Forensics Compendium

Volume 1

The Power of Precision

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Introduction

SCIEX Forensics Compendium Volume 1

Evidence can hold crucial clues vital to any investigation, trace level forensic drug compounds may be present in your sample which could determine the verdict of your case. Gathered evidence must be subjected to comprehensive testing which profiles the sample and leaves no doubt or objections.

The risk of reporting data could be disputed in the courtroom is something your crime lab cannot afford. Erroneous results not only have a financial cost but also can have a more lasting impact on the reputation of the evidence processing lab.

Mass Spectrometry enables highly accurate trace level detection of forensic compounds in a variety of complex matrices. From surface swabs for explosives testing through to dried blood spots analysis looking for the ever-growing list of Novel Psychoactive Substances (NPS), the LC-MS/MS solutions from SCIEX equip you in your pursuit to deliver true, accurate and concise results.

Our portfolio of analytical instruments, workflows, software, service and training are key to unlocking your laboratories potential to grow and become a leader in forensic analysis.
In this compendium of forensic applications we present details of key workflows and useful pieces of information to make the leap to LC-MS/MS.

Oscar G. Cabraices

Global Staff Scientist, Forensics Testing

The world of forensic science has drastically evolved over the years. Once upon a time, a magnifying glass and a microscope were all the tools an investigator had at their disposal, yet by the dawn of the 21st century, whole divisions, such as criminalistics and toxicology, had been remodeled around new technologies.

These revolutionary pieces of equipment opened up entire new areas of potential evidence. Cases of injustice that would previously have been left open could now be resolved on the basis of trace materials alone; even simple clothing fibers could convict a murderer.

But all this would not be possible without the organizations that spearhead these technologies. Grand ideas of computational detection are important, but it's research, development, and commercialization that actually bring these advances to the forensic labs that need them. And great ideas are worth sharing.

The following compendium is a detailed account of some of the recent advancements developed by the forensics department at SCIEX. If adopted and utilized by forensic laboratories, these successes have the potential to advance certain forensic screenings and expedite results crucial to cases of justice. From the record analysis of ethanol metabolites, to the rapid detection of novel psychoactive substances, these innovations join the growing body of new forensic techniques required for the changing worlds of explosives and substance abuse.

"The SCIEX X500R QTOF System is just the type of revolutionary screening technology the forensic community needed for the rapid detection of potential threats, keeping our world safe."

A Changing World

And substance abuse is changing. New challenges, such as the rising use of novel psychoactive substances (NPS), increasing rates of alcohol poisoning, and the opioid crisis, necessitate new technologies to help better protect the public. The increasing prevalence of NPS, in particular, will certainly dominate the direction of forensic toxicology for years to come. Formally known as 'legal highs', these compounds are usually manufactured in unregulated, underground drug laboratories, and can contain chemicals hitherto unfamiliar to a forensic investigator. One week a substance might be white, the next it might be yellow, and contain completely different molecules. In fact, over 560 NPS are currently being monitored by the European Monitoring Centre for Drugs and Drug Addiction, with 100 new agents identified in 2015 alone[1]. And with NPS-related death rates rising, the time it takes to detect these metabolites really is of the essence. Therefore, novel detection methods such as the robust and fast SCIEX LC-MS/MS forensic screening technique, which can reliably determine 664 forensic drugs (including NPS) in blood and urine matrices are so critical in the global efforts to tackle this issue. It's projects such as this that will help us better understand emerging drugs, and in the future, help international bodies to save lives.

But toxicology is not the only focus of our forensics division. As SCIEX's long history and success within the pharmaceutical and food industries shows, we are nothing if not adaptive. After all, it's our ultimate goal to strive to break new ground in scientific discovery. Thus, in the past year we have successfully begun our expansion into the field of criminalistics. As the threats of explosive weaponry still persist, both in war zones and civilian spaces around the world, so does the need to better assess and understand the devastation these objects can leave behind. We are only just beginning our foray into these new forensic divisions, but already our mass spectrometry capabilities have lent us a great start. For example (as later detailed in this compendium), the SCIEX X500R QTOF System is just the type of selective screening technology needed for organic explosive analysis. It provides acquisition rates at up to 100 MS/MS per second and has the ability to analyze 14 analytes typically left behind from organic explosives in under three minutes. Bolstered by successes such as this and the keen interest of international security and counter terrorism organizations, we will continue to innovate our technologies and match these new forensic endeavors. By expanding our resources and expertise into areas such as explosive analyses, and even fiber and DNA analyses, we can offer scientists the high-accuracy screenings they need, and help to make a difference in the security of the world.

The Forensics of the Future

Of course, we are not alone in our innovations. As the drugs and explosives of the world advance, so does the technology that detects them. Technological developments in this area are now so rapid it can sometimes feel as though forensic science has become science fiction. Emerging techniques, such as DNA phenotyping, where suspect profiles are visualized from their DNA alone, are already bearing results. And previously untapped sources of evidence are at last beginning to be utilized. The diversity of bacteria in the human intestinal tract (the microbiome), for example, is even being explored as a potential identifier for criminal cases. Recently, forensic scientists in China assisted in a murder case by matching the microbial life of residual soil from a suspect's shovel to that of the microbes present at the gravesite of the body. It's truly an incredible time to be working in the field of forensics.

But while these futuristic trends may not be the focus of our attention for now, one aspect that will be key to future analytical success is usability. As technology becomes more interwoven with our professional and personal lives, it's this accessible quality that will really determine the tools of tomorrow. And just as commercial laptops are not the hulking contraptions they once were, mass spectrometers have changed too. When once only the biggest and most lucrative laboratories could afford the latest analytical technology, now every forensic organization with three feet to spare can make room for equipment such as the SCIEX TripleTOF® 6600 System. But usability is about more than just size. At SCIEX we are also investing heavily into the user interfaces of our machines. Because it's one thing to make a novel, high-complexity tool, but to ensure a scientist will get results, it has to be accessible. From fresh graduates to established experts, everyone can benefit from a friendly interface. It's this insight that helps to make us unique when it comes to providing these services. We understand how forensic investigators think, what their priorities are, and the short timescales that they work to. So, as a solution-driven company, we will continue to increase the usability of our equipment and do what we do best: provide solutions.

Forensics Testing

Forensics, as with every avenue we explore at SCIEX, is a fascinating field. It goes with the purpose of our vision: to improve the future of science and, ultimately, to help our customers realize life's potential. For when we look at forensics, we look at the potential of life and how important it is to safeguard it. And by giving definite answers to investigators, whether they be in the lines of toxicology or counter terrorism, we help drive this security. Thus, it has been a fantastic year for the forensics team here at SCIEX. We are incredibly proud of our achievements at this time, and I hope you enjoy reading about them as much as we enjoyed working on them.

DO YOUR EVIDENCE JUSTICE WITH SCIEX OS

Your evidence has a story to tell, let the intuitive software from SCIEX be your narrator.

- **What if the analyte of interest in your sample is not yet known to your lab?**
- **What if it isn't on your target list, would you still see it?**
- **What if you needed to reanalyze a sample that is years old?**
- **What if you could remove the element of risk associated with manual interpretation?**
- **Would a degraded sample still show the trace level analyte that could be vital to your case?**
- **Do you want to report accurate results that are clear and concise?**

"**SCIEX OS**, offers straightforward method development, acquisition, data handling, and evaluation of the instrument's mass calibration. Performance is also very simple, just two calibration solutions are required, one negative and one positive, and reports are pre-installed, making life in an accredited environment easier. The easy-to-use app- style software is more straightforward too. Applications such as **PeakView®**, **MasterView™** and **MultiQuant™** are included as part of a single package, which is less complex than using separate software solutions to run the experiment and analyze the data, and well suited to routine activities."

Whether you are analyzing for forensic drugs or explosive residues our software platforms can assist to deliver the results your investigation needs in a clear and easy to understand format. SCIEX OS software was designed exclusively for the X-Series QTOF systems so that operators, the expert user and the novice in LC-MS, can build high-quality methods, acquire and process data, analyze results, publish reports and manage the instrument, all within the same point-and-click interface for enhanced productivity.

DOWNLOAD

Download a free trial of SCIEX OS Software

LIVE DEMONSTRATION

See your data, your way - Request a live demo to get hands-on experience

Technology

Overview

In modern times, the role of a forensic investigator can sometimes feel like that of a computer programmer. A typical workday can be spent moving from one high-computational analytical machine to the next, checking digital displays for the data that could progress a case.

Thus, the efficacy, accuracy, and reliability of such instruments have become almost as important to a forensic lab's output as the investigators themselves.

To ensure these qualities in our analytical equipment and the dependable results they generate, we at SCIEX have designed and developed some of the foremost advanced forensic mass spectrometers in the industry. Through our own research, these tools have been tested in a variety of forensic contexts, and have even borne unique and enlightening results.

Here is an overview of some of our latest technologies used in the rapidly expanding field of forensics and the exciting results they can produce.

The X500R QTOF Series

Traditionally, the analytical methods used to detect explosives and drug analytes involve gas chromatography (GC) or high-performance liquid chromatography (HPLC). However, these techniques alone lack the high-throughput capability forensic investigators need to identify the key analytes of a crime scene. In part to meet this need, SCIEX developed the revolutionary X500R Series of QTOF mass spectrometers. Designed with the needs of food, environmental and forensic labs in mind, its key features include enhanced mass accuracy to deliver improved selectivity for routine mass spectrometry (MS) quantification of targeted compounds, and sensitivity combined with high speed MS/MS data acquisition to enhance quality of data and ensure comprehensive detection of unknowns. This accuracy is due to the X500R series' Turbo V™ ion source, which has a renowned ionization performance among industry professionals.

In addition, the SCIEX X500R Series has an independent calibrant delivery path, which maintains highly reliable mass calibration through long runs. Together, these features provide the platform with the speed and mass accuracy for the high throughput identification and quantitation of forensic analytes. Some of the most recent and exciting results that have been acquired using this series include the obtainment of retention times for 664 forensic compounds. For more detail on this accomplishment and others from the X500R Series, please see the relevant Technology Notes compiled in this compendium.

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The CESI 8000

Since its inception in the 1990s, CE has been applied in a range of scientific domains, from biochemistry to pharmacology, and biomedicine. But in recent years it is the field of forensics that has seen the greatest and most exciting use of CE. Used as a tool for illicit drug analysis in clandestine preparations, CE equipment such as the SCIEX CESI 8000 have both assisted in numerous toxicology cases and borne new analytical techniques.

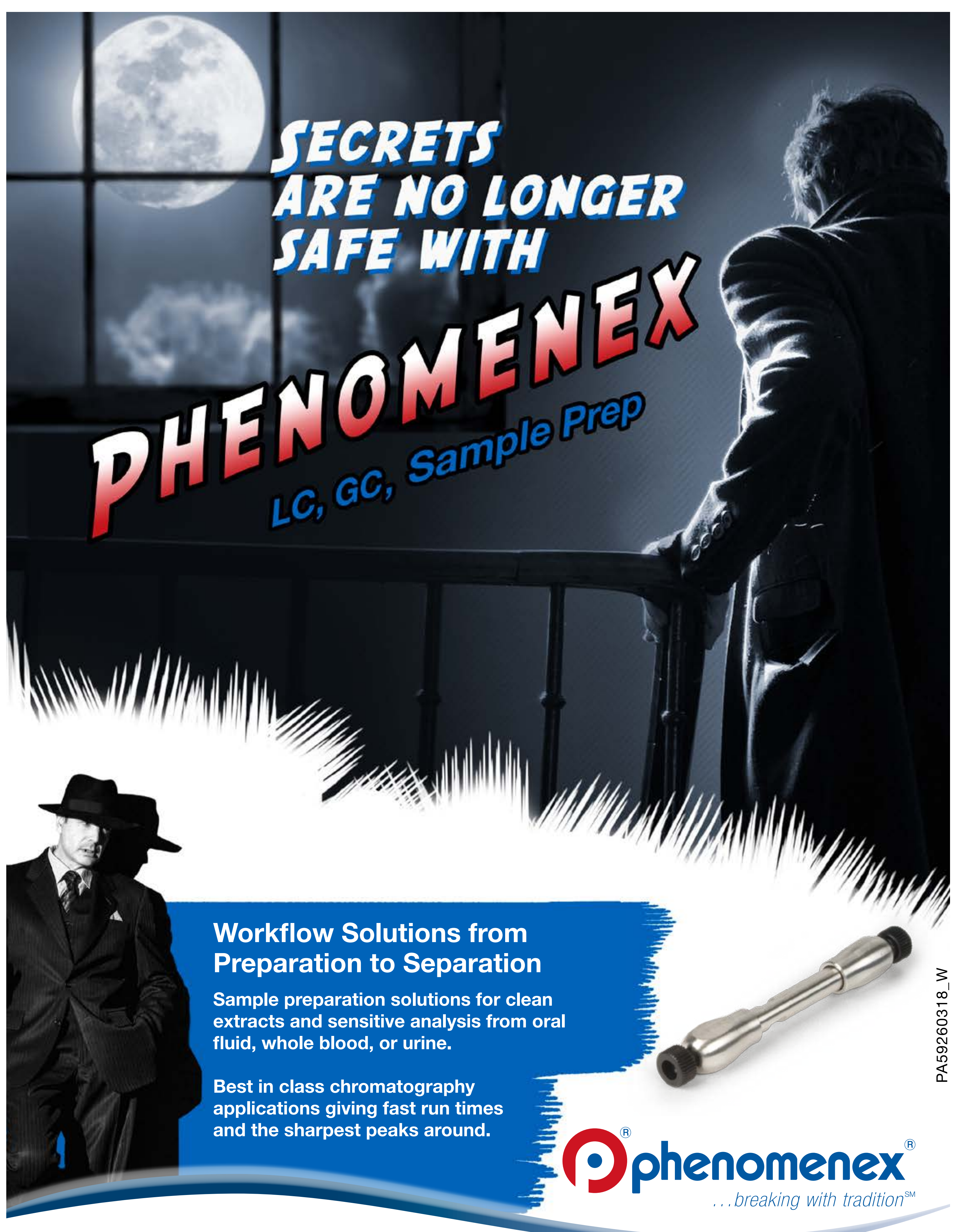
The SCIEX CESI 8000, in particular, has been used as a platform for transformative chiral separation techniques. As detailed later in this compendium, SCIEX researchers used the equipment in conjunction with mass spectrometry to separate the enantiomers of methamphetamine from its amphetamine metabolite in a single run, with great sensitivity. With regards to specifications, the CESI 8000 also provides a unique multi-segment injection system, which increases the throughput of an analysis by ten times. Plus, the platform provides extraordinary sensitivity and a high resolution that enables maximum information with minimal sample consumption (~50 nL per injection).

This type of Capillary Electrophoresis Electrospray Interface for Mass Spectrometry (CESI-MS) is particularly valuable for precious samples, such as subcellular fractions, needle biopsies, and highly toxic samples such as venoms.

The QTRAP® 4500 Series

Not all LC-MS/MS systems are made equal, and only one is known as The Workhorse: the SCIEX QTRAP® 4500 LC-MS/MS system. Intelligently re-engineered from the 4000 QTRAP platform, the 4500 offers forensic investigators vigorous and reliable high-throughput screening for a wide range of analytes. This hardiness and flexibility makes the system well suited to forensic laboratories that daily screen multiple compounds in varied samples. As detailed later in this compendium, SCIEX researchers displayed this feature by the successful testing of over 800 continuous injections of urine samples.

Designed for robustness, rapidity, and the quantitative sensitivity needed for the detection of trace analytes, the QTRAP® 4500's dominance in delivering productivity for demanding applications lies in its impressive technology. Like the X500R Series, it possesses a Turbo V ionization source - the gold standard for LC-MS/MS ionization. Plus, with the 4500, investigators can maximize the capacity of their laboratories and deliver quality quantitation every time. This is due to its powerful Scheduled MRM™ algorithm and Curved LINAC® collision cell design, which, together, improve the quality of forensic data to ensure fewer peaks are missed and optimal sensitivity is achieved.



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An Overview of SCIEX Technology Notes

High Throughput Platform for Confident Identification and Quantitation of Organic Explosives

The detonations and blast impacts of organic explosives pose significant dangers to the public worldwide. These alarming explosions, both in warzones and civilian locations, have led forensic scientists to better their screening technologies, to become even more selective in identifying key composites at crime scenes.

The SCIEX X500R QTOF System is an example of just the kind of selective screening technology needed for organic explosive analysis. Providing high-resolution mass spectrometry at fast acquisition rates (up to 100 MS/MS per second), it enables the quick identification of analytes typically left behind from organic explosives.

As a further boon for forensic investigators, the X500R QTOF System also helps to shorten case turnaround times, as it is capable of analyzing 14 explosives in under three minutes.

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

New synthetic drugs are on the rise. At the dawn of the millennium the UN Office on Drugs and Crime (UNODC) listed only a handful of “new psychoactive substances”. By 2008 the number was up to 26. By 2014 it was 451. These new drugs often contain unknown substances, which can be difficult to detect in drug screening.

Fortunately, the SCIEX X500R QTOF System can provide the high-resolution and accurate-mass data ideal for fully scanning all product ions. This means that the X500R QTOF can retrospectively mine for additional analytes that were missed in initial screens.

Elevating the Forensic Laboratory Performance

Forensic laboratories, like many places of work, are often pushed for time. However, considering cases of justice can hang in the balance, the pressure to deliver results quickly can be very high. The goal, then, is to complete a case investigation in as short a time as possible after receiving a sample.

For many cases dealing with illicit drug use, GC/MS has been the staple screening method, despite its lengthy sample preparation time. But now forensic investigators are turning to another technique: liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). LC-MS/MS technologies, such as the SCIEX Triple Quad 3500 LC-MS/MS System, can complete comprehensive drug analyses in rapid times. In one instance, 285 samples from a urine sample were processed within a 24-hour time frame.

Efficiently Designed Workflows Provide Accurate Results in Forensic Analysis of THC-COOH in Hair Samples

It has been reported that as many as 238 million people worldwide annually consume cannabis, making it the most widely used recreational drug in the world. Its main metabolite - THC-COOH - can be detected in urine, blood and saliva. But these sources only retain the metabolite for a limited time. For accurate detection of long-term cannabis use, hair samples are far more reliable sources.

However, using hair for detecting cannabis does provide its own analytical challenge: characteristically low THC-COOH concentrations. Fortunately, the combination of a solid phase extraction procedure with the SCI-EX Triple Quad 4500 LC-MS/MS System allows for the efficient detection of levels of THC-COOH as low as 0.2 pg/mg.

Ultra-Fast Forensic Toxicological Screening and Quantitation under 3 Minutes using SCIEX X500R QTOF System and SCIEX OS 1.0 Software

The world is currently experiencing a dramatic acceleration of technological capabilities - an acceleration that has the potential to revolutionize the forensics field. In the coming years, the beginnings of this revolution may arrive in the form of a fast technique that can detect an unlimited number of analytes with mass accuracy, LC retention time and MS/MS spectral library matching.

And such a technique may be available sooner than expected. Using new SCIEX X500R QTOF systems and SCIEX OS 1.0 software, a team at SCIEX has developed an ultra-fast forensic toxicological screening method, which has an LC runtime of under three minutes.

Fast and simultaneous analysis of ethanol metabolites and barbiturates using the QTRAP® 4500 LC-MS/MS system

Alcohol-related disorders are common. So common, in fact, that six people die every day in the US alone because of alcohol poisoning, according to the Centers for Disease Control and Prevention. To understand more about this disease it is crucial to know more about how alcohol is broken down by the body and into which metabolites. To do this, highly sensitive analyses are required.

A SCIEX method to simultaneously analyze ethanol metabolites and barbiturates in human urine, using a QTRAP/Triple Quad 4500 LC-MS/MS system, is an example of such a highly sensitive analysis. In one test of the method, more than 800 continuous injections of human urine were performed in five minutes, with no evident deterioration in performance.

LC-MS/MS Screening of 64 New Psychoactive Substances Using Dried Blood Spots

Novel psychoactive substances (NPS) are often chemically quite similar to more established drugs, to mimic their effects. However, NPS' metabolites are sufficiently different to that of their contemporaries, meaning that international drug laws do not cover them. Therefore, to better regulate these substances, better classification techniques are required.

As part of these needed efforts, SCIEX researchers have developed a robust, fast and sensitive LC-MS/MS screening method that can reliably determine 64 of these new psychoactive substances in dried blood spots. Using a SCIEX QTRAP 5500 LC-MS/MS System, the method can extract all these data from relatively low volumes of blood, meaning any sample extraction could be minimally invasive.

Chiral Analysis of Methamphetamine and Its Metabolite, Amphetamine in Urine by CESI-MS

The abuse of Amphetamine-type stimulants (ATS) is growing substantially in many East Asian and Oceanic countries. Fortunately, the effective chiral separation of Methamphetamine can help forensic investigators better understand the criminal operations of synthetic laboratories.

As a demonstration of its chiral separation techniques, SCIEX researchers adapted the Partial Filling Technique to a low flow Capillary Electrophoresis Electrospray Interface for Mass Spectrometry (CESI-MS). This new technique separated the enantiomers of methamphetamine and its metabolite, amphetamine, in a single run, with great sensitivity.

TRAINING AND SERVICES FROM SCIEX

It is paramount that every piece of equipment in active forensic laboratories is running at peak performance.

If your laboratory is considering a transition from GC-MS to LC-MS/MS, but your staff are unfamiliar with liquid chromatography, how can you be fully confident your equipment is functioning at its best? How can you be confident in your results?

To help you and your staff get the best possible results from your LC-MS/MS assays, **we offer the following training packages:**

Personalized Forensics LC-MS Training

Our most powerful and effective courses, the Personalized Success Programs cover the workings of LC-MS systems, from the basics all the way to advanced workflows. Customized to your instruments and your staff's experience level, these programs consist of a blend of online learning, instructor-led and hands-on training. This unique approach will help your staff stay more engaged and retain their learning, so your lab can see better results right away.

Courses Available:

- Basic, Intermediate, or Advanced Forensics Quantitation on SCIEX Triple Quad™ or QTRAP systems (2 or 3 days onsite)
- Basic, Intermediate, or Advanced Forensics Screening on SCIEX Triple Quad or QTRAP systems (2 or 3 days onsite)
- Basic, Intermediate, or Advanced Forensics Quantitation and Screening on SCIEX X500 QTOF systems (2 or 3 days onsite)

Request a Quote for Personalized Forensics LC-MS Training >

LC-MS Maintenance And Troubleshooting Courses

You can reduce your lab costs by self-maintaining and self-diagnosing your common LC-MS issues. All you need is the right training. Build your staff's skills with the SCIEX University Success Programs, which offer a unique blend of self-paced online learning, instructor-led and hands-on training to maximize knowledge retention.

Courses Available:

- One-day Introduction to MS Maintenance at Your Site
- One-day Introduction to LC Maintenance at Your Site
- Two-day Advanced LC-MS Quantitation Troubleshooting at Your Site

Request a Quote for LC-MS Maintenance and Troubleshooting courses >

Remote Monitoring

Your lab needs to be running at peak performance. **StatusScope® Remote Monitoring Service** enables you to connect to your lab from anywhere securely, allowing you to stay ahead of potential instrument problems. With the **Remote Monitoring Service** you can respond to issues quickly and efficiently, and reduce your work downtime.

Connect to Your Lab from Anywhere with StatusScope Remote Monitoring Service >

Increase Your Throughput, Without Adding More People

SCIEX Laboratory Optimization Services help analytical labs to identify and eliminate unproductive waste in sample processing, method optimization, and data processing. When many of our clients have reported productivity increases as high as 30-50%, why wait to unlock your lab's productivity?

Unlock Your Lab's Hidden Productivity with SCIEX Laboratory Optimization Services >

Technical Notes

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High Throughput Platform for Confident Identification and Quantitation of Organic Explosives

High Resolution Mass Spectrometry Analysis Workflows using the SCIEX X500R QTOF System

Oscar G. Cabrices[1], Xiang He[1] ,Scott Krepich[2] and Adrian M. Taylor[3]
[1]SCIEX, USA; [2]Phenomenex, USA, [3]SCIEX, Canada

The daily occurrence of explosive materials found in crime scenes or geographical warzones is a rising safety concern for legal authorities around the world. This has led forensic scientists to develop rapid and accurate screening techniques to detect explosive occurrence as part of an investigation.

Traditional analytical methods for detecting explosives frequently involve GC or HPLC based approaches. However, these techniques are often challenging for forensics as they don't provide the essential throughput and selectivity required to identify key components of a crime scene involving explosive use.

High resolution mass spectrometry offers forensic investigators a greater level of explosive occurrence information (e.g., acquisition of analyte specific MS/MS spectra) leading to increased confidence in compound identification and acquisition of accurate mass information at low analyte concentrations.

In this technical note, the multiple uses of the SCIEX X500R QTOF System for the fast, specific and sensitive analysis of the most common organic explosives encountered in forensic analytical settings are demonstrated.

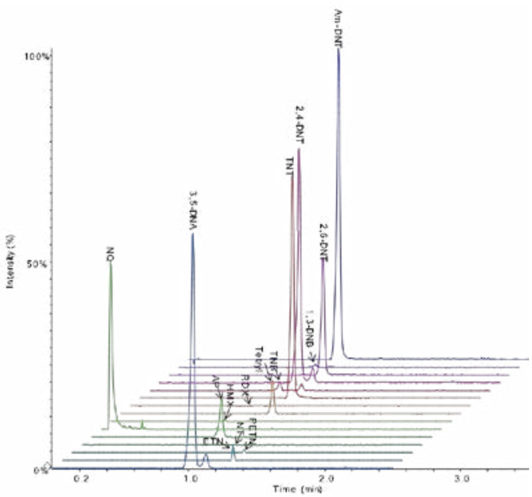

Figure 1. Obtain Ultra-Fast and Confident Organic Explosive Identification. Extracted Ion Chromatogram shows a rapid LC separation (2.5 min) and identification of 14 organic explosives using SWATH® acquisition.

Features of SCIEX X500R QTOF System for Forensic Analysis of Explosives

- The X500R QTOF System provides high resolution MS at fast acquisition rates (up to 100 MS/MS per second) to enable the quick identification of organic explosives.
- Easily build and optimize targeted methods (MRM^{HR} workflow) on different analytes using streamlined method optimization features in SCIEX OS software 1.3.
- SWATH® Acquisition provides the comprehensive detection and quantitation of every detectable compound in a forensic sample (MS/MS^{ALL}). This increases confidence for the accurate detection of explosives eliminating the risk of missing a critical component in the analysis.
- Industry leading robustness of Turbo V™ source with Twin Sprayer APCI Probe for sensitive detection of organic explosives.

Experimental Details

Materials: Organic explosive standards were acquired from Accustandard Inc.(New Haven, CT) and Cambridge Isotope Laboratories (Tewksbury, MA). All standards were serially diluted in acetonitrile for analysis. Table 1 specifies all the analytes evaluated in this study. ^{13}C-2,4,6-trinitrotoluene (^{13}C-TNT) was used as internal standard.

Table 1. List of the Organic Explosive Compounds Analyzed in a Single injection using the SCIEX X500R QTOF System

Analyte	Abbreviation
Nitroguanidine	*NQ*
Ammonium Picrate	*AP*
Octahydro-1,3,5,7-tetranitro-1,3,5,7-triazine	*HMX*
Hexahydro-1,3,5-trinitro-1,3,5-triazine	*RDX*
1,3,5-Trinitrobenzene	*TNB*
1,3-Dinitrobenzene	*1,3-DNB*
2,4,6,N-tetranitro-N-methylaniline	*Tetryl*
2,4,6-trinitrotoluene	*TNT*
4-Amino-4,6-dinitrotoluene	*Am-DNT*
2,6-Dinitrotoluene	*2,6-DNT*
2,4-Dinitrotoluene	*2,4-DNT*
Pentaerythritol tetranitrate	*PETN*
Erythritol tetranitrate	*ETN*
3,5-Dinitroaniline	*3,5-DNA*

Liquid Chromatography: HPLC separation was performed using SCIEX ExionLC™ AC system with two different separation approaches. A method development gradient separation was performed using a Phenomenex Luna Omega 3 µm Polar C18 column (100 x 3.00 mm) with an LC runtime of 6.5 minutes. An alternate fast gradient method for high throughput sample processing was performed using a Phenomenex 2.5 µm Mercury MS Cartridge Hydro-RP (20 x 0.30 mm) with an LC runtime of 2.5 minutes. Mobile phases used were buffered water and methanol/acetonitrile with appropriate additives. Injection volume for both approaches was 7 µL.

Mass Spectrometry: MS and MS/MS data were collected using Turbo V™ source with Twin Sprayer APCI Probe on the benchtop SCIEX X500R QTOF System and the SWATH® acquisition and MRM^{HR} workflow modes with the SCIEX OS software 1.3 were used for data acquisition.

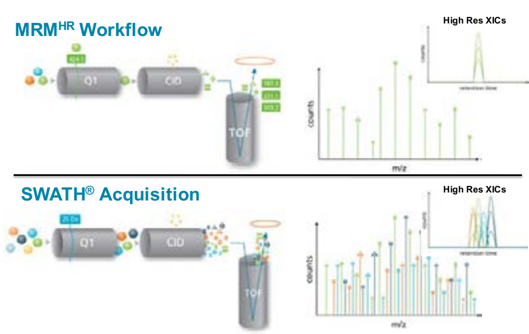

Figure 2. Apply different analysis workflows for explosives analysis with the SCIEX X500R QTOF system. (Top) MRM^{HR} workflow is a targeted data acquisition mode for analyte quantification. (Bottom) SWATH® Acquisition is data independent acquisition technique that allows comprehensive detection and quantitation of virtually every detectable compound in a sample (MS/MS^{ALL}).

Guided MRM^{HR} mode in the SCIEX OS software was used to determine the optimal fragment ions for different target compounds and to optimize the compound dependent parameters for each fragment.

SWATH® Acquisition workflow consisted of a TOF MS scan with 7 looped MS/MS experiments. The Q1 windows (50 Da each) covering 50-400 m/z were automatically populated by SCIEX OS software 1.3 for a total cycle time of 0.32 sec. Figure 3. displays the data acquisition methods and source conditions.

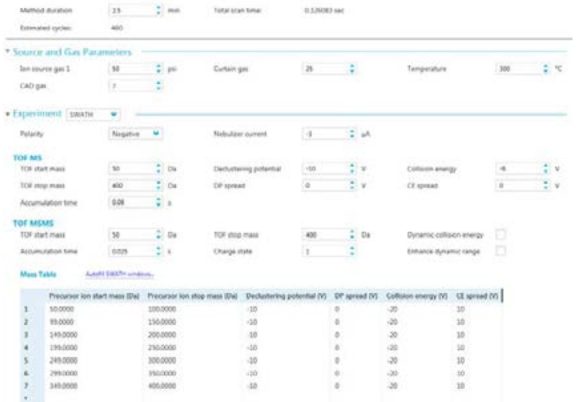

Figure 3. SCIEX OS Software 1.3 Provides Straightforward Acquisition Method Programming. Mass spectrometry parameters used for the analysis of explosives using SWATH® acquisition. Seven 50Da wide Q1 windows covering 50-400m/z were used to collect MS/MS on all detectable species.

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Table 2. List of the Organic Explosive Compounds Analyzed in a Single injection using the SCIEX X500R QTOF System

Compound	Formula	Precursor Mass	Molecular Species	Found at Mass	Mass Error (ppm)
NQ	$CH_4N_4O_2$	104.0334	$[M-H]^-$	103.0261	-0.2
AP	$C_6H_6N_4O_7$	246.0237	$[M-NH_4]^-$	227.9898	0.6
HMX	$C_4H_8N_8O_8$	312.0414	$[M+CH_3COO]^-$	355.0604	0.5
RDX	$C_3H_6N_6O_6$	222.0349	$[M+CH_3COO]^-$	218.0487	1.2
TNB	$C_6H_3N_3O_6$	213.0022	$[M-NO]^-$	183.0047	1.0
1,3-DNB	$C_6H_4N_2O_4$	168.0171	$[M-NO]^-$	138.0197	0.1
Tetryl	$C_7H_5N_5O_8$	287.0138	$[M-NO_2]^-$	241.0215	0.7
TNT	$C_7H_5N_3O_6$	227.0178	$[M-NO_2]^-$	181.0256	0.8
Am-DNT	$C_7H_9N_3O_2$	197.0437	$[M-H]^-$	196.0364	0.9
2,6-DNT	$C_7H_6N_2O_4$	182.0328	$[M-H]^-$	181.0255	1.0
2,4-DNT	$C_7H_6N_2O_4$	182.0328	$[M-H]^-$	181.0255	1.0
PETN	$C_5H_8N_4O_{12}$	316.0139	$[M-NO_2 + CH_2O_2]^-$	316.0270	0.7
ETN	$C_4H_6N_4O_{12}$	301.9982	$[M-NO_2 + CH_2O_2]^-$	302.0113	0.6
3,5-DNA	$C_6H_5N_3O_4$	183.0280	$[M-H]^-$	182.0159	0.4

Developing a High Throughput Strategy for Accurate Mass Analysis of Organic Explosives

The initial compound survey using the long column enabled the separation of all analytes in less than 8 min and allowed for detailed method development. However, analysis speed and resolution are crucial for high throughput and confident identification of explosives, which helps forensic investigators improve their case turnaround times.

Figure 1 shows an extracted ion chromatogram of the 14 explosives analyzed with the short cartridge under 3 minutes. The high resolution and speed of the X500R QTOF System allowed for MS/MS characterization of the explosives analyzed, even with such a fast separation.

Table 2 lists the observed spectral precursor mass and corresponding proposed molecular species, associated mass error (ppm). Most explosives were observed as deprotonated molecular form $[M-H]^-$. A number of adducts and alternative molecular species were also observed. Figure 4 shows XIC chromatograms and TOF MS spectra of the acetate adducts for nitramine explosives (HMX and RDX).

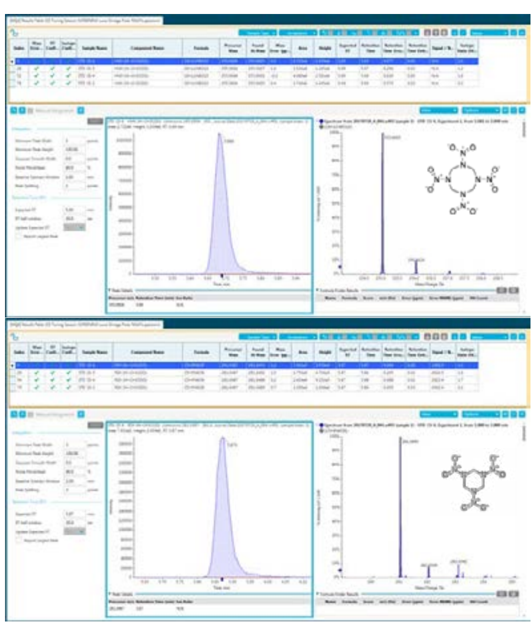

Figure 4. Quickly Obtain Accurate Mass Spectral Information for Reliable Identification of Organic Explosives. Nitramine explosives XIC chromatograms and TOF MS spectra for HMX (Top) and RDX (Bottom) demonstrate the high quality separation achieved on these compounds and the spectral quality underlying.

High quality MS/MS leads to accurate explosive compound characterization and quantitation.

SWATH® acquisition generates comprehensive and high quality MS/MS spectra for quantitation and identification using spectral library database searching. Figure 5 shows extracted ion chromatograms for Am-DNT and 2,6-DNT. The MS/MS spectra allowed the positive identification of both explosives through spectral library searching.

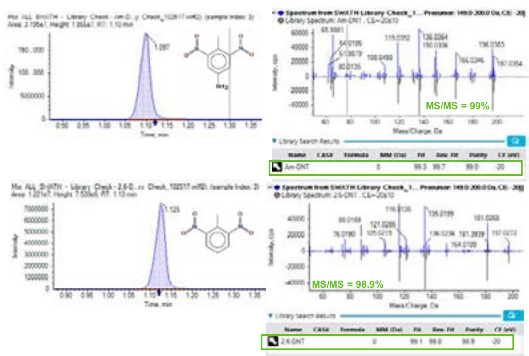

Figure 5. Automatic MS/MS library search. XICs of Am-DNT (Top) and 2,6-DNT (Bottom) with MS/MS spectra and library match score.

In addition, to help transition from the familiarity of MRM performed on a triple quadrupole system, the MRM^{HR} Workflow on the SCIEX X500R QTOF system was also evaluated. Here, the accurate mass product ions were used for quantitation, which included common fragment ions such as $[NO2]^-$ (m/z 45.9923) particular of nitro-based-explosives

Figure 4 shows calibration curves and XICs obtained for TNT and Tetryl using ^{13}C-TNT as the internal standard for quantitation. Linear dynamic range of the explosives analyzed was evaluated and most analytes showed linearity between 1-1000 µg/L.

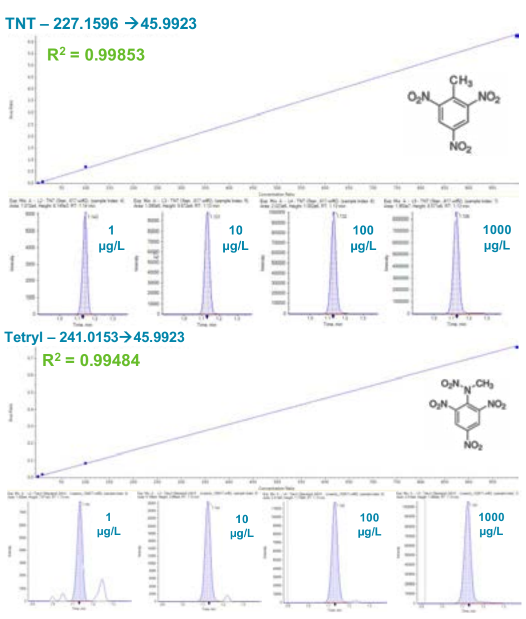

Figure 6. Good Linearity was Achieved across the Targeted Compounds using the MRM^{HR} Workflow. Calibration curves for TNT and Tetryl are shown, with linearity between 1-1000 µg/L, as well a few representative XIC traces at different concentration levels.

Conclusions

- The SCIEX X500R QTOF System provides the speed and mass accuracy for the high throughput identification and quantitation of organic explosives in forensic analytical settings.
- SWATH® acquisition generates comprehensive and high--quality MS/MS spectra, enabling reliable fragmentation for accurate explosive compound identification through spectral library search.
- MRM^{HR} workflow enables sensitive quantitation of organic explosives utilizing selective high resolution accurate mass MS/MS information.

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Contents

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

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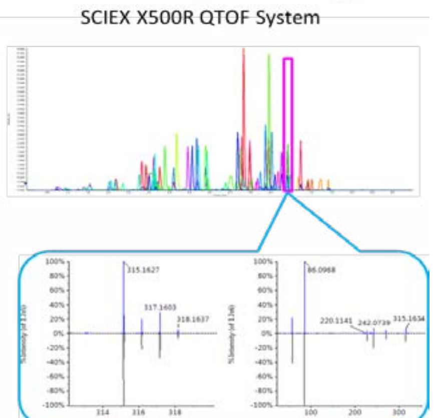
Introduction

Quadrupole Time-of-flight mass spectrometry (QTOF-MS) provides high-resolution, accurate-mass data for full-scan information of both precursor ion and all product ions. This is an ideal approach for forensic toxicology screening where unknown compounds in complex samples must be identified from information-rich data sets. The SCIEX X500R Q-TOF system provides the capability of switching between MS and MS/MS scans instantly, enabling fast acquisition of detailed structural information for easier compound identification. Designed for routine use, the benchtop SCIEX X500R QTOF system could also be used for high-specificity, targeted quantitation as well as for non-targeted screening from single sample sets in a routine testing laboratory environment. Due to its straightforward design and intuitive software workflows, non-targeted data obtained on the X500R can be retrospectively mined for additional analytes missed in initial screens, which is important with the constant emergence of new synthetic drugs. Also, the availability of retrospective analysis on X500R has become increasingly popular in forensic work.

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

Herein, we present a single-injection method for screening 664 most up-to-date forensic compounds using the SCIEX X500R QTOF system and SCIEX OS 1.2. The obtained data provides both structural information and retention times to enhance identification accuracy, especially for structurally similar isomers. Sample preparation procedures for urine and whole blood samples and library-search settings recommended here can help



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

automate and confidently establish the identification of unknowns in an efficient, all-in-one workflow.

Experimental conditions

Sample preparation

The stock standard mixtures in neat solutions were diluted with methanol: water (20:80, v/v) to appropriate concentrations. These

diluted solutions were used to determine the retention time of the 664 compounds.

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

For whole blood samples, 10.0 µL of stock standard solutions were spiked into 90.0 µL of human whole blood matrix. The samples were extracted by using a protein precipitation procedure. Basically, 900 µL of Methanol: MeCN (50:50, v/v) were added into the above mixture and vortexed for 1 min then follow by 3 min sonication and another 1 min vortex. Then the samples were centrifuged for 5 min at 8,000 rpm. The supernatant was transferred out and completely dried down under nitrogen gas. The residues were reconstituted with 500 µL methanol: water (20:80, v/v).

LC separation

Analytes (10 µL sample injection volume) were chromatographically separated using a Phenomenex Kinetex® 2.6 µm phenyl-hexyl (50 x 4.6 mm) column. 10 mM ammonium formate in water was used as mobile phase A and 0.05% formic acid in methanol was employed as mobile phase B. The mobile phases were replaced every 2 days. A linear gradient (600 µL/min) from 10% B to 98% B in 7.0 min followed by 1.5 min of 98% B and 1.0 min of 10% B was employed.

Acquisition method settings

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

Processing method settings

To identify compounds in the analyzed samples, a targeted screening approach was employed using SCIEX OS software version 2.0. Samples were evaluated against a list of parameters containing the names, molecular formulas and retention times (RTs) for all targeted compounds. Appropriate integration parameters were defined for each component. For example, the compound, hydromorphone, was defined as the peak at 2.35 min (Figure 2) with a 30 second half time window. An MS/MS library [2] was used for MS/MS library matching.

The confidence criteria used for screening were mass error, RT error, isotope ratio difference, and library score. A traffic light system where different colors were assigned to different performance levels provided a way to assess the quality of the match. For example, in the case of mass error, green represented mass errors less than 5 ppm; orange, mass errors between 5 and 10 ppm; and red, mass errors larger than 10 ppm. Color representation for all the four criteria are shown in Figure 3. A representative search result is also shown (Figure 4).

Results and Discussion

Optimization of LC conditions

The performance of separation was evaluated with different mobile phases (acidic and neutral), gradient conditions, and column types. Results indicate that a majority of the isomeric compounds was fully resolved with neutral Buffer A and a 10 min linear gradient using a Phenomenex phenyl-hexyl column (Part Number: 00B-4495-E0). Figure 2 shows an example of full chromatographic separation for 4 isomers, including Morphine, Hydromorphone, Norcodeine and Norhydrocodone, with the optimized LC condition. Figure 5 show example extracted ion chromatograms for 80 out of the 664 compounds using the optimized LC condition.

Reproducibility of retention time measurements

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

The reproducibility tests indicate that the RTs generated from our optimized LC conditions are consistent and reproducible. RTs measured on three separated analytical columns all have %CVs of less than 5% for each of the 664 compounds. RT inter-day reproducibility (tested on 80 compounds) resulted in %CVs less than 5% over 3 days. Lastly, RT variability in human whole blood and urine samples (tested on 80 compounds) indicated that the %CV for 3 individual lots is less than 5%. In addition, the RT difference between neat solutions and matrix is less than 5% for all tested compounds, as determined using the following equation:

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

Enhanced ability of compound identification

The retention time determined by the optimized LC condition combined with high-resolution mass spectrometry (HRMS) and HR-MS/MS information [2], enable more accurate compound identification. For example, the Noroxycodone (Figure 6 A) and Oxymorphone (Figure 6 B) have exactly same precursor ion and very similar MS/MS spectra. However, these two compounds were fully resolved by using the LC condition in this method. The retention time is 3.05 min for Noroxycodone and 2.10 min for Oxymorphone. Therefore, it is easier and more accurate to distinguish these two compounds by using retention time combined with MS and MS/MS information.

In addition, because the data was acquired in a non-targeted approach the processing method designed here for screening targeted compounds can be quickly adjusted and used for unknown compound identification using non-targeted data processing. Users can retrospectively analyze previously acquired MS and MS/MS data sets to screen for new compounds without having to re-inject samples, allowing data sets to be re-processed when newly identified forensic targets are discovered. For instance, initial screening results with a five-compound list was shown in Figure 7-A. For retrospective data analysis, a new process method was built for 10 compounds including 5 initial compounds and 5 new compounds by using search parameters that included compounds name, their formula and their retention times. The updated processing method was then used to re-analyze data sets for the new compound. And the retrospective screening results with new compound list are shown in Figure 7-B.

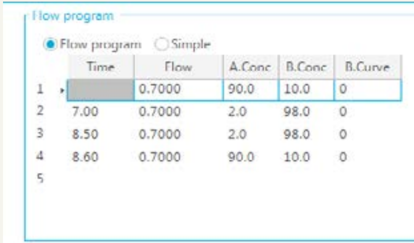
Conclusion

We have developed an LC-MS/MS-based toxicological screening method that includes the Retention Times for 664 forensic compounds. When combined with high-resolution mass spectrometry (HRMS) and HR-MS/MS information [2], the retention time identified herein enable more accurate compound identification. Overall, the ability to identify structural similar isomers was largely enhanced.

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

References

[1] SCIEX vMethod™ - Forensic Toxicology Screening on X500R QTOF, part number: 5058220
[2] SCIEX Forensics High Resolution MS/MS Spectral Library 2.1, part number: 5059566 (To be available in September 2017)



Flow program

Flow program / Simple

	Time	Flow	A.Conc	B.Conc	B.Curve
1		0.7000	90.0	10.0	0
2	7.00	0.7000	2.0	98.0	0
3	8.50	0.7000	2.0	98.0	0
4	8.60	0.7000	90.0	10.0	0
5					

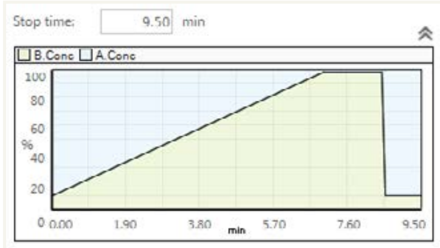


Figure 1. LC gradient

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

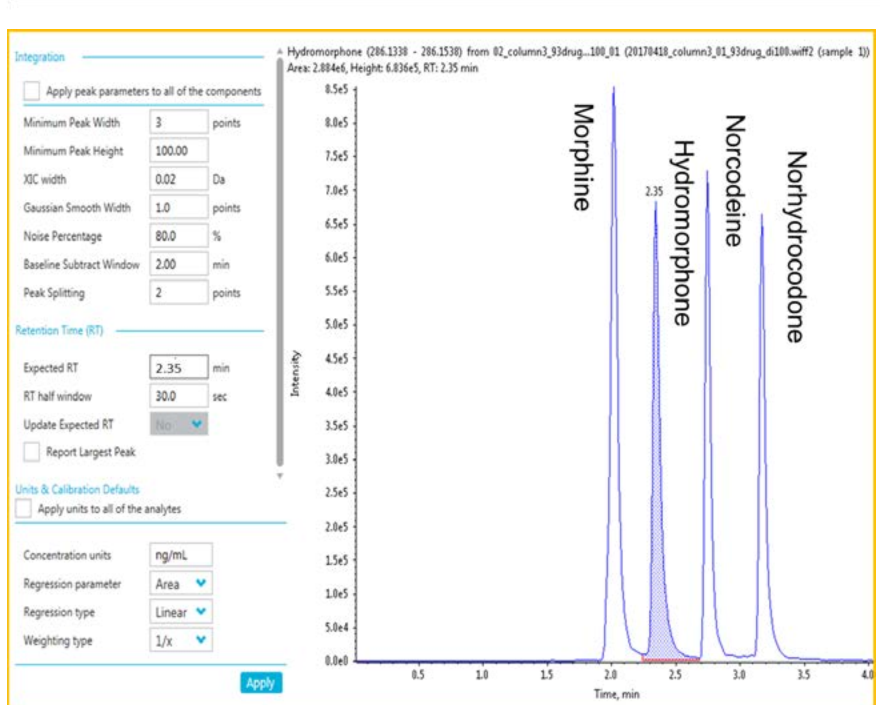


Figure 2. The parameters used for the integration of hydromorphone.

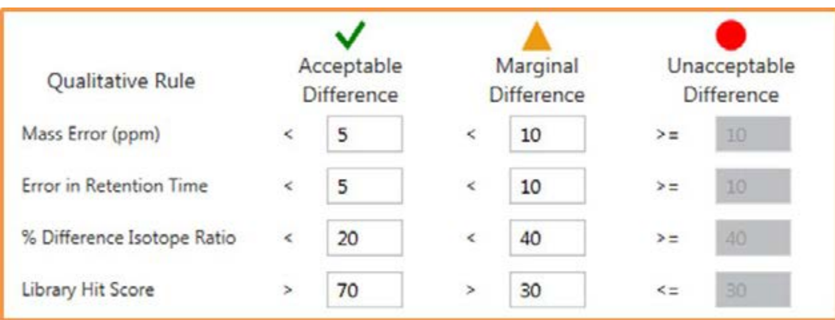


Figure 3. Confidence criteria for data processing using Sciex OS 2.0 software

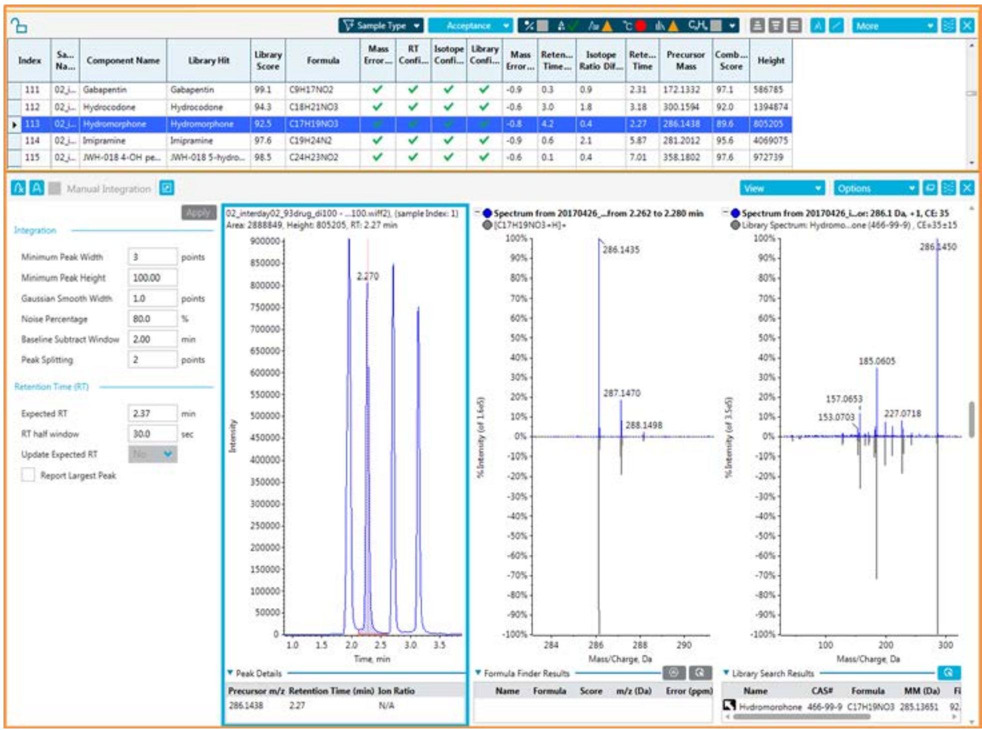


Figure 4 Representative search results obtained after using a targeted screening approach to identify compounds in urine samples.

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

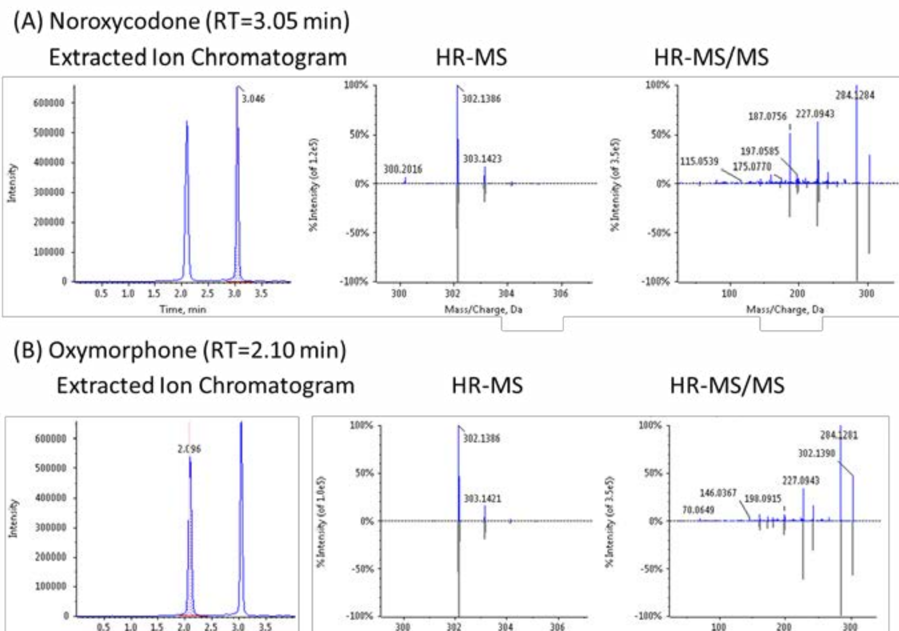
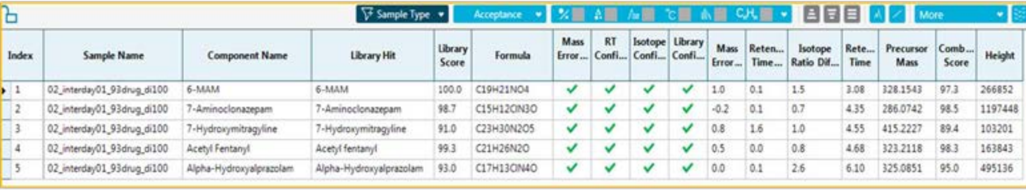


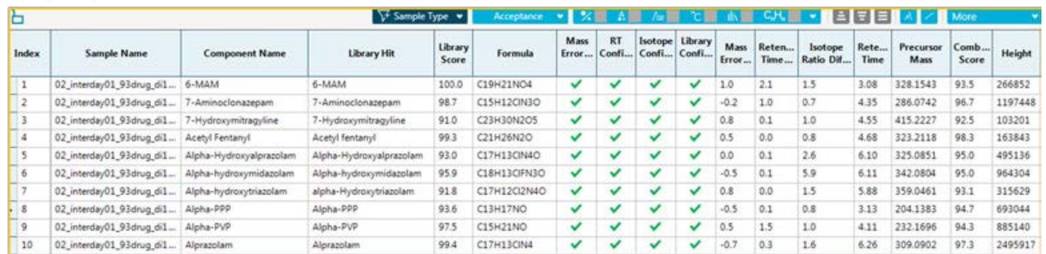
Figure 6 Representative XIC, HR-MS and HR-MS/MS spectra for Noroxycodone and Oxymorphone

(A) Original data analysis with 5 compounds



Index	Sample Name	Component Name	Library Hit	Library Score	Formula	Mass Error...	RT Confi...	Isotope Confi...	Library Confi...	Mass Error...	Reten... Time...	Isotope Ratio Dif...	Rete... Time	Precursor Mass	Comb... Score	Height
1	02_interday01_93drug_di100	6-MAM	6-MAM	100.0	C19H21NO4	✓	✓	✓	✓	1.0	0.1	1.5	3.08	328.1543	97.3	266852
2	02_interday01_93drug_di100	7-Aminoclonazepam	7-Aminoclonazepam	98.7	C15H12ClN3O	✓	✓	✓	✓	-0.2	0.1	0.7	4.35	286.0742	98.5	1197448
3	02_interday01_93drug_di100	7-Hydroxymitragyline	7-Hydroxymitragyline	91.0	C23H30N2O5	✓	✓	✓	✓	0.8	1.6	1.0	4.55	415.2227	89.4	103201
4	02_interday01_93drug_di100	Acetyl Fentanyl	Acetyl fentanyl	99.3	C21H26N2O	✓	✓	✓	✓	0.5	0.0	0.8	4.68	323.2118	98.3	163843
5	02_interday01_93drug_di100	Alpha-Hydroxyalprazolam	Alpha-Hydroxyalprazolam	93.0	C17H13ClN4O	✓	✓	✓	✓	0.0	0.1	2.6	6.10	325.0851	95.0	495136

(B) Retrospective data analysis with 10 compounds



Index	Sample Name	Component Name	Library Hit	Library Score	Formula	Mass Error...	RT Confi...	Isotope Confi...	Library Confi...	Mass Error...	Reten... Time...	Isotope Ratio Dif...	Rete... Time	Precursor Mass	Comb... Score	Height
1	02_interday01_93drug_dil...	6-MAM	6-MAM	100.0	C19H21NO4	✓	✓	✓	✓	1.0	2.1	1.5	3.08	328.1543	93.5	266852
2	02_interday01_93drug_dil...	7-Aminoclonazepam	7-Aminoclonazepam	98.7	C15H12ClN3O	✓	✓	✓	✓	-0.2	1.0	0.7	4.35	286.0742	96.7	1197448
3	02_interday01_93drug_dil...	7-Hydroxymitragyline	7-Hydroxymitragyline	91.0	C23H30N2O5	✓	✓	✓	✓	0.8	0.1	1.0	4.55	415.2227	92.5	103201
4	02_interday01_93drug_dil...	Acetyl Fentanyl	Acetyl fentanyl	99.3	C21H26N2O	✓	✓	✓	✓	0.5	0.0	0.8	4.68	323.2118	98.3	163843
5	02_interday01_93drug_dil...	Alpha-Hydroxyalprazolam	Alpha-Hydroxyalprazolam	93.0	C17H13ClN4O	✓	✓	✓	✓	0.0	0.1	2.6	6.10	325.0851	95.0	495136
6	02_interday01_93drug_dil...	Alpha-hydroxymidazolam	Alpha-hydroxymidazolam	95.9	C18H13ClFN3O	✓	✓	✓	✓	-0.5	0.1	5.9	6.11	342.0804	95.0	964304
7	02_interday01_93drug_dil...	Alpha-hydroxytriazolam	alpha-Hydroxytriazolam	91.8	C17H12Cl2N4O	✓	✓	✓	✓	0.8	0.0	1.5	5.88	359.0461	93.1	315629
8	02_interday01_93drug_dil...	Alpha-PPP	Alpha-PPP	93.6	C13H17NO	✓	✓	✓	✓	-0.5	0.1	0.8	3.13	204.1383	94.7	693044
9	02_interday01_93drug_dil...	Alpha-PVP	Alpha-PVP	97.5	C15H21NO	✓	✓	✓	✓	0.5	1.5	1.0	4.11	232.1696	94.3	885140
10	02_interday01_93drug_dil...	Alprazolam	Alprazolam	99.4	C17H13ClN4	✓	✓	✓	✓	-0.7	0.3	1.6	6.26	309.0902	97.3	2495917

Figure 7 Example for retrospective data analysis

Table 2. Retention time reproducibility for forensic compounds (partial list)

Component name	RT (min)	%CV				Difference (%) between neat and matrix	
		Column (n=3)	Inter-day (n=3)	Whole blood (n=3)	Urine (n=3)	Whole blood	Urine
6-MAM	3.05	1.5	0.3	0.0	0.2	1.0	0.8
7-Aminoclonazepam	4.35	0.6	0.4	0.0	0.1	0.2	0.2
7-Hydroxymitragyline	4.50	1.5	0.6	0.1	0.2	1.7	1.5
Acetyl Fentanyl	4.63	1.1	0.3	0.0	0.2	0.6	0.4
Alpha-Hydroxyalprazolam	6.09	0.3	0.1	0.0	0.2	0.0	0.0
Alpha-hydroxymidazolam	6.11	0.7	0.5	0.0	0.1	0.6	0.5
Alpha-hydroxytriazolam	5.87	0.2	0.2	0.1	0.1	-0.1	-0.1
Alpha-PPP	3.11	1.9	0.5	0.0	0.2	1.0	0.7
Alpha-PVP	4.05	1.5	0.4	0.0	0.1	0.5	0.3
Alprazolam	6.26	0.2	0.1	0.1	0.2	0.1	0.0
Amitriptyline	5.87	1.0	0.3	0.1	0.1	0.4	0.2
Amphetamine	2.79	2.1	0.5	0.0	0.2	0.7	0.8
Benzoylecgonine	3.95	0.3	0.1	0.0	0.1	0.3	0.1
Buphedrone	3.10	1.6	0.5	4.8	0.2	3.8	9.3
Buprenorphine	5.24	1.1	0.5	0.1	0.2	1.4	1.1
Carisoprodol	5.62	0.2	0.1	0.1	0.2	0.1	0.0
Clomipramine	6.24	1.1	0.3	0.1	0.2	0.4	0.3
Codeine	2.81	1.4	0.4	0.2	0.2	0.9	0.8
Cotinine	2.89	2.1	1.6	0.2	0.2	2.8	2.6
Cyclobenzaprine	5.73	1.0	0.3	0.0	0.2	0.5	0.3
Desalkylflurazepam	6.16	0.2	0.2	0.0	0.1	0.0	-0.1
Desipramine	5.78	1.1	0.3	0.1	0.2	0.5	0.3
Desmethyldoxepin	5.34	1.1	0.3	0.1	0.2	0.5	0.4
Dextromethorphan	5.16	1.2	0.3	0.0	0.1	0.6	0.4
Diazepam	6.72	0.2	0.1	0.0	0.1	0.0	-0.1
Dihydrocodeine	2.73	1.6	0.6	0.2	0.4	0.8	0.7
Doxepin	5.34	1.1	0.4	0.1	0.2	0.5	0.4
EDDP	5.20	1.1	0.3	0.1	0.2	0.5	0.4
MDA	3.07	1.9	0.5	0.2	0.2	0.5	0.4
MDEA	3.56	1.5	0.4	0.0	0.2	0.6	0.4
MDMA	3.27	1.7	0.5	0.2	0.2	0.5	0.4
MDPV	4.32	1.3	0.3	0.1	0.2	0.6	0.5
Meperidine	4.26	1.3	0.2	0.0	0.1	0.5	0.3
Mephedrone	3.37	1.7	0.4	0.0	0.2	0.6	0.4
Meprobamate	4.53	0.3	0.1	0.0	0.1	0.2	0.1
Methadone	5.80	1.1	0.3	0.0	0.1	0.3	0.2
Methamphetamine	3.03	1.9	0.5	0.2	0.2	0.9	0.8

Component name	RT (min)	%CV				Difference (%) between neat and matrix	
		Column (n=3)	Inter-day (n=3)	Whole blood (n=3)	Urine (n=3)	Whole blood	Urine
Methedrone	3.27	1.1	0.5	2.5	2.7	2.4	2.2
Methylone	2.85	1.7	0.5	0.0	0.3	0.7	0.7
Methylphenidate	4.09	1.3	0.4	0.0	0.1	0.5	0.3
Midazolam	5.84	1.8	1.3	0.1	0.2	2.6	2.5
Nortriptyline	5.87	1.1	0.3	0.0	0.1	0.3	0.2
O-Desmethyltramadol	3.02	1.8	0.3	0.0	0.2	0.6	0.4
Oxazepam	6.12	0.3	0.1	0.0	0.1	0.2	0.1
Oxycodone	3.03	1.5	0.4	0.0	0.2	0.6	0.4
Oxymorphone	2.07	1.9	0.6	0.0	0.5	1.0	1.3
Pregablin	2.20	2.0	1.4	0.3	0.8	-2.4	-2.3
Propoxyphene	5.58	1.1	0.3	0.0	0.2	0.4	0.2
Protriptyline	5.87	0.5	0.3	0.0	0.1	0.3	0.2
Ritalinic acid	3.58	0.5	0.2	0.0	0.2	0.0	-0.2
Sufentanil	5.55	0.9	0.3	0.1	0.1	0.8	0.6
Tapentadol	4.05	1.3	0.2	0.0	0.1	0.5	0.3
Temazepam	6.39	0.2	0.1	0.1	0.2	0.1	-0.1
Tramadol	3.93	1.5	0.2	0.0	0.1	0.5	0.3
Zolpidem	4.64	1.6	0.7	0.1	0.1	2.0	1.8

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Contents

Elevating the Forensic Laboratory Performance

Application of the SCIEX Triple Quad™ 3500 LC-MS/MS System for Rapid Analysis of Drugs of Abuse

Oscar G. Cabrices[1], Xiang He[1], Alexandre Wang[1], Matthew Clabaugh[1], and Adrian M. Taylor[2]
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Forensic Laboratories around the world share a common objective: to quickly complete their case investigations after receiving a sample (e.g., Driving Under the Influence). GC/MS has long been the technique of choice for illicit drug analysis. Nonetheless, extensive sample preparation is required as well as multiple lengthy analysis methods to achieve the required selectivity and sensitivity. This often increases sample turnaround time, and results in large case backlogs.

Liquid Chromatography coupled to Tandem Mass Spectrometry (LC-MS/MS) offers a significant benefit to forensic investigators by providing the simultaneous analysis of multiple classes of drugs of abuse. This analytical tool operated in Multiple Reaction Monitoring (MRM), enables the quantitation of low levels of compounds in biological matrices, often with much reduced sample preparation and analysis times.

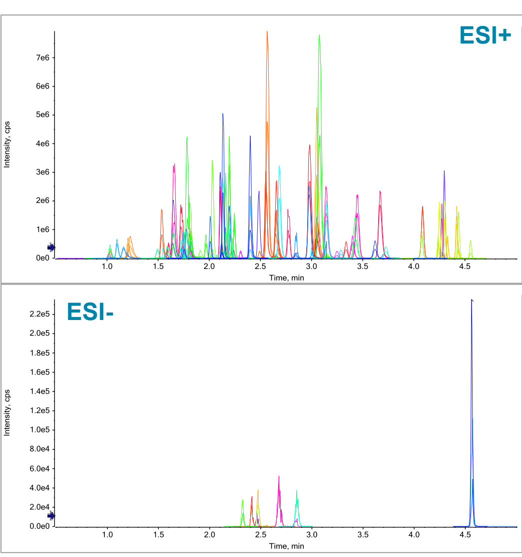

Figure 1. Rapidly analyze multiple classes of drugs of abuse from a single injection. Extracted Ion Chromatograms showing the comprehensive analysis of forensic compounds mixed in a urine sample. A rapid polarity switching method is used to include THC-COOH and Barbiturates (Bottom) using negative electrospray ionization, as well as other drugs (Top) that ionize well in positive electrospray mode.

In this study a rapid and reliable method for the comprehensive analysis of forensic drugs in urine samples using the SCIEX Triple Quad™ 3500 LC-MS/MS system is described. Owing to the inclusion of several barbiturates in the method which efficiently ionize in negative mode, a polarity switching method has been implemented. Due to a high number of MRM transitions (Over 200 MRMs, including the internal standards) and a short LC runtime (6.5 min), an optimized *Scheduled* MRM™ algorithm is used.

Features of the SCIEX Triple Quad™ 3500 LC-MS/MS System for Forensic Drug Analysis

- Industry leading Turbo V™ source efficiently ionizes compounds, delivering highly efficient desolvation for stable and sensitive performance while analyzing complex biological matrices.
- Proprietary Curtain Gas™ interface reduces the need for routine maintenance and ensures maximum productivity.
- *Scheduled* MRM™ algorithm and Curved LINAC® collision cell design improves data quality to ensure fewer peaks are missed and optimal sensitivity is achieved for all drugs of abuse.
- MultiQuant™ Software allows fast data processing, with less manual intervention and quick flagging of outliers, so forensic laboratories can release results faster.

Methods

Sample Preparation: Calibration curves and QCs with a mix of known compound standards (Cerilliant, Round Rock, Texas) were prepared in human drug free urine. The Internal Standards used were: 6-MAM-d3, Amphetamine-d5, Benzoylecgonine-d3, Buprenorphine-d4, Carisoprodol-d7, Codeine-d6, Fentanyl-d5, Hydrocodone-d6, Hydromorphone-d6, JWH 018 4-OH pentyl-d5, JWH 019 6-OH hexyl-d5, MDPV-d8, Meperidine-d4, Mephedrone-d3, Meprobamate-d7, Methadone-d3, Methamphetamine-d5, Methylone-d3, Mitragynine-d3, Morphine-d6, Nordiazepam-d5, Nortriptyline-d3, Oxycodone-d6, Oxymorphone-d3, THC-COOH-d3, Butalbital-d5, Secobarbital-d5 These were mixed and diluted in methanol as the IS spiking solution. The sample preparation workflow used in this study is detailed in Figure 2.

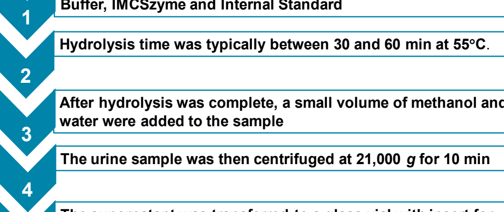

Figure 2. Urine Sample Preparation Workflow. A straightforward urine hyrdrolysis was for glucuronide-conjugated analytes for comprehensive forensic drug analysis with the Triple Quad™ 3500 LC-MS/MS System.

LC conditions: HPLC separation was performed on Phenomenex Kinetex Phenyl-hexyl (50 × 4.6 mm, 2.6 µm, 00B-4495-E0) on the SCIEX ExionLC™ AC system. Mobile phase A (MPA) and mobile phase B (MPB) were ammonium formate in water and methanol with formic acid, respectively. The LC flowrate was 1 mL/min, and the total LC runtime was 6.5 min. Injection Volume was 20 µL.

MS and MS/MS conditions: Curtain gas (CUR): 30, Collision gas (CAD): Medium. IonSpray Voltage (IS): 2500 V (positive) and -4500 V (negative). Temperature (TEM): 650°C. Ion Source Gas 1 (GS1): 60. Ion Source Gas 2 (GS2): 50. The Declustering Potential (DP), Collision Energy (CE), and Collision Cell Exit Potential (CXP) voltages were optimized for each individual component, as described in Table 1.

***Data Processing**:* Data was acquired with Analyst 1.6.3 and processed with MultiQuant™ Software 3.0. Linear dynamic range was evaluated through calibration curves with analyte concentrations ranging from 1-1000 ng/mL.

Table 1. List of the Target Compounds Analyzed in a Single Method using the *Scheduled* MRM algorithm.

6-MAM	*Hydromorphone*	*Nordiazepam*
7-Aminoclonazepam	*Imipramine*	*Norfentanyl*
7-Hydroxymitragynine	*JWH-018 4-OH pentyl*	*Norhydrocodone*
Acetyl Fentanyl	*JWH-018 pent acid*	*Normeperidine*
Alpha-Hydroxyalprazolam	*JWH-019 6-OH hexyl*	*Noroxycodone*
Alpha-Hydroxymidazolam	*JWH-073 3-OH butyl*	*Norpropoxyphene*
Alpha-Hydroxytriazolam	*JWH-073 but acid*	*Nortriptyline*
Alpha-PPP	*JWH-081 5-OH pentyl*	*O-D-methyltramadol*
Alpha-PVP	*JWH-122 5-OH pentyl*	*Oxazepam*
Alprazolam	*JWH-210 5-OH pentyl*	*Oxycodone*
AM-2201 4-OH pentyl	*JWH-250 4-OH pentyl*	*Oxymorphone*
Amitriptyline	*Lorazepam*	*PCP*
Amphetamine	*MDA*	*Pregabalin*
Benzoylecgonine	*MDEA*	*Propoxyphene*
Buphedrone	*MDMA*	*Protriptyline*
Buprenorphine	*MDPV*	*RCS4-4-OH-pentyl*
Carisoprodol	*Meperidine*	*Ritalinic Acid*
Clomipramine	*Mephedrone*	*Sufentanil*
Codeine	*Meprobamate*	*Tapentadol*
Cotinine	*Methadone*	*Temazepam*
Cyclobenzaprine	*Methamphetamine*	*Tramadol*
Desalkylflurazepam	*Methedrone*	*Zolpidem*
Desipramine	*Methylone*	*Amobarbital*
Desmethyldoxepin	*Methylphenidate*	*Butabarbital*
Dextromethorphan	*Midazolam*	*Butalbital*
Diazepam	*Mitragynine*	*Phenobarbital*
Dihydrocodeine	*Morphine*	*Secobarbital*
Doxepin	*Naloxone*	*Pentobarbital*
EDDP	*Naltrexone*	*THC-COOH*
Fentanyl	*N-desmethyltapentadol*	
Gabapentin	*Norbuprenorphine*	
Hydrocodone	*Norcodeine*	

Implementing a rapid and selective comprehensive drug analysis method

Using the Phenomenex Kinetex Phenyl-Hexyl column enabled the rapid separation of various isobaric compounds targeted in this method. Under the conditions used, peak front-tailing for early eluting compounds like gabapentin, morphine and oxymorphone was not noticed. Figure 3 shows the LC separation of several forensic isobaric compounds targeted in this method.

The optimized *Scheduled* MRM™ algorithm in Analyst® 1.6.3 ensured optimal data quality even during regions of the chromatogram when the MRM concurrency was very high. Most MRM transitions had 15 or more data points across the LC peaks. A minimum of 10 data points was achieved across every peak.

Figure 1 shows the elution profile of all the compounds, in which the top trace shows the extracted ion chromatograms (XICs) for the barbiturates and THC-COOH (negative mode) and the bottom trace the XICs for the remaining compounds in positive mode.

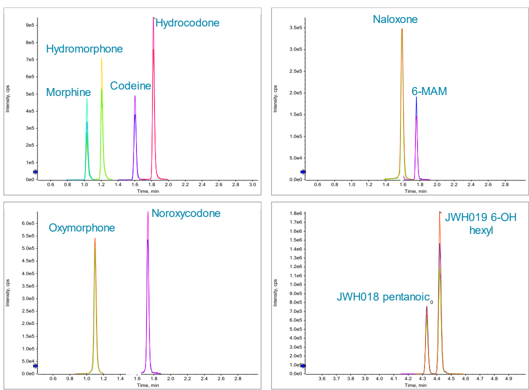

Figure 3. Obtain complete separation of isobaric compounds. The combination of the selectivity of the Phenomenex Kinetex Phenyl-Hexyl column and speed of the *Scheduled* MRM™ algorithm enable the separation and definite analysis of compounds that share the same MS/MS characteristics.

It was essential to employ rapid polarity switching to accommodate more all the analytes (more than 200 MRM transitions) within a single, rapid data acquisition method. Compared to two separate injections (one for each polarity), this rapid polarity switching method offers a significant improvement in sample throughput.

Table 2. Drug Analysis Method Sample Throughput Comparison

	GC/MS	*SCIEX 3500 Triple Quad™ LC-MS/MS System*
Analysis run time (min)	*15*	*5*
Sample Throughput in 24 hours	*96*	*288*

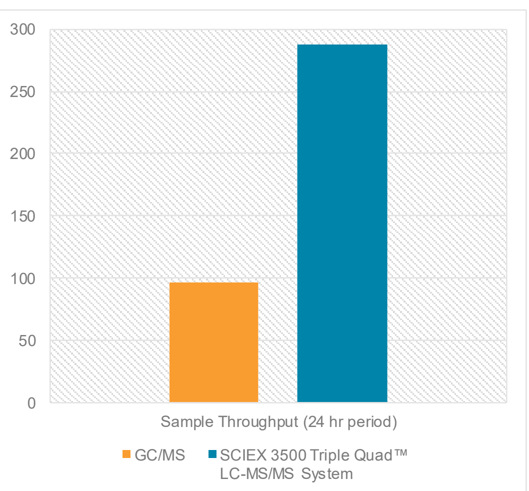

Figure 4. Maximize laboratory sample throughput. The combination of all target compounds in a comprehensive LC-MS/MS method helps the performance of the forensic laboratory by analyzing more than 280 samples within a 24 hour period.

Forensic analysis methods for drugs of abuse using GC/MS range between 15-17 minutes per sample, due to analyte volatility, derivatization technique, capillary column dimensions, carrier gas flow rates and physicochemical properties.

In the current LC-MS/MS method the runtime was approximately 6.5 minutes to accommodate all drugs of abuse in the same run. With a smaller target list (e.g. < 60 analytes), the LC runtime can be reduced to approximately 5 minutes or even further with the additional LC separation pumps, column switching valves as part of the LC multiplexing capabilities of the MPX™ Software.

Figure 4 shows the Sample throughput comparison of the SCIEX 3500 Triple Quad™ LC-MS/MS System and GC/MS. More than 285 samples can be processed within a 24 hour time frame using a 5 minute LC run, which would significantly help the forensic laboratory increase sample turnaround time, and reduce large case backlogs.

Analytical Performance of the SCIEX 3500 Triple Quad™ LC-MS/MS System for forensic drug analysis

The processed urine sample had a final dilution factor of 10. With only 20 µL injection volume (equivalent of 2 µL unprocessed urine), all compounds were detected with ease. Limits of Quantitation (LOQ) ranged between 1 to 100 ng/mL, depending on compound class. Figure 5 shows a few representative calibration curves generated with this comprehensive method.

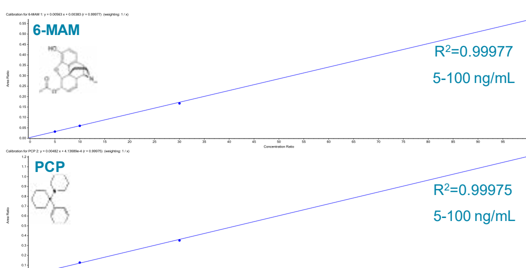

Figure 5. Good Linearity was achieved for drugs of abuse in urine samples. Calibration curves are shown for 6-MAM (top) and PCP (bottom) R^2 values were equal to 0.99 all compounds in the comprehensive method.

In addition to the good linearity obtained, method reproducibility was evaluated across the entire concentration ranges assessed. Figure 6 shows representative XICs of the quantifier MRMs of notable drugs of abuse and metabolites measured in this method (THC-COOH, Methadone and Norfentanyl).

Three replicate set of injections were performed, obtaining excellent reproducibility with RSD values less than 10% for most compounds. All quantitation results were processed with MultiQuant™ Software 3.0, designed for easy, quick, versatile and streamlined data processing with accurate and reliable quantitation.

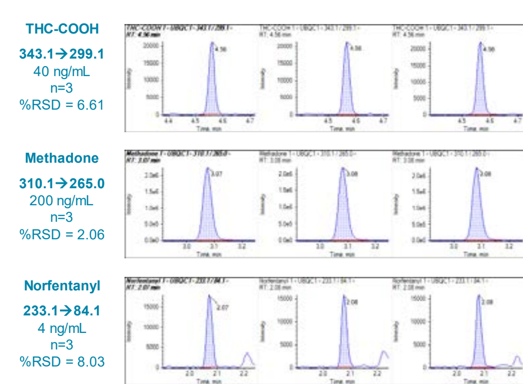

Figure 6. Excellent reproducibility was achieved for drugs of abuse in urine. Representative XIC traces of different forensic compounds are shown to demonstrate reliable quantitation at different concentrations.

Conclusions

A rapid and comprehensive analysis method for the LC-MS/MS analysis of drugs of abuse in urine samples was developed using the SCIEX Triple Quad™ 3500 LC-MS/MS System, enabling the platform to be readily adaptable by forensic laboratories.

- The combination of the selectivity of the Phenomenex Kinetex Phenyl-Hexyl column, speed of the *Scheduled* MRM™ algorithm and rapid polarity switching enable the rapid separation and definite analysis of forensic compounds.
- The combination of all target compounds in a comprehensive LC-MS/MS method maximizes laboratory sample throughput with more than 285 samples processed within a 24 hour time frame.
- Successful quantitative analysis was achieved through MultiQuant™ Software 3.0 enabling streamlined and accurate data processing of the all compounds analyzed. Excellent linearity and reproducibility were observed for all analytes across the relevant calibration range.

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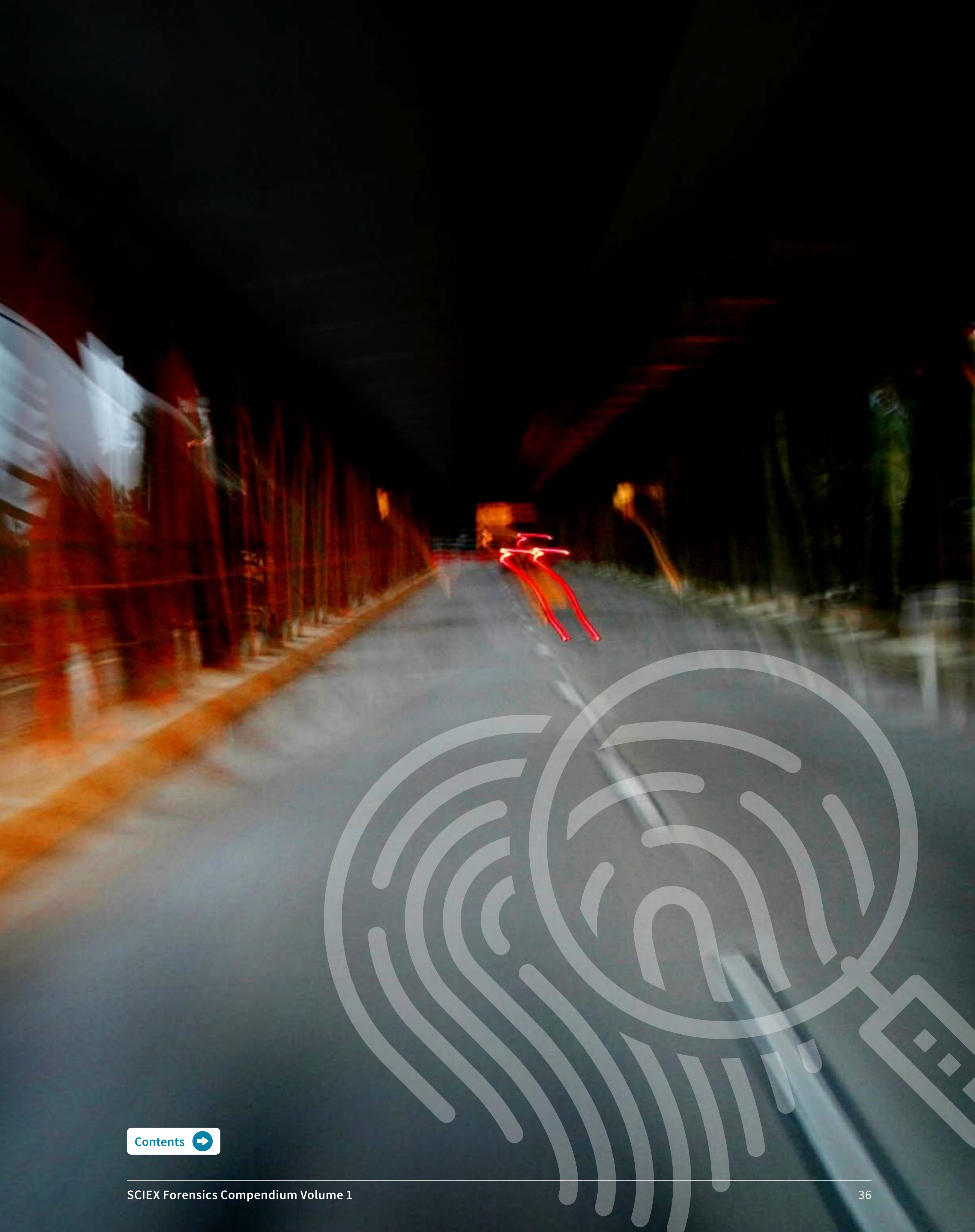


Contents

Efficiently Designed Workflows Provide Accurate Results in Forensic Analysis of THC-COOH in Hair Samples

Sensitive detection of the marijuana metabolite with the Triple Quad™ 4500 LC-MS/MS System

Xiang He[1], Oscar G. Cabrices[1], Alexandre Wang[1], Matthew Clabaugh[1], and Adrian M. Taylor[2]
[1]SCIEX, Redwood City, USA; [2]SCIEX, Concord, Canada

Marijuana is one of the most popular recreational drugs abused worldwide. Detection of its use can be done in many biological matrices, such as urine, blood, oral fluid and hair. While urine and oral fluid are very useful for determining marijuana use in short term, hair samples are extremely valuable in testing the long-term use. Additional benefits of hair testing include but are not limited to (1) sample collection being non-invasive; and (2) little risk of sample adulteration.

Presence of the main marijuana metabolite (i.e. THC-COOH) in hair indicates active drug usage. However, there are two major analytical challenges associated with detecting THC-COOH in hair samples: The concentration of THC-COOH in hair samples is very low and the high abundance of matrix interferences associated within hair samples that specifically interfere with the detection of THC-COOH.

We have previously shown the successful applicability of the QTRAP® 5500 or 6500+ systems for THC-COOH analysis in hair. Using MS/MS/MS or MRM[3], very low level of THC-COOH (~ 0.1 pg/mg) in hair samples can be detected without exhaustive sample preparation.

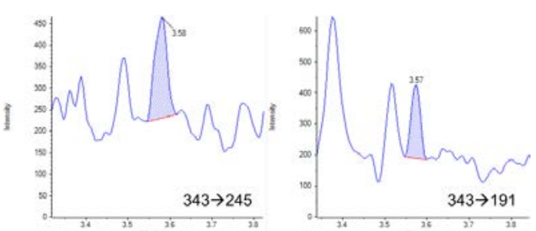


Figure 1. Detect THC-COOH in hair down to 0.2 pg/mg trace concentration levels with an efficient SPE sample preparation procedure. Using fast MRM cycle times (~100 msec) allowed the accurate quantitation (Quantifier Ion shown on left) and confirmation (Qualifier ion shown on right) of THC-COOH extracted from 25 mg of a hair sample.

In the absence of a QTRAP® System, forensic laboratories must efficiently design analysis approaches for the accurate detection of marijuana metabolite present in hair samples. In this technical note, we present a workflow that combines the Triple Quad™ 4500 LC-MS/MS System with a solid phase extraction procedure that allows the reliable and sensitive detection of trace levels of THC-COOH in hair matrix.

Features of the Triple Quad™ 4500 LC-MS/MS System for Forensic Hair Analysis

- Turbo V™ source and Curtain Gas™ interfaces delivers highly efficient desolvation for stable and sensitive performance while analyzing complex biological matrices.
- Ultra-fast MRM cycle times (With minimum dwell times of 1 msec for MRM acquisition) increasing sample throughput and data quality for trace levels of THC-COOH.
- Achieve up to 5 orders of dynamic range for high performance quantitation, reducing the needs for repeat analyses for increased productivity.
- MultiQuant™ Software allows fast data processing, with less manual intervention and quick flagging of outliers, so forensic laboratories can release results faster.

Contents

Methods

Hair Sample preparation and digestion: Hair samples were washed according to accepted laboratory procedure, dried and cut into segments of ~ 2 mm lengths. Approximately 25 mg of each hair sample was transferred into suitable and sealable container with cap. 20 µL THC-COOH-d9 internal standard solution in methanol and ~ 1.1 mL 1N potassium hydroxide solution was added, and the container was capped and gently agitated to suspend the hair segments in the solution. The containers were placed at 70ºC for 1 hour with gentle agitation every 20 min (to keep hair segments fully suspended in digestion solution) for complete digestion of the hair samples. The containers were allowed to cool to room temperature. Contents of the containers were transferred to 2-mL microcentrifuge tubes for ultra-centrifugation at 15,000 rpm for 5 min.

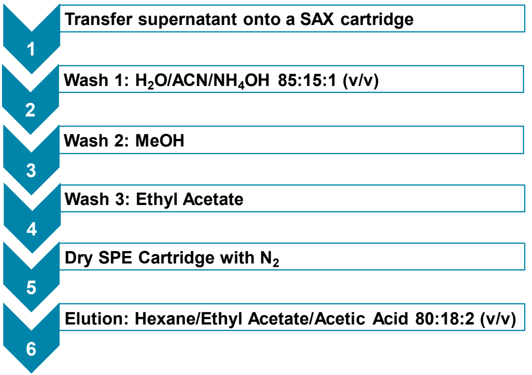


Figure 2. Strong Anion Exchange (SAX) Solid Phase Extraction workflow. A 6-step extraction protocol can be rapidly implemented and optimized for selectively extracting THC-COOH from hair samples for analysis with the Triple Quad™ 4500 LC-MS/MS System.

LC conditions: HPLC separation was performed on Phenomenex Kinetex Phenyl-hexyl column (50 × 3 mm, 2.6 µm, 00B-4495-E0) on the SCIEX ExionLC™ AC system. Mobile phase A (MPA) and mobile phase B (MPB) were 0.01% acetic acid in water and methanol, respectively. The LC flowrate was 0.75 mL/min, column temperature was held at 40ºC, and the total LC runtime was 5.5 min.

MS and MS/MS conditions: Source conditions were in Table 1 and MRM conditions were listed in Table 2.

Table 1: Source Parameters

Curtain gas	*25*
CAD	*10*
Spray voltage (V)	*-4500*
Temperature (C)	*650*
GS 1	*60*
GS 2	*60*

Table 2: MRM Transitions used

Analyte	Q1	Q3	DP	CE
THC-COOH (1)	*342.9*	*245.1*	*-100*	*-39*
THC-COOH (2)	*342.9*	*191.0*	*-100*	*-45*
THC-COOH-d9 (1)	*351.9*	*254.1*	*-100*	*-39*
THC-COOH-d9 (2)	*351.9*	*194.0*	*-100*	*-45*

Data Processing: Data was acquired with Analyst 1.6.3 and processed with MultiQuant™ Software 3.0. Linear dynamic range was evaluated through calibration curves with analyte concentrations ranging from 0.2 – 2 pg/mg.

Designing an efficient sample preparation workflow to maximize THC-COOH recovery

One of the biggest challenges in getting the clean extracts of THC-COOH from hair for detection was the presence of complex matrix contents, some of which were structurally similar to THC-COOH. To remove these interferences, a SAX SPE procedure was suggested and tested.

Usually in the SAX procedure, the sample need to be basified first before being applied to the SPE cartridge, so the target analytes can bind to the oppositely charged SAX stationary phase strongly. Because hair samples usually were digested in highly alkaline solutions for more complete release of analytes from the hair samples into the extraction media, the sample solutions were already basified and can be, in theory, directly applied onto the SPE cartridge.

It was discovered that an extra ultra-centrifugation step was needed before the samples were applied to the SPE cartridge, mainly to remove the insoluble particulates in the digested hair samples. Failure to remove these particulates rendered a very long sample application step as the SPE cartridge would be clogged or partially clogged during this process.

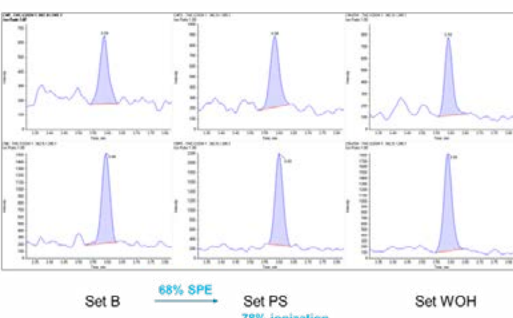


Figure 3. Obtain maximized analyte recovery performance and reduced matrix effects. The combination of SPE, LC separation and highly efficient ionization through the Turbo V™ source delivered high analyte recovery, allowing consistent quantitation of THC-COOH at low picogram levels.

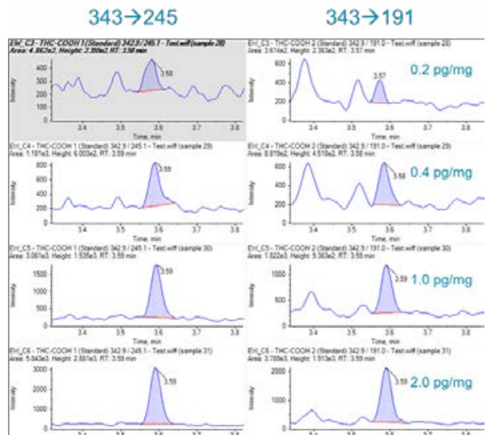
To test the sample preparation recovery and matrix effects, three sets of samples were prepared:

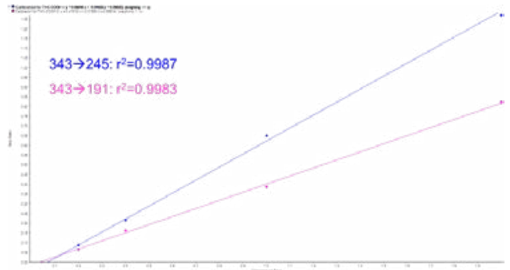
- Set B was spiked hair samples with 0.4 and 1 pg/mg THC-COOH processed with SCX SPE.
- Set PS was similar to B but THC-COOH was not spiked before SPE step was completed, so we could measure the sample preparation recovery.
- Set WOH was also similar to B but the hair samples (in solution) were replaced with 1 N potassium hydroxide, so the signal difference between B and WOH solely indicated ion suppression or enhancement.

It was observed that the sample preparation recovery was at 68% and the matric effects showed 22% loss of signal (or 78% signal recovery due to ion suppression, Figure 3). This allows the reliable quantitation of THC-COOH at low picogram concentration levels, which is only possible through the implementation of the SAX SPE procedure designed.

Evaluating the analytical sensitivity of the Triple Quad™ 4500 LC-MS/MS System

Six levels of calibrators were prepared at 0.04, 0.1, 0.2, 0.4, 1 and 2 pg/mg for THC-COOH in hair. Figure 3 showed both the quantifier (343→245) and qualifier (343→191) transitions for samples from 0.2 to 2 pg/mg samples.





Row	Component Name	Actual Concentration	Num. Values	Mean	Standard..	Percent CV	Accuracy
1	THC-COOH 1	0.20	3 of 3	0.194	0.025	12.80	96.99
2	THC-COOH 1	0.40	3 of 3	0.417	0.046	10.93	104.30
3	THC-COOH 1	1.00	3 of 3	0.984	0.074	7.54	98.39
4	THC-COOH 1	2.00	3 of 3	2.004	0.136	6.78	100.19
5	THC-COOH 2	0.20	3 of 3	0.191	0.024	12.54	95.67
6	THC-COOH 2	0.40	3 of 3	0.423	0.045	10.62	105.81
7	THC-COOH 2	1.00	3 of 3	0.979	0.138	14.07	97.88
8	THC-COOH 2	2.00	3 of 3	2.004	0.087	4.35	100.21

Figure 4. Good Linear Dynamic Range, accuracy and precision was achieved for THC-COOH in hair. Calibration curves are shown as well a few representative XIC traces to demonstrate reliable quantitation from 0.2 to 2 pg/mg.

We determined 0.2 pg/mg as our LOQ for THC-COOH in hair samples. The assay showed excellent accuracy (>95%) and precision (< 15%), and the R^2 values for quantifier and qualifier were 0.9987 and 0.9983, respectively.

All quantitation results were processed with MultiQuant™ Software 3.0, designed for easy, quick, versatile and streamlined data processing with accurate and reliable quantitation.

Conclusions

The combination of a solid phase extraction procedure with the Triple Quad™ 4500 LC-MS/MS System allowed the efficient and sensitive detection of trace levels of THC-COOH (0.2 pg/mg) in hair samples, making the workflow to be readily adaptable into a forensic toxicology laboratory.

- A 6-step extraction protocol using SAX SPE can be rapidly implemented and optimized for selective analysis of THC-COOH.
- The design of the hair analysis workflow resulted in efficient ionization through the Turbo V™ source delivered high analyte recovery for stable and sensitive performance.
- Successful quantitation of THC-COOH was performed using MultiQuant™ Software 3.0 allowing streamlined and accurate data processing of trace level concentrations (0.2 to 2 pg/mg).

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Contents

Ultra-Fast Forensic Toxicological Screening and Quantitation under 3 Minutes using SCIEX X500R QTOF System and SCIEX OS 1.0 Software

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Overview

Recently, there has been increasing use of high resolution and accurate mass LC-MS/MS systems for drug screening in forensic toxicological settings. More notably, a fast method that can detect an unlimited number of analytes with all information afforded by this technology, such as mass accuracy, LC retention time, and MS/MS spectral library matching, is desirable. In this technical note, we present an ultra-fast forensic toxicological screening method using the new SCIEX X500R QTOF system and SCIEX OS 1.0 software. The LC runtime of this method is 2.5 minutes. Two different non-targeted data acquisition methods were used and compared, both of which yielded data that can be analyzed retrospectively.

Introduction

There are several critical attributes of a high-confidence and high-efficient forensic screening method: (1) the method must generate all necessary information to unequivocally identify a compound, (2) retrospective analysis can be performed without re-injecting the sample when new targeted compounds are being added to the screened panel, (3) the method should be robust and does not require lengthy sample preparation procedures, and (4) data processing should be fast and straight-forward so even inexperienced laboratory personnel should be able to process data without much difficulty.

Forensic toxicological screening is challenging, however, because: (1) usually there are more than hundreds of compounds to be screened with drastically varying chemical properties, (2) new compounds are constantly introduced to evade the current targeted methodologies, (3) current analytical techniques such as immunoassay are slow and inflexible to adapt to the new analytes, lack specificities, and often require multiple individual tests to complete the entire panel of targeted compounds.

Mass spectrometry (MS), especially the high resolution accurate mass system such as Time-of-flight (TOF) mass spectrometer, is a great fit for such screening applications because the data generated from these systems provides structural information for every possible analyte. Typically, scanning across the full mass range provides the fine spectral details of the precursor ions of the analytes. After coupling to a quadrupole mass filter, such a mass spectrometer (Quadrupole-TOF or QTOF) is capable of providing the full-scan information of not only the precursors ions, but also all the product ions in very high resolving power. Modern QTOF systems provide the capability of switching between MS and MS/MS scans instantly, enabling structural information to be obtained very quickly.

There are mainly two approaches to acquire MS/MS information for screening purpose: Information-dependent-acquisition (IDA) or Data-dependent-acquisition (DDA), and MS/MS^{All}. For IDA-MS/MS, a survey scan is performed to collect the information on the precursor ions, followed by multiple dependent MS/MS scans for several of the most abundant precursor/candidate ions. Choices of these candidate ions are made with artificial intelligence in the software program by sorting through the observed intensities of the precursor ions. As each MS/MS scan is performed after mass filtration (by Q1) of single precursor ion, the IDA-MS/MS spectrum is free of interfering species.

IDA-MS/MS is biased, however, because it favors the ions with higher intensities. An unbiased MS/MS data acquisition approach that collects MS/MS information for everything, all the time

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(MS/MS^{All}), has become more popular in forensic screening. 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.

MS/MS^{All} with SWATH® acquisition is a novel MS/MS^{All} technique. In every data cycle, the instrument acquires TOF-MS information first, and then it sequentially acquires MS/MS information of all precursor ions across a specified mass range in pre-divided Q1 mass isolation windows. SWATH® acquisition records MS/MS information of everything all the time, and it significantly improves the MS/MS data quality by allowing sequentially programed Q1 isolations therefore more selective MS/MS data collection compared to other MS/MS^{All} techniques.

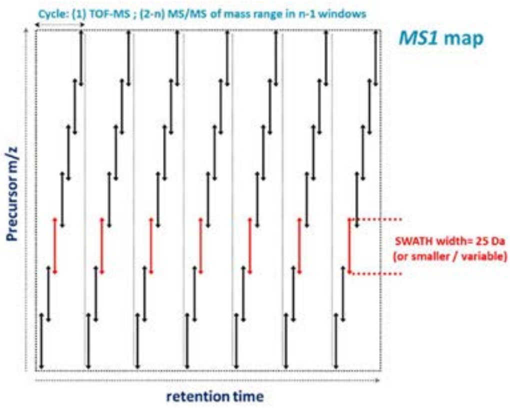

Figure 1: Principle of SWATH® acquisition in fixed window size.

LC-MS/MS technology combines separation (LC) and detection (MS, and MS/MS) and is widely used for screening applications. Mostly, the duration of LC runtime in these studies vary from 5 to 20 min. For high-throughput laboratories, a fast screening method is desired.

In this study, we aimed to develop an ultra-fast screening method in a forensic toxicological setting using the SCIEX X500R LC-MS/MS system with SCIEX OS software 1.0. We also aimed to compare IDA-MS/MS and MS/MS^{All} with SWATH® acquisition for this fast screening method.

Experimental

Sample Preparation

Dilute and Shoot. Blank urine samples were spiked with multiple drugs commonly found in forensics setting at different concentration levels. Typically, samples were diluted 10-fold in 10% methanol and centrifuged. The clear supernatants were transferred to autosampler vials and 10 µL of each sample was injected.

List of Target Compounds

In this study, we have evaluated two sets of samples with two different groups of compounds for screening. Spiked compounds in Sample Set 1 are shown in Table 1.

Samples

Sample Set 1: Urine samples spiked with compounds in Table 1. Four calibrators and two QC samples were prepared. The concentrations in the calibrators were 50%, 100%, 300% and 1000% of cutoff (CO1) concentration. The two QC samples were 200% and 600% of CO1 concentrations respectively. Injection volume was 10 µL.

Sample Set 2: Second set of urine samples contained a calibrator set spiked with compounds similar to what is described in Table 1 with minor differences. Cutoff concentrations in Sample 2 (CO2) were slightly higher than Sample Set 1. There are 7 levels of calibrators: 40%, 80%, 100%, 200%, 300%, 500% and 1000% of CO2 concentration. There were also 20 urine samples with unknown number of compounds. Injection volume was 5 µL. Dilution factor for Sample set 2 was 4.

LC Condition

HPLC separation was on Phenomenex Synergi Hydro-RP column (20 × 2 mm, 2.5 µm). Mobile phase A was 10 mM ammonium formate in water and mobile phase B was 0.1% formic acid in methanol. Figure 2 shows the gradient information.

Figure 2. LC Gradient Information

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Table 1: Compound list for Set 1.

Compounds list/Cutoff concentrations

Compounds	(ng/mL)	Compounds	(ng/mL)	Compounds	(ng/mL)
6-MAM	10	EDDP	100	Mitragynine	10
7-Aminoclonazepam	50	Fentanyl	2	Morphine	50
7-Hydroxymitragyline	10	Gabapentin	100	Naloxone	50
Acetyl Fentanyl	2	Hydrocodone	50	Naltrexone	50
Alpha-Hydroxyalprazolam	50	Hydromorphone	50	N-desmethyltapentadol	50
Alpha-hydroxymidazolam	50	Imipramine	50	Norbuprenorphine	20
Alpha-hydroxytriazolam	50	JWH-018 4-OH pentyl	10	Norcodeine	50
Alpha-PPP	10	JWH-018 pentanoic acid	10	Nordiazepam	50
Alpha-PVP	10	JWH-019 6-OH hexyl	10	Norfentanyl	2
Alprazolam	50	JWH-073 3-OH butyl	10	Norhydrocodone	50
AM-2201 4-OH pentyl	10	JWH-073 butanoic acid	10	Normeperidine	50
Amitriptyline	50	JWH-122 5-OH pentyl	10	Noroxycodone	50
Amphetamine	100	JWH-210 5-OH pentyl	10	Norpropoxyphene	100
Benzoylecgonine	50	JWH-250 4-OH pentyl	10	Nortriptyline	50
Buphedrone	10	Lorazepam	50	O-Desmethyltramadol	50
Buprenorphine	20	MDA	100	Oxazepam	50
Carisoprodol	100	MDEA	100	Oxycodone	50
Clomipramine	50	MDMA	100	Oxymorphone	50
Codeine	50	MDPV	10	PCP	25
Cotinine	50	Meperidine	50	Pregablin	100
Cyclobenzaprine	50	Mephedrone	10	Propoxyphene	100
Desalkylflurazepam	50	Meprobamate	100	Protriptyline	50
Desipramine	50	Methadone	100	Ritalinic acid	50
Desmethyldoxepin	50	Methamphetamine	100	Sufentanil	2
Dextromethorphan	50	Methedrone	10	Tapentadol	50
Diazepam	50	Methylone	10	Temazepam	50
Dihydrocodeine	50	Methylphenidate	50	Tramadol	50
Doxepin	50	Midazolam	50	Zolpidem	50
				THC-COOH	20

MS and MS/MS Conditions

Source conditions used are detailed in Table 2. Figure 3 shows the method settings for IDA-MS/MS and SWATH® acquisition methods.

Data Analysis: Confidence Settings and Screening Criteria

Data was processed in SCIEX OS software version 1.0. In this study post acquisition data processing was performed in a targeted screening approach, in which samples were evaluated against a targeted list of compounds.

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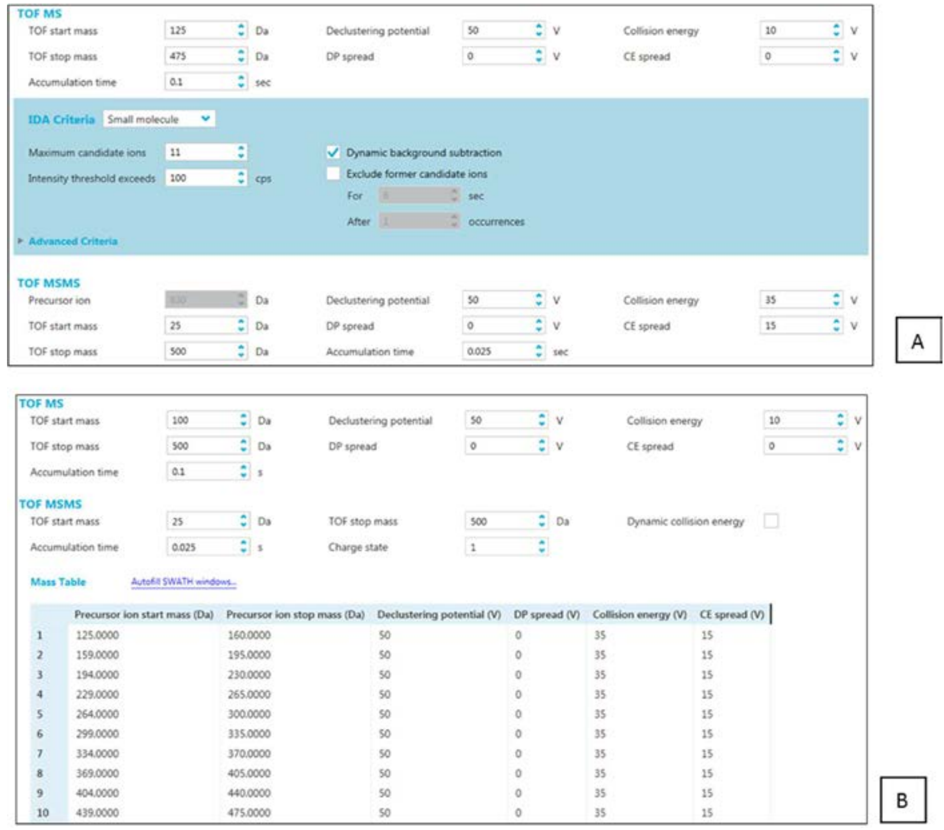
The user simply needs to enter the names, molecular formulas and retention times for all the targeted compounds. An MS/MS library (SCIEX Forensics High Resolution MS/MS Spectral Library) was used for MS/MS library matching. Both IDA-MS/MS and SWATH® acquisition are non-targeted data acquisition methods, therefore the generated data (MS and MS/MS) from

these two approaches can be retrospectively analyzed. This means that compounds that were not monitored previously can be screened at a later time without the need for re-injecting the samples.

The main confidence criteria for screening that were used were mass error, RT error, isotope ratio difference, and library score. A traffic light system was used in which different colors were assigned to different performance levels. For instance, for mass error, green represents mass error less than 5 ppm, orange represents mass error between 5 and 10 ppm, and red represents mass error larger than 10 ppm. Color representation for all the four criteria are shown in Figure 4.

Either some or all criteria can be used together to determine positive identification. All the qualifying compounds are automatically made visible (positive identification). The user has the option to further interrogate the data and manually remove potential false positives, thus rendering highly confident screening results. Figure 5 is an exemplary setting for positive identification. What this means is that any preliminary qualifying positive identification needs to satisfy the following criteria: 1) Peak is detected, 2) mass error better than 5 ppm, 3) RT error better than 10%, and 4) library matching score better than 30%.

For quantitation, the TOF-MS data from both IDA and SWATH® acquisition can be used. It is worth mentioning that the MS/MS data from SWATH® acquisition can also be used for quantitation and compound identification through ion ratios, but we did not test that in this study but has been demonstrated in a study described in a previous technical note [1].



	Precursor ion start mass (Da)	Precursor ion stop mass (Da)	Declustering potential (V)	DP spread (V)	Collision energy (V)	CE spread (V)
1	125.0000	160.0000	50	0	35	15
2	159.0000	195.0000	50	0	35	15
3	194.0000	230.0000	50	0	35	15
4	229.0000	265.0000	50	0	35	15
5	264.0000	300.0000	50	0	35	15
6	299.0000	335.0000	50	0	35	15
7	334.0000	370.0000	50	0	35	15
8	369.0000	405.0000	50	0	35	15
9	404.0000	440.0000	50	0	35	15
10	439.0000	475.0000	50	0	35	15

Figure 3. IDA-MS/MS (A) and SWATH® acquisition (B) settings.

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Figure 6 is an example of defining a component for integration. In this example, EDDP was defined at 1.54 minutes, while its isomer amitriptyline was the peak on the right at 1.65 minutes.

Table 2: Source parameters.

Polarity	Positive ionization
Source temperature (°C)	600
GS1 (psi)	60
GS2 (psi)	60
Curtain gas (psi)	30
CAD gas (psi)	7
Spray voltage (V)	2500

Figure 4. Example of configuration of confidence levels.

Figure 5. Typical setting to determine positive identification.

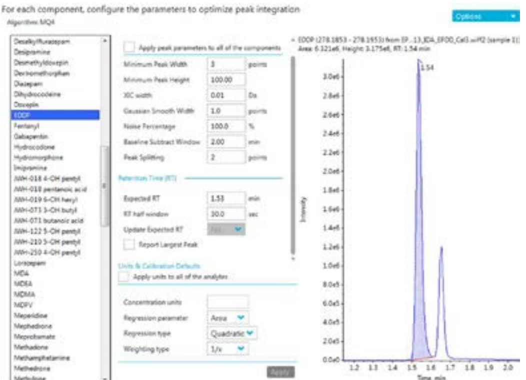

Figure 6. Peak integration and definition for EDDP.

MS/MS spectral library matching is the most integral part of the screening workflow as an MS/MS spectrum provides the fingerprint-like structural details of a compound and a carefully designed spectral matching adds the most powerful piece of evidence for a confident identification. A composite of fragment-rich MS/MS spectra from different compounds is the foundation for this critical step. To obtain a spectrum with rich fragment ion information, when building the MS/MS library, a gradual ramping of collision energy was performed during MS/MS scan from 20 to 50 V. In this study, for both IDA and SWATH® acquisition, the MS/MS data was collected in the same way for the best match between the acquired data and the spectral library.

Results

LC Performance

Previously we have reported a 2-minute LC method using a similar 20 × 2.0 mm dimension column for a similar fast screening method [2]. In this study, we have optimized the condition and extended the LC runtime by half a minute and used a different column (Hydro-RP) for better retention of polar species. Sufficient retention of those very polar species was important to allow diversion of the salt-containing eluates in the beginning of the gradient to waste. We also aimed to evenly distribute all the eluting analytes throughout the duration of the data window to reduce the number of co-eluting analytes therefore minimizing ion suppression and matrix effects. Sufficient organic wash at the end of the gradient was needed along with ample aqueous equilibration so we could maintain RT reproducibility and elongate column life. Within the 2.5-min LC runtime, we were able to resolve several groups of isomers (Figure 7). Morphine (0.47 min), hydromorphone (0.98 min), norcodeine (1.08 min) and norhydrocodone (1.18 min) were shown in Insert A, oxymorphone

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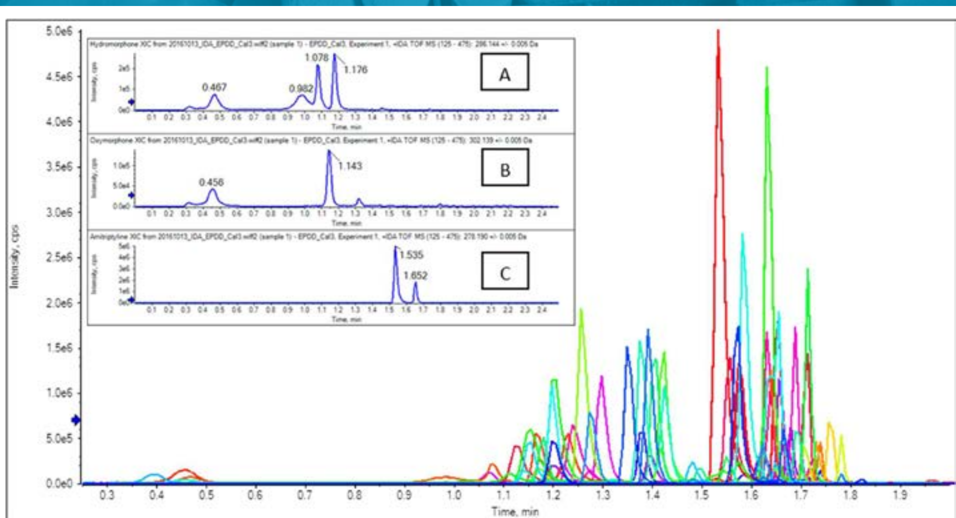

Figure 7. Extracted ion chromatograms of multiple drugs (Compound list 1).

Samples | Components and Groups
Options
EPDD_Cal1
EPDD_Cal2
EPDD_Cal3
EPDD_Cal4
EPDD_QC1
EPDD_QC2

Samples | Components and Groups
Options
All Components
6-MAM
7-Aminoclonazepam
7-Hydroxymitragyline
Acetyl Fentanyl

Figure 8. Reviewing data in sample and compound levels.

(0.46 min) and noroxycodone (1.14 min) were shown in Insert B, and amitriptyline (1.54 min) and EDDP (1.65 min) were shown in Insert C. Accurate mass TOF MS full scan provides the advantage of a generic methodology. It is a non-targeted method that allows later data re-interrogation to serach for unanticipated drugs. This is particularly important in the scenario of designer drugs where new drugs emerge on a monthly basis. But full scan TOF MS approach, in a lot of cases, is not selective enough when analyzing biological samples where matrix interference is common. SWATH® acquisition is a non-targeted method providing selective MS/MS detection of every single analyte in addition to the full scan TOF MS data.

Sample Set 1

Sample set 1 were urine samples spiked with all the compounds in Compound List 1 (Table 1) at various concentrations. Post-acquisition processing involved performing simultaneous quantitation and targeted identification. Quantitation was based on TOF-MS information of the parent ions. For targeted identification, mass error, retention time error and library score were used (Figure 4 and 5). In Analytics portion of SCIEX OS 1.0, the user can view the processed results either in sample level or compound level. This allowed the evaluation of both quantitation and screening results easily and quickly (Figure 8).

At sample level, the user could easily see what was determined as positive identifications. We consider the samples spiked with these 85 analytes "challenged" samples because there would not be any real case sample containing all these analytes but it provides a good number of compounds to use in the evaluation of these two acquisition methods as screening tools. For IDA-MS/MS data acquisition, due to the confidence setting and criteria for qualifying components, there were a few analytes that did not meet all the criteria. The main reason was either skipped MS/MS information or poor MS/MS library matching. This was

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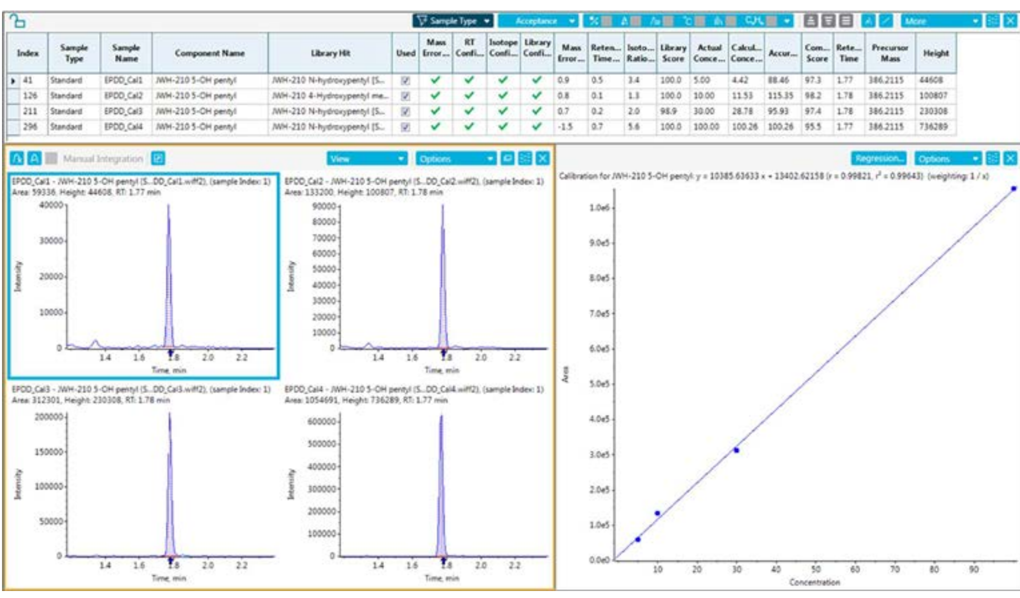

Figure 9. Quantitation and identification results in the same interface for JWH 210 5-OH metabolite in urine.

understandable considering the ultra-fast chromatography which increased the number of co-eluting substances at any moment during the LC run, thus reduced the quality of MS/MS spectra or caused the skipping of MS/MS acquisition for some analytes. With a longer 6.5-min gradient, all analytes were detected with matching to all criteria at 100% CO1 concentration.

Using SWATH® acquisition, at the same concentration level, the positive rate was improved thanks to the, *always-available*, MS/MS information. Table 3 shows the true positive rates in the calibrators for both data acquisition methods.

.Figure 9 shows the quantitation results for JWH 210 5-OH metabolite in urine. The user has the flexibility to review quantitation and screening results in one place. With user-inputs such as the compound name, expected *m/z* and retention time, the software outlines the screening and quantitation performances in the table on the top of this interface. In the center portion of the table, the user can easily see the "Traffic light" system where more green colors indicated higher identification performance. On the right of the "Traffic lights", the user can see the individual performances at each concentration level, such as mass error etc. Next to the "Library Score" column which lists the library matching score,

Table 3. True positive rate in Set 1 mixture in urine for IDA-MS/MS and SWATH acquisition.

		True Positive rate (%)	
Sample	Concentration	IDA-MS/MS	SWATH acquisition
Set 1 Level 1	50% CO1	86%	91%
Set 1 Level 2	100% CO1	91%	95%
Set 1 Level 3	300% CO1	96%	99%
Set 1 Level 4	1000% CO1	100%	96%

quantitation performance can be reviewed by comparing the "Calculated concentration" to the "Actual concentration" and "Accuracy". In the same interface, the user can choose to show the XICs and also the calibration curve associated with each analyte.

Screening and quantitation performance for all the 84 compounds was overall satisfactory with this ultra-fast LC method for diluted urine samples. The mass errors for all these analytes at all the tested concentrations were mostly within 2 ppm. The retention time consistency was excellent. As shown in Table 4, the library

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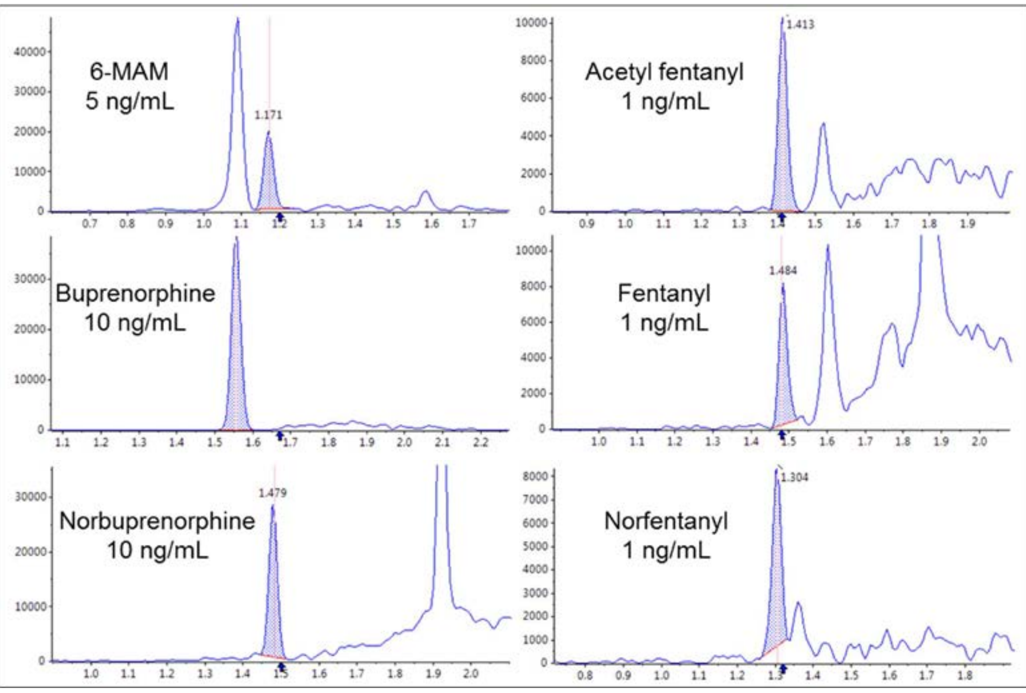

Figure 10. Extracted chromatograms of parent ions of selected analytes in urine at Level 1.

matching for both IDA and SWATH® acquisition was satisfactory especially at higher concentrations from CO1.

For a few analytes with much lower cutoff concentrations such as 6-MAM, acetyl fentanyl, buprenorphine, fentanyl, norbuprenorphine and norfentanyl, Figure 10 shows the extracted ion chromatograms with a 10 mDa extraction window around their accurate masses at 50% CO1 concentrations.

Sample Set 2

Sample set 2 were also urine samples with both calibrators and unknown samples. The "challenged" standard mixtures were blank urine samples spiked with various concentrations of a different compound panel from Table 1.

A similar statistical analysis was performed to compare the positive rate in the Set 2 standard mixtures in urine from IDA and SWATH® acquisition data (Table 4). Similar to what was observed in sample set 1, the positive rate was slightly better for SWATH® acquisition data.

20 unknown urine samples were used to further demonstrate the screening performance of this ultra-fast method. Unknown 1 and 2 were quality control urine samples spiked with all the analytes at different levels. Sample 3 through 20 were true unknown samples. There was a total of 82 true positive identifications from Sample 3 to Sample 20. In a previous test, all the calibrators and unknowns were tested with a 6.5-minute LC method using both IDA-MS/MS and SWATH® acquisition. With the 6.5-minute method, the true positive rates were both 96.3% for IDA-MS/MS and SWATH® acquisition, respectively. As previously observed in the first sample set, the imperfection in positive identification was either due to skipped MS/MS scan (IDA) or poor library match (SWATH® acquisition). True positive rates were lower (84.2%) for the IDA-MS/MS with the shorter 2.5-minute method due to the higher possibility of missing MS/MS scans. For the 2.5-minute SWATH ® acquisition, the true positive rate was significantly improved at 93.9% (Table 5).

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Table 4. True positive rate in Set 2 mixtures in urine for IDA-MS/MS and SWATH® acquisition.

		True Positive rate (%)	
Sample	Concentration	IDA-MS/MS	SWATH acquisition
Set 2 Level 1	40% CO2	93%	96%
Set 2 Level 2	80% CO2	95%	100%
Set 2 Level 3	100% CO2	95%	99%
Set 2 Level 4	200% CO2	98%	100%
Set 2 Level 5	300% CO2	99%	100%
Set 2 Level 6	500% CO2	99%	99%
Set 2 Level 7	1000% CO2	99%	99%

Quantitation was again performed with TOF-MS information and the results from the 6.5-minute and 2.5-minute methods agreed (data not shown).

Discussion

Choice of data acquisition approach

The main question to ask is when to use which data acquisition approach for screening applications.

In situations that throughput is the priority, and a short LC method is used, the results from this study suggests the preferred data acquisition approach would be SWATH® acquisition. The results from Sample set 2 clearly demonstrated the nearly equal screening performance between the 6.5-minute and 2.5-minute methods when SWATH® acquisition was used.

Based on the results from the two sample sets, it was clear that for ultra-fast LC method, SWATH® acquisition yielded better positive rate than IDA due to its complete coverage of MS/MS information. When a longer LC method was used, IDA and SWATH® acquisition gave equally excellent performances. The size of data files of SWATH acquisition was usually more than twice the size of the corresponding IDA-MS/MS data. Therefore, in situations when throughput was not the primary consideration, the IDA-MS/MS approach should be considered as the primary data acquisition approach for screening.

Conclusion

In this technical note, we have developed a super-fast screening/quantitation method in forensic setting; under 3 minutes using the SCIEX X500R QTOF LC-MS/MS system. Two non-targeted data acquisition methods: IDA-MS/MS and MS/MS^{All} with SWATH® acquisition were both tested and compared. Depending on the specific requirement of the screening method, both IDA-MS/MS and SWATH® acquisition have their advantage and disadvantage in certain areas and user should be flexible to adopt either approach with appropriate method settings.

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Acknowledgements

The authors would like to thank Joanna Porter from Precision Labs for providing the unknown urine samples for this study.

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Table 5: Screening performance for unknown samples.

Sample	6.5 min IDA	6.5 min SWATH	2.5 min IDA	2.5 min SWATH
Unknown 03	Gabapentin	Gabapentin	Gabapentin	Gabapentin
	Oxazepam	Oxazepam	Oxazepam	Oxazepam
Unknown 04	Cotinine	Cotinine	Cotinine	Cotinine
	Gabapentin	Gabapentin	Gabapentin	Gabapentin
	Naltrexone	Naltrexone	Naltrexone	Naltrexone
Unknown 05	Cotinine	Cotinine	Cotinine	Cotinine
	Gabapentin	Gabapentin	Gabapentin	Gabapentin
	Oxazepam	Oxazepam		Oxazepam
	Temazepam	Temazepam		Temazepam
	Nordiazepam	Nordiazepam		
		mCPP	mCPP	mCPP
Unknown 06	7-Aminoclonazepam	7-Aminoclonazepam	7-Aminoclonazepam	7-Aminoclonazepam
	Acetaminophen	Acetaminophen	Acetaminophen	Acetaminophen
	Alpha-hydroxyalprazolam	Alpha-hydroxyalprazolam	Alpha-hydroxyalprazolam	Alpha-hydroxyalprazolam
	Benzoylecgonine	Benzoylecgonine	Benzoylecgonine	Benzoylecgonine
	Cotinine	Cotinine	Cotinine	Cotinine
	Naltrexone	Naltrexone		Naltrexone
		Alprazolam		Alprazolam
Unknown 07	Acetaminophen	Acetaminophen	Acetaminophen	Acetaminophen
	Buprenorphine	Buprenorphine	Buprenorphine	Buprenorphine
	Cotinine	Cotinine	Cotinine	Cotinine
	Gabapentin	Gabapentin		Gabapentin
	Naloxone	Naloxone	Naloxone	Naloxone
	Norbuprenorphine	Norbuprenorphine	Norbuprenorphine	Norbuprenorphine
Unknown 08	Cotinine	Cotinine	Cotinine	Cotinine
	mCPP	mCPP		mCPP
Unknown 09	Cotinine	Cotinine	Cotinine	Cotinine
Unknown 10	Cotinine	Cotinine	Cotinine	Cotinine
	Quetiapine	Quetiapine	Quetiapine	
Unknown 11 and 12	Acetaminophen	Acetaminophen	Acetaminophen	Acetaminophen
	Buprenorphine	Buprenorphine	Buprenorphine	Buprenorphine
	Cotinine	Cotinine	Cotinine	Cotinine
	Naloxone	Naloxone	Naloxone	Naloxone
	Norbuprenorphine	Norbuprenorphine	Norbuprenorphine	Norbuprenorphine

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Unknown 14	7-Aminoclonazepam	7-Aminoclonazepam	7-Aminoclonazepam	7-Aminoclonazepam
	Acetaminophen			
	Benzoylecgonine	Benzoylecgonine	Benzoylecgonine	Benzoylecgonine
	Buprenorphine	Buprenorphine	Buprenorphine	Buprenorphine
	Cotinine	Cotinine	Cotinine	Cotinine
	Lorazepam	Lorazepam	Lorazepam	Lorazepam
	Naloxone	Naloxone	Naloxone	Naloxone
	Norbuprenorphine	Norbuprenorphine	Norbuprenorphine	Norbuprenorphine
	Norfentanyl			
Unknown 15	Acetaminophen	Acetaminophen	Acetaminophen	Acetaminophen
	Cotinine	Cotinine	Cotinine	Cotinine
	mCPP	mCPP		
Unknown 16	Cotinine	Cotinine	Cotinine	Cotinine
	Naltrexone	Naltrexone	Naltrexone	Naltrexone
Unknown 17	Acetaminophen	Acetaminophen		Acetaminophen
	Buprenorphine	Buprenorphine	Buprenorphine	Buprenorphine
	EDDP	EDDP	EDDP	EDDP
	Fentanyl	Fentanyl	Fentanyl	Fentanyl
	Hydrocodone	Hydrocodone		Hydrocodone
	Hydromorphone	Hydromorphone	Hydromorphone	Hydromorphone
	Meperidine	Meperidine	Meperidine	Meperidine
	Methadone	Methadone	Methadone	Methadone
	Norbuprenorphine	Norbuprenorphine	Norbuprenorphine	Norbuprenorphine
	Norfentanyl	Norfentanyl		Norfentanyl
	Oxycodone	Oxycodone	Oxycodone	Oxycodone
	Tapentadol	Tapentadol	Tapentadol	Tapentadol
	Tramadol	Tramadol	Tramadol	Tramadol
		Codeine	Codeine	Codeine
Unknown 18 and 19	6-MAM	6-MAM	6-MAM	6-MAM
	Codeine	Codeine	Codeine	Codeine
	Cotinine	Cotinine	Cotinine	Cotinine
	Gabapentin	Gabapentin	Gabapentin	Gabapentin
Unknown 20	Acetaminophen	Acetaminophen	Acetaminophen	Acetaminophen
	Cotinine		Cotinine	Cotinine
Detected Positives/True positives	79/82	79/82	69/82	77/82
True positive rate	**96.3%**	**96.3%**	**84.2%**	**93.9%**

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Contents

Fast and simultaneous analysis of ethanol metabolites and barbiturates using the QTRAP® 4500 LC-MS/MS system

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Overview

In this technical note, we describe a fast and sensitive method to analyze ethanol metabolites (ethyl glucuronide and ethyl sulfate) and barbiturates (amobarbital, butabarbital, butalbital, pentobarbital, phenobarbital and secobarbital) in human urine using the SCIEX QTRAP®/Triple Quad™ 4500 LC-MS/MS system (Figure 1). Sample preparation is based on a simple "dilute and shoot" methodology. We evaluated both analytical performance and method robustness.

Introduction

Liquid Chromatography coupled to Tandem Mass Spectrometry (LC-MS/MS) is a widely used analytical tool for quantitation of compounds in forensic samples. While most analytes in forensic applications analyze well with positive ionization, there are analytes that show better ionization efficiency with negative ionization, for example acidic compounds. These analytes include ethanol metabolites such as ethyl glucuronide (ETG), ethyl sulfate (ETS), and the barbiturates such as amobarbital, butabarbital, butalbital, pentobarbital, phenobarbital and secobarbital. Typically, for LC-MS/MS analysis of a comprehensive forensic analytical panel, detection of urinary barbiturates is done in negative ionization mode, and majority of other compound classes are detected in positive ionization mode. In a previous technical note, we have described a method for analysis of a comprehensive forensic drug panel in one injection using polarity switching. The sample preparation of that method has a hydrolysis step because many analytes in the panel formed phase II conjugates that need to be de-conjugated with hydrolysis back to the parent drug that typically gives better analytical performance. However, if ETG and ETS are included in the panel, then a separate injection for ETG and ETS detection is required because they cannot undergo hydrolysis.

As a two-sample-preparation/two-injection approach is inevitable, we investigated an experimental design to run one injection in positive mode for most analytes after performing a hydrolysis step in the sample preparation. Then perform a second sample preparation, that doesn't include the hydrolysis, on a separate aliquot of the sample. A second injection in negative mode for ETG/ETS and barbiturates was therefore performed.

Figure 1: SCIEX ExionLC™ AC HPLC and QTRAP®/Triple Quad™ 4500 LC-MS/MS System

In this study, we describe a fast and sensitive method to analyze ETG, ETS, amobarbital, butabarbital, butalbital, pentobarbital, phenobarbital and secobarbital in human urine in a single injection with SCIEX QTRAP®/Triple Quad™ 4500 LC-MS/MS system. Sample preparation is based on a simple "dilute and shoot" methodology without hydrolysis. Analytical performance was evaluated. In addition, a robustness test for the method was done with over 800 continuous injections of urine samples

Experimental

Materials

Compounds of interest include ETG, ETS, amobarbital, butabarbital, butalbital, pentobarbital, phenobarbital and secobarbital. Internal standards are ETG-D5 and ETS-D5 for ETG and ETS, and butalbital-D5 and secobarbital-D5 for the barbiturates. All the standards were procured from Cerilliant.

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Table 1: MRM Transitions (Period 1:ETG and ETS, 0-1.8 min; Period 2: barbiturates, after 1.8 min)

Analyte	Q1	Q3	Time (msec)	DP	EP	CE	CXP
ETG 1	221	85	50	-60	-10	-20	-5
ETG 2	221	75	50	-60	-10	-20	-5
ETS 1	125	97	50	-52	-3	-21	-7
ETS 2	125	80	50	-52	-3	-40	-6
ETG D5	226	85	20	-60	-10	-20	-5
ETS D5	130	98	20	-52	-3	-21	-7
Amobarbital 1	225.2	42	10	-60	-10	-40	-10
Amobarbital 2	225.2	182	10	-60	-10	-18	-10
Butabarbital 1	211	42	10	-65	-10	-40	-10
Butabarbital 2	211	168	10	-65	-10	-18	-10
Butalbital 1	223.1	42	10	-65	-10	-40	-10
Butalbital 2	223.1	180	10	-65	-10	-16	-10
Pentobarbital 1	225.1	42	10	-70	-10	-40	-10
Pentobarbital 2	225.1	182.1	10	-70	-10	-19	-10
Phenobarbital 1	231.1	42.1	10	-70	-10	-40	-10
Phenobarbital 2	231.1	188	10	-70	-10	-14	-10
Secobarbital 1	237.1	42.1	10	-70	-10	-40	-10
Secobarbital 2	237.1	194.1	10	-70	-10	-17	-10
Butalbital D5	228.1	42	10	-65	-10	-40	-10
Secobarbital D5	242.1	42	10	-70	-10	-40	-10

Calibrator Preparation

Blank human urine was used to prepare calibrators. Four levels of calibrators in human urine were prepared (50, 100, 300 and 1000 ng/mL).

Sample Preparation

- 100 µL urine sample was mixed with 10 µL internal standards solution, and then diluted with 890 µL water.
- The mixture was then centrifuged at 21,000 rcf for 10 min.
- The supernatant was transferred to a glass vial with insert for LC-MS/MS analysis.

Liquid Chromatography

HPLC separation was performed using a SCIEX ExionLC™ AC HPLC system at 30°C. Phenomenex Kinetex Phenyl-hexyl column (50 × 4.6 mm, 2.6 µm, 00B-4495-E0), Phenomenex SecurityGuard ULTRA UHPLC Phenyl (AJ0-8774) and ULTRA holder (AJ0-9000) were used. Mobile phase A (MPA) and mobile phase B (MPB) were water and methanol with modifier. The LC flowrate was 0.75 mL/min and the total LC runtime was 5 min. Injection volume was 10 µL.

For autosampler, the needle rinse solution was methanol:ACN:isopropanol (1:1:3, v/v/v). Rinse sequence was:

1. Rinsing volume: 1 mL
2. Needle stroke: 54 mm
3. Rinsing speed: 35 µL/sec
4. Sampling speed: 15 µL/sec
5. Rinse dip time: 5 sec
6. Rinse mode: before and after aspiration

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Table 2: MS Timings and Source Conditions

Source Parameters	Period 1 (0.0-1.8 min)	Period 2 (1.8 to 5.0 min)
Curtain gas	30	30
CAD	10	9
Spray voltage (V)	-2000	-4500
Temperature (C)	600	650
GS 1	50	60
GS 2	50	50

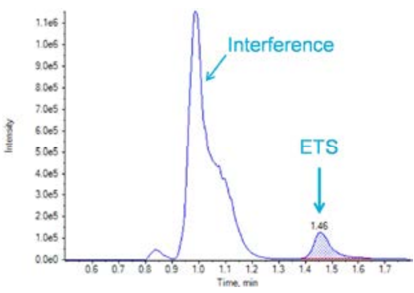
MS/MS Conditions

The SCIEX QTRAP® 4500 was operated in Multiple Reaction Monitoring (MRM) mode. Two selective MRM transitions were monitored for each target analyte and one MRM transition for each internal standard (Table 1). The Turbo V™ source was used with an Electrospray Ionization (ESI) probe in negative polarity and parameters were optimized for optimum sensitivity (Table 2). Analyst® software version 1.6.3 was used for data acquisition. LC-MS/MS data was processed using the MultiQuant™ software version 3.0.

Results and Discussion

A Phenomenex Kinetex Phenyl-hexyl column (50 × 4.6 mm, 2.6 µm) was used for LC separation and a guard column was used for LC column protection. A fast LC gradient with a 5-min runtime was used in this method. Overall, both ETG and ETS had good retention using the developed LC conditions. In addition, we were able to achieve baseline separation between a frequently-observed strong interference for one of the monitored MRM transitions for ETS (125/80 m/z) in urine samples (Figure 2).

Figure 2: LC separation of ETS



(ETS; 1.46 min, 125à 80 *m/z*, 100 ng/mL) and a frequently observed interference in human urine (found at 1 min).

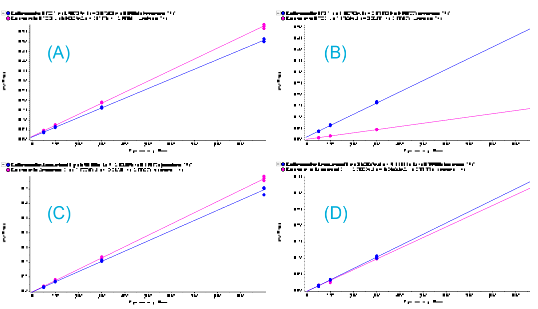
Analytical Sensitivity

The processed urine sample had a final dilution factor of 10. With 10 µL injection volume (equivalent of 1 µL unprocessed urine), we were able to detect all the analytes at the lowest concentration (50 ng/mL) with ease (Figure 3).

Calibration Curves

Figure 4 shows some typical calibration curves of a few analytes; ETG, ETS, amobarbital and secobarbital (n=3).

Figure 4: Representative Calibration Curves

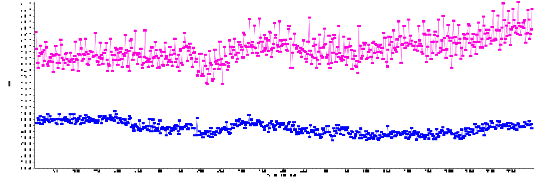


(A) ETG; (B) ETS; (C) amobarbital and (D) secobarbital (n=3)

Robustness

It is critical to prove the method robustness with real human urine samples. Over 840 injections of diluted urine samples spiked with various amount of these analtyes were performed during a >3 day period. No deterioration in either chromatographic separation or sensitivity was observed. Figure 5 shows the signals of ETG-d5 and secobarbital-d5 over 55 hours of 600 continuous injections of urine samples (blank, calibrators and QCs). Figure 6 shows the consistency of the retention times of all the internal standards during this period.

Figure 5. ETG-d5 and Secobarbital-d-5 Signal Stability



600 Continuous injections of urine (~55 hours)

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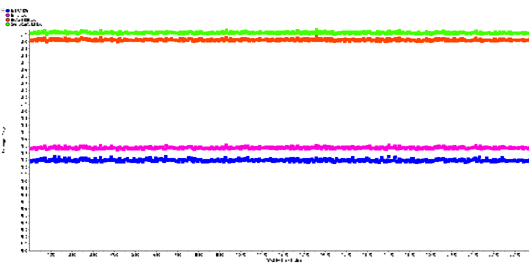
Figure 3. Extracted ion chromatograms (XICs) of both quantifying and qualifying analyte MRM transitions at 50 ng/mL in urine.

ETG (A, B), ETS (C, D), amobarbital (E, F), butabarbital (G, H), butalbital (I, J), pentobarbital (K, L), phenobarbital (M, N) and secobarbital (O, P).

Figure 6. Internal Standard Retention Time Stability



600 Continuous injections (from injection #241 to #840) of urine samples in 55 hours

Conclusion

In this technical note, we demonstrated a method to simultaneously analyze ethanol metabolites and barbiturates in human urine using QTRAP®/Triple Quad 4500 LC-MS/MS system. Sample preparation is based on a simple “dilute and shoot” methodology. The method has a total runtime of 5 minutes, shows good sensitivity and is very robust. More than 800 continuous injections of human urine samples were performed on a single LC column with no deterioration in performance evident.

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Contents

LC-MS/MS Screening of 64 New Psychoactive Substances Using Dried Blood Spots

Dried Blood Spots as an Alternative to Whole Blood

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Overview

A robust, fast, sensitive and specific LC-MS/MS screening method for the determination of 64 new psychoactive substances (NPS) in dried blood spots (DBS) has been developed and validated using a SCIEX QTRAP® 5500 LC-MS/MS System. DBS provide fast and efficient sample preparation and are easy to handle in terms of further preparation steps and shipping. Only low sample volumes of capillary blood are required which can be obtained through minimally invasive techniques compared to blood sampling via venipuncture.

Introduction

In recent years there has been a significant increase of new designer drugs alongside the classic drugs of abuse. The number of new substances reported for the first time to the European Monitoring Centre for Drugs and Drug Abuse (EMCDDA) has risen from 13 substances in 2008 to 73 new compounds in 2012 (Figure 1). Those new psychoactive substances often consist of a broad range of substances that are not controlled under international drug laws. Frequently, they are chemically similar to controlled drugs to mimic the effects of existing controlled drugs. However they are sufficiently different

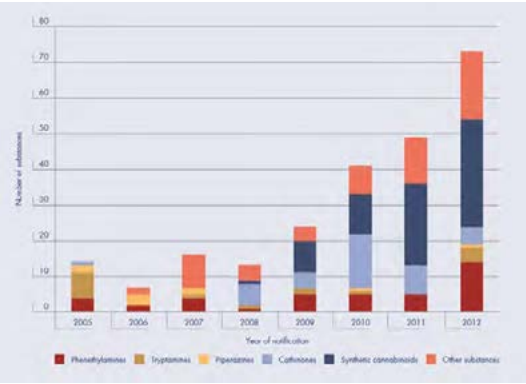

Figure 1: Number of new psychoactive substances notified to the European EWS, 2005–2012. Source: EMCDDA/EWS

Figure 2: SCIEX QTRAP® 5500 LC-MS/MS System

that they are not covered by national drug laws.

The main groups of substances monitored by the early warning system (EWS) operated by the EMCDDA besides synthetic cannabinoids are the phenethylamines (with stimulant, entactogenic or hallucinogenic effects, such as PMMA and 2C-I), tryptamines (which have predominantly hallucinogenic effects, such as AMT and 5-MeO-DALT), piperazines (which exhibit predominantly stimulant effects, such as mCPP and BZP) and cathinones [1].

Dried blood spots can be an interesting alternative to serum/plasma or whole blood analysis for which only one or few drops of blood are sampled on filter paper. After drying the paper, it can be stored until preparation and analysis or can easily be shipped. The low required blood volumes, the fast, easy and minimally invasive sampling, as well as the stabilization of the analytes, all form the main advantages of this technique.

Since the sample volume is very low, a highly sensitive and selective LC-MS/MS system is required to apply this sampling method for the detection of low concentrations of the designer drugs [2].

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Experimental

Analytes and Internal Standards

The following classes of designer drugs were included in the method:

Amphetamine derivatives: 2,5-DMA; 3,4-DMA; 3,4,5-TMA; 4-MTA; Amphetamine; DOB; DOET; DOM; Ethylamphetamine; MDA; MDDMA; MDEA; MDMA; Methamphetamine; PMA; PMMA; TMA-6.
2C family: 2C-B; 2C-D; 2C-E; 2C-H; 2C-I; 2C-P; 2C-T-2; 2C-T-4; 2C-T-7.
Aminoindanes: 5-IAI; MDAI.
Cathinones: 3-FMC; 4-MEC; Butylone; Cathinone; Ethcathinone; Ethylone; Flephedrone; MDPPP; MDPV; Mephedrone; Methcathinone; Methedrone; Methylone; Naphyrone; Pentylone; Pyrovalerone.
Piperazines: BZP; mCPP; MDBP; MeOPP; p-fluoro-BZP; TFMPP.
Tryptamines: 5-MeO-DALT; 5-MeO-DMT; AMT; DiPT; DMT; DPT; MiPT.
Other Substances: Desoxypipradol; Ephedrine; Pseudoephedrine; Ketamine; Norephedrine; Norpseudoephedrine; PCP.

The following **internal standards** were included in the method: Amphetamine-D5; Cocaine-D3; DMPP; Ethylone-D5; Fenfluramine-D10; Ketamine-D4; MDA-D5; MDEA-D5; MDMA-D5; MDPV-D8; Mephedrone-D3; Methamphetamine-D5, PCP-D5.

Sample Preparation

For the method development and validation venous blood was used which was applied on the blood spot cards.

10 µL of blood were pipetted onto the center of a printed circle on 226–1004 Bioanalysis cards (PerkinElmer, Greenville, SC, USA) and dried for at least 3 h at room temperature prior to further analysis (Figure 3). The whole DBS was punched out using a 1-cm-diameter hole-puncher and collected in an Eppendorf tube. The extraction of the analytes was performed by adding 500 µL of methanol and 10 µL of the IS working solution (10 ng/mL) and subsequent vortexing for 15 min. The methanolic solution was transferred into a vial containing 10 µL of 0.25 % HCl in methanol and dried under a gentle stream of nitrogen at room temperature. The samples were then reconstituted with 100 µL of water/formic acid (99.9/0.1; v/v) solution and shaken for 1 min. The reconstituted solution was transferred into 200 µL restricted volume vials.

Spiked blood samples were prepared by mixing 20 µL of the appropriate working solution with 180 µL of blank blood, before spotting them onto the Bioanalysis cards.

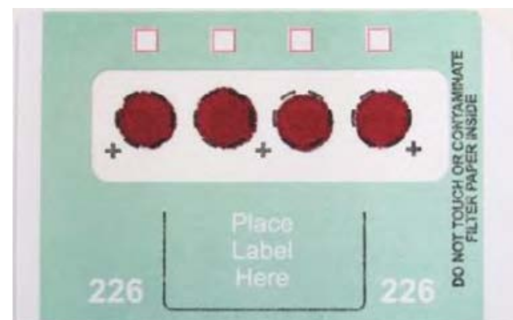

Figure 3: Spot card with four dried blood spots

LC-MS/MS Method

LC separation was achieved on an UltiMate 3000 HPLC system (Thermo Scientific Dionex) using a 10 minute gradient on a Phenomenex Synergi Polar-RP (100 × 2.0 mm, 2.5 µm) at 50 °C. Mobile phase A was water and mobile phase B acetonitrile both containing 0.1% formic acid. The injection volume was 5 µL.

Time (min)	Flow (mL/min)	Solvent A (%)	Solvent B (%)
0	0.4	97.5	2.5
1	0.4	97.5	2.5
6	0.4	70	30
6.1	0.4	5	95
7.1	0.4	5	95
7.2	0.4	97.5	2.5
10	0.4	97.5	2.5

Table 1: HPLC gradient conditions

A SCIEX QTRAP® 5500 LC-MS/MS System was used (Figure 2) equipped with Turbo V™ source and ESI probe set in positive mode at an ionspray voltage of 5000 V. The gas settings were as follows: curtain gas 30, collision gas 6, gas 1 40 and gas 2 to 60 psi. The ion source temperature was set to 700 °C.

For optimal sensitivity the LC-MS/MS was operated in Multiple Reaction Monitoring mode using the *Scheduled* MRM™ algorithm; with two transitions for each analyte and one transition for each IS. For the analytes with isobaric precursor mass-to-charge ratio and fragments mass-to-charge ratio, three transitions were monitored. The *Scheduled* MRM™ detection

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window was set to ±30 s around the expected retention time, and the total cycle time of the MRM mode was 1.0 s including a pause time between the MRM transitions of 5 ms. Mass-to-charge ratios of target and qualifier transitions as well as corresponding potentials and collision energies are summarized in Table 2 [2].

Results and Discussion

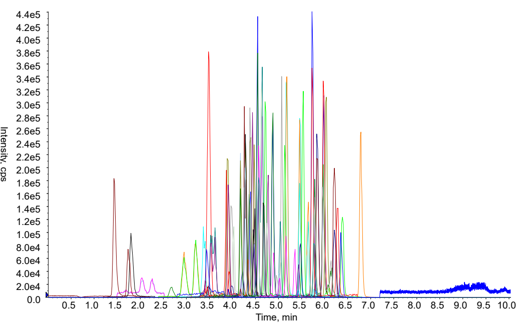

Figure 4: Chromatogram of spiked DBS sample

Figure 4 shows a chromatogram of a spiked DBS sample at a concentration of 20 ng/mL in whole blood. All compounds elute between 1.4 and 7 minutes. All isobaric compounds could be separated except flephedrone and 3-FMC, and MDDMA and MDEA. The LODs ranged from 1 to 10ng/mL and no interfering signals were observed analyzing eight different blank DBS samples and two blank samples spiked with all internal standards.

The extraction efficiency was higher than 60 % for all compounds except for the tryptamine derivatives 5-MeO-DALT (31.4 %), 5-MeO-DMT (45.2 %), DMT (57.7 %) and DPT (57.1 %) with an overall mean of 84.6 %.

All analytes proved to be stable for at least one week if the spot cards were stored at 4°C in polyethylene bags with desiccant. Flephedrone and 3-FMC, and MDDMA and MDEA were not included into the assessment of the stability.

Matrix effects were investigated by post-column infusion of the analytes during analysis of blank samples. No significant ion suppression or enhancement could be observed in the elution window of the analytes.

Summary

The results of this validated screening method for 64 novel designer stimulants show that DBS can be an interesting alternative to whole blood analysis. Capillary blood can easily be sampled e.g. from the fingertip in a minimally invasive way and shipped for analysis.

The sample preparation method described is very fast, easy, efficient and non-selective which allows the expansion of this method as the number of designer drugs is constantly increasing.

References

[1] http://www.emcdda.europa.eu/attachements.cfm/att_194336_EN_TD3112366ENC.pdf (assessed February 2014)

[2] Ambach L, Hernández Redondo A, König S, Weinmann W. Drug Test Anal. 2013 Jul 19. doi: 10.1002/dta.1505. [Epub ahead of print]

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Compound Name	Q1 Mass (amu)	Q3 Masses (amu)	RT (min)	DP (V)	CE (V)
2,5-DMA	196.1	179.2 / 151.1 / 121.1	4.5	80	16 / 22 / 37
2C-B	260.1	243.0 / 228.0 / 213.0	5.1	80	15 / 30 / 41
2C-D	196.2	179.1 / 164.1 / 149.1	4.8	80	16 / 25 / 35
2C-E	210.2	193.2 / 178.2 / 163.2	5.4	80	16 / 24 / 38
2C-H	182	165.1 / 150.1 / 135.1	4.2	80	15 / 25 / 39
2C-I	308.1	291.0 / 276.0 / 261.0	5.4	80	18 / 29 / 40
2C-P	224.1	207.1 / 192.1 / 163.1	5.9	80	17 / 25 / 37
2C-T-2	242.1	225.1 / 210.1 / 195.1	5.4	80	17 / 27 / 31
2C-T-4	256.1	197.1 / 239.2 / 182.1	5.7	80	20 / 28 / 36
2C-T-7	256.1	239.2 / 197.1 / 167.1	5.9	80	20 / 29 / 40
3,4,5-TMA	226.1	209.2 / 194.2 / 181.1	4.1	80	17 / 25 / 26
3,4-DMA	196.1	179.1 / 151.1 / 107.0	3.8	80	14 / 27 / 50
3-FMC	182.2	149.1 / 164.2 / 103.1	3.5	80	21 / 29 / 39
4-MEC	192.1	174.2 / 144.1	4.7	80	20 / 43
4-MTA	182.1	165.1 / 137.1 / 117.1	4.9	80	18 / 26 / 27
5-IAI	260	116.0 / 243.0	5	80	38 / 23
5-MeO-DALT	271.2	110.1 / 174.2	5.7	80	19 / 24
5-MeO-DMT	219.1	58.0 / 174.2	4.4	80	21 / 34
Amphetamine	136.1	91.0 / 119.0	3.3	50	21 / 11
Amphetamine-D5	141.2	93.1	3.3	80	26
AMT	175.1	158.1 / 143.1	4.2	80	16 / 38
Butylone	222.1	174.2 / 204.2 / 146.2	4.4	80	26 / 17 / 34
BZP	177.2	91.1 / 65.0	1.4	80	32 / 62
Cathinone	150.1	117.1 / 132.1	2.6	80	33 / 15
Cocaine-D3	307.2	185.2	5.4	80	25
Desoxypipradrol	252.2	91.1 / 167.2	6.2	80	47 / 30
DiPT	245.2	144.1 / 114.1 / 117.1	5.8	80	28 / 20 / 53
DMPP	191.2	133.1	5	80	35
DMT	189.1	58 / 144.1	4.3	80	27 / 29
DOB	274.1	257.1 / 229.0	5.4	80	20 / 28
DOET	224.2	207.1 / 192.1 / 177.1	5.7	80	18 / 28 / 37
DOM	210.2	193.1 / 165.1 / 178.1	5.2	80	18 / 25 / 27
DPT	245.2	114.1 / 144.1 / 86.1	6.1	80	22 / 31 / 38
Ephedrine	166.1	148.1 / 133.1 / 115.1	2.9	80	19 / 31 / 39
Ethcathinone	178.1	160.1 / 130.1 / 117.1	3.9	80	18 / 44 / 38
Ethylamphetamine	164.1	91.0 / 119.0 / 65.0	4.4	80	29 / 17 / 61
Ethylone	222.1	174.2 / 204.2 / 146.2	4.2	80	27 / 21 / 37
Ethylone-D5	227.2	179.2	4.2	80	27

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Fenfluramine-D10	242.2	161.1	5.5	80	33
Flephedrone	182.1	164.2 / 149.1 / 123.1	3.6	80	19 / 30 / 30
Ketamine	238.1	125.1 / 220.2	4.7	80	40 / 22
Ketamine-D4	242.2	129	4.7	80	38
mCPP	197.1	118.1 / 119.0	5	80	50 / 34
MDA	180.1	133.0 / 105.0	3.8	50	28 / 24
MDA-D5	185.1	168.1	3.8	80	13
MDAI	178.1	161.1 / 131.1 / 103.0	3.4	80	18 / 26 / 41
MDBP	221.2	135.1 / 77.0	1.7	80	23 / 57
MDDMA	208.1	163.1 / 105.1 / 135.1	4.5	80	22 / 37 / 32
MDEA	208.1	163.1 / 105.1	4.5	80	16 / 34
MDEA-D5	213.2	163.1	4.5	80	19
MDMA	194.1	163.1 / 105.1	4.2	80	14 / 30
MDMA-D5	199.1	165.1	4.2	80	19
MDPPP	248.2	98.1 / 177.1	4.6	80	35 / 23
MDPV	276.2	126.1 / 135.1	5.6	80	37 / 27
MDPV-D8	284.3	134.2	5.6	80	38
MeOPP	193.1	150.1 / 119.1	3.9	80	35 / 27
Mephedrone	178.1	160.1 / 145.1 / 91.1	4.4	80	28 / 18 / 47
Mephedrone-D3	181.1	163.1	4.4	80	16
Methamphetamine	150.1	91.0 / 119.1	3.8	80	30 / 13
Methamphetamine-D5	155.1	92	3.8	80	30
Methcathinone	164.1	146.1 / 130.1 / 105.1	3.3	80	19 / 43 / 32
Methedrone	194.1	176.2 / 161.1	4.1	80	21 / 28
Methylone	208.1	160.1 / 132.1 / 190.1	3.9	80	24 / 37 / 17
MiPT	217.2	86.1 / 144.1	5.1	80	18 / 25
Naphyrone	282.2	141.1 / 211.1	6.7	80	37 / 23
Norephedrine	152.1	134.1 / 117.1	2	80	15 / 25
Norpseudoephedrine	152.1	134.1 / 117.1	2.2	80	15 / 25
PCP	244.2	91.1 / 159.2	6.3	80	51 / 19
PCP-D5	249.2	164.2	6.3	80	22
Pentylone	236.2	188.2 / 175.1	5	80	23 / 31
p-Fluoro-BZP	195.1	109.0 / 83.0	1.8	80	33 / 64
PMA	166.1	149.1 / 121.0 / 91.1	4	80	14 / 25 / 44
PMMA	180.2	149.2 / 121.0	4.3	80	19 / 29
Pseudoephedrine	166.1	148.1 / 133.1 / 115.1	3.1	80	19 / 31 / 39
Pyrovalerone	246.2	105.1 / 175.2	6	80	32 / 24
TFMPP	231.1	188.1 / 118.1	5.3	80	30 / 57
TMA-6	226.1	209.2 / 181.1 / 121.1	5	80	18 / 28 / 39

Table 2. Mass-to-charge ratios of target and qualifier transitions as well as corresponding potentials and collision energies. Cell exit potential (CXP) was 10 V for all transitions.

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Contents

Chiral Analysis of Methamphetamine and Its Metabolite, Amphetamine in Urine by CESI-MS

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SCIEX, Regina, SK, Canada

Introduction

Chiral separation of drug enantiomers is essential in order to show that the active enantiomer is in fact present in forensic specimens. This avoids legal arguments and simplifies challenges to analytical findings. Chiral analysis of Methamphetamine (Meth) "street" samples yields information on the clandestine lab synthetic route (1). Chiral analysis is also of great importance in Pharma and Drug Discovery for the detection of chiral impurities and for quantitative determinations.

In the past, chiral analysis has been done using a combination of processes, starting with the drug confirmation by hyphenated mass spectrometry (CE-MS, GC-MS or LC-MS). This was followed by separation of the enantiomers and impurities of the drug by a specific chiral separation technique such as chiral capillary electrophoresis or chiral chromatography.

Direct connection of chiral separation technology with mass spectrometry can be problematic. The use of chiral GC and LC columns alone or with mass spectrometry provides, at best, marginal separation capability. Furthermore, the addition of neutral or highly sulfated cyclodextrin additives in chromatographic and electro-driven separation modes can cause contamination and ion suppression in the electrospray process.

In 2005, Rudaz and Veuthey (2) showed that adequate chiral separations and identification of enantiomers could be done using a sheath-liquid CE-MS technique. Their Partial Filling Technique (PFT) under countercurrent conditions, employed highly sulfated cyclodextrin additives to a simple background electrolyte (BGE) to separate the enantiomers of Amphetamine (Amp) derivatives (see Figure 1).

In this work, a low flow Capillary Electrophoresis Electrospray Interface for Mass Spectrometry (CESI-MS) was used with the Partial Filling Technique as illustrated in Figure 1, to generate the chiral separation and produce the quantitative data.

Materials and Methods

Chemicals: All chemicals were Reagent Grade and were purchased on-line from VWR Int. HS-γ-CD, 20% solution in water, was obtained from Beckman Coulter Inc., Brea, CA, USA.

Drug and Metabolite Standards: Meth±, Amp± and their D11deuterated internal standards, all at 1 mg/mL were purchased from Cerilliant Corporation, Round Rock, TX, USA. These standard solutions in methanol were diluted and spiked into volunteer pooled urine samples. Standard solutions for mass spectrometry and extractions were prepared at 1 ng/μL in 5 to 50 mM Ammonium Formate (pH 2.85).

Urine Calibration Standards: Urine samples were prepared at 2000 ng/mL. These spiked urine sample were diluted with blank urine to prepare calibrators from 0.5 to 1000 ng/mL per enantiomer. The samples were kept at ~4°C until the time of analysis. Spiked urine samples and blanks were prepared by liquid-liquid extraction after the addition of internal standards at 50 ng/mL of each deuterated enantiomer.

Instrument Conditions and Extraction Protocol: Figures 2 and 3 outline CESI-MS parameters and the liquid-liquid extraction process.

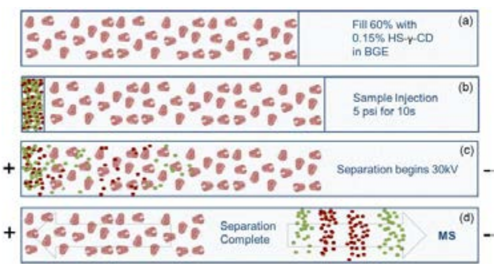

Figure 1: PFT and Counterion Flow, (a) rinse with BGE followed by 25 psi/60s injection to partially fill the capillary, (b) inject sample (~8.5 nL) with 5 psi for 10s, (c) voltage separation at 30 kV and (d) separation complete.

OptiMS® Capillary Interface	90cm bare fused silica capillary, 150 μm OD, 30 μm ID with conductive emitter tip.
CE Instrument	CESI 8000
MS Instrument 1	SCIEX 6600 Triple TOF®, with Analyst® 1.7 Software.
MS Instrument 2	Waters Xevo with MassLynx 4.1 Software.
ESI Voltage	1.25 kV
Sample Introduction	Hydrodynamic 5 psi for 10 s
Capillary Conditioning	Initial conditioning with MeOH, water, 0.1N NaOH, water and BGE.
Background Electrolyte (BGE)	25 mM Ammonium Formate pH 2.85
Separation	25kV, 277 v/cm, 2.3 μamp
Temperatures	Capillary 25°C Samples 10°C

Figure 2: CESI 8000 with Opti-MS ® Conditions

To 1 mL of urine (or serum, plasma oral fluid):

1. Add 50 μL of Mixed D11 Internal Standards to 1 mL of urine followed by 0.2 mL of conc. NH_4OH and vortex.
2. Add 5 mL of 1-chlorobutane and shake for 10 min.
3. Centrifuge at 0°C for 10 min. at 3000 rpm.
4. Evaporate at 40°C under N_2 for 10 min. to remove any NH_4OH, then add 10 μL of 1% HCl in MeOH. Vortex and continue to evaporate with N_2.
5. Add 200 μL of 5 mM BGE to each tube and vortex.
6. Transfer to a 200 μL Microfuge tube (Beckman Coulter)..
7. Pressure inject the sample for 10 seconds at 5 psi.

Figure 3: Liquid-Liquid Extraction Protocol for Bio-fluids

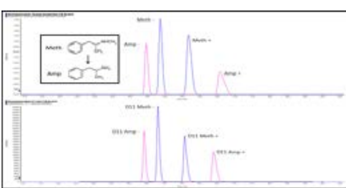

Figure 4: Chiral Separation of Amp±, Meth±, D11-Amp±and D11- Meth± with a SCIEX TripleTOF® 6600 System and Analyst® 1.7 Software.

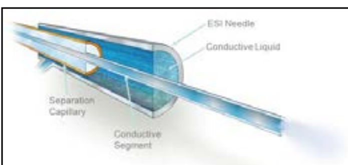

Figure 5: OptiMS - Sheathless ESI Interface.

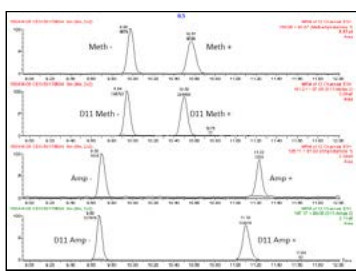

Figure 6: MRM Analysis (~20 fg Injected).

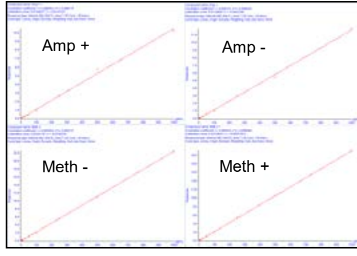

Figure 7: Linear Regression Analysis for Amp and Meth Enantiomers.

Results

Spiked urine samples for Meth and Amp using D11, deuterated Internal Standards (IS) were prepared and analyzed using a liquid-liquid extraction protocol (Figure 3).

The Partial Filling Technique (Figure 1), in which the capillary is 40% filled with BGE containing 0.15% HS-γ-CD, was used to affect the chiral separation of Meth and Amp (Figure 4).

The CESI 8000 with Opti-MS (Figure 5) was used to interface CE and MS, providing the required sensitivity on injections of only 8.5 nL of the extract reconstituted in 200 µL (~20 fg injected).

Multiple Reaction Monitoring (MRM) was used for the quantitative processing (Meth: 150.2 → 119.1, Amp: 136.2 → 119.2, D11-Amp 147.2 → 98.1, D11-Meth 161.2 → 97.1).

The Chiral CESI-MS separation for the 0.5 ng/mL spiked urine extract is shown in Figure 6. LOD/LOQ was the low calibrator, 0.5 ng/mL of each enantiomer.

Ten point calibrations in triplicate over three orders of magnitude for each enantiomer, were linear with R^2 >0.995 for both Meth and Amp from 0.5 to 1000 ng/mL of urine (Figure 7).

Conclusions

A Partial Filling Technique (PFT) was adapted to a low flow Capillary Electrophoresis Electrospray Interface for Mass Spectrometry (CESI-MS).

Chiral separation and confirmation of the enantiomers of methamphetamine and its metabolite, amphetamine, in a single run, were demonstrated as Proof of Principle at the sensitivity which forensic toxicologists require in even the most challenging case work.

References

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2. Rudaz, S., Geiser, L., Souverain, S., Prat, J. and Jean-Luc Veuthey "Rapid stereoselective separations of amphetamine derivatives with highly sulfated cyclodextrin", Electrophoresis 2005, *26* 3910-3920.

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[1] Khaled SM, Hughes E, Bressington D, et al. The prevalence of novel psychoactive substances (NPS) use in non-clinical populations: a systematic review protocol. Systematic Reviews. 2016;5:195. doi:10.1186/s13643-016-0375-5.

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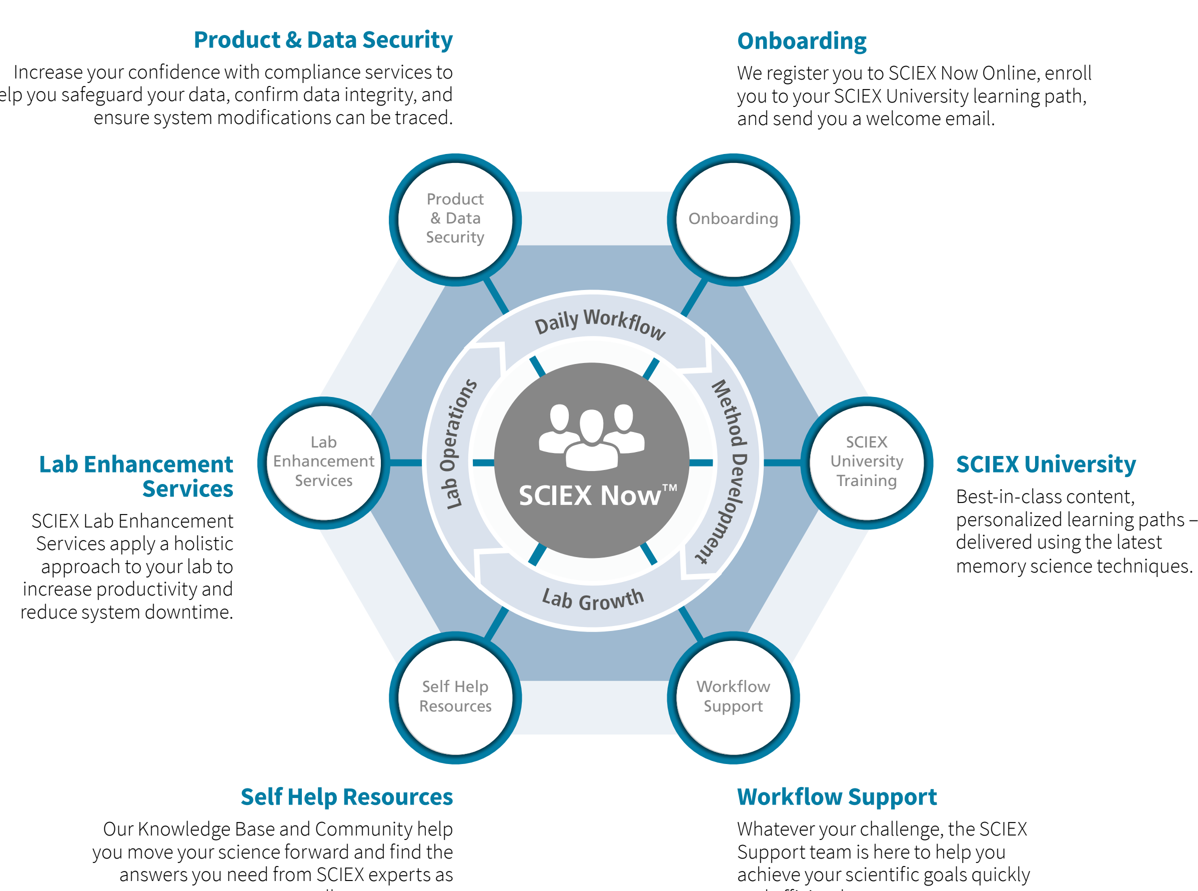

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