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™ 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™ 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).

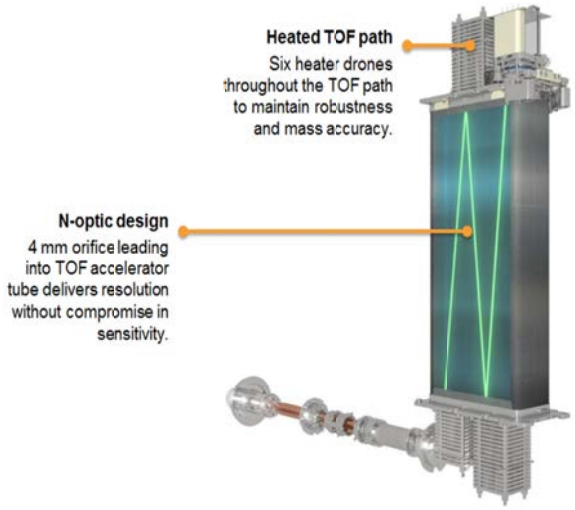

Figure 2. SCIEX X500R QTOF System and Technology Advances

The system has been designed to maximize robustness and uptime

- Integrated Calibrant Delivery System and Turbo V™ Source with TwinSpray probe (Figure 3), allows seamless mass accuracy auto-calibrations during long runs.
- Service Accessibility
  - Easy QJet® 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.

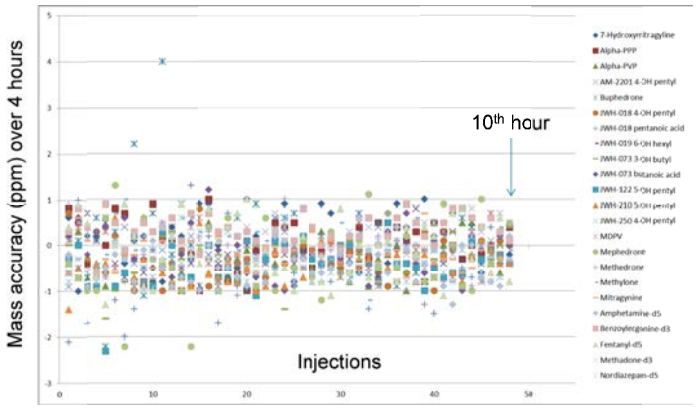

Figure 4. Mass Accuracy Stability of the SCIEX X500R QTOF System in the Analysis of Urine Samples

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.

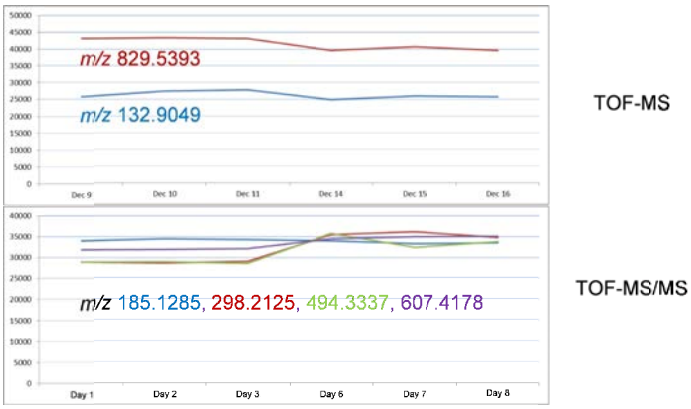

Figure 5. Resolution of the SCIEX X500R QTOF System Over a Week's Period for Selected *m/z*; both TOF-MS and TOF-MS/MS

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.

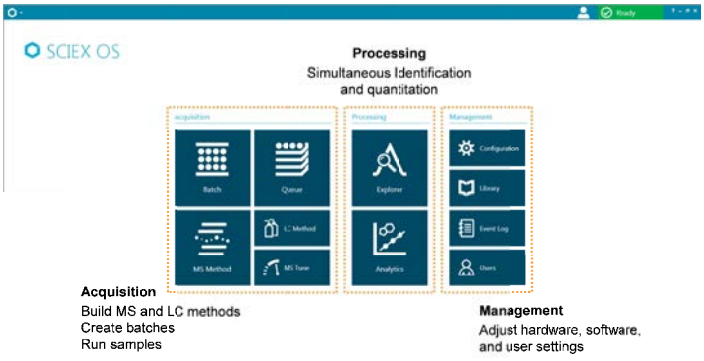

Figure 7. Home Page of SCIEX OS Software. Single Software Platform for LC/MS Control, Data Processing and Reporting.

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).

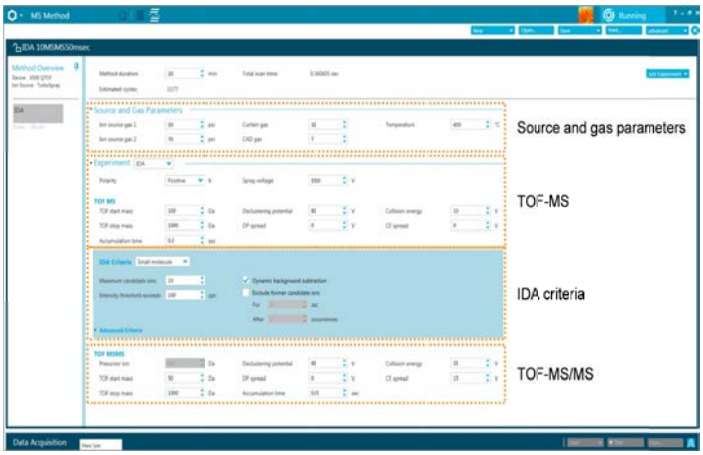

Figure 8. SCIEX OS Software MS Acquisition Method

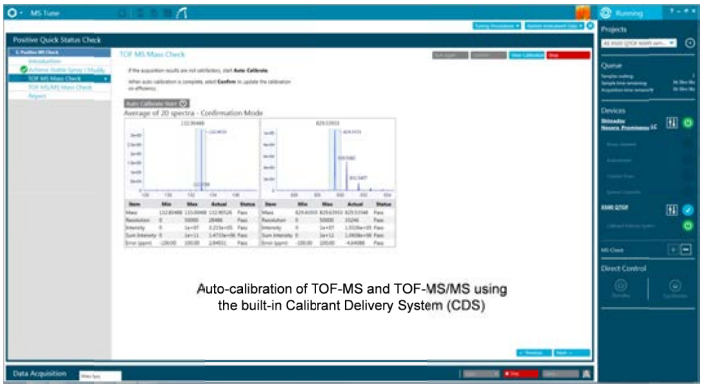

Figure 9. SCIEX OS Software MS Tune Allows for Quick Instrument Status Check via Simple Step by Step Instructions

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).

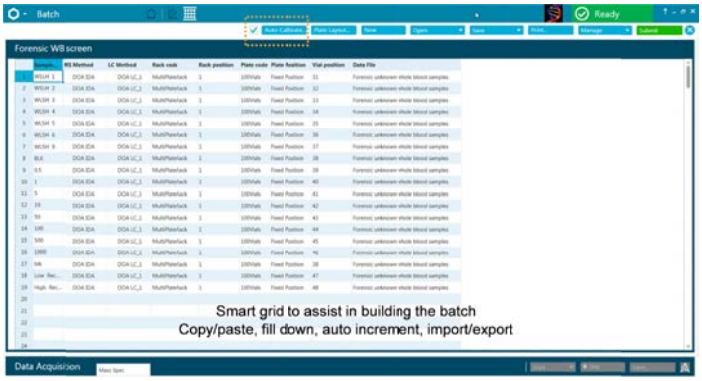

Figure 10. SCIEX OS Software Batch Editor and Setup for Auto-Calibration

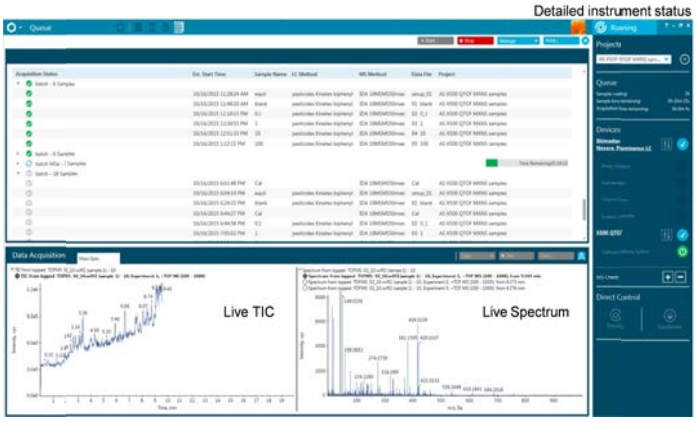

Figure 11. SCIEX 0S Software Queue Manager with Inserted Auto-Cal Samples and Detailed Instrument Status Panel

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.

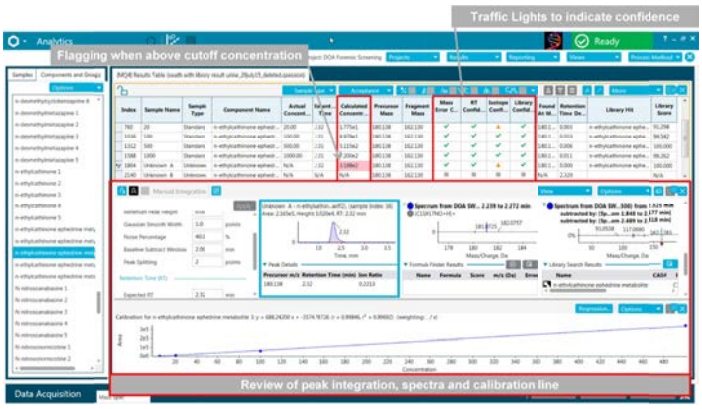

Figure 12. SCIEX OS Software Allows the Simultaneous Review of Qualitative and Quantitative Results

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)

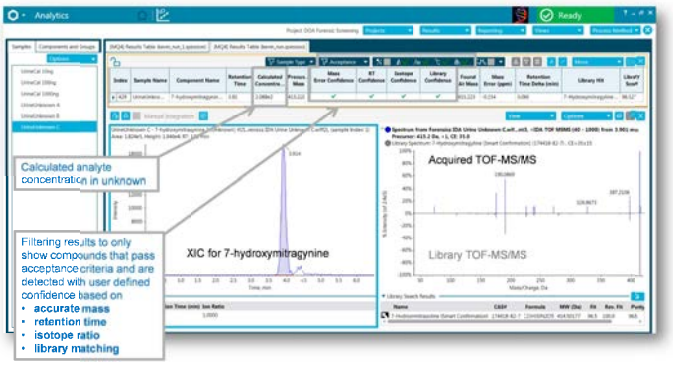

Figure 13. SCIEX OS Software Filtering Criteria

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.

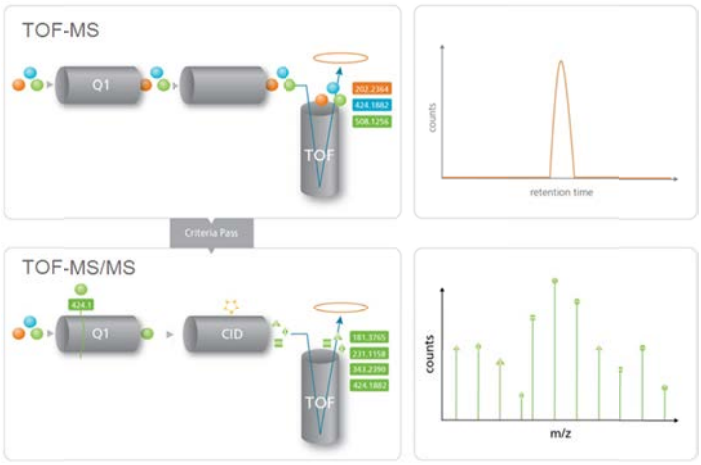

Figure 15. Information Dependent Acquisition

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.

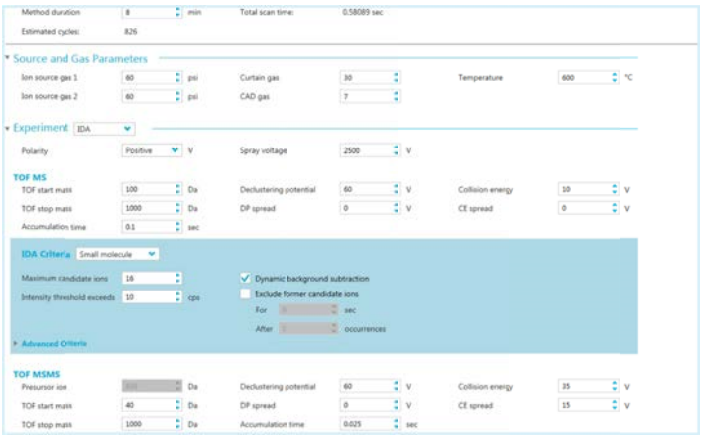

Figure 16. SCIEX OS Software Information Dependent Acquisition Method Editor

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^{All}) will solve this potential concern.

SWATH® Acquisition

SWATH® acquisition (Figure 17) is non-targeted and provides MS/MS information for everything in the sample, all the time. Each scan cycle in SWATH® 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^{All} 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.

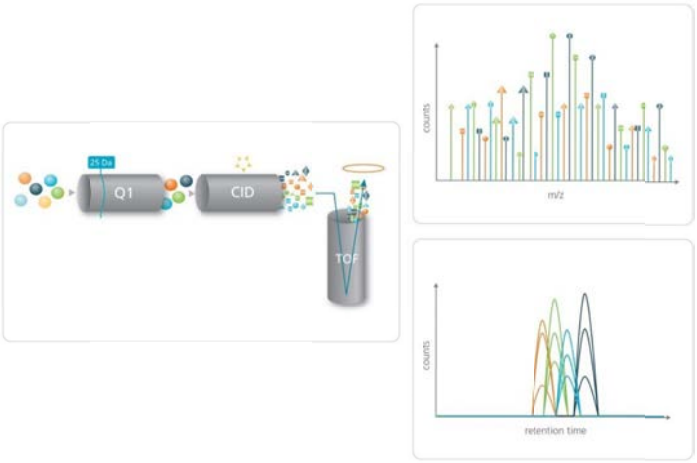

Figure 17. SWATH® Acquisition

SWATH® 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® 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*).

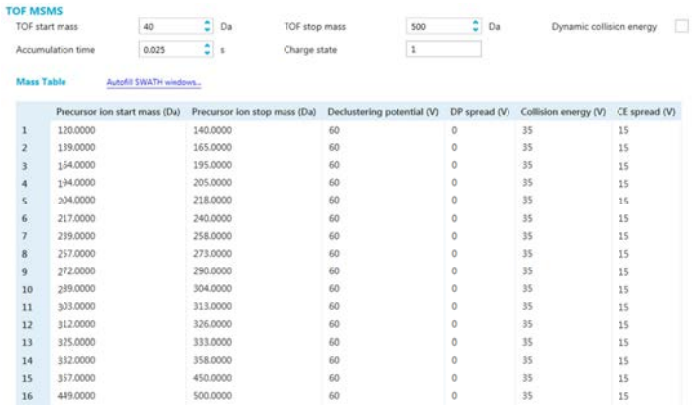

Figure 18. SCIEX OS Software SWATH® Acquisition Method Editor

By varying the Q1 isolation window for each TOF-MS/MS experiment we are able to separate compounds with similar mass into different SWATH® 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).

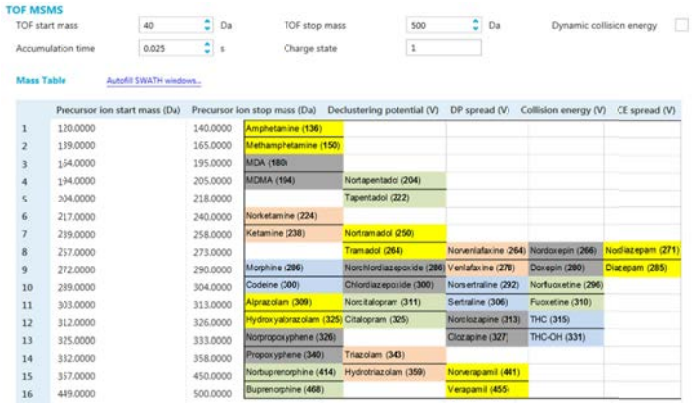

Figure 19. Constructing Variable SWATH® Acquisition Window Sizes for each Looped TOF-MS/MS Experiment to Minimize Convolution in the SCIEX OS Software

MRM^HR

MRM^{HR} (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^{HR}.

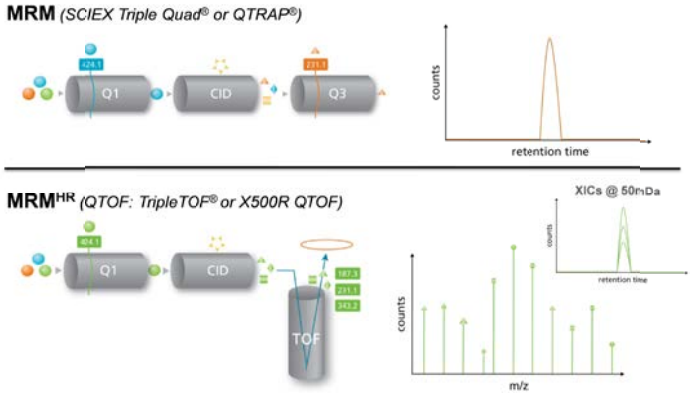

Figure 20. Comparison of MRM^{HR} with traditional (unit resolution) MRM

To help transition familiarity of MRM performed on a triple quadrupole to MRM^{HR} performed on the SCIEX X500R QTOF system, the SCIEX OS Software has a unique way of building the MRM^{HR} 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.

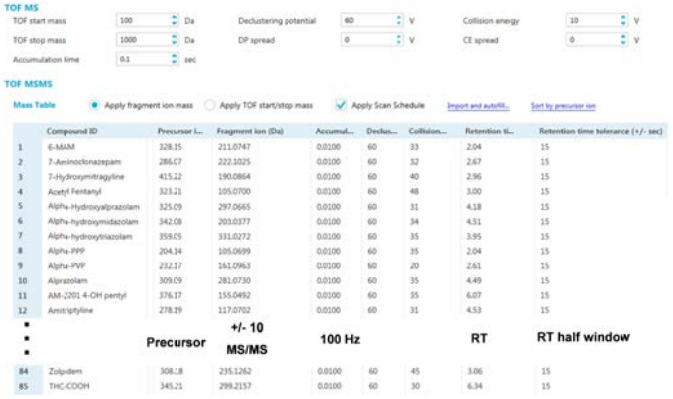

Figure 21. SCIEX OS Software *Scheduled* MRM^{HR} Method Editor, Fragment Ion Mass ± 10 *m/z*

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

Row	IS	Group	Name	Precursor Ma...	Fragment Mass (Da)	XIC Width (Da)	Retention Time (min)	IS...	Experiment Index
1		6-MAM	6-MAM	328.154	211.0747	0.02	2.04		2 TOF MSMS of 328.2 (40 - 500)
2		7-Aminoclonazepam	7-Aminoclonazepam	286.074	222.1025	0.02	2.67		3 TOF MSMS of 286.1 (40 - 500)
3		7-Hydroxymitragyline	7-Hydroxymitragyline	4:5.223	190.0864	0.02	2.96		4 TOF MSMS of 415.2 (40 - 500)
4		Acetyl Fentanyl	Acetyl Fentanyl	323.212	105.07	0.02	3.00		5 TOF MSMS of 323.2 (40 - 500)
5		Alpha-Hydroxyalprazolam	Alpha-Hydroxyalprazolam	325.085	297.0665	0.02	4.19		6 TOF MSMS of 325.1 (40 - 500)
6		Alpha-hydroxymidazolam	Alpha-hydroxymidazolam	342.08	203.0377	0.02	4.51		7 TOF MSMS of 342.1 (40 - 500)
7		Alpha-hydroxytriazolam	Alpha-hydroxytriazolam	359.046	331.0272	0.02	3.94		8 TOF MSMS of 359.0 (40 - 500)
8		Alpha-PPP	Alpha-PPP	294.138	105.0699	0.02	2.04		9 TOF MSMS of 204.1 (40 - 500)
9		Alpha-PVP	Alpha-PVP	212.17	161.0963	0.02	2.61		10 TOF MSMS of 232.2 (40 - 500)
10		Alprazolam	Alprazolam	309.09	281.073	0.02	4.49		11 TOF MSMS of 309.1 (40 - 500)
11		AM-2201 4-OH pentyl	AM-2201 4-OH pentyl	376.171	155.0492	0.02	6.07		12 TOF MSMS of 376.2 (40 - 500)
12		Amitriptyline	Amitriptyline	278.19	117.0702	0.02	4.53		13 TOF MSMS of 278.2 (40 - 500)
13		Amphetamine	Amphetamine	136.112	91.0553	0.02	1.63		14 TOF MSMS of 136.1 (40 - 500)
14		Benzoylecgonine	Benzoylecgonine	290.139	168.1021	0.02	2.36		15 TOF MSMS of 290.1 (40 - 500)
15		Buphedrone	Buphedrone	178.123	131.07	0.02	1.98		16 TOF MSMS of 178.1 (40 - 500)
16		Buprenorphine	Buprenorphine	468.311	414.2636	0.02	3.67		17 TOF MSMS of 468.3 (40 - 500)
17		Carisoprodol	Carisoprodol	261.181	55.0565	0.02	3.66		18 TOF MSMS of 261.2 (40 - 500)
18		Clomipramine	Clomipramine	3:5.162	86.0959	0.02	5.36		19 TOF MSMS of 315.2 (40 - 500)
19		Codeine	Codeine	340.159	215.1109	0.02	1.87		20 TOF MSMS of 300.2 (40 - 500)
20		Cotinine	Cotinine	177.102	80.0496	0.02	1.83		21 TOF MSMS of 177.1 (40 - 500)
21		Cyclobenzaprine	Cyclobenzaprine	276.175	215.0878	0.02	4.28		22 TOF MSMS of 276.2 (40 - 500)
22		Desalkylflurazepam	Desalkylflurazepam	289.054	140.0264	0.02	4.42		23 TOF MSMS of 289.1 (40 - 500)
23		Desipramine	Desipramine	267.186	72.0823	0.02	4.28		24 TOF MSMS of 267.2 (40 - 500)
24		Desmethyldoxepin	Desmethyldoxepin	246.154	107.0493	0.02	3.60		25 TOF MSMS of 266.2 (40 - 500)
25		Dextromethorphan	Dextromethorphan	272.201	215.1458	0.02	3.44		26 TOF MSMS of 272.2 (40 - 500)
26		Diazepam	Diazepam	285.079	154.0424	0.02	5.43		27 TOF MSMS of 285.1 (40 - 500)

Figure 22. Automatically generating the SCIEX OS Software MRM^{HR} Quantification Method from the SCIEX OS Software MRM^{HR} Acquisition Method

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

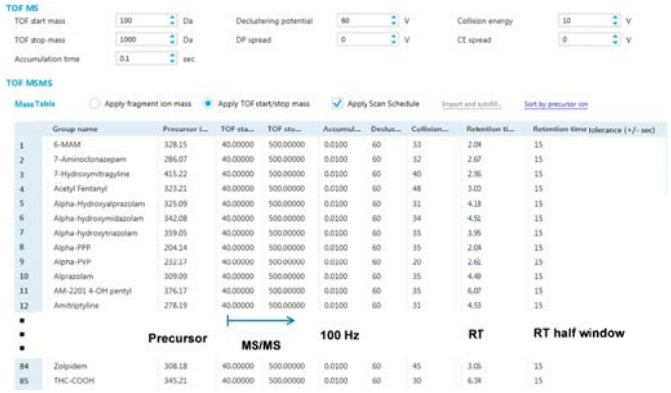

Figure 23. *Scheduled* MRM^{HR} Method Editor, MS/MS Full Scan

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

1. 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.
2. 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™ 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*	*THC-COOH*	*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.

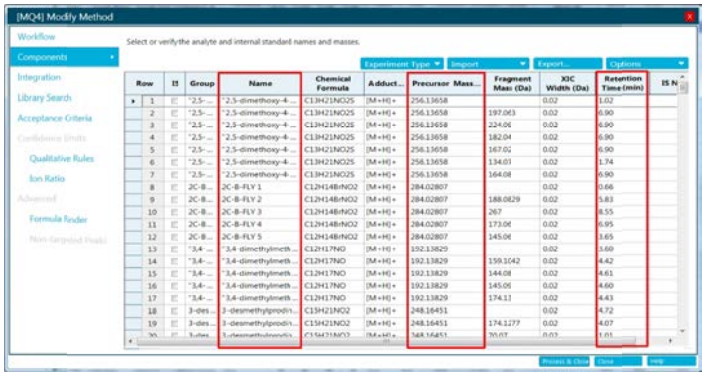

Figure 24. Defining the Retention Time, Accurate mass of Precursor and Fragment Ions

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).

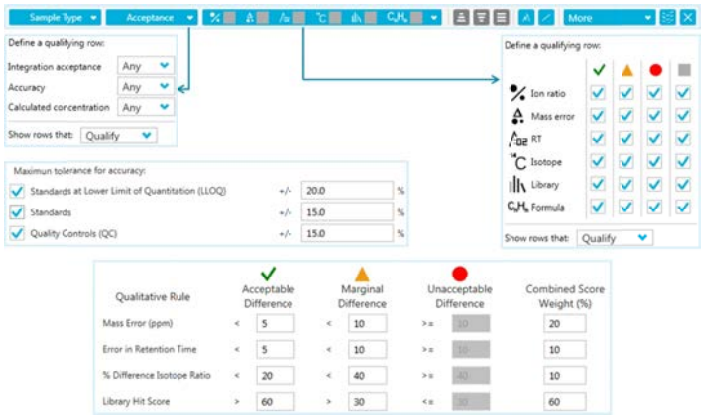

Figure 25. Defining the Identification and Quantification Qualifying Components in the SCIEX OS Software

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^{HR} concurrency for quality of data (*Scheduled* MRM^{HR}), 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.

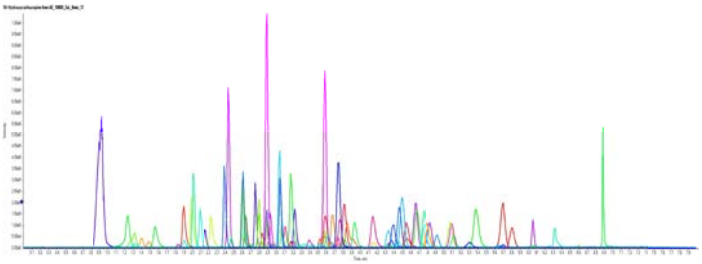

Figure 26. Extracted Ion Chromatograms for Analytes from a Urine Analysis using an 8.0 Minute LC Runtime

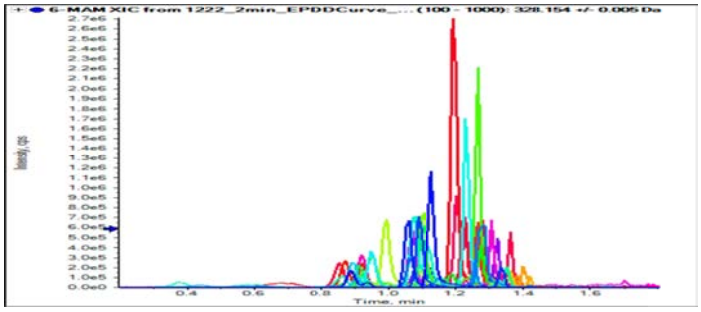

Figure 27. Extracted Ion Chromatograms for Analytes from a Urine Analysis using an 2.0 Minute LC Runtime

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.

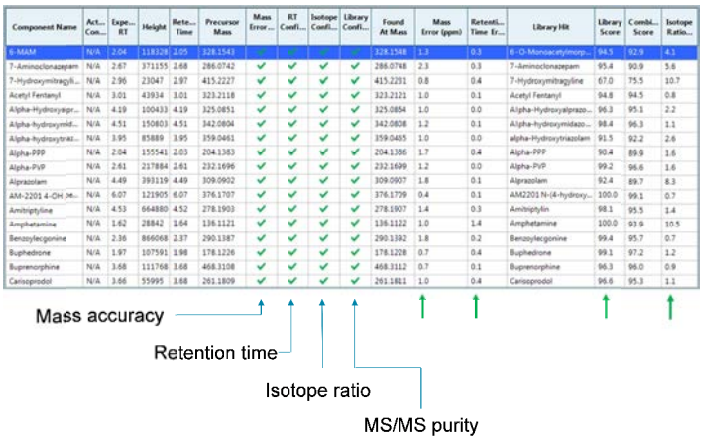

Figure 28 Screening and Identification Results from an IDA Experiment

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.

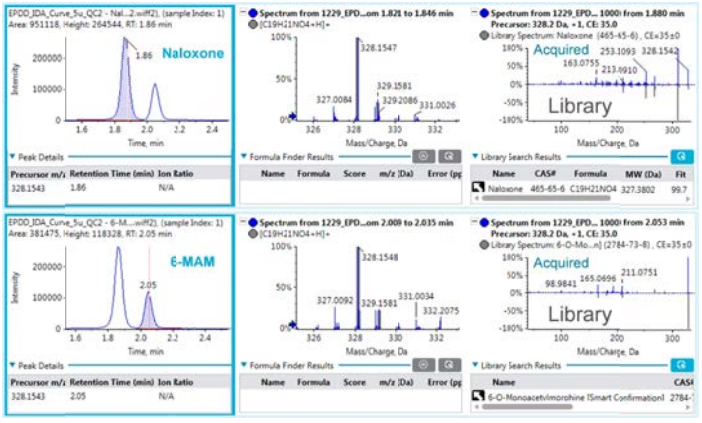

Figure 29. High Confidence Identification of Naloxone and 6-MAM Isobaric Compounds Gained through Library MS/MS Comparisons

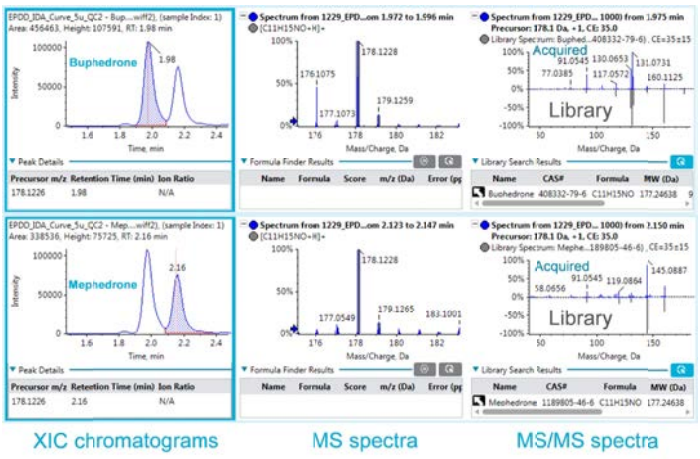

Figure 30. High Confidence Identification of Buprenorphine and Mephedrone Isobaric Compounds Gained through Library MS/MS Comparisons

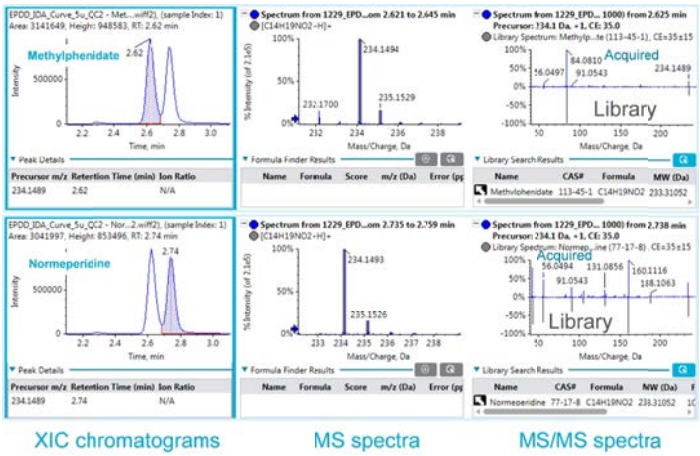

Figure 31. High Confidence Identification of Methylphenidate and Normeperidine Isobaric Compounds Gained through Library MS/MS Comparisons

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.

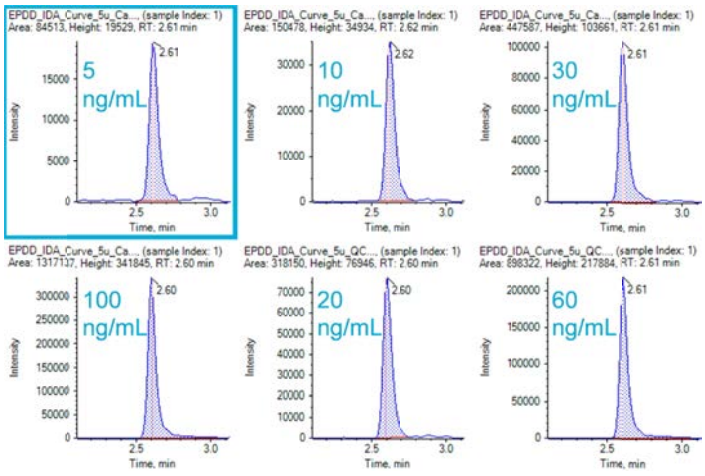

Figure 32. XICs of α-PVP in Urine from TOF-MS information (Urine was diluted 10-fold; 10 µL injection)

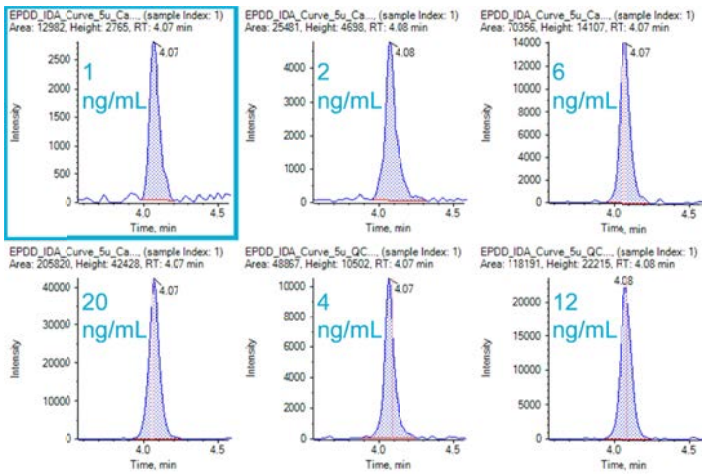

Figure 33. XICs of Sufentanil in Urine from TOF-MS information (Urine was diluted 10-fold; 10 µL injection)

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

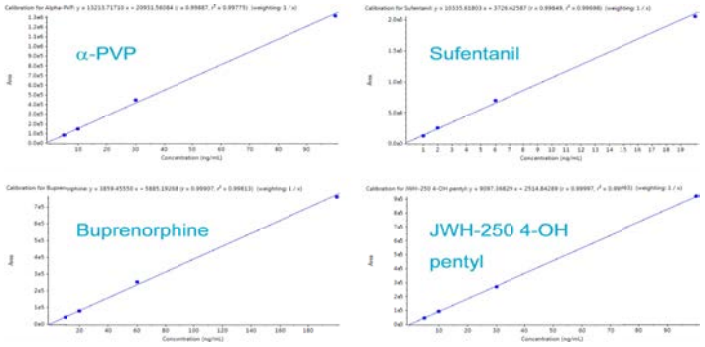

Figure 34. Representative Calibration Curves for Selected Compounds Showing that the TOF-MS information can be used for Quantification in an IDA Workflow

SWATH® Acquisition Results

SWATH® 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%.

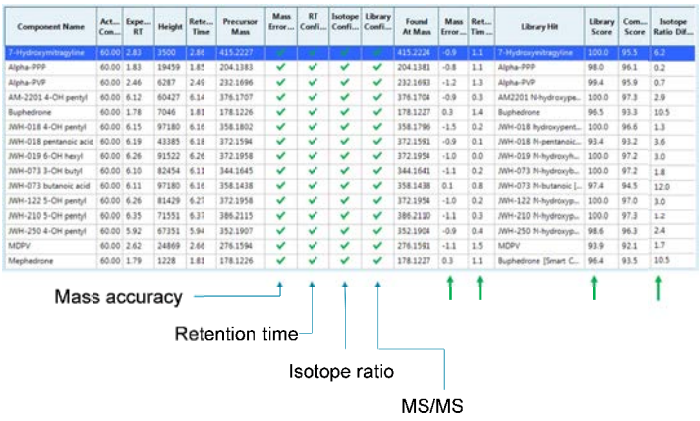

Figure 35. Processed SWATH® Acquired Data using Multiple Identification Criteria; including MS/MS Library Matching

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® 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.

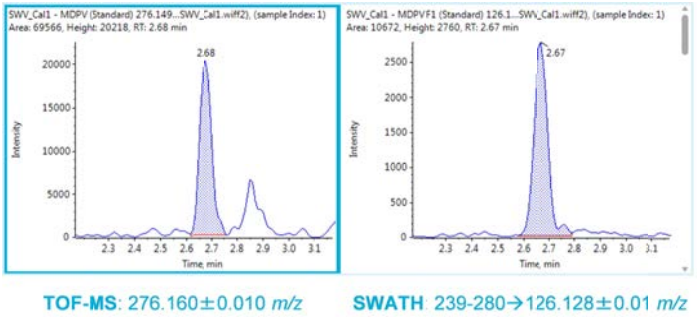

Figure 36. Gains in Selectivity with the Ability to Extract Out a Specific Fragment Ion From Variable Window SWATH® Acquired Data Compared to Extracted Accurate Mass of the Precursor Ion

Figure 37 shows identification and quantification results for a synthetic drug obtained from SWATH® 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.

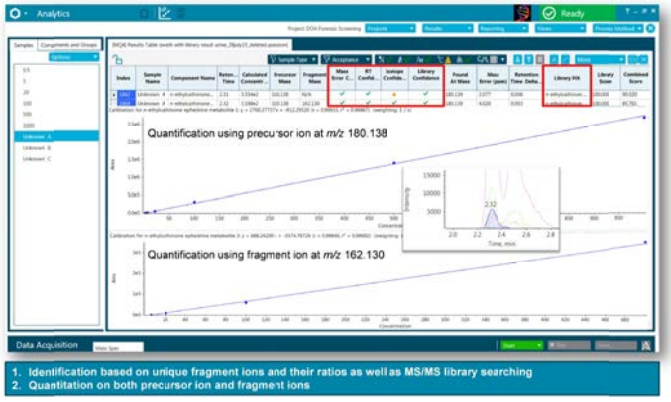

Figure 37. Identification and Quantification Results for n-Ethylcathinone Ephedrine Metabolite Compound Anlaysed by SWATH® Acquisition

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® 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® 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.

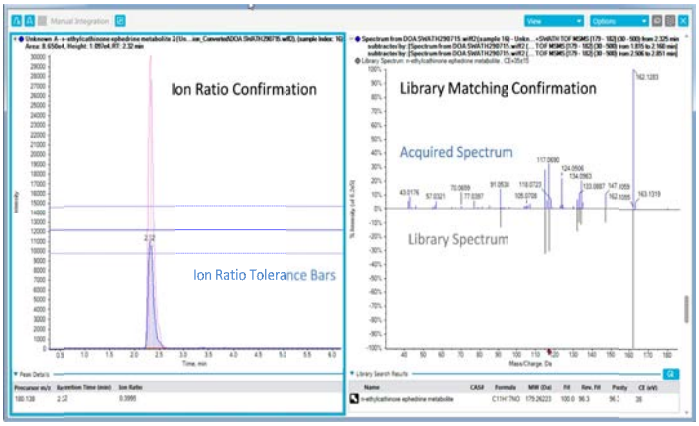

Figure 38. Extraction of Unique Fragment Ions From SWATH® Acquisition and Using Both Ion Ratio and Library Matching to Confirm Presence of n-Ethylcathinone Ephedrine metabolite in an Unknown Urine Sample

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

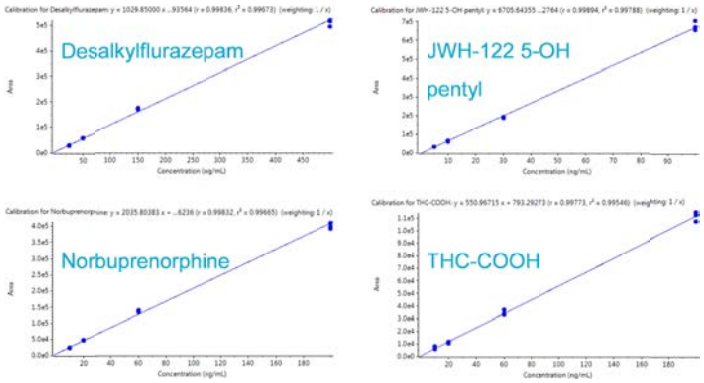

Figure 39. Representative Calibration Curves Generated from the SWATH® Acquisition using a 2.0 minute LC Runtime (n=3)

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

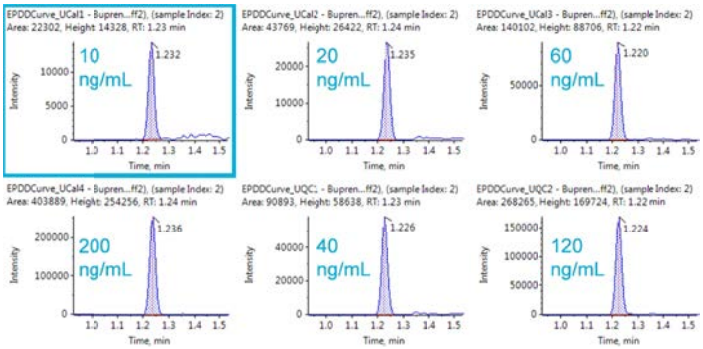

Figure 40. XICs of Buprenorphine at Various Concentrations in Urine (Diluted 10-fold, 10 µL injection)

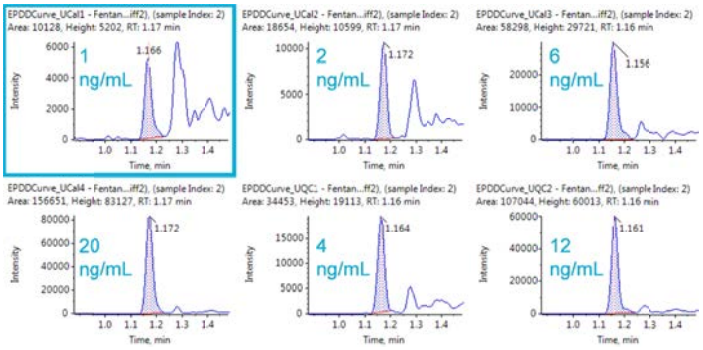

Figure 41. XICs of Fentanyl at Various Concentrations in Urine (Diluted 10-fold, 10 µL injection)

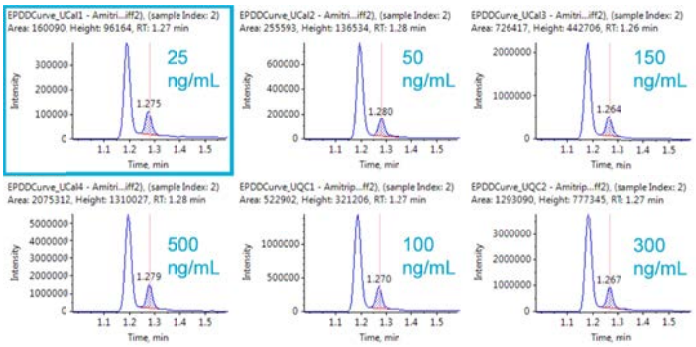

Figure 42. XICs of Amitriptyline at Various Concentrations in Urine (Diluted 10-fold, 10 µL injection)

In the SWATH® 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.

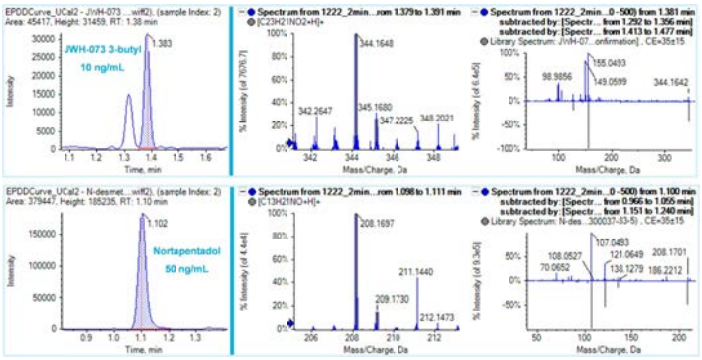

Figure 43. Confident Identification of JWH-073 3-Butyl and Nortapentadol from SWATH® Acquisition Through Library Searching

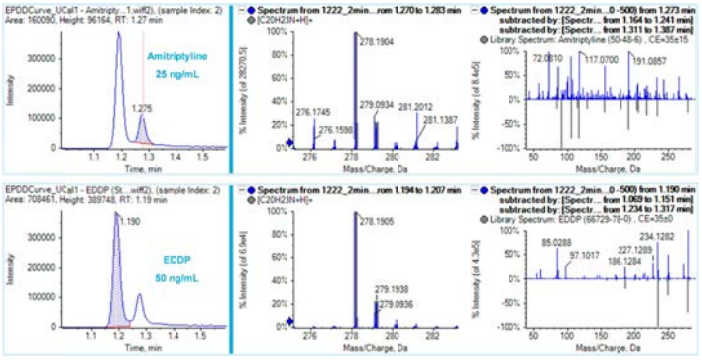

Figure 44. Confident Identification of Amitriptyline and EDDP from SWATH® Acquisition Through Library Searching; Showing LC Separation Between Isomers was Still Achievable with this Fast Method

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 At Mass	Mass Error...	Ret... Tim...	Library Hit	Library Score	Combi... Score	Isotope Ratio Dif...
EPDDCurve_UCal2	Alpha-Hydroxyal...	50.00	39322	1.31	325.0851	✔	✔	✔	✔	325.0851	0.0	0.00	Alpha-hydroxya...	100.0	95.1	18.6
EPDDCurve_UCal2	Alpha-hydroxym...	50.00	54742	1.32	342.0804	✔	✔	✔	✔	342.0804	0.0	0.00	Alpha-hydroxy...	100.0	99.3	1.8
EPDDCurve_UCal2	Buprenorphine	20.00	26422	1.24	468.3108	✔	✔	✔	✔	468.3107	-0.3	0.01	Buprenorphine	100.0	97.9	2.9
EPDDCurve_UCal2	Desalkylflurazep...	50.00	39350	1.33	289.0538	✔	✔	✔	✔	289.0539	0.2	0.01	Desalkylflurazep...	100.0	98.2	1.4
EPDDCurve_UCal2	EDDP	100.00	768373	1.20	278.1903	✔	✔	✔	✔	278.1906	0.8	0.00	EDDP	100.0	96.1	1.3
EPDDCurve_UCal2	Fentanyl	2.00	10599	1.17	337.2274	✔	✔	✔	✔	337.2276	0.5	0.01	Fentanyl	100.0	97.3	0.7
EPDDCurve_UCal2	Hydrocodone	50.00	61526	0.92	300.1594	✔	✔	✔	✔	300.1597	0.9	0.01	Hydrocodone	100.0	94.9	3.3
EPDDCurve_UCal2	JWH-018 4-OH...	10.00	29593	1.39	358.1802	✔	✔	✔	✔	358.1801	-0.1	0.01	JWH-018 hydro...	100.0	98.0	4.6
EPDDCurve_UCal2	JWH-019 6-OH h...	10.00	44558	1.40	372.1958	✔	✔	✔	✔	372.1959	0.3	0.01	JWH-019 N-hyd...	100.0	96.7	4.4
EPDDCurve_UCal2	JWH-073 3-OH...	10.00	31459	1.38	344.1645	✔	✔	✔	✔	344.1648	0.9	0.01	JWH-073 N-hyd...	100.0	94.3	5.1
EPDDCurve_UCal2	JWH-122 5-OH...	10.00	44558	1.40	372.1958	✔	✔	✔	✔	372.1959	0.3	0.01	JWH-019 N-hyd...	100.0	96.7	4.4
EPDDCurve_UCal2	Methamphetami...	100.00	33875	0.92	150.1277	✔	✔	✔	✔	150.1277	-0.4	0.00	Methamphetam...	100.0	98.0	0.6
EPDDCurve_UCal2	Methylphenidate	50.00	195247	1.07	234.1489	✔	✔	✔	✔	234.1490	0.8	0.00	Methylphenidate	100.0	96.4	0.7
EPDDCurve_UCal2	Norbuprenorphi...	20.00	27123	1.17	414.2639	✔	✔	✔	✔	414.2641	0.5	0.00	Norbuprenorphi...	100.0	97.5	2.3
EPDDCurve_UCal2	Nordiazepam	50.00	44694	1.35	271.0633	✔	✔	✔	✔	271.0635	0.8	0.00	Nordiazepam	100.0	95.5	3.7
EPDDCurve_UCal2	Noroxycodone	50.00	23823	0.90	302.1387	✔	✔	✔	✔	302.1386	-0.2	0.01	Noroxycodone	100.0	97.9	4.3
EPDDCurve_UCal2	Tapentadol	50.00	203639	1.09	222.1852	✔	✔	✔	✔	222.1854	0.7	0.00	Tapentadol	100.0	96.0	1.1
EPDDCurve_UCal2	Temazepam	50.00	58735	1.34	301.0738	✔	✔	✔	✔	301.0738	-0.2	0.01	Temazepam	100.0	98.1	3.2
EPDDCurve_UCal2	Tramadol	50.00	169458	1.06	264.1958	✔	✔	✔	✔	264.1961	1.0	0.00	Tramadol	100.0	95.9	0.6
EPDDCurve_UCal2	Zolpidem	50.00	277586	1.13	308.1757	✔	✔	✔	✔	308.1760	0.8	0.00	Zolpidem	100.0	96.1	1.9
EPDDCurve_UCal2	7-Aminoclonaze...	50.00	76113	1.12	286.0742	✔	✔	✔	✔	286.0744	0.9	0.01	7-Aminoclonaze...	99.8	95.0	3.2
EPDDCurve_UCal2	Alprazolam	50.00	123684	1.32	309.0902	✔	✔	✔	✔	309.0904	0.8	0.00	Alprazolam	99.8	95.7	4.0
EPDDCurve_UCal2	Diazepam	50.00	110405	1.36	285.0789	✔	✔	✔	✔	285.0791	0.7	0.01	Diazepam	99.7	95.5	3.4
EPDDCurve_UCal2	Naltrexone	50.00	56359	0.91	342.1700	✔	✔	✔	✔	342.1698	-0.4	0.00	Naltrexone	99.7	97.3	3.0
EPDDCurve_UCal2	Oxymorphone	50.00	6763	0.41	302.1387	✔	✔	✔	✔	302.1389	0.8	0.02	Oxymorphone	99.7	94.2	3.5
EPDDCurve_UCal2	Amphetamine	100.00	3665	0.88	136.1121	✔	✔	✔	✔	136.1120	-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^HR

MRM^{HR} 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^{HR} 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^{HR} 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^{HR} approach.

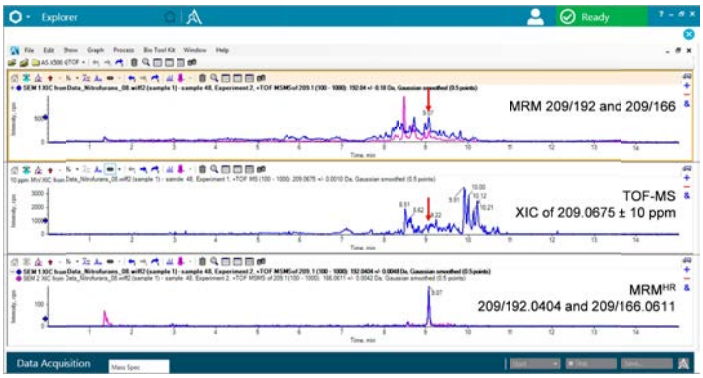

Figure 46. Increased Selectivity with MRM^{HR}; Avoiding False Negatives. Example given is a Feed Sample Tested Positive for NP Semicarbazide

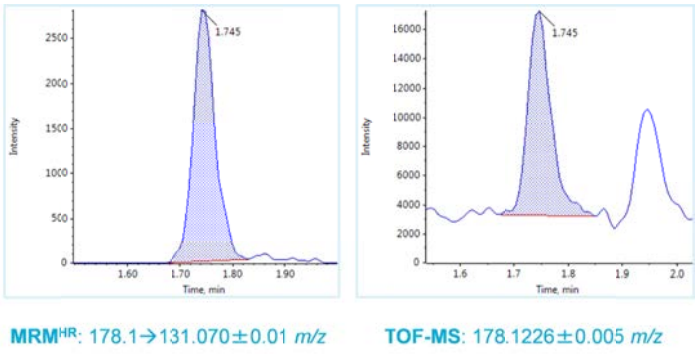

Figure 39. Scheduled MRM^{HR} Selectivity Compared to TOF-MS; Buprenorphine (5ng/mL in urine, 10 fold dilution, 10 µL injection)

Quantification performance of the MRM^{HR} is demonstrated in Figure 40 for the 8.0 minute LC-MS/MS method.

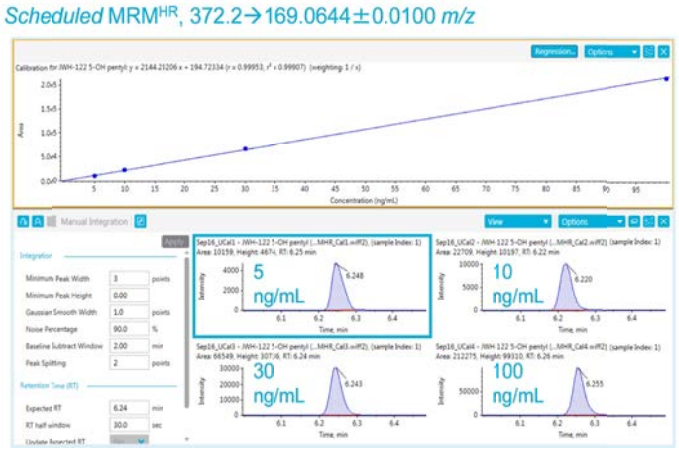

Figure 40. MRM^{HR} Quantification Results for JWH-122 5-OH Pentyl in urine (Urine was diluted 10-fold, 10 µL injection)

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™ 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 V-geometry, 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^{HR}
        - Targeted data acquisition for quantitation purpose
        - Can be performed unscheduled or scheduled
    - SWATH® 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^{HR} concurrency for quality of data (*Scheduled* MRM^{HR}). The library searching worked well for the SWATH® Acquisition in the 2.0 minute LC runtime with MS/MS information always being available with this MS/MS^{ALL} approach.

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