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

Oscar G. Cabrices[1], Alexandre Wang[1], Xiang He[1], Holly McCall[1], Laura Baker[1], Adrian Taylor[2].

[1]SCIEX, 1201 Radio Rd, Redwood City, CA USA 94065.
[2]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.

SCIEX X500R QTOF System

EXPERIMENTAL

Sample Preparation for Urine Matrix

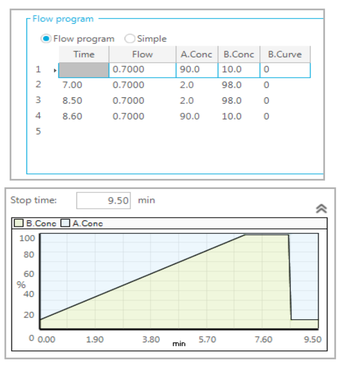
The stock standard mixtures in neat solutions were diluted with methanol: water (20:80, v/v) to appropriate concentrations. These diluted solutions were used to determine the retention time of the 664 compounds. Subsequently, the urine and whole blood samples were prepared to confirm the retention times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

Sample Preparation for Blood Matrix

For whole blood samples, 10.0 µL of stock standard solutions were spiked into 90.0 µL of human whole blood matrix. The samples were extracted by using a protein precipitation procedure. Basically, 900 µL 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 µL methanol: water (20:80, v/v).

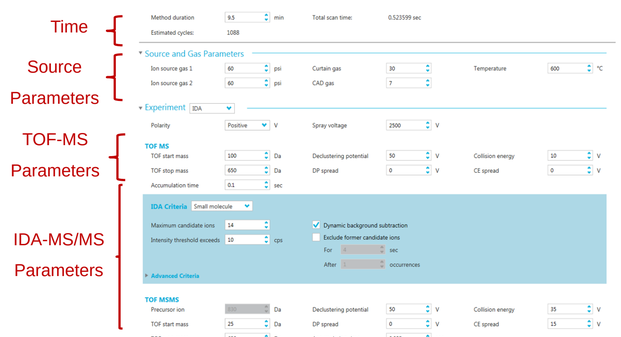
LC Conditions

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.

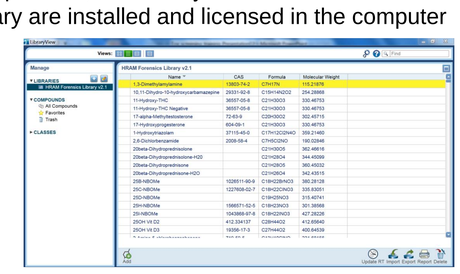


MS/MS Conditions

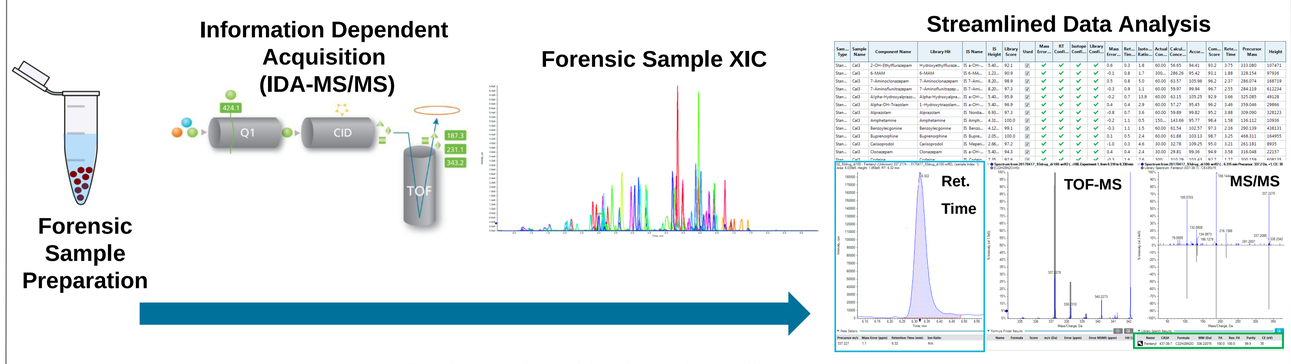
Source conditions and the method settings for non-targeted, IDA-MS/MS acquisition methods are listed below. Those settings allow screening for the 664 targeted, as well as the additional non-targeted compounds.



Prior to data process, the LibraryView™ software and HRAM forensic library are installed and licensed in the computer system.



664 Forensic Compound vMethod™ Analysis Workflow



Identification with multi-criteria including MS/MS

Index	Sample Name	Sample Type	Component Name	Used	Mass Error Confidence	RT Confidence	Isotope Confidence	Library Confidence	Mass Error (ppm)	Library Hit	Library Score	Isotope Ratio Difference
28	1	Unkno...	Nitenpyram		✔	✔	✔	✔	0.7	Nitenpyr...	87.9	1.6
34	2	Unkno...	Nitenpyram	✔	✔	✔	✔	✔	0.4	Nitenpyr...	91.9	5.5
58	20	Unkno...	Nitenpyram	✔	✔	✔	✔	✔	0.5	Nitenpyr...	92.8	2.5
64	50	Unkno...	Nitenpyram		✔	✔	✔	✔	-0.7	Nitenpyr...	95.1	5.7
70	100	Unkno...	Nitenpyram		✔	✔	✔	✔	0.0	Nitenpyr...	94.8	3.2

Mass accuracy — Retention time — Isotope ratio — MS/MS purity

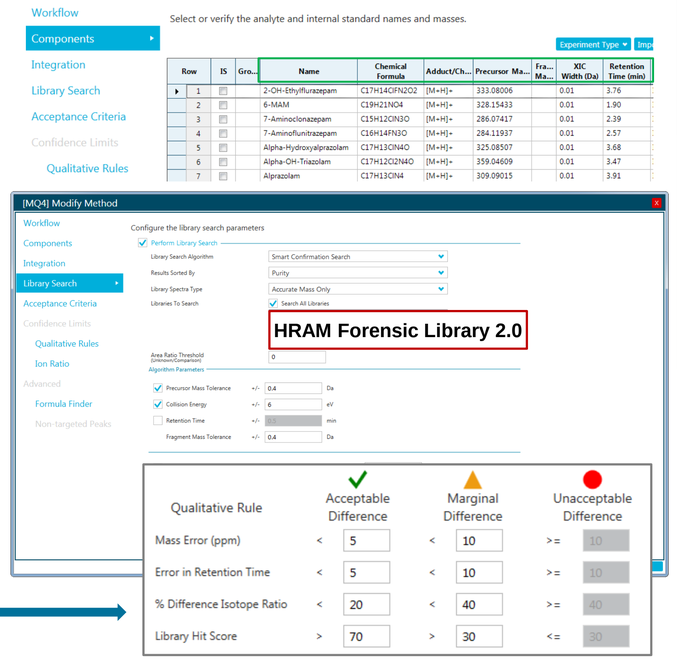
RESULTS AND DISCUSSION

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.

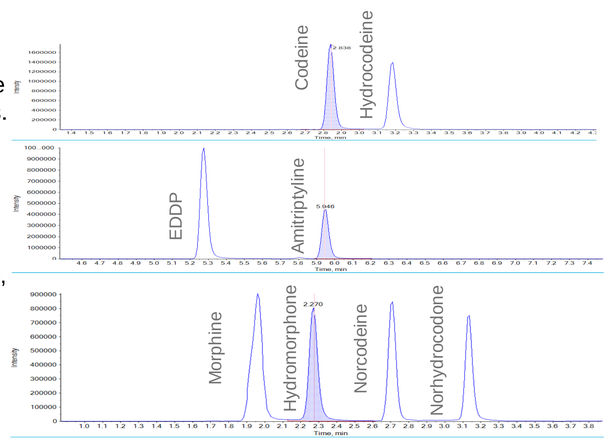
The confidence criteria used for screening were mass error, RT error, isotope ratio difference, and library score. A traffic light system where different colors were assigned to different performance levels provided a way to assess the quality of the match. For example, in the case of mass error, green 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:



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.

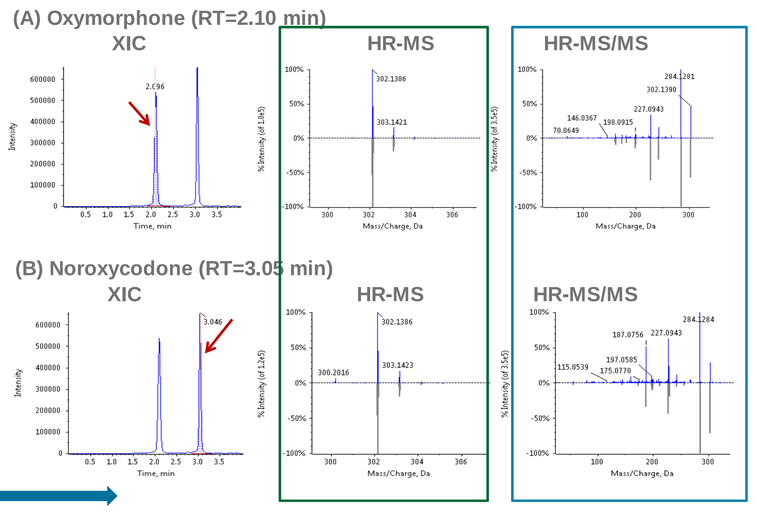


Enhanced Ability of Compound Identification

The retention time determined by the optimized LC condition combined with high-resolution mass spectrometry (HRMS) and HR-MS/MS information enable more accurate compound identification.

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.



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

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	✔	✔	✔	✔	1.0	0.1	1.5	3.08	328.1543	97.3	266852
2	02_interday01_93drug_di100	7-Aminoclonazepam	7-Aminoclonazepam	98.7	C15H12ClN3O	✔	✔	✔	✔	-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	✔	✔	✔	✔	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	✔	✔	✔	✔	0.5	0.0	0.8	4.68	323.2118	98.3	163843
5	02_interday01_93drug_di100	Alpha-Hydroxyalprazolam	Alpha-Hydroxyalprazolam	93.0	C17H13ClN4O	✔	✔	✔	✔	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

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_di1 ...	6-MAM	6-MAM	100.0	C19H21NO4	✔	✔	✔	✔	1.0	2.1	1.5	3.08	328.1543	93.5	266852
2	02_interday01_93drug_di1 ...	7-Aminoclonazepam	7-Aminoclonazepam	98.7	C15H12ClN3O	✔	✔	✔	✔	-0.2	1.0	0.7	4.35	286.0742	96.7	1197448
3	02_interday01_93drug_di1 ...	7-Hydroxymitragyline	7-Hydroxymitragyline	91.0	C23H30N2O5	✔	✔	✔	✔	0.8	0.1	1.0	4.55	415.2227	92.5	103201
4	02_interday01_93drug_di1 ...	Acetyl Fentanyl	Acetyl fentanyl	99.3	C21H26N2O	✔	✔	✔	✔	0.5	0.0	0.8	4.68	323.2118	98.3	163843
5	02_interday01_93drug_di1 ...	Alpha-Hydroxyalprazolam	Alpha-Hydroxyalprazolam	93.0	C17H13ClN4O	✔	✔	✔	✔	0.0	0.1	2.6	6.10	325.0851	95.0	495136
6	02_interday01_93drug_di1 ...	Alpha-hydroxymidazolam	Alpha-hydroxymidazolam	95.9	C18H13ClFN3O	✔	✔	✔	✔	-0.5	0.1	5.9	6.11	342.0804	95.0	964304
7	02_interday01_93drug_di1 ...	Alpha-hydroxytriazolam	alpha-Hydroxytriazolam	91.8	C17H12Cl2N4O	✔	✔	✔	✔	0.8	0.0	1.5	5.88	359.0461	93.1	315629
8	02_interday01_93drug_di1 ...	Alpha-PPP	Alpha-PPP	93.6	C13H17NO	✔	✔	✔	✔	-0.5	0.1	0.8	3.13	204.1383	94.7	693044
9	02_interday01_93drug_di1 ...	Alpha-PVP	Alpha-PVP	97.5	C15H21NO	✔	✔	✔	✔	0.5	1.5	1.0	4.11	232.1696	94.3	885140
10	02_interday01_93drug_di1 ...	Alprazolam	Alprazolam	99.4	C17H13ClN4	✔	✔	✔	✔	-0.7	0.3	1.6	6.26	309.0902	97.3	2495917

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)

Component name	RT (min)	%CV				Difference (%) between neat and matrix	
		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
Desalkylflurazepam	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
Desmethyldoxepin	5.34	1.1	0.3	0.1	0.2	0.5	0.4
Dextromethorphan	5.16	1.2	0.3	0.0	0.1	0.6	0.4
Diazepam	6.72	0.2	0.1	0.0	0.1	0.0	-0.1
Dihydrocodeine	2.73	1.6	0.6	0.2	0.4	0.8	0.7
Doxepin	5.34	1.1	0.4	0.1	0.2	0.5	0.4
EDDP	5.20	1.1	0.3	0.1	0.2	0.5	0.4
MDA	3.07	1.9	0.5	0.2	0.2	0.5	0.4
MDEA	3.56	1.5	0.4	0.0	0.2	0.6	0.4
MDMA	3.27	1.7	0.5	0.2	0.2	0.5	0.4
MDPV	4.32	1.3	0.3	0.1	0.2	0.6	0.5
Meperidine	4.26	1.3	0.2	0.0	0.1	0.5	0.3
Mephedrone	3.37	1.7	0.4	0.0	0.2	0.6	0.4
Meprobamate	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
Methamphetamine	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

For a complete list of compounds, please refer to the SCIEX vMethod™ 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 non-targeted 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.
- 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™ - 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)

TRADEMARKS/LICENSING

AB Sciex is doing business as SCIEX.

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