gas 1 Ion source gas 2</li> </ul>	60 psi 60 psi	Curtain gas CAD gas	30 <b>*</b> 7 <b>*</b>	Temperature	600 C
	• Experiment IDA	Positive      V	Spray voltage	2500 🗘 V		
TOF-MS	TOF MS	100 Da	Declustering potential	50 <b>*</b> V	Collision energy	10 C V
Parameters ]	TOF stop mass Accumulation time	650 Da	DP spread	0 🗘 V	CE spread	0 <b>*</b> V
	IDA Criteria Small mo	lecule 👻				
IDA-MS/MS	Maximum candidate ions Intensity threshold exceed	14 * s 10 * cps	<ul> <li>Dynamic background</li> <li>Exclude former candi</li> <li>For</li> </ul>	subtraction date ions sec		
Parameters	Advanced Criteria		After 1	occurrences		
	TOF MSMS Precursor ion	830 🗘 Da	Declustering potential	50 🗘 V	Collision energy	35 🗘 V
l	TOF start mass	25 🗘 Da	DP spread	0 🗘 V	CE spread	15 🗘 V
	TOF stop mass	650 🗘 Da	Accumulation time	0.025 🗘 sec		

Prior to data process, the LibraryView<sup>™</sup> software and HRAM forensic library are installed and licensed in the computer

Manage	HRAM Forensics Library v2.1				
	Name *	CAS	Formula	Molecular Weight	
HRAM Forensics Library v2.1	1,3-Dimethylamylamine	13803-74-2	C7H17N	115.21876	
and Find Am Forenaics Elorary 42.1	10,11-Dihydro-10-hydroxycarbamazepine	29331-92-8	C15H14N2O2	254.28868	
▼ COMPOUNDS	11-Hydroxy-THC	36557-05-8	C21H30O3	330.46753	
48 All Compounds	11-Hydroxy-THC Negative	36557-05-8	C21H30O3	330.46753	
Favorites	17-alpha-Methyltestosterone	72-63-9	C20H30O2	302.45715	
ilash	17-Hydroxyprogesterone	604-09-1	C21H30O3	330.46753	
► CLASSES	1-Hydroxytriazolam	37115-45-0	C17H12Cl2N4O	359.21460	
	2,6-Dichlorbenzamide	2008-58-4	C7H5CI2NO	190.02846	
	20beta-Dihydroprednisolone		C21H30O5	362.46616	
	20beta-Dihydroprednisolone-H20		C21H28O4	344.45099	
	20beta-Dihydroprednisone		C21H28O5	360.45032	
	20beta-Dihydroprednisone-H2O		C21H26O4	342.43515	
	25B-NBOMe	1026511-90-9	C18H22BrNO3	380.28128	
	25C-NBOMe	1227608-02-7	C18H22CINO3	335.83051	
	25D-NBOMe		C19H25NO3	315.40741	
	25H-NBOMe	1566571-52-5	C18H23NO3	301.38568	
	25I-NBOMe	1043868-97-8	C18H22INO3	427.28226	
	250H Vit D2	412.334137	C28H44O2	412.65640	
	250H Vit D3	19356-17-3	C27H44O2	400.64539	
	2 Amine 5 ablerabenens	740 50 5	01211100110	224 00450	

#### **Retrospective Analysis Simplified**

In addition, because the data was acquired in a non-targeted approach the processing method designed here for screening targeted compounds can be quickly adjusted and used for unknown compound identification using non-targeted data processing. Users can retrospectively analyze previously acquired MS and MS/MS data sets to screen for new compounds without having to re-inject samples, allowing data sets to be re-processed when newly identified forensic targets are discovered. For instance, initial screening results with a five-compound list was shown below:

(A) Original data analysis with 5 compounds

Ъ			<b>√</b> <sup>+</sup> Sample Type	•	Acceptance 🔹 👻	%	<b>A</b>	/22 🔡 '	'c 📃 I	k C	"H" 📃 👻	23		\ ∕ Mo	re	•
Index	Sample Name	Component Name	Library Hit	Library Score	Formula	Mass Error	RT Confi	Isotope Confi	Library Confi	Mass Error	Reten Time	Isotope Ratio Dif	Rete Time	Precursor Mass	Comb Score	Height
1	02_interday01_93drug_di100	6-MAM	6-MAM	100.0	C19H21NO4	<ul> <li>Image: A set of the set of the</li></ul>	<ul> <li>Image: A start of the start of</li></ul>	<ul> <li>Image: A start of the start of</li></ul>	<ul> <li>Image: A set of the set of the</li></ul>	1.0	0.1	1.5	3.08	328.1543	97.3	266852
2	02_interday01_93drug_di100	7-Aminoclonazepam	7-Aminoclonazepam	98.7	C15H12CIN3O	<ul> <li>Image: A second s</li></ul>	<ul> <li>Image: A second s</li></ul>	<ul> <li>Image: A second s</li></ul>	<ul> <li>Image: A second s</li></ul>	-0.2	0.1	0.7	4.35	286.0742	98.5	1197448
3	02_interday01_93drug_di100	7-Hydroxymitragyline	7-Hydroxymitragyline	91.0	C23H30N2O5	<ul> <li>Image: A second s</li></ul>	<ul> <li>Image: A set of the set of the</li></ul>	<ul> <li></li> </ul>	<ul> <li>Image: A second s</li></ul>	0.8	1.6	1.0	4.55	415.2227	89.4	103201
4	02_interday01_93drug_di100	Acetyl Fentanyl	Acetyl fentanyl	99.3	C21H26N2O	<ul> <li>Image: A set of the set of the</li></ul>	<ul> <li>Image: A start of the start of</li></ul>	<ul> <li>Image: A set of the set of the</li></ul>	<ul> <li>Image: A second s</li></ul>	0.5	0.0	0.8	4.68	323.2118	98.3	163843
5	02_interday01_93drug_di100	Alpha-Hydroxyalprazolam	Alpha-Hydroxyalprazolam	93.0	C17H13CIN4O	<ul> <li>Image: A second s</li></ul>	<ul> <li>Image: A second s</li></ul>	~	<ul> <li>Image: A start of the start of</li></ul>	0.0	0.1	2.6	6.10	325.0851	95.0	495136

For retrospective data analysis, a new process method was built for 10 compounds including 5 initial compounds and 5 new compounds by using search parameters that included compounds name, their formula and their retention times. The updated processing method was then used to re-analyze data sets for the new compound. And the retrospective screening results with new compound list are shown below:

(B) Retrospective data analysis with 10 compounds

							••	7-32		IIIX	∽n' 'n				more	· · · ·
Index Sam	nple Name	Component Name	Library Hit	Library Score	Formula	Mass Error	RT Confi	Isotope Confi	Library Confi	Mass Error	Reten Time	Isotope Ratio Dif	Rete Time	Precursor Mass	Comb Score	Height
1 02_interday	y01_93drug_di1	6-MAM	6-MAM	100.0	C19H21NO4	~	~	~	~	1.0	2.1	1.5	3.08	328.1543	93.5	266852
2 02_interday	y01_93drug_di1	7-Aminoclonazepam	7-Aminoclonazepam	98.7	C15H12CIN3O	~	~	~	~	-0.2	1.0	0.7	4.35	286.0742	96.7	1197448
3 02_interday	y01_93drug_di1	7-Hydroxymitragyline	7-Hydroxymitragyline	91.0	C23H30N2O5	~	~	~	~	0.8	0.1	1.0	4.55	415.2227	92.5	103201
4 02_interday	y01_93drug_di1	Acetyl Fentanyl	Acetyl fentanyl	99.3	C21H26N2O	~	~	~	~	0.5	0.0	0.8	4.68	323.2118	98.3	163843
5 02_interday	y01_93drug_di1	Alpha-Hydroxyalprazolam	Alpha-Hydroxyalprazolam	93.0	C17H13CIN4O	~	~	~	~	0.0	0.1	2.6	6.10	325.0851	95.0	495136
6 02_interday	y01_93drug_di1	Alpha-hydroxymidazolam	Alpha-hydroxymidazolam	95.9	C18H13CIFN3O	~	~	~	~	-0.5	0.1	5.9	6.11	342.0804	95.0	964304
7 02_interday	y01_93drug_di1	Alpha-hydroxytriazolam	alpha-Hydroxytriazolam	91.8	C17H12Cl2N4O	~	~	~	~	0.8	0.0	1.5	5.88	359.0461	93.1	315629
8 02_interday	y01_93drug_di1	Alpha-PPP	Alpha-PPP	93.6	C13H17NO	~	~	~	~	-0.5	0.1	0.8	3.13	204.1383	94.7	693044
9 02_interday	y01_93drug_di1	Alpha-PVP	Alpha-PVP	97.5	C15H21NO	~	~	~	~	0.5	1.5	1.0	4.11	232.1696	94.3	885140
10 02_interday	y01_93drug_di1	Alprazolam	Alprazolam	99.4	C17H13CIN4	~	~	~	~	-0.7	0.3	1.6	6.26	309.0902	97.3	2495917

Users can retrospectively analyze previously acquired MS and MS/MS data sets to screen for new compounds without having to re-inject samples, allowing for data sets to be re-processed preparation procedures for urine and whole blood.

## REFERENCES

[1] SCIEX vMethod<sup>™</sup> - Forensic Toxicology Screening on X500R QTOF, part number: 5058220

[2] SCIEX Forensics High Resolution MS/MS Spectral Library 2.1, part number: 5059566 (To be available in September 2017)

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