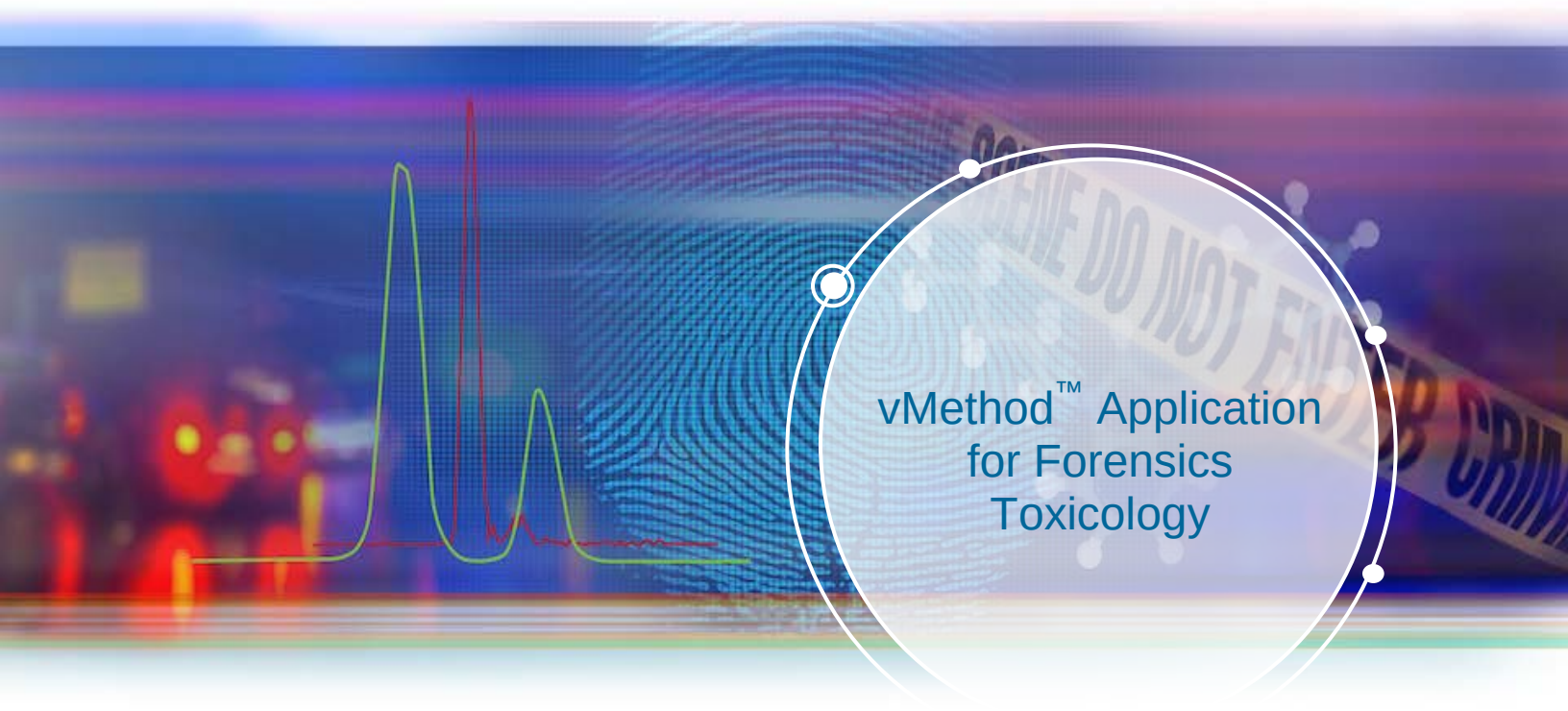
SCIEX
Answers for Science.
Knowledge for Life.™



Single-Injection Screening of 664 Forensic Toxicology Compounds on a SCIEX X500R QTOF System

Single-Injection Screening of 664 Forensic Toxicology Compounds on a SCIEX X500R QTOF System

Lijuan Fu, Xiang He, Holly McCall, Alexandre Wang, Xiong Lei, Adrian Taylor, Juan Chen, Laura Baker, Chris Borton

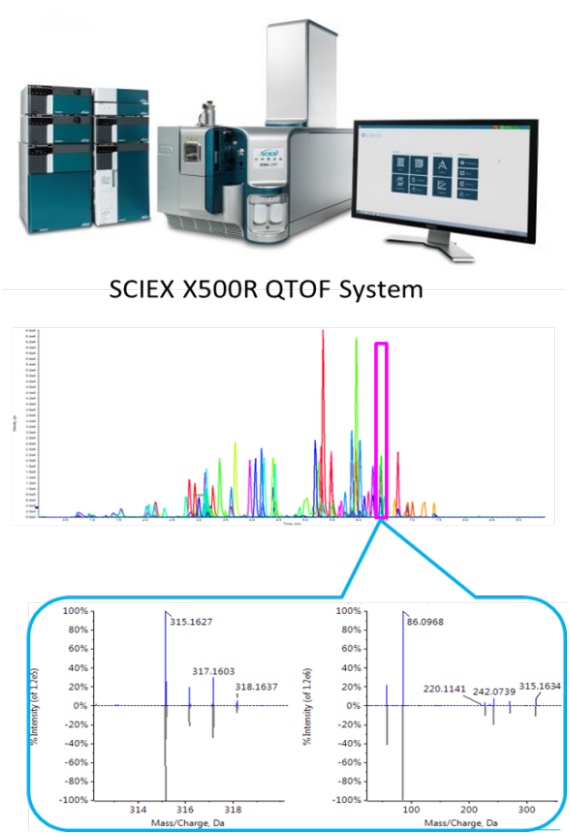
SCIEX Redwood City, California (USA)

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. The SCIEX X500R Q-TOF system provides the capability of switching between MS and MS/MS scans instantly, enabling fast acquisition of detailed structural information for easier compound identification. 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. Due to its straightforward design and intuitive software workflows, non-targeted data obtained on the X500R can be retrospectively mined for additional analytes missed in initial screens, which is important with the constant emergence of new synthetic drugs. Also, the availability of retrospective analysis on X500R has become increasingly popular in forensic work.

Information-dependent acquisition (IDA), also called data-dependent acquisition (DDA), is a widely-used approach for acquiring MS/MS information for screening purposes. In IDA-MS/MS mode, a survey scan is performed to collect information on precursor ions, followed by multiple, dependent MS/MS scans on several of the most abundant precursor/candidate ions. To efficiently evaluate these complex and data-rich scans, SCIEX OS 1.2 software platform was developed to automatically choose candidate ions by sorting through the observed intensities of precursor ions. Each MS/MS scans are performed after mass filtration (by Q1) of single precursor ion, resulting in IDA-MS/MS spectra that are free of interfering species aiding in accurate MS/MS library spectral matching.

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 1.2. 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 samples and library-search settings recommended here can help automate and confidently establish the identification of unknowns in an efficient, all-in-one workflow.



SCIEX X500R QTOF System, Representative XIC and library matching results.

Experimental conditions

Sample preparation

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.

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 separation

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. The mobile phases were replaced every 2 days. A linear gradient (600 µL/min) from 10% B to 98% B in 7.0 min followed by 1.5 min of 98% B and 1.0 min of 10% B was employed.

Acquisition method settings

Source conditions and the method settings for non-targeted, IDA-MS/MS acquisition methods are listed in Table 1. Those settings allow screening for the 664 targeted, as well as the additional non-targeted compounds.

Processing method settings

To identify compounds in the analyzed samples, a targeted screening approach was employed using SCIEX OS software version 2.0. 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 (Figure 2) with a 30 second half time window. An MS/MS library [2] 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 Figure 3. A representative search result is also shown (Figure 4).

Results and Discussion

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). Figure 2 shows an example of full chromatographic separation for 4 isomers, including Morphine, Hydromorphone, Norcodeine and Norhydrocodone, with the optimized LC condition. Figure 5 show example extracted ion chromatograms for 80 out of the 664 compounds using the optimized LC condition.

Reproducibility of retention time measurements

Because retention time (RT) is a critical element for accurate identification of each forensic analyte using this screening method, the following RT reproducibility tests were conducted for each compound to evaluate the robustness of the LC condition in this method: (1) reproducibility on 3 separate columns; (2) the inter-day (n=3) reproducibility; (3) the reproducibility in neat versus matrix samples. Results are shown for 80 out the 664 compounds (Table 2). For a complete list of compounds, please refer to the SCIEX vMethod™ application [1].

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, as determined using the following equation:

$$\text{Difference \%} = \frac{(\text{Average Retention Time in urine} - \text{Retention Time in Neat})}{\text{Retention Time in Neat}} \times 100$$

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 [2], enable more accurate compound identification. For example, the Noroxycodone (Figure 6 A) and Oxymorphone (Figure 6 B) 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.

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 in Figure 7-A. 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 in Figure 7-B.

Conclusion

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 when newly identified forensic targets are discovered.

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)

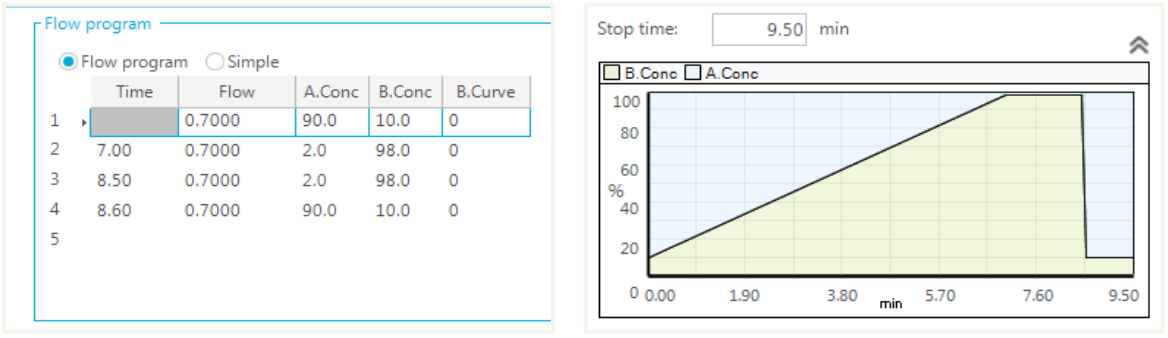


Figure 1. LC gradient

Table 1. X500R Q-TOF system parameters and settings for operation	
Source parameters	
Source	Turbo Spray
CUR	30
CAD	7
IS	2500
TEM	600
GS1	60
GS2	60
Duration	9.5 min
Compound parameters	
Polarity	Positive
Experiment mode	IDA (Information-dependent acquisition)
TOF MS	
TOF start mass	100
TOF stop mass	650
Accumulation time	0.1 s
DP	50
DP Spread	0
CE	10
CE Spread	0
IDA Criteria	
Small molecule	Selected
Maximum Candidate Ion	14
Intensity Threshold exceeds	10
Dynamic background subtraction	Selected
TOF MS/MS	
TOF start mass	25
TOF stop mass	650
DP	50
DP Spread	0
CE	35
CE Spread	15
Accumulation time	0.025 sec

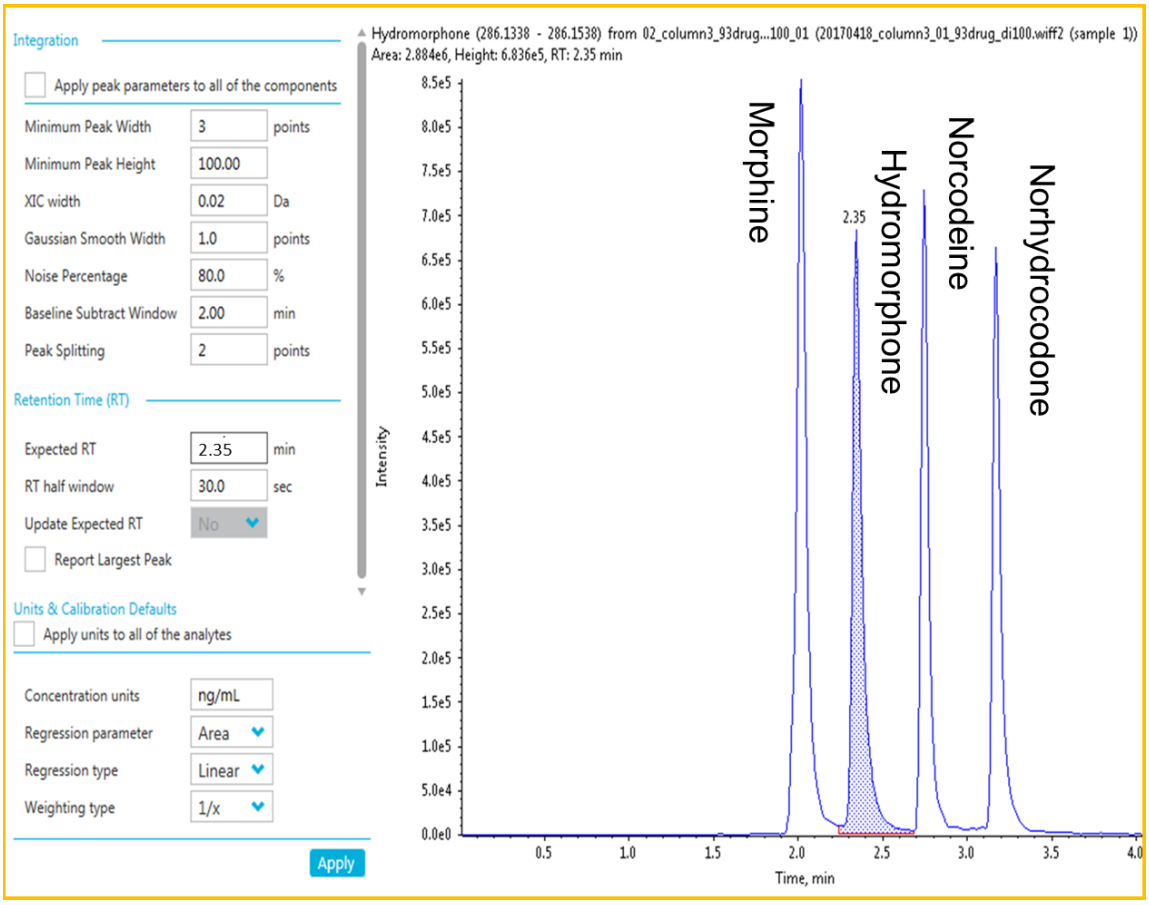


Figure 2. The parameters used for the integration of hydromorphone.

Qualitative Rule	✓ Acceptable Difference		▲ Marginal Difference		● Unacceptable Difference
Mass Error (ppm)	<	5	<	10	>= 10
Error in Retention Time	<	5	<	10	>= 10
% Difference Isotope Ratio	<	20	<	40	>= 40
Library Hit Score	>	70	>	30	<= 30

Figure 3. Confidence criteria for data processing using Sciex OS 2.0 software

Figure 4 Representative search results obtained after using a targeted screening approach to identify compounds in urine samples.

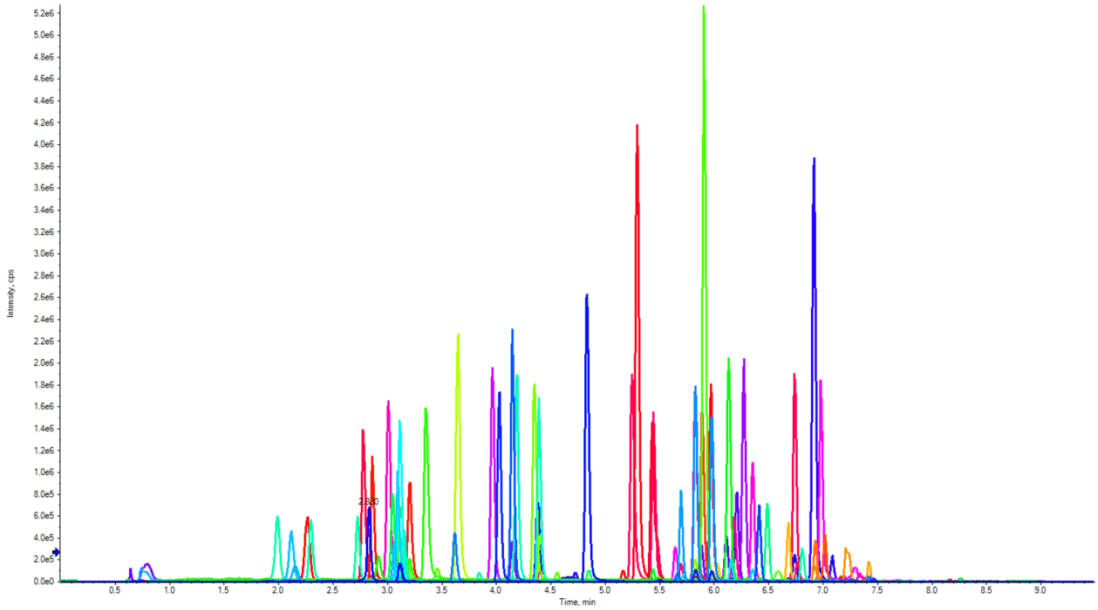
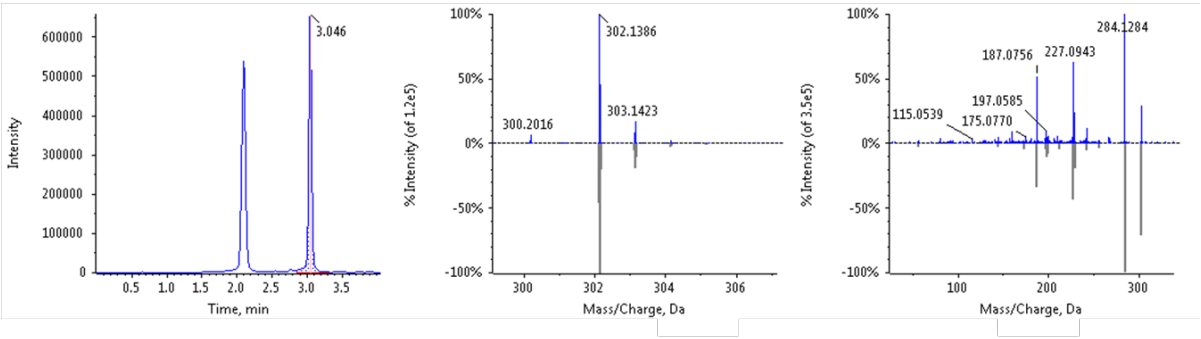


Figure 5. Extracted ion chromatograms (XICs) for multiple analytes (80 out of 600) show optimal peak separation.

(A) Noroxycodone (RT=3.05 min)

Extracted Ion Chromatogram HR-MS HR-MS/MS



(B) Oxymorphone (RT=2.10 min)

Extracted Ion Chromatogram HR-MS HR-MS/MS

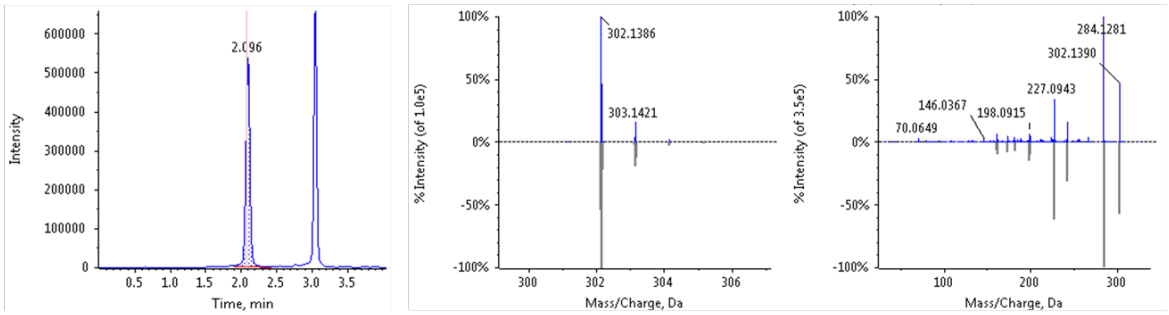


Figure 6 Representative XIC, HR-MS and HR-MS/MS spectra for Noroxycodone and Oxymorphone

(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

(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

Figure 7 Example for retrospective data analysis

Table 2. 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

vMethod™ Application for Forensics Toxicology

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
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

Document Number: RUO-MKT-08-6395-A

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

International Sales
For our office locations please call the division headquarters or refer to our website at
sciex.com/offices