



# Forensic Identification and Quantification Workflows Delivered on a Revolutionary Designed QTOF and SCIEX OS Software

Igniting your routine forensic testing with the new SCIEX X500R QTOF System

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## **Overview**

Quadrupole Time-of-Flight (QTOF) mass spectrometry is becoming the desired technology for sensitive and selective screening workflows in a forensic toxicological setting. The technology overcomes many challenges faced when using traditional techniques and more significantly captures all information about the sample in one injection to allow for retrospectively mining the data. Using the accurate mass and mass resolution information from both TOF-MS and TOF-MS/MS acquired data allows for simultaneous highly specific targeted quantitation and non-targeted screening. Here we describe a new benchtop QTOF system with revolutionary N geometry TOF designed flight path and new, intuitive software for easy adoption of accurate mass technology to forensic testing. We demonstrate that the new hardware and software combined allow a high level of confidence for compound identification and quantification from urine samples in one seamless workflow.

## Introduction

Liquid Chromatography coupled to Tandem Mass Spectrometry (LC-MS/MS) is a widely used analytical tool for the screening of compounds and metabolites. Triple quadrupole based mass analyzers operated in Multiple Reaction Monitoring (MRM) mode have become the preferred method to routinely deliver highly selective and sensitive quantitative results, but are limited to targeted screening only.

With an increasing demand for retrospective and non-targeted analyses of forensic toxicological samples, high resolution, accurate mass and full scan mass analyzers are gaining popularity. The adoption of the technology has been restricted by more complicated to use and more expensive instrumentation compared to their nominal mass counterparts. Here we introduce a revolutionary new Quadrupole Time-of-Flight (QTOF) mass spectrometer that contains advances in engineering design to bring the high performance TOF-MS and TOF-MS/MS capabilities into a compact benchtop platform.



Figure 1: The SCIEX ExionLC<sup>™</sup> AC HPLC system (left), the SCIEX X500R QTOF System (middle) and SCIEX OS Software (right).

The SCIEX X500R QTOF mass spectrometer is part of a complete workflow from the fully integrated SCIEX ExionLC<sup>™</sup> Systems to the freshly designed SCIEX OS software; a new user interface for simultaneous identification and quantification workflows (Figure 1.)

## SCIEX X500R QTOF System

The new benchtop SCIEX X500R QTOF System with revolutionary N geometry TOF designed flight path has been engineered for simplicity, service accessibility and minimized footprint. N TOF geometry, versus V geometry, gives the same effective flight path length for ions and therefore resolution, but in a smaller overall foot print. This has been accomplished with an extra mirror in the TOF chamber without a loss in transmission (Figure 2). To maintain stable mass accuracy the system uses a simple heated TOF vacuum chamber design. This consists of 6 discreet heater drones maintaining a constant TOF chamber temperature, insulating against ambient temperature changes (Figure 2).



Figure 2. SCIEX X500R QTOF System and Technology Advances

The system has been designed to maximize robustness and uptime

- Integrated Calibrant Delivery System and Turbo V<sup>™</sup> Source with TwinSpray probe (Figure 3), allows seamless mass accuracy auto-calibrations during long runs.
- Service Accessibility
  - Easy QJet<sup>®</sup> and Turbo pump access for fast and efficient maintenance, increasing system uptime
  - Segmented TOF vacuum chamber allows easy access to detector while protecting sensitive accelerator.



Figure 3. Integrated Calibrant Delivery System and Turbo V™ Source with TwinSpray probe

Figure 4 shows the mass accuracy stability of the SCIEX X500R QTOF System when analyzing multiple urine samples, spiked with various concentrations of analytes, without auto-calibration, over a ten hour period. The majority of compounds are shown to be within a 1 ppm mass accuracy over this time period.



Figure 4. Mass Accuracy Stability of the SCIEX X500R QTOF System in the Analysis of Urine Samples

Figure 5 shows the resolution for both TOF-MS and TOF-MS/MS masses sampled over a seven day time period on a SCIEX X500R QTOF System.



Figure 5. Resolution of the SCIEX X500R QTOF System Over a Week's Period for Selected m/z; both TOF-MS and TOF-MS/MS

Figure 6 shows a representative linear dynamic range of the SCIEX X500R QTOF System showing 4 orders for the Asenapine compound.

# **SCIEX OS Software**

SCIEX OS Software is a single software platform for LC and MS control, data processing as well as reporting.



Figure 6. Linearity of the SCIEX X500R QTOF System shown for Asenapine (0.5 ng/mL to 1000 ng/mL)

The SCIEX OS software is intuitive and logical, segregated into Acquisition, Processing and Management work spaces (Figure 7). In the Acquisition work space there are separate method editors for the LC and MS parameters as well as batch creation and queue panes. The Processing allows for simultaneous identification and quantification. The Management workspace allows the adjustment of hardware, software and user settings.



Figure 7. Home Page of SCIEX OS Software. Single Software Platform for LC/MS Control, Data Processing and Reporting.

# Acquisition

The SCIEX OS software has a simplified step by step acquisition method setup with only relevant parameters being visible. Figure 8 shows the setup for an Information Dependent Acquisition method for the analysis of small molecules and the intuitive steps that are taken to input the MS parameter values.

For a quick instrument status check, the Manual Tune guides the user through the steps to perform a quick review of the system performance, perform an auto-calibration and report out the test result prior to running a batch (Figure 9).



Figure 8. SCIEX OS Software MS Acquisition Method



Figure 9. SCIEX OS Software MS Tune Allows for Quick Instrument Status Check via Simple Step by Step Instructions

Building a batch is assisted by the smart grid design allowing copy/paste, fill down, auto increment and import/export. Figure 10 shows the batch editor and the link to the auto-calibration setup.

Once the batch has been submitted to the queue the Auto-Cal samples are inserted as shown in the Queue Manager in Figure 11. The SCIEX OS software allows for detailed instrument status including monitoring and recording of LC pressure traces as well as direct control of the individual components of the system (Figure 11).

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Fore	nsic WB	screen				· ····									
	terry to	RS Method	LC Method	Rack cells	Rack pesition	Plate code	Plate Austrian	Vial position	Date Hite		_				
4	WILH 1	DOA DA	00AUC.1	Muthheelack	3	100 March	Field Position	н	Parentic unknown	where bioast samples					
1	WEH 2	1014.054	004163	MultiParelack	1	300Mate	Field Particle	30	Personal junction	whether (related suggests					
	WUSH 3	1004.004	DOA1C,3	Mutchare/ack	3	Littinete	Farest Partners	33	Parental ambravat	whole travid samples					
۰.	WORK 4	1004.004	00410,1	thatfurfack	1	100Mat	Parti Position.	34	General Laboration	shale blood camples					
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۰.	WISH &	004304	DO41/23	Matheelack	3	10mm	Field Partiest	36	Torrest advant	whether belowed samples					
r,	WISH R	1004.004	D04103	MultiPlanefack	2	3209446	Parel Posttein	37	Parencia unknown	while blood samples					
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۰.	0.5	DOAIDA.	00A(C.1	MultiPatelack	3	100Mes	Fined Position	38	Parynaic unknown	while block samples.					
	1	004.04	00416,1	MARPINE'sch	1	300946	Field Postice	40	Forenal selected	where blood samples					
11	5	004304	00410.3	Mathanlak	3	1/Dives	Parti Politica	-0.	Porefect; unknown	whole block samples					
12	38.	1004 EH	00410.1	shatthatchick	1	107Mail	Famil Posticon	42	Reported and real	which bened tarrying					
13	16	DOA SIA	DOA1C.1	MURPHICK	1.	1001046	Parel Partner	40	Parents ( previous	which becard samples					
24	100	DOA DA	004163	Mathanlack	1	300946	Fixed Postton	44	Passan attended	whole proved samples					
23	500	DOA DA	00410.3	Muthheaduck	3	107944	Fixed Postton	45	Aprenaic anticipant	whole blood samples					
26	1000	posizie.	DOALC,1	Mattheofack	1.	100vium	Facel Parmer.	-14	Patricia and series	where bland samples					
17	18	DOAIDA.	00A\C.1	MultiPate/ark	1	100Vitete	Fired Postfrier	38	Parenti primare	which blood samples.					
28	tim feet	1014.014	00416,3	Matthewise.	1	3003/who	Field Particle	.47	Terring and services	whethe Select Lastspilled					
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21															
21					Sm	art o	rid to a	assist i	n buildi	ng the ba	tch				
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21				C	opy/pa	ste, f	III dow	n, auto	increm	ent, impo	rvexpo	n -			
24															

Figure 10. SCIEX OS Software Batch Editor and Setup for Auto-Calibration



Figure 12. SCIEX OS Software Allows the Simultaneous Review of Qualitative and Quantitative Results

The SCIEX OS Software allows the user to filter the results to only show compounds that pass acceptance criteria and are detected with user defined confidence (Figure 13)



Figure 11. SCIEX 0S Software Queue Manager with Inserted Auto-Cal Samples and Detailed Instrument Status Panel

# **Processing- Analytics**

Once a results table is generated, quantitative and qualitative results can be reviewed in the same panel (Figure 12). A Traffic light system indicates the confidence of the identification based on accurate mass, retention time, isotopic pattern and library matching. Compounds calculated to be above the cutoff concentration in unknown samples are flagged. In the same work space the peak integration, spectra and calibration lines can be displayed.



Figure 13. SCIEX OS Software Filtering Criteria

Finally results can be reported out using the *Create Report* functionality (Figure 14)



Figure 14. SCIEX OS Software Report Generation

# Acquisition Workflows on the SCIEX X500R QTOF System with SCIEX OS Software

#### Information Dependent Acquisition

Information Dependent Acquisition (IDA) is a non-targeted data acquisition (Figure 15). It allows for TOF-MS quantification and provides high confidence in screening with MS/MS information that uses high selectivity through unit Q1 resolution. IDA-MS/MS provides the most interference-free fragmentation information.



Figure 15. Information Dependent Acquisition

When creating an IDA acquisition, MS and MS/MS settings are all contained in a single User Interface. Figure 16 shows the parameters used in the IDA experiments described in this technical note. In this example, one TOF-MS survey scan and up to 16 dependent TOF-MS/MS scans are triggered from the survey scan, in each data cycle.

Method duration			min	Total scan time:	0.58089 s	ec 👘					
Estimated cycles:	826										
Source and Gas Para	neters -										
Ion source ges 1	60	- 1	psi	Curtain gas	30	;		Temperature	600	0	۰с
Ion source-ges 2	60	- :	pel	CAD gas	7	:					
Experiment IDA	• -										
Polarity	Positive	٣	v	Spray voltage	2500	;	V.				
TOF MS											
TOF start mass	100	- :	Da	Declustering potential	60	:	v	Collision energy	10	٥	٧
10F stop mats	1000	:	Da	DP spread	0	:	v	CE spread	0	۰	٧
Accumulation time	0.1	:	sec.								
IDA Criteria Small mole	cule 💌										
Maximum candidate ions	16	:		Oynamic background	subtraction						
Intensity threshold exceeds	10	:	CD0	Exclude former candi	date ions						
				For	sec						
<ul> <li>Advenced Offente</li> </ul>				After 1	C occurrent	DES					
TOT MEME											
Presursor ion	411	1	Da	Declustering potential	60	:	v	Collision energy	35	۵	٧
TOF start mass	40	- :	De	DP spread	ø	;	v	CE spread	15	0	v
10F stop math	1000	-	De	Accumulation time	0.025	:	sec				

Figure 16. SCIEX OS Software Information Dependent Acquisition Method Editor

Due to the high scanning speed (up to 100 Hz for single collision energy) on SCIEX X500R QTOF systems, almost all potential

compound targets in the samples can be confirmed with confident MS/MS library matching.

IDA-MS/MS is a non-targeted data acquisition method and the user needs to define the maximum number of candidates in each data cycle. More intense ions take higher priority within any data cycle, so for less abundant species especially in complex sample matrices, the associated MS/MS information might be missed. Therefore, an unbiased MS/MS data acquisition approach that collects MS/MS information for everything at all times (MS/MS<sup>AII</sup>) will solve this potential concern.

# SWATH<sup>®</sup> Acquisition

SWATH<sup>®</sup> acquisition (Figure 17) is non-targeted and provides MS/MS information for everything in the sample, all the time. Each scan cycle in SWATH<sup>®</sup> Acquisition starts with a TOF-MS experiment. The acquisition approach therefore allows for screening and quantification from both TOF-MS and TOF-MS/MS acquired data.

Most of the existing MS/MS<sup>AII</sup> techniques collect MS and MS/MS information for all ions in an alternating fashion, i.e. MS scan of all precursor ions, followed by MS/MS scan of the fragments of all precursor ions. Without precursor ion selection, such approaches suffer from insufficient sensitivity, selectivity and narrower linear range compared to IDA-MS/MS.



#### Figure 17. SWATH<sup>®</sup> Acquisition

SWATH<sup>®</sup> acquisition uses either a fixed or a variable Q1 isolation window, as part of a TOF-MS/MS experiment, which is stepped across the mass range of interest. Figure 18 shows the SWATH<sup>®</sup> acquisition method editor in the SCIEX OS Software, with the example of 16 looped TOF-MS/MS experiments, each with a different (variable) Q1 isolation window, that are required to cover the mass range of interest (120 to 500 *m/z*).

TOF st	art mass	40	0	Da	TOF stop	mass	500	\$	Da	Dynamic collin	sion energy
Accum	ulation time	0.025	0	\$	Charge s	tate	1				
lass	Table	Autofill SWATH winds									
	Precursor	ion start mass (Du)	Precu	rsor lon	stop mass (Da)	Declusterin	g potential (V)	DP spre	ad (V)	Collision energy (V)	CE spread (V
1	120.0000		140.0	000		60		0		35	15
2	139.0000		165.0	000		60		0		35	15
3	154.0000		195.0	000		60		0		35	15
£	194.0000		205.0	000		60		0		35	15
÷	204.0000		218.0	000		60		0		35	15
6	217.0000		240.0	000		60		0		35	15
6.	239.0000		258.0	000		60		0		35	15
ŝ.	257.0000		273.0	000		60		0		35	15
Ê.	272.0000		290.0	000		60		0		35	15
0	239.0000		304.0	000		60		0		35	15
11	303.0000		313.0	000		60		0		35	15
12	312.0000		326.0	000		60		0		35	15
13	325.0000		333.0	000		60		0		35	15
14	332.0000		358.0	000		60		0		35	15
15	357.0000		450.0	000		60		0		35	15
16	449.0000		500.0	000		60		0		35	15

Figure 18. SCIEX OS Software SWATH<sup>®</sup> Acquisition Method Editor

By varying the Q1 isolation window for each TOF-MS/MS experiment we are able to separate compounds with similar mass into different SWATH<sup>®</sup> Acquisition windows so that we minimize the amount of convolution (multiple precursor ions generating common fragment ions at the same time) in each TOF-MS/MS experiment (Figure 19).

OF M	SMS									
TOF st	art mass	40	\$	Da	TOF stop mas	is 500	0	Da	Dynamic co	Ilisicn energy
Accum	ulation time	0.025	0	5	Charge state	1				
Mass	Table	Autofill SWATH winds								
	Precursor	ion start mass (Da)	Precu	irsor i	ion stop mass (Da) De	clustering potential (V	DP spre	ead (V)	Collision energy (V	) CE spread (V)
1	120.0000		140.0	000	Amphetanine (136)				11	
2	139.0000		165.0	0000	Methamphetamine (150)					
3	154.0000		195.0	0000	MDA (180)					
4	194.0000		205.0	000	MDMA (194)	Nortapentadol (204)				
5	204.0000		218.0	000		Tapentadol (222)				
6	217.0000		240.0	0000	Norketamine (224)					
7	239.0000		258.0	0000	Ketamine (238)	Nortramadol (259)				
8	257.0000		273.0	0000		Tramadol (264)	Novenia	facine (264	Nordoxepin (266)	Nodiazepam (271
9	272.0000		290.0	000	Morphine (206)	Norchlordiazepoxide (28	6) Venlafax	ine (276)	Doxepin (290)	Diacepam (285)
10	239.0000		304.0	0000	Codeine (300)	Chlordiazeposide (300)	Norsertra	aline (292)	Norfuczetine (296)	)
11	303.0000		313.0	000	Alprazolan (309)	Norcitaloprarr (311)	Setralin	e (306)	Fuoxetine (310)	
12	312.0000		326.0	000	Hydroxyalorazolam (325)	Citalopram (325)	Norciaza	spine (313)	THC (315)	
13	325.0000		333.0	0000	Norproposyphene (326)		Clozapin	ie (327)	THC-OH (331)	
14	332.0000		358.0	0000	Propoxyphene (340)	Triazolam (343)				
15	357.0000		450.0	000	Norbuprenorphine (414)	Hydrotriazolam (359)	Norverap	amil (441)		
16	449.0000		500.0	0000	Buprenorphine (468)		Verapart	nil (455)		

Figure 19. Constructing Variable SWATH<sup>®</sup> Acquisition Window Sizes for each Looped TOF-MS/MS Experiment to Minimize Convolution in the SCIEX OS Software

## **MRM**<sup>HR</sup>

MRM<sup>HR</sup> (High Resolution Multiple Reaction Monitoring) is a targeted data acquisition for quantification purposes and can be unscheduled or scheduled. Compound dependent parameters can be optimized for each MRM<sup>HR</sup>.





Figure 20. Comparison of  $\text{MRM}^{\text{HR}}$  with traditional (unit resolution) MRM

To help transition familiarity of MRM performed on a triple quadrupole to MRM<sup>HR</sup> performed on the SCIEX X500R QTOF system, the SCIEX OS Software has a unique way of building the MRM<sup>HR</sup> method to have the look and feel of performing traditional MRM experiments by allowing the input of the precursor ion mass (MRM Q1 equivalent mass) and accurate fragment mass (MRM Q3 equivalent nominal mass) (Figure 21). These transitions can easily be imported from the SCIEX high resolution 1700 compound MS/MS forensic spectral library to include up to 5 transitions per compound.

TOF M	s								
TOF st	tart mass 100	Da 🕄 Da	Declustering potentia	a) 60	:	V	Collision energy	10	C V
TOF st	top mass 100	10 🗘 Da	DP spread	Φ	:	V	CE spread	0	5 V
Accum	vulation lime 0.1	a sec							
TOF M	SMS								
Mass	Table   Apply f	ragment ion mass	Apply TOF start/stop mass		ipply Scan Sc	hedule	Import and autofit.	Sort by precursor ion	
	Compound ID	Pressesor i	Fragment ion (Da)	Accumul	Declus	Collision		Retention time tol	varce (+/- see
1	6-MMM	328.15	211.0747	0.0100	60	33	2.04	15	
2	7-Aninoclonazepam	286.07	222.1025	0.0100	60	32	2.67	15	
3	7-Hydroxymitragyline	415.22	190.0864	0.0100	60	40	2.96	15	
4	Acetyl Fentanyl	323.21	105.0700	0.0100	60	45	3.00	15	
5	Alpha-Hydroxyalprazola	im 325.09	297.0665	0.0100	60	31	4.18	15	
6	Alpha-hydroxymidazola	m 342.08	203.0377	0.0100	60	34	4.51	15	
7	Alpha-hydroxytriazolam	359.05	331.0272	0.0100	60	35	3.95	15	
8	Alphy-PPP	204.14	105.0699	0.0100	60	35	2.04	15	
9	Alphu-PVP	232.17	161.0963	0.0100	60	20	2.61	15	
10	Alprazolam	309.09	281.0730	0.0100	60	35	4.49	15	
11	AM-2201-4-OH pentyl	376.17	155.0492	0.0100	60	35	6.07	15	
12	Amitiptyline	278.29	117.0702	0.0100	60	31	4.53	15	
			+/- 10						
:		Precursor	MS/MS	100 H	z		RT	RT half wind	low
84	Zolpdem	308.18	235.1262	0.0100	60	45	3.06	15	
85	THC-COOH	345.21	299.2157	0.0100	60	30	6.34	15	

Figure 21. SCIEX OS Software Scheduled MRM<sup>HR</sup> Method Editor, Fragment Ion Mass  $\pm$  10 m/z

The quantification method is then generated automatically from the acquisition method (Figure 22).

8	ow	15	Group	Name	Precursor Ma	Fragment Mass (Da)	XIC Width (Da)	Retention Time (min)	B	Experiment Index
	1	10	6-MAM	6-MAM	328.154	211.0747	0.02	2.04	-	2 TOF M5M5 of 328.2 (40 - 500)
	2	10	7-Aminoclonazepam	7-Aminoclonazepam	246.074	222.1025	0.02	2.67		3 TOF MSM5 of 286.1 (40 - 500)
	3	10	7-Hydroxymitragyline	7-Hydroxymitragyline	4:5.223	190.0864	0.02	2.96		4 TOF MSMS of 415.2 (40 - 500)
	4	10	Acetyl Feritanyl	Acetyl Fentanyl	323.212	105.07	0.02	3.00		5 TOF MSMS of 323.2 (40 - 500)
	5	12	Alpha-Hydroxyalpracolam	Alpha-Hydroxyalprazolam	325.085	297.0665	0.02	4.19		6 TOF M5M5 of 325.1 (40 - 500)
	6	10	Alpha-hydroxymidacolam	Alpha-hydroxymidazolam	342.08	203.0377	0.02	4.51		7 TOF MSMS of 342.1 (40 - 500)
	7	10	Alpha-hydroxytriadolam	Alpha-hydroxytriazolam	359.046	331.0272	0.02	3.94		8 TOF MSMS of 359.0 (40 - 500)
	8	12	Alpha-PPP	Alpha-999	214.138	105.0699	0.02	2.04		9 TOF MSM5 of 204.1 (40 - 500)
	9	12	Alpha-PvP	Alpha-PVP	202.17	161.0963	0.02	2.61		10 TOF MSMS of 232.2 (40 - 500)
	10	10	Alprazolam	Alprazolam	319.09	281.073	0.02	4.49		11 TOF MSMS of 309.1 (40 - 500)
	11	10	AM-2201 4-OH pentyl	AM-2201 4-OH pentyl	3*6.171	155.0492	0.02	6.07		12 TOF MSMS of 376.2 (40 - 500)
	12	10	Amitriptyline	Amitriptyline	278.19	117.0702	0.02	4.53		13 TOF MSMs of 278.2 (40 - 500)
	13	10	Amphetamine	Amphetamine	136.112	91.0553	0.02	1.63		14 TOF MSMS of 136.1 (40 - 500)
	14	10	Benapylecgonine	Benzoylecgonine	290.139	168.1021	0.02	2.36		15 TOF MSMS of 290.1 (40 - 500)
	15	15	Buphedrone	Buphedrone	1'8.123	131.07	0.02	1.98		16 TOF MSMS of 178.1 (40 - 500)
	16	10	Bupresorphine	Buprenorphine	448.311	414.2636	0.02	3.67		17 TOF MSMS of 468.3 (40 - 500)
	17	10	Carisoprodol	Carisoprodol	261.181	55.0565	0.02	3.66		18 TOF MSMS of 261.2 (40 - 500)
	18	10	Oomipramine	Clomipramine	3,5.162	86.0959	0.02	5.36		19 TOF MSMS of 315.2 (40 - 500)
	19	10	Codeine	Codeine	3#0.159	215.1109	0.02	1.87		20 TOF MSMS of 300.2 (40 - 500)
	20	10	Cotinine	Cotinine	177.102	80.0495	0.02	1.83		21 TOF MSMS of 177.1 (40 - 500)
	21	10	Cyclobenzaprine	Cyclobenzaprine	2'6.175	215.0878	0.02	4.28		22 TOF MSMS of 276.2 (40 - 500)
	22	12	DesalkyHuracepam	Desalkylfluracepam	219.054	140.0264	0.02	4.42		23 TOF MSMS of 289.1 (40 - 500)
	23	10	Desipramine	Desipramine	247.186	72.0823	0.02	4.28		24 TOF MSMS of 267.2 (40 - 500)
	24	10	Desmethyldoxepin	Desmethyldaxepin	216.154	107.0493	0.02	3.60		25 TOF MSMS of 266.2 (40 - 500)
	25	10	Dextromethorphan	Dextromethorphan	2'2.201	215.1458	0.02	3.44		26 TOF MSMS of 272.2 (40 - 500)
	26	15	Diazepam	Diageparts	215.079	154.0424	0.02	5.43		27 TOF MSMS of 285.1 (40 - 500)

Figure 22. Automatically generating the SCIEX OS Software MRM<sup>HR</sup> Quantification Method from the SCIEX OS Software MRM<sup>HR</sup> Acquisition Method

Alternatively, if the fragment masses are not known at the time of the acquisition method creation, then the traditional MRM<sup>HR</sup> setup is still achievable by inputting the TOF start and stop masses (Figure 23).

OF MS	100	· Da	Dealler	and an an advector of	60	-		Collision anarrai	10	• •
10.0 8.0	11 Product 1	• •	C-PC-LA	and because		-		country install		
TOF sto	ip mass 1000	Da Da	DP spr	bed	0	5	v	CE spread	0	C V
Accurta	dation time 0.1	391								
OF MS	MS									
Mass T	able O Apply frage	nent ion mass	Apply TOF a	tart/stop mass	V App	Scan Sche	dule 🔤	urt and substitu-	fort by precursor ion	
	Group name	Precursor L.	TOF sta	TOF sto	Accumul_	Declus	Collision	Retention ti	Retention time (c	Aerance (+/- se
1	6-MAM	328.15	40.00000	500.00000	0.0100	60	33	2.04	15	
2	7-Aminoclonazepam	286.07	40.00000	500.00000	0.0100	60	32	2.67	15	
3	7-Hydroxymitragyline	415.22	40.00000	500.00000	0.0100	60	40	2.96	15	
4	Acetyl Fentanyl	323.21	40.00000	500.00000	0.0100	60	48	3.00	15	
5	Alpha-Hydroxyalprazolam	325.09	40.00000	500.00000	0.0100	60	31	4.18	15	
6	Alpha-hydroxymidazolam	342.08	40.00000	500.00000	0.0100	60	34	4.52	15	
7	Alpha-hydroxytriazolam	359.05	40,00000	500.00000	0.0100	60	35	3.95	15	
8	Alpha-PPP	204.14	40.00000	500.00000	0.0100	60	35	2.04	15	
9	Alpha-PvP	232.17	40.00000	500.00000	0.0200	60	20	2.62	15	
10	Alprazolam	309.09	40.00000	500.00000	0.0100	60	35	4.00	15	
11	AM-2201 4-OH pentyl	376.17	40.00000	500.00000	0.0100	60	35	6.07	15	
12	Amitriptyline	278.19	40.00000	500.00000	0.0100	60	31	4.53	15	
			-	$\rightarrow$						
:		Precursor	MS	MS	100 Hz			RT	RT half wi	ndow
84	Zolpidem	308.18	40.00000	500.00000	0.0100	60	.45	3.05	15	
85	THC-COOH	345.22	40.00000	500.00000	0.0100	60	30	6.34	15	

Figure 23. Scheduled MRM<sup>HR</sup> Method Editor, MS/MS Full Scan

## **Materials and Methods**

#### Compound list and spiking solutions

Table 1 lists the 93 compounds plus internal standards. All were procured from Cerilliant Corporation (Round Rock, TX). Two spiking solutions in methanol were prepared: one for analytes (**SA**) and the other for internal standards (**SIS**). Concentrations of all the analytes in the spiking solution **SA** are listed in Table 1.

Compounds in black font are in the regular panel (72 analytes) and the ones in blue font are the additional 21 analytes in the extended panel (93 analytes). Internal standards are shown in grey background.

#### Calibrator preparation

Blank human urine was spiked with solution **SA** to prepare calibrators. Four levels of calibrators were prepared. Actual concentrations varied for each compound, however the concentration ratio between these calibrators was always (in descending order): 20:6:2:1. For instance, the four different concentrations (in descending order) for fentanyl in the calibrators were: 20, 6, 2 and 1 ng/mL, while those of gabapentin were: 1000, 300, 100 and 50 ng/mL.

#### Sample preparation

- 100 μL urine sample was mixed with 25 μL IMCS Rapid Hydrolysis Buffer, 20 μL IMCSzyme and 10 μL SIS. Both IMCS Rapid Hydrolysis Buffer and IMCSzyme were acquired from IMCS (Columbia, SC). Hydrolysis time was typically between 30 and 60 min at 55°C.
- After hydrolysis was complete, 0.2 mL methanol and 0.625 mL water were added to the mixture.
- 3. The mixture was then centrifuged at 21,000 g for 10 min.
- 4. The supernatant was transferred to a glass vial with insert for analysis by LC-MS/MS.

#### Liquid Chromatography

Liquid Chromatography analysis was performed on the SCIEX ExionLC<sup>TM</sup> AC HPLC system at 30°C. Separation was achieved using a Phenomenex Kinetex Phenyl-Hexyl column (50 × 2.1 mm, 2.6 µm, 00B-4495-E0), with a Phenomenex SecurityGuard ULTRA UHPLC Phenyl (AJ0-8774) and ULTRA holder (AJ0-9000). Mobile phase A (MPA) was ammonium formate in water. Mobile phase B (MPB) was formic acid in methanol. The LC flow rate was 0.5 mL/min and the LC run-times investigated were 8.0 and 2.0 minutes. Injection volume was 10 µL.

#### Table 1: List of analytes and internal standards, and their concentrations in spiking solution (for preparation of calibrators)

Compounds	(ng/mL)	Compounds	(ng/mL)	Compounds	(ng/mL)	Compounds	(ng/mL)
6-MAM	1000	Gabapentin	10000	Naloxone	5000	Pentobarbital	10000
7-Aminoclonazepam	5000	Hydrocodone	5000	Naltrexone	5000	Secobarbital	10000
7-Hydroxymitragynine	1000	Hydromorphone	5000	N-desmethyltapentadol	5000	ТНС-СООН	2000
Acetyl Fentanyl	200	Imipramine	5000	Norbuprenorphine	2000	6-MAM-d3	
Alpha-Hydroxyalprazolam	5000	JWH 122 5-OH pentyl	1000	Norcodeine	5000	Amphetamine-d5	
Alpha-Hydroxymidazolam	5000	JWH 19 6-OH hexyl	1000	Nordiazepam	5000	Benzoylecgonine- d3	
Alpha-Hydroxytriazolam	5000	JWH 210 5-OH-pentyl	1000	Norfentanyl	200	Buprenorphine-d4	
Alpha-PPP	1000	JWH-018 4-OH pentyl	1000	Norhydrocodone	5000	Carisoprodol-d7	
Alpha-PVP	1000	JWH-018 pentanoic acid	1000	Normeperidine	5000	Codeine-d6	
Alprazolam	5000	JWH-073 3-OH butyl	1000	Noroxycodone	5000	Fentanyl-d5	
AM-2201 4-OH pentyl	1000	JWH-073-butanoic acid	1000	Norpropoxyphene	10000	Hydrocodone-d6	
Amitriptyline	5000	JWH-250-N-4-OH pentyl	1000	Nortriptyline	5000	Hydromorphone-d6	
Amphetamine	10000	JWH-073-butanoic acid	1000	O-Desmethyltramadol	5000	JWH 018 4-OH pentyl-d5	
Benzoylecgonine	5000	JWH-250-N-4-OH pentyl	1000	Oxazepam	5000	JWH 019 6-OH hexyl-d5	
Buphedrone	1000	Lorazepam	5000	Oxycodone	5000	MDPV-d8	
Buprenorphine	2000	MDA	10000	Oxymorphone	5000	Meperidine-d4	
Carisoprodol	10000	MDEA	10000	PCP	2500	Mephedrone-d3	
Clomipramine	5000	MDMA	10000	Pregabalin	10000	Meprobamate-d7	
Codeine	5000	MDPV	1000	Propoxyphene	10000	Methadone-d3	
Cotinine	5000	Meperidine	5000	Protriptyline	5000	Methamphetamine- d5	
Cyclobenzaprine	5000	Mephedrone	1000	RCS4-4-OH-pentyl	1000	Methylone-d3	
Desalkylflurazepam	5000	Meprobamate	10000	Ritalinic Acid	5000	Mitragynine-d3	
Desipramine	5000	Methadone	10000	Sufentanil	200	Morphine-d6	
Desmethyldoxepin	5000	Methamphetamine	10000	Tapentadol	5000	Nordiazepam-d5	
Dextromethorphan	5000	Methedrone	1000	Temazepam	5000	Nortriptyline-d3	
Diazepam	5000	Methylone	1000	Tramadol	5000	Oxycodone-d6	
Dihydrocodeine	5000	Methylphenidate	5000	Zolpidem	5000	Oxymorphone-d3	
Doxepin	5000	Midazolam	5000	Amobarbital/pentobarbital	10000	THC-COOH-d3	
EDDP	10000	Mitragynine	1000	Butabarbital	10000	Butalbital-d5	
Fentanyl	200	Morphine	5000	Butalbital	10000	Secobarbital-d5	

Grey background: IS

# SCIEX OS Software Processing

### Identification and Quantification Results

Defining the retention time and accurate precursor and fragment mass for each analyte is performed first (Figure 24) followed by setting up the library searching parameters.

Workflow	Sele	ct or v	erity ti	te analyte	and internal standard is	ames and masses.						
Components •						1	Experimen	t Type * Import		Export_	Options	
Integration		ow	15	Group	Name	Chemical Formula	Adduct	Precursor Mass	Fragment Mass (Da)	XIC Width (Da)	Retention Time (min)	ISN
Library Search		1	10	-2.5	2.5-dimethoxy-4-	C13H21N025	[M+H]+	256.13658		0.07	1.02	_
and the second se		2	12	12.5	2.5-dimethoxy-4	C13H21N025	[M+H]+	256.13658	197.063	0.02	6.90	
Acceptance Otterna		3	12	12.5	2,5-dimethoxy-4 _	C13H21N025	[M+H]+	256.13658	224.06	0.02	6.90	
		4	12	*2.5	'2.5-dimethoxy-4-	C13H21N025	[M+H]+	256.13658	182.04	0.02	6.90	
		5	10	-2.5	"2.5-dimethoxy-4	C13H21N025	[M+H]+	256.13658	167.02	0.02	6.90	
Qualitative Rules		6.	10	-2.5-	'2,5-dimethoxy-4-	C13H21N025	[M+H]+	256.13658	134.03	0.02	1.74	
Ine Patio		7	12	*2.5	"2.5-dimethoxy-4	C13H21NO25	[M+H]+	256.13658	164.08	0.02	6.90	
aut rating		8	12	20-8	2C-8-FLY 1	C12H14BrNO2	[M+H]+	284.02807		0.02	0.66	
		9	12	20-8	2C-B-FLY 2	C12H14BrNO2	[M+H]+	284.02807	188.0629	0.02	5.83	
		10	12	20-8	2C-8-FLY 3	C12H148/NO2	[M+H]+	284.02807	267	0.02	8.55	
Formula Neider		11	10	20-8-	2C-B-FLY 4	C12H14BrNO2	[M+H]+	284.02907	173.06	0.02	6.95	
		22	12	20-8	2C-8-FLY 5	C12H148/NO2	[M+H]+	284.02807	145.06	0.02	1.65	
		3.5	12	*3,4	3,4-dimethylmeth	C12H17NO	[M+H]+	192.13829	Stores	0.02	3.60	
		14	12	*3.4	"1.4-dimethylmeth	C12H17NO	[M+H]+	192.13829	159.1042	0.02	4.42	
		15	11	*3,4	"3,4-dimethylmeth	C12H17NO	[M+H]+	192.13829	144.08	0.02	4.61	
		16	12	*3,4	"3.4-dimethylmeth	C12H17NO	[M+H]+	192.13829	145.05	0.02	4.60	
		17	12	13,4	"3,4-dimethylmeth	C12H17NO	[M+H]+	192.13829	174.13	0.02	4.43	
		10	12	3-des	3-desmethylprodin	C15H21N02	[M+H]+	248.16451		0.02	4.72	
		19	80	3-des	3-desmethylprodin	C15H21NO2	[M+H]+	248.16451	174.1177	0.02	4.07	
	125	20	10	3.des	L-riesmethylogodia	C15421N/02	DA+HI+	14816451	70.07	0.02	1.01	

Figure 24. Defining the Retention Time, Accurate mass of Precursor and Fragment lons

Defining the qualifying components includes setting accuracy tolerance levels for calibrants and controls as well as flagging integration discrepancies. Qualifying definitions also includes defining the identification criteria and setting the confidence levels at which mass error, error in retention time, isotope pattern and library matching scores are deemed an acceptable difference, marginal difference or unacceptable difference (Figure 25).



Figure 25. Defining the Identification and Quantification Qualifying Components in the SCIEX OS Software

# **Results and Discussion**

As part of evaluating the new SCIEX X500R QTOF to perform simultaneous identification and quantification of compounds from forensically related samples routinely, we investigated two LC gradients. We evaluated each methods capabilities to elute all analytes throughout the entire gradient as evenly as possible in order to maximize triggering IDA MS/MS for all components, reduce the MRM<sup>HR</sup> concurrency for quality of data (*Scheduled* MRM<sup>HR</sup>), resolve isobaric species and alleviate ion suppression caused by co-elution of excessive number of analytes. Figure 26 shows the Extracted Ion Chromatograms (XICs) for the 8.0 minute run and Figure 27 show the XICs for the 2.0 minute run.



Figure 26. Extracted Ion Chromatograms for Analytes from a Urine Analysis using an 8.0 Minute LC Runtime



Figure 27. Extracted Ion Chromatograms for Analytes from a Urine Analysis using an 2.0 Minute LC Runtime

#### Information Dependent Acquisition

With the ability to provide the most interference free fragmentation information for library searching in a non-targeted acquisition, the IDA workflow provides the highest confidence screening using MS/MS information. Figure 28 shows the multiple screening criteria that are used for identification purposes in the SCIEX OS Software's easy to understand user interface.



Figure 28 Screening and Identification Results from an IDA Experiment

The importance of acquiring quality MS/MS data for identification purposes, and not to solely rely on the accurate mass of the precursor ion, is demonstrated in Figures 29, 30 and 31. Each figure demonstrates how, by acquiring MS/MS data, we can distinguish between structural isobaric compounds. In each example shown, isobaric compounds are barely chromatographically separated and so the presence of either or both the compounds cannot be identified by either accurate mass of the precursor ions or confidently by retention time if there is any drift in retention of the compounds. The highest confidence is gained through library MS/MS comparisons.



Figure 29. High Confidence Identification of Naloxone and 6-MAM Isobaric Compounds Gained through Library MS/MS Comparisons



Figure 30. High Confidence Identification of Buprenorphine and Mephedrone Isobaric Compounds Gained through Library MS/MS Comparisons



Figure 31. High Confidence Identification of Methylphenidate and Normeperidine Isobaric Compounds Gained through Library MS/MS Comparisons

Figures 32 and 33 show selected compound examples of XICs from the TOF-MS information acquired as part of the IDA workflow. This information can be used for quantification purposes.



Figure 32. XICs of  $\alpha$ -PVP in Urine from TOF-MS information (Urine was diluted 10-fold; 10 µL injection)



Figure 33. XICs of Sufentanil in Urine from TOF-MS information (Urine was diluted 10-fold; 10  $\mu L$  injection)

Figure 34 shows representative calibration curves obtained from the IDA experiment.



Figure 34. Representative Calibration Curves for Selected Compounds Showing that the TOF-MS information can be used for Quantification in an IDA Workflow

# SWATH<sup>®</sup> Acquisition Results

SWATH<sup>®</sup> Acquired data can be processed in a similar way to processing IDA data for screening purposes. Again this uses multiple criteria for confidence in identification; most importantly using MS/MS library matching. Figure 35 shows a result of this from the 8.0 minute LC run which resulted in a high true positive rate of 98%.

Component Name	Act Con	Expe	Height	Rete Time	Precursor Mass	Mass Error	RT Conti	Isotope Confi	Library Confi	Found At Man	Mass Error_	Ret Tim	Library Hit	Library Score	Com Score	Isotope Ratio Dif.
7-Hydroxymitragyline	60.00	2.83	3500	2.68	415.2227	4		24	Ŧ	415.2224	-0.9	1.1	7-Hydroxyevbragyline	100.0	95.5	6.2
Alpha-999	60.00	1.83	19459	1.85	204.1383	~	~	4	~	204.1381	-0.8	1.1	Alpha-PPP	98.0	96.1	0.2
Alpha-PVP	60.00	2.46	6287	2.49	232.1696	~	~	~	~	232.1653	-1.2	1.3	Alpha-PVP	99.4	95.9	0.7
AM-2201 4-OH pentyl	60.00	6.12	60427	6.14	376.1707	~	~	~	~	376.1704	-0.9	0.3	AM2201 N-hydroxype	100.0	97.3	2.9
Buphedrone	60.00	1.78	7046	1.81	178.1226	~	~	~	~	178.1237	0.3	1.4	Buphedrone	96.5	93.3	10.5
MH-018 4-OH pentyl	60.00	6.15	97180	6.16	358.1802	~	~	~	~	358.1796	-1.5	0.2	JWH-018 hydroxypent_	100.0	96.6	1.3
/WH-018 pentanoic acid	60.00	6.19	43385	6.18	372.1594	~	~	~	~	372.1591	-0.9	0.1	/WH-018 N-pentanoic	93.4	93.2	3.6
JWH-019 6-OH heryl	60.00	6.26	91522	6.28	372.1958	~	*	~	~	372.1954	+1.0	0.0	JWH-019 N-hydroxyft	100.0	97.2	3.0
JWH-073 3-OH butyl	60.00	6.10	82454	6.11	344.1645	~	~	~	~	344.1641	-1.1	0.2	JWH-073 N-hydroxyb	100.0	97.2	1.8
IWH-073 butanoic acid	60.00	6.11	97180	6.16	358.1438	~	~	~	~	358.1438	0.1	0.8	JWH-073 M-butanoic [	97,4	94.5	12.0
/WH-122 5-OH pentyl	60.00	6.26	81429	6.21	372.1958	~	×.	~	~	372.1954	-1.0	0.2	/WH-122 N-hydroxyp	100.0	97.0	3.0
JWH-210 5-OH pentyl	60.00	6.35	71551	6.31	386.2115	*	*	*	*	386.2110	-1.1	0.3	/WH-210 N-hydroxyp	100.0	97.3	1.2
7WH-250 4-OH pentyl	60.00	5.92	67351	5.94	352.1907	~	~	~	~	352.1904	-0.9	0.4	/WH-250 N-hydroxyp	98.6	96.3	2.4
MDPV	60.00	2.62	24869	2.64	276.1594	~	~	~	~	276.1591	-1.1	1.5	MOPV	93.9	92.1	1.7
Mephedrone	60.00	1.79	1228	1.81	178.1226	~	~	~	~	178.1227	0.3	1.1	Buphedrone [Smart C	96.4	93.5	10.5
Mas	s ac	cur	acy F	lete	ntion	t time	, <b>]</b>	Ť	Ì		1	1		t		1
						ŀ	sotc	ppe i	ratio	)						
										MS/	MS					

Figure 35. Processed SWATH® Acquired Data using Multiple Identification Criteria; including MS/MS Library Matching

With traditional IDA-MS/MS, quantitation can only be performed from TOF-MS mode and not from the *in situ* sporadic TOF-MS/MS data points. In contrast, due to the continual and looped MS/MS scan function, quantification from fragment ions is achievable from SWATH<sup>®</sup> acquisition. Better selectivity from the fragment ion information (Figure 36) relative to parent ion information, allows more sensitive detection in MS/MS mode of lower concentration species in complex matrices.



Figure 36. Gains in Selectivity with the Ability to Extract Out a Specific Fragment Ion From Variable Window SWATH<sup>®</sup> Acquired Data Compared to Extracted Accurate Mass of the Precursor Ion

Figure 37 shows identification and quantification results for a synthetic drug obtained from SWATH<sup>®</sup> Acquisition using the 8.0 min LC run time. This compound was not in the original targeted list but retrospective interrogation of the data from this unknown

sample allowed for its identification without having to re-inject the sample again.



Figure 37. Identification and Quantification Results for n-Ethylcathinone Ephedrine Metabolite Compound Anlaysed by SWATH<sup>®</sup> Acquisition

The n-ethylcathinone ephedrine metabolite compound was identified based on unique fragment ions and their ratios as well as a library searching match (Figure 38). In a SWATH<sup>®</sup> acquisition experiment, not only can confirmation of the presence of compounds be made through MS/MS library matching and ion ratio calculations but because of the ability to extract out many unique fragment ions from the SWATH<sup>®</sup> acquired MS/MS data we can also determine the concentration based on quantification of either or both the precursor and fragment ions depending on which has less interferences.



Figure 38. Extraction of Unique Fragment Ions From SWATH<sup>®</sup> Acquisition and Using Both Ion Ratio and Library Matching to Confirm Presence of n-Ethylcathinone Ephedrine metabolite in an Unknown Urine Sample

When investigating using a 2.0 minute LC run time as part of the SWATH<sup>®</sup> acquisition, we were able to accomplish good quantification results. Figure 39 shows representative calibration curves obtained from the ultra-fast screening experiment.



Figure 39. Representative Calibration Curves Generated from the SWATH  $^{\otimes}$  Acquisition using a 2.0 minute LC Runtime (n=3)

Sensitivity examples are shown in Figures 40, 41 and 42 for selected compounds.







Figure 41. XICs of Fentanyl at Various Concentrations in Urine (Diluted 10-fold, 10  $\mu L$  injection)



Figure 42. XICs of Amitriptyline at Various Concentrations in Urine (Diluted 10-fold, 10  $\mu L$  injection)

In the SWATH<sup>®</sup> Acquisition, MS/MS information is always available and so we can confirm the presence of the compound through MS/MS library matching (Figures 43 and 44) at the same time as determining how much of the compound is present.







Figure 44. Confident Identification of Amitriptyline and EDDP from SWATH<sup>®</sup> Acquisition Through Library Searching; Showing LC Separation Between Isomers was Still Achievable with this Fast Method

At the cutoff concentrations, library matching worked well with 80% of compounds yielding greater than 70% hit score (Figure 45).

Sample Name	Component Na	Actual Conce	Height	Rete Time	Precursor Mass	Mass Error	RT Confi	Isotope Confi_	Library Confi	Found AtMass	Mass Error_	Ret Tim	Library Hit	Library Score	Combi. Score	Isotope Ratio Dif.
EPODCurve_UCal2	Alpha-Hydroxyal	50.00	39322	1.31	325.0851	~	~	~	~	3250851	0.0	0.00	Alpha-Hydroxya	100.0	95.1	18.6
EPODCurve_UCal2	Alpha-hydroxym	50.00	54742	1.32	342.0804	~	4	~	~	3420804	0.0	0.00	Alpha-hydroxy_	100.0	99.3	1.8
EPODCurve_UCal2	Buprenorphine	20.00	26422	1.24	468.3108	~	~	~	~	4683107	-0.3	0.01	Buprenorphine	100.0	97.9	2.9
EPODCurve_UCal2	Desallytturatep	50.00	39350	1.33	289,0538	4	~	~	~	2890539	0.2	0.01	Desalkytflurapep	100.0	98.2	1.4
EPODCurve_UCal2	E004	100.00	768373	1.20	278.1903	~	~	~	~	2781906	0.8	0.00	EDOP	100.0	96.1	1.3
EPODCurve_UCal2	Fentaryl	2.00	10599	1.17	337.2274	~	~	~	~	3372276	0.5	0.01	Fentanyl	100.0	97.3	0.7
EPOOCurve_UCal2	Hydrocodone	50.00	61526	0.92	300.1594	~	~	~	~	3001597	0.9	0.01	Hydrocodone	100.0	94.9	3.3
EPODCurve_UCal2	/WH-018 4-OH	10.00	29593	1.39	358,1802	~	~	~	~	3581801	-0.1	0.01	JWH-018 hydro	100.0	98.0	4.6
EPODCurve_UCal2	/WH-019 6-OH h_	10.00	44558	1.40	372.1958	~	~	~	~	3721959	0.3	0.01	/WH-019 N-byd.	100.0	96.7	4.4
EPODCurve_UCal2	/WH-073 3-OH_	10.00	31459	1.38	344.1645	~	~	~	~	3441648	0.9	0.01	MH-013 N-hyd	100.0	94.3	5.1
EPODCurve_UCal2	/WH-122 5-DH	10.00	44558	1.40	372.1958	~	~	~	~	3721959	0.3	0.01	JWH-019 N-hyd	100.0	96.7	4.4
EPODCurve_UCal2	Methamphetami	100.00	33875	0.92	150.1277	~	~	~	~	1501277	-0.4	0.00	Methamphetam	100.0	98.0	0.6
EPODCurve_UCal2	Methylphenidate	50.00	195247	1.07	234.1489	~	~	~	~	2341490	0.8	0.00	Methylphenidate	100.0	96.4	0.7
EPODCurve_UCal2	Norhuprenorphi	20.00	27123	1.17	414,2639	~	~	~	~	4142641	0.5	0.00	Norbuprenorphi	100.0	97.5	2.3
EPODCurve_UCal2	Nordiazepam	50.00	44694	1.35	271.0633	~	~	~	~	271.0635	0.8	0.00	Nordiasepam	100.0	95.5	3.7
EPODCurve_UCal2	Norosycodone	50.00	23623	0.90	302.1387	~	~	~	~	3021386	-0.2	0.01	Noroxycodone	100.0	97.9	4.3
EPODCurve_UCel2	Tepentedol	50.00	203639	1.09	222.1852	*	*	*	*	2221854	0.7	0.00	Tepentadol	100.0	95.8	1.1
EPDOCurve_UCal2	Тетнаграт	50.00	58735	1.34	301.0738	~	~	~	~	3010738	-0.2	0.01	Ternazeparts	100.0	98.1	3.2
EPODCurve_UCal2	Tramadol	50.00	169458	1.06	264.1958	~	~	~	~	2641961	1.0	0.00	Tramacol	100.0	95.9	0.6
EPODCurve_UCal2	Zolpidem	50.00	277566	1.13	308.1757	~	~	~	~	3081760	0.8	0.00	Zolpidem	100.0	96.1	1.9
EPODCurve_UCal2	7-Aminoclonaze	50.00	76113	1.12	286.0742	~	~	~	~	2850744	0.9	0.01	7-Amisocionaze	99.8	95.0	3.2
EPOOCurve_UCal2	Alprozolam	50.00	123684	1.32	309,0902	~	~	~	~	3090904	0.8	0.00	Alprazolam	99.8	95.7	4.0
EPODCurve_UCal2	Diapipam	50.00	110405	1.36	285.0789	~	~	~	~	2850791	0.7	0.01	Diszepum	99.7	95.5	3.4
EPODCurve_UCal2	Nathexone	50.00	56359	0.91	342.1700	~	~	~	~	3421698	-0.4	0.00	Nattresone	99.7	97.3	3.0
EPODCurve_UCal2	Ovymorphone	50.00	6763	0.41	302.1387	~	~	~	~	3021389	0.8	0.02	Oxymorphone	99.7	94.2	3.5
EPODCurve_UCal2	Amphetamine	100.00	3665	0.88	136.1121	~	~	~	~	1361129	-0.3	0.00	Amphetamine	99.6	97.1	4.1

Figure 45. Library Searching and Identification of Compounds in the 2.0 Minute Method at Cutoff Concentration Levels

## **MRM**<sup>HR</sup>

MRM<sup>HR</sup> is a purely targeted data MS/MS acquisition and can be unscheduled or scheduled. The only non-targeted and therefore retrospective capability is through the TOF-MS experiment which is performed at the beginning of every scan. The power of the workflow however, is its selectivity capabilities through the accurate mass of unique fragment ions for quantification purposes. This is demonstrated in Figure 46 where MRM<sup>HR</sup> is compared to the MRM analysis, extracted at nominal mass, and the extraction of the accurate mass of the precursor ion from a TOF-MS experiment. The compound is not able to be distinguished from the high background and interferences of the nominal mass experiment and not even by the extraction of the accurate mass of the precursor ion from the full scan TOF-MS experiment. It is not until we extract out two unique accurate mass fragment ions from the MRM<sup>HR</sup> experiment that we achieve the selectivity required to detect this compound by removal of the background and interferences and increase the S/N; improving the quantification capabilities. Another example of this selectivity gain over the accurate mass of the precursor ion is demonstrated in Figure 47 where a visible improvement in S/N is gained for the analysis of buprenorphine by the MRM<sup>HR</sup> approach.



Figure 46. Increased Selectivity with MRM<sup>HR</sup>; Avoiding False Negatives. Example given is a Feed Sample Tested Positive for NP Semicarbazide





TOF-MS: 178.1226±0.005 m/z

Figure 39. Scheduled MRM<sup>HR</sup> Selectivity Compared to TOF-MS; Buprenorphine (5ng/mL in urine, 10 fold dilution, 10 µL injection)

Quantification performance of the MRM<sup>HR</sup> is demonstrated in Figure 40 for the 8.0 minute LC-MS/MS method.

#### Scheduled MRM<sup>HR</sup>, 372.2→169.0644±0.0100 m/z



Figure 40. MRM^R Quantification Results for JWH-122 5-OH Pentyl in urine (Urine was diluted 10-fold, 10  $\mu L$  injection)

#### Negative Mode Performance

Figures 41 and 42 show a couple of examples of negative mode performance of the SCIEX X500R QTOF System.



Figure 41. Negative Mode Performance of SCIEX X500R QTOF System for Analysis of Amo/pentobarbital



Figure 42. Negative Mode Performance of SCIEX X500R QTOF System for Analysis of THC-COOH

## Conclusion

The arrival of the next generation QTOF, with the launch of the SCIEX X500R QTOF System and SCIEX OS Software, brings the powerful performance capabilities of the high resolution accurate mass technology to the routine identification and quantification forensic workflows.

- Hardware
  - SCIEX ExionLC<sup>™</sup> Systems
    - Fully controlled by SCIEX OS software
    - Improved software integration for better stability
  - SCIEX X500R QTOF System
    - N-geometry design (same effective flight path length for ions and therefore resolution than Vgeometry, but in a smaller overall footprint)
    - Heated TOF path for mass accuracy stability

 Minimized footprint, engineered for simplicity and service accessibility

Software

- SCIEX OS Software
  - Intuitive and logical single software platform for LC control, MS control, data processing and reporting.
  - New user interface
  - Simultaneous identification and quantitation

We have described the screening and quantification workflows of the SCIEX X500R QTOF System. Each workflow is straightforward to setup in the newly designed SCIEX OS Software and depending on the end users requirements we have demonstrated in this technical note the strengths of each workflow. Each provides TOF-MS and TOF-MS/MS analysis, both data being crucial in confidently identifying and quantifying forensic compounds.

- TOF-MS
- TOF-MS/MS
  - IDA
    - Non-targeted data acquisition
    - MS quantitation
    - Highest confidence screening with MS/MS information
  - MRM<sup>HR</sup>
    - Targeted data acquisition for quantitation purpose
    - Can be performed unscheduled or scheduled
  - SWATH<sup>®</sup> Acquisition (with variable windows)
    - Non-targeted data acquisition
    - MS/MS for everything all the time
    - Screening and quantitation (MS/MS)
      - Library Searching and Ion Ratio

We evaluated different LC runtime methods. The longer method aided eluting all analytes throughout the entire gradient as evenly as possible in order to maximize triggering IDA MS/MS for all components and reduce the MRM<sup>HR</sup> concurrency for quality of data (*Scheduled* MRM<sup>HR</sup>). The library searching worked well for the SWATH<sup>®</sup> Acquisition in the 2.0 minute LC

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runtime with MS/MS information always being available with this MS/MS<sup>ALL</sup> approach.