Novel psychoactive substances: staying ahead of the curve

Screening, identifying, and quantifying with mass spectrometry

SCIEX

The Power of Precision

Approaches to drug screening using Triple Quadrupole, QTRAP® and QTOF technologies

With the emergence of novel psychoactive substances (NPS), forensic toxicology labs have undergone an evolutionary change in their analytical testing and technology usage as the demands in the detection and identification of these new compounds have required different testing regimes.

No longer is the modern forensic toxicology lab able to solely utilize targeted screening even with a panel of a few hundred drugs. Nowadays, comprehensive screenings often require targeting for more than 1000 drugs including monitoring of their metabolites. The increased potency of these new substances has demanded rapid and comprehensive analytical methods that can provide identification of these drugs with high confidence and quantify them at low concentrations with good accuracy and reproducibility in a broad range of biological matrices.

Liquid chromatography (LC) coupled to tandem Mass Spectrometry (MS/MS) is a powerful analytical tool used in many forensic testing laboratories to detect drugs and their metabolites from a variety of biological matrices. When identifying and quantifying hundreds of compounds in challenging samples, the increased sensitivity of the latest generation mass spectrometers enables simplified workflows by allowing extensively dilution of sample extracts or the ability to utilize less sample volume when sample limited. This is an effective way to eliminate ion suppression caused by matrix components and the extended linear dynamic range allows quantification of more compounds to meet the most challenging forensic toxicology workflows.

For targeted screening, triple quadrupole and QTRAP mass spectrometers are the gold standard for routine high sensitivity detection and quantification of drug analytes. Multiple Reaction Monitoring (MRM) is the most common mode of employing triple quadrupole MS/MS for quantitative analysis. MRM functionality of these systems provide selective and sensitive quantification with the lowest limits of detection, excellent reproducibility and linear range. Using MRM ratios is a way to identify compounds with high confidence, that includes the ratio of quantifier and qualifier MRM transition. Despite the high selectivity of MRM detection, there is however always a risk of false positive findings due to interfering matrix signals. Acquiring full scan MS/MS data in an Enhanced Product Ion (EPI) experiment, using QTRAP® functionality, allows for searching against mass spectral libraries and can significantly increase confidence in identification. The combination therefore of triple quadrupole and QTRAP system functions allows for quantification and identification with MS/MS spectra in a single LC run.

The SCIEX Triple Quad™ 7500 LC-MS/MS System – QTRAP® Ready is the latest offering of nominal systems that builds on the SCIEX legacy of groundbreaking innovation for quantitative performance. The continuing advancements in mass spectrometric technology from the ionization source all the way through the ion guide enabled improvement in the efficiency of ion capture and transmission, resulting in more sensitivity through sampling more ions with no sacrifice in robustness and reliability. The improved ion generation and sampling results in higher sensitivity and up to 6 orders of linear dynamic ranges, allowing quantification of more compounds across a wider range of chemical properties without the requirement for extensive sample preparation.

Forensic scientists are also concerned about screening for and identifying non-targeted compounds, including metabolites. High resolution and accurate mass LC-MS/MS systems are capable of performing highly sensitive and fast MS scanning experiments to search for unknown molecular ions while also performing selective and characteristic MS/MS scanning for further compound structural elucidations and, therefore, is the instrument

of choice for this challenging task. General unknown screening workflows do not use a target analyte list and compound detection is not based on any prior knowledge, including retention times and information on possible molecular and fragment ions. Therefore, acquired chromatograms are information-rich and can easily contain thousands of ions from both any compounds present in the sample as well as from the sample matrix. Powerful software tools are required to allow the exploration of such data and aid in the efficient data reduction to the significant components and identification of the unexpected compounds. Data processing include a combination of automated sample-control-comparisons followed by MS/MS library searching, empirical formula finding, and structural database searching.

For these untargeted workflows, the combination of the SCIEX X500R QTOF System and SCIEX OS Software provide a comprehensive solution designed for routine testing to deliver reliable and sensitive results in the forensic toxicology laboratory. The X500R QTOF System was designed with performance in mind and engineered to simplify screening and quantification of unknowns in complex biological samples. The X500R QTOF System is a flexible system that can be used for both high specificity, targeted quantification as well as non-targeted screening using acquisition methods such as IDA or SWATH® Acquisition to collect high resolution spectra from single sample sets in a routine testing laboratory environment. These non-targeted data acquisition strategies enable generation of high quality TOF MS and TOF-MS/MS spectra, which provide comprehensive compound fragmentation on all the analytes present in the sample. Because these fragments are acquired in high resolution, the detected compounds can be accurately identified through extraction of specific accurate mass fragment ions. These fragment ions can in turn be matched for identification through spectral library matching using the spectral database searching functionality of the software. In addition to providing the ability to optimize, acquire, process and review the data in a streamlined and integrated fashion, SCIEX OS Software also enables retrospective data analysis (or data mining) of additional analytes missed in initial screens, which is becoming extremely relevant with the constant flux of new synthetic substances on the drug market. Full quantitative and qualitative analysis can be performed in one centralized platform that provides quick, intuitive and streamlined data processing power to produce accurate and reliable results.

To conclude, forensic testing has seen the transition into the adoption of tandem MS workflows with the routine use of triple quadrupole and QTRAP instrumentation. Developments in this technology in combination with continual software improvements have allowed for more compound coverage in a single workflow and helped streamline the process of getting to the right result, every time. As focus turns to identifying the significant components in a forensic sample in an untargeted workflow, high resolution and accurate mass LC-MS/MS systems such as the SCIEX TripleTOF® and QTOF Systems are quickly developing as the tool of choice with the capability to capture all information about a sample. That data can be processed using a targeted approach to identify known compounds and still quantify them at low concentrations with good accuracy and reproducibility. Most significantly, the same data can be processed using non-targeted approaches to identify the new, unknown compounds – all from a single instrument.

Table of contents

Harnessing the power of mass spectrometry for early novel psychoactive substances (NPS) detection

SCIEX solutions for both targeted an non-targeted screening. Left: SCIEX Triple Quad™ 7500 LC-MS/MS System – QTRAP® Ready
Right: SCIEX X500R QTOF System.

The increased prevalence of novel psychoactive substances (NPS) in the recreational drug market has been a major contributor to the ongoing opioid crisis. NPS are newly emerging compounds designed to mimic existing recreational drugs that have emerged as potent alternatives to controlled opioids and frequently used as adulterants or cutting agents to commonly abused drugs. Continuous abuse of these substances can result in severe intoxication and, in some cases, fatal overdose.

Over the years, the surge of NPS and other synthetic drug classes has dramatically shifted the landscape of the drug market. What was previously characterized as a small subset of illicit drugs has now turned into a plethora of novel substances comprised of various chemistries — each inducing unique physiological effects. The dynamics of this growing interplay continues to pose serious safety concerns for public health and law enforcement officials alike that has resulted in a global public health crisis. The nature of this transformative shift has critical implications for the effective monitoring of these emerging substances. Since their potency and composition is highly variable, fast and comprehensive drug screening approaches are critically needed to enable accurate and timely identification of these emerging novel substances.

Traditionally, the detection of illicit substances has been performed using immunoassays or gas chromatography-mass spectrometry (GC-MS), however these techniques have their limitations. The use of immunoassays for designer drug screening is limited by the need to develop antibodies specific to an increasingly wide array of new chemical structures, proving a challenge for the dynamic and rapidly evolving nature of the NPS market. In addition, immunoassays are renowned for low specificity, cross-reactivity and are prone to a high rate of false negative results. Furthermore, immunoassays often need multiple panels to detect the wide range of NPS, because of the ever-expanding panels of pharmacologically active and toxicologically hazardous NPS. This disadvantages the speed at which the analytical process can be carried out. GC-MS, by contrast, requires lengthy sample preparation which slows the analytical process significantly. Overall, the similarity in molecular composition, transformative nature over time and the ever-expanding panel of pharmacologically active and toxicologically hazardous NPS makes their identification increasingly difficult for forensic toxicologists.

Liquid chromatography tandem mass spectrometric analysis (LS-MS/MS) is providing forensic toxicologists the speed and confidence required to reliably identify NPS and other novel synthetic drugs of abuse. Over the years, the gain in sensitivity compared to GC-MS, and the highly accurate analytical nature of tandem MS has become the preferred method for analysis of NPS over traditional techniques, for both screening and confirmation. Mass spectrometry enables characterization of NPS by assessing their mass, molecular weight and fragmentation pattern, providing the necessary information to elucidate their ever-evolving molecular structure. The data acquired by mass spectrometers provides analyte specific results which enables accurate quantification with far greater sensitivity and specificity than previously used techniques.

More recently, high-resolution mass spectrometry (HRMS) has emerged as a powerful and comprehensive tool for the characterization of NPS by reliably providing accurate mass, isotope pattern and MS/MS fragments that can be used to identify designer drugs using spectral library matching. These attributes have enabled toxicologists to specifically correlate mass measurements and molecular formulas to elucidate the molecular profile of an NPS. Where other nominal mass instruments rely heavily on fragmentation of these substances as a chemical fingerprint, HRMS provides an additional level of specificity by incorporating the chemical formula into criteria for positive identification. Likewise, acquisition of accurate mass MS/MS fragments is enabling toxicologists to reliably piece together the chemical structure of an NPS based on the accurate mass data acquired during HRMS experiments. The acquisition of full scan, high-resolution mass spectra in both MS and MS/MS modes also enables retrospective data analysis without the need to re-run the sample. This strategy is very attractive considering the ever-changing landscape of NPS in the drug market.

In recent years, the MS expertise developed by forensic toxicology laboratories for the early identification and detection of NPS has provided public health professionals and law enforcement agencies with a clearer picture of the emergence of NPS on the drug market.

This collective effort has proven to be an effective, team-based approach to staying ahead of the transformative NPS trends and continuously monitoring their evolution.

This critical information will strengthen existing responses to the emergence of NPS and provide the level of scientific intelligence to support NPS surveillance, monitoring, response efforts and drug policy formulation.

This eBook, brought to you by SCIEX, serves as a comprehensive resource for NPS-related content. In addition to general NPS information, it contains a repository of technical notes and webinars highlighting some of the recent scientific advancements developed by the forensics team at SCIEX and their collaborators. The portfolio of analytical instruments, workflows and integrated software solutions is presented as a comprehensive arsenal of tools available for forensic laboratories conducting NPS screening and identification. Also included in this eBook are testimonials from current passionate scientists describing how they leverage SCIEX technology in their laboratory and the challenges they face. Overall, this eBook has been designed to bring together all the necessary tools and resources to make the leap to LC-MS/MS for NPS screening and identification.

References

1. List of Announcements. (2020). Retrieved 26 June 2020, from https://www.unodc.org/LSS/Announcement?type=NPS

2. Hagan, A., & Smith, C. (2017). A New Beginning: An Overview of New Psychoactive Substances. Retrieved 26 June 2020, from https://medcraveonline.com/FRCIJ/a-new-beginning-an-overview-of-new-psychoactive-substances.html

3. Peacock, A., Bruno, R., Gisev, N., Degenhardt, L., Hall, W., & Sedefov, R. et al. (2019). New psychoactive substances: challenges for drug surveillance, control, and public health responses. The Lancet, 394(10209), 1668-1684. doi: 10.1016/s0140-6736(19)32231-7

Detecting and identifying NPS: a brief history

1929
- Mephedrone (M-CAT) first synthesized in China

1970s
- Illicit laboratories in China and Indonesia were creating synthetic drugs that imitate the effects of controlled substances, by altering the chemical structure to escape legislative controls

1979-1980
- When toxicological analysis showed no trace of drugs in overdose cases, law enforcement officials seized street samples sold as heroin, and identified a potent narcotic that had not undergone scientific evaluation

1988
- The first 'classical cannabinoid' analog of THC, HU-120, was manufactured in Israel

2004
- Reports of B2P use and harms emerge in New Zealand

2005
- New Zealand makes B2P restricted access

2007
- First reports of mephedrone availability and use in Europe

2008
- Detection of synthetic cannabinoid JWH-018 in Spice mix in Europe

2011
- Online darknet marketplace Silk Road opens– major platform for selling drugs

2010
- Once banned, online availability of mephedrone plummeted from 33% to 1% and the rise of street dealers began
- Ireland passes first blanket ban on NPS via Criminal Justice Act
- UK and then EU controls mephedrone

2012
- US passes Synthetic Drug Abuse Protection Act

2014
- Synthetic cannabinoid intoxication spikes in Russia and Eastern Europe
- ECDD conducts first major review of various NPS

2013
- UNODC launch Early Warning Advisory on NPS
- New Zealand passes Psychoactive Substances Act

2015
- CND (places 10 NPS (eg., BZP, JWH-018) under international control

2016
- New York mass intoxication from synthetic cannabinoid AMD-FUBINACA
- UK Passes New Psychoactive Substances Act
- Up until 2016, NPS were available to purchase in head stores that specialize in drug paraphernalia. NPS were contained in attractive packets, with piercing identities such as, Spice, China White and Benzo Fury

2017
- Chief Coroner in New Zealand issues warning after cluster of synthetic cannabinoid deaths

2018
- Cryptomarket Dream Market bans sale of fentanyl and analogues

2020
- Isotonitazene, a new synthetic opioid, emerges in Europe and North America
- The 'Synthetic Drugs in East and Southeast Asia' report is launched

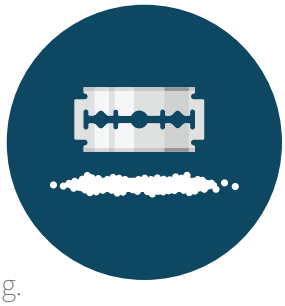
1. List of Announcements. (2020). Retrieved 26 June 2020, from https://www.unodc.org/LSS/Announcement?type=NPS
2. Hagan, A., & Smith, C. (2017). A New Beginning: An Overview of New Psychoactive Substances. Retrieved 26 June 2020, from https://medcraveonline.com/FRCIJ/a-new-beginning-an-overview-of-new-psychoactive-substances.html
3. Peacock, A., Bruno, R., Gisev, N., Degenhardt, L., Hall, W., & Sedefov, R. et al. (2019). New psychoactive substances: challenges for drug surveillance, control, and public health responses. The Lancet, 394(10209), 1668-1684. doi: 10.1016/s0140-6736(19)32231-7

Novel Psychoactive Substances

In the last decade, there has been a surge in the circulation of, and demand for, novel psychoactive substances (NPS). These compounds are designed to mimic the effects of existing – and illegal – recreational drugs, yet due to a lack of regulation and knowledge about their constituents, there is widespread concern about their safety. This makes providing effective treatment, recovery and support a challenge.[1,2]

NPS can be split into four main categories:

Stimulant-type drugs



Mimic the effects of amphetamine, cocaine and ecstasy, increasing alertness and producing a sense of euphoria and wellbeing.

Can cause:
Anxiety | Agitation | Stroke
Psychosis | Hyperthermia
Depression | Seizures

Examples include:
Bath salts | Plant food | M-cat | 2C-series

Depressants or "downers"

Synthetic opioids are similar to recreational opioids, however they have longer durations of action. Benzodiazepine-type NPS, by contrast, have sedative, anxiolytic, hypnotic and anticonvulsant properties.

Can cause:
Overdose | Impaired cognition | Confusion
Seizures (after withdrawal) | Addiction

Examples include:
Novel fentanyls, AH-7921, MT-45 (opioids)
Diclazepam & Flubromazepam (benzodiazepines)

Psychedelics and dissociatives

Psychedelics produce perceptual alterations and quasi-mystical experiences. They can also have stimulatory effects. Dissociatives cause euphoria that is often accompanied with a sense of disconnection from the physical body.

Can cause:
Psychosis | Agitation | Confusion | Seizures
Hypertension | Psychological dependency
Tachycardia | Addictive potential

Examples include:
5-MeO-DALT, NBOMe-series, 2C-series (psychedelic)
Methoxetamine (mexxy) (dissociative)

Synthetic cannabinoid receptor agonists

Synthetic cannabinoid receptor agonists (SCRAs) are often laced into herbal products and sold as Spice, K2, Kronic, etc. They are structurally similar to cannabis and therefore mimic its effects in the brain, typically producing a pleasant state of relaxation and of feeling "stoned".

Can cause:
Psychosis | Agitation | Confusion | Seizures
Hypertension | Psychological dependency
Tachycardia | Addictive potential

Examples include:
Spice | Noids

1. 1.The BMJ. 2017. Novel Psychoactive Substances (NPS). [online] Available at: https://www.bmj.com/content/356/bmj.i6848/infographic [Accessed 3 February 2020]

2. Robinson, J., 2016. Novel Psychoactive Substances: What Are They And What Implications Can They Have For Pharmacists?. [online] Your Royal Pharmaceutical Society. Available at: https://www.pharmaceuticaljournal.com/your-rps/novel-psychoactive-substances-what-are-they-and-what-implications-can-theyhave-for-pharmacists/20201674.article?firstPass=false [Accessed 3 February 2020].

The age of novel psychoactive substances

Novel psychoactive substances (NPS) are compounds which are designed to mimic existing recreational drugs. The emergence of NPS has changed the landscape of the synthetic drug market. Previously, the market had a limited number of compounds which belonged to a small number of chemical groups; now NPS has shifted the market to one which possesses hundreds of compounds. The European Monitoring Centre for Drugs and Drug Addiction is currently monitoring 730 substances, with more being identified each year.

In the past, NPS used to evade anti-drug laws and were therefore called "legal highs". Manufacturers achieved this by tweaking the pharmacological structures of existing compounds to create new substances. To tackle this problem and ensure that "legal highs" became illegal, different countries passed laws to create a blanket ban on NPS. For example, in the UK and Ireland, the 2016 Psychoactive Substances Act makes it an offence to produce or supply, but not possess (unless an individual is in prison) current and future NPS. In the United States, the Synthetic Drug Abuse Prevention Act of 2012 bans synthetic cannabinoids, synthetic cathinone and hallucinogenic drugs. This Act puts these NPS drugs under Schedule 1 of the Controlled Substance Act. A Schedule 1 substance is defined as a drug that has a high potential for abuse, no accepted medical use in the US and is not classed as safe. Despite these laws, the supply, use or possession of these substances has not decreased. In fact, the strength and price of NPS have increased since the act.

NPS can be categorized into six groups based on their similarity to established recreational drugs. The six groups of NPS are: stimulants, cannabinoids, classic hallucinogens*, dissociatives*, sedatives/hypnotics** and opioids**. Stimulants and cannabinoids being the most common.[1,2]

Stimulants

Stimulants mimic the effects of traditional psycho-stimulants such as 3,4-methylenedioxymethamphetamine (MDMA), cocaine and amphetamines, producing a sense of euphoria and wellbeing by increasing the synaptic levels of serotonin, dopamine and/or noradrenaline. Stimulants include synthetic cathinones or "SCs" (Mephedrone, bath salts), substituted phenylethylamines (2C agents) and piperazines (BZP). Most stimulants are typically sold in a powder or pill format, however, SCs are often sold under the disguise of plant food or bath salts —hence the street name. SCs are normally snorted or ingested orally (wrapped in cigarette paper "bombing" or dissolved in water "whizzy water").[1,2,3]

Synthetic cannabinoids

NPS variants of cannabinoids are termed synthetic cannabinoid receptor agonists (SCRAs). The common street names for SCRAs include "Spice" and "K2" these synthetic cannabinoids are often packaged into foil sachets and sold as incense. SCRAs are usually solids or oils sprayed onto

* dissociatives and classical hallucinogens can be merged into a Hallucinogenic NPS group
**note opioid and sedatives can be merged into depressant NPS

herbal mixtures which are smoked. Liquid SCRAs can be used in electronic cigarettes and vaporizers.[1,2,3]

Classic hallucinogens/psychedelics

Psychedelics are known as classic hallucinogenics, however despite the name they do not produce hallucinations but a range of "psychedelic effects". These effects include perceptual alternations and quasi-mystical experiences that can be categorized under oceanic boundlessness (positive emotions ranging from heightened mood to sublime happiness and serenity or grandiosity) and anxious ego-dissolution (thought disorder and loss of autonomy and self-control associated with arousal, anxiety and paranoid ideations).[1]

Dissociatives

Dissociatives are a type of hallucinogen that distort visual and auditory perceptions causing the perception of an absence of time, weightlessness and disconnection from the physical body. All of these effects lead to detachment and potent psychedelic experiences. Dissociatives can be inhaled, swallowed or injected, with effects that can range from milder effects than ketamine to stronger effects experienced with phencyclidine (PCP).[1,3]

Sedatives/ hypnotics

Sedatives are known as central nervous system depressants and are designed to slow down the function of the human brain. These drugs have a significant inhibitory and relaxing effect on the brain and mimic varying sedating and anti-anxiety drugs. They are the least understood of the NPS. One of the reasons for this is that the clinical symptoms are so similar to the established recreational drug that it is difficult to identify their exposure in a clinical setting.[2,4,5]

Synthetic opioids

Little is known about the specific subjective effects of novel opioids compared to the established recreational opioids. Fewer NPS opioids appear in isolation, and they are normally sold as part of cannabinoid smoking mixtures. Those that do appear in isolation are AH-7921, doxylam, nortilidine, and desomorphine; normally these opioids are sold as "research chemicals" or "legal opioids". All of them have opioid receptor activity, with AH-7921 having the same potency as morphine.[1,3]

Novel psychoactive substances – Availability, trends and concerns

The darknet uses custom software and hidden networks superimposed onto the architecture of the Internet. It can be used for the sale of restricted goods as it has a low risk of detection, making it an "attractive" platform for obtaining NPS,[6] with online purchases of NPS increasing according to a 2016 Global Drug Survey (GDS).[7]

According to the last World Drug Report 2018, synthetic cannabinoids and SCs represent the largest class of NPS. This poses a problem as synthetic cannabinoids are most likely to lead to emergency medical treatment than any other.[7]

In summary, NPS are on the rise globally and with it an increase in the incidence of intoxication and death. The major limitation of these drugs is the lack of identification tools – which exacerbates the difficulty for medical practitioners to identify the best treatment route and for forensic staff to identify illegal substances. A lack of prosecution encourages an increase in NPS drug use.

References

1. UNODC. *Global Smart Update*. **19**. 1-12. (2018) https://www.unodc.org/documents/scientific/Global_Smart_Update_2018_Vol.19.pdf

2. D.K. Tracy *et al*. *BMJ*. **356**. 1-7. (2017). https://www.bmj.com/content/356/bmj.i6848

3. G. Dignam. *BJA Educ.* **17 (5)**. 172-177 (2017). https://academic.oup.com/bjaed/article/17/5/172/2999280

4. Home Office. 1-31. (2016). https://www.gov.uk/government/publications/new-psychoactive-substances-nps-resource-pack

5. Randox testing service. (2018) https://randoxtestingservices.com/wp-content/uploads/2018/06/four-types-of-nps.pdf

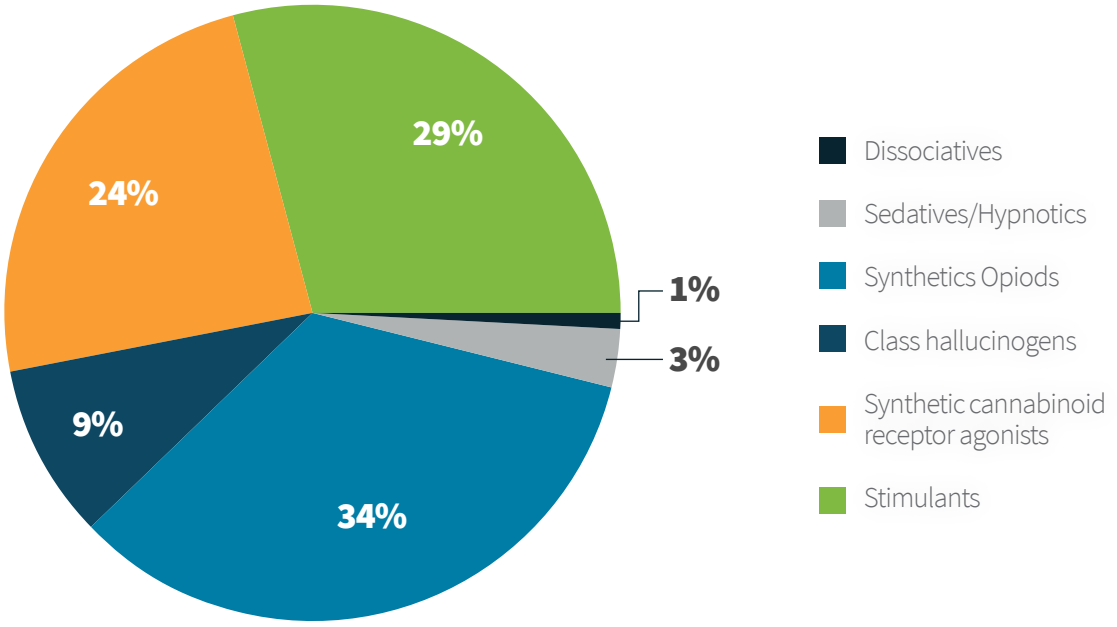
6. EMCDDA. Europol. 1-88. (2017) http://www.emcdda.europa.eu/system/files/publications/6585/TD0417834ENN.pdf

7. A.R. Winstock. http://www.globaldrugsurvey.com/past-findings/the-global-drug-survey-2016-findings/ (2016)

The growing problem of NPS

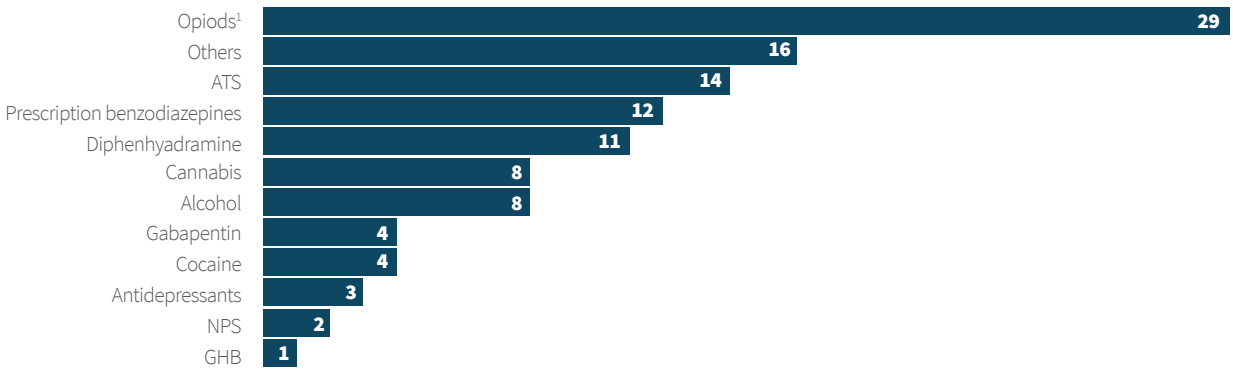
The United Nations Office on Drugs and Crime (UNODC) Early Warning Advisory (EWA) monitors, analyzes and reports on NPS trends to help provide effective evidence-based policy responses and improve the understanding of NPS distribution patterns and use worldwide.

As of January 2020, 120 countries and territories reported the cumulative emergence of 950 individual NPS.[1]
The distribution of new cases reported to the UNODC EWA since the beginning of 2018 is shown below:



Between 2016 and 2018, just over half of all NPS toxicology cases reported to the Tox-Portal involved opioids or synthetic cannabinoids. However, the most recent information from 2019 indicates that benzodiazepine-type NPS now account for most cases, demonstrating the dynamic nature of NPS trends.[1,2]

Additionally, poly-drug use is very common; in 2019, a high proportion of reported NPS fatalities involved kratom and in all these cases, additional substances were detected. This presents a significant challenge when trying to assess the significance and contribution of a particular drug in a person's death.[1,2]



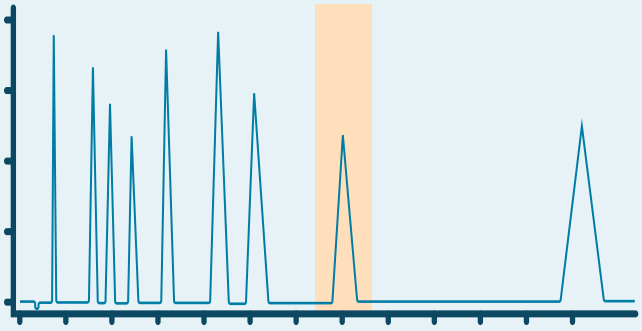
**Kratom is the colloquial name of the plant Mitragyna speciose which has some opioid and stimulant properties, containing pharmacologically active alkaloids especially mitragynine and 7-hydroxymitragynine.*

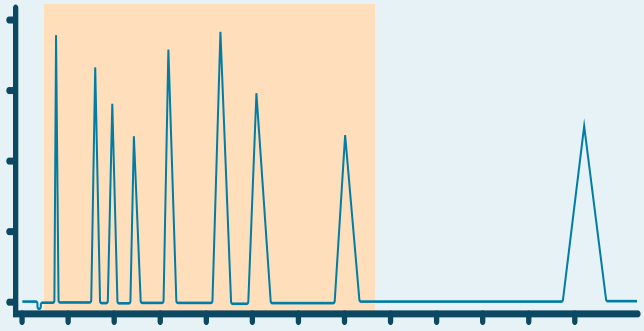
1. United Nations Office on Drugs and Crime. 2019. Current NPS Threats Volume II. [online] Available at: https://www.unodc.org/documents/scientific/Current_NPS_Threats_Volume_II_Web.pdf [Accessed 3 February 2020].
2. United Nations Office on Drugs and Crime. 2019. Current NPS Threats Volume I. [online] Available at: https://www.unodc.org/pdf/opioids-crisis/Current_NPS_Threats_-_Volume_I.pdf

Overcoming NPS screening challenges in the forensic laboratory

Novel psychoactive substances (NPS) are synthetic compounds that are designed to mimic the effects of traditional prescription drugs. The use of these highly potent substances can lead to severe intoxication and overdose fatalities. The detection of NPS poses a challenge to forensic laboratories due to the variable nature of their composition and potency. As a result, these structurally-related compounds often go undetected since they are not part of the panel of drugs routinely screened for in targeted workflows. As a result, non-targeted approaches are often required to detect the presence of these emerging substances. The differences between these two approaches are listed below:

Analysis window





Targeted screening approach

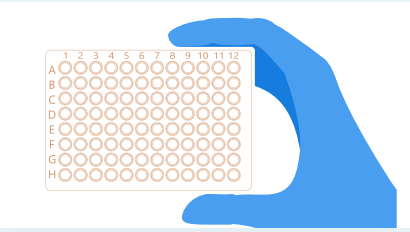
- Monitor well-defined compound list
- Positively identify compounds on the list using appropriate criteria. New compounds can be added to the list to extend screening capabilities

Non-targeted screening approach

- No list of targeted compounds is available
- Better with comparison to look at differences and propose their identities
- Confirm and add to target list
- Newly discovered compounds can be added after they have been characterized
- Retrospective analysis (or data mining) of previously-acquired data can be performed to look for the presence of newly-added/characterized compounds

Detecting novel psychoactive substances the workflow

There are different ways to detect known and unknown NPS.



Screening → **Confirmation**

Immunoassay → **GC-MS** → **LC-MS/MS**

Positives:

- Easy sample prep
- Lower costs
- Lower sample volume
- Fast results

Negatives:

- Not good for specificity
- Cross reactivity
- Multiple assays required for each class of drugs
- Do not always cover new NPS in the panels

Positives:

- More specific than immunoassay
- Analyte specific
- Very sensitive

Negatives:

- Requires derivatisation
- Identification based on library spectra, this may not be readily available for NPS
- Long GC times

Positives:

- Robustness, reliability and versatility
- Accuracy and precision
- Fast and sensitive
- Requires less sample preparation and is compatible with generic sample preparation methods

Negatives:

- Standards may not be available (But this *can* be overcome by using SCIEX Triple Quad™, QTRAP®, or TOF systems).

Different types of LC-MS/MS for forensic screening

SCIEX Triple Quad systems

4500 Series

5500 Series

6500+ and SelexION® Differential Mobility Separation Technology

7500 Series

QTRAP systems

Push the limits

TOF systems

SCIEX TripleTOF systems

TripleTOF® 5600 LC-MS/MS System

TripleTOF® 6600 LC-MS/MS System

SCIEX QTOF systems

X500R QTOF System and X500B QTOF System

Challenges of screening and identifying NPS in the forensic laboratory

An interview with Dr Alex Krutolski, Research Scientist at the Center for Forensic Science Research and Education (CFSRE)

Left: A sneak peak into Alex's laboratory and working environment. Right: SCIEX TripleTOF® 6600 LC-MS/MS System for non-targeted screening of NPS.

The prevalence of novel psychoactive substances (NPS) has increased over the last few decades. The challenges relating to NPS screening and identification are impacting scientists globally.

Dr. Alex J. Krotulski serves as a Research Scientist at the Center for Forensic Science Research and Education (CFSRE) and the Program Manager for NPS Discovery – which is a collaborative flagship program for the identification of new synthetic drugs and the dissemination of information surrounding their impact. His current research and casework focus heavily on aspects related to the detection and characterization of NPS, including studies that examine NPS positivity, trends, metabolism, and effects through intelligence, surveillance, monitoring, and response efforts.

In this interview, Alex shares his insights on the scope of the global NPS issue, the challenges associated with NPS screening and detection and the work that is being conducted in his laboratory to overcome these challenges.

Q: Can you provide some context as to why designer drugs and NPS are an issue and why is it important to detect these substances?

A: Novel psychoactive substances (NPS) (sometimes referred to as designer drugs, synthetic drugs, or research chemicals), are chemical substances that are specifically designed to act like traditional drugs of abuse by targeting endogenous receptor systems within the body. There are several reasons why different or new NPS can emerge, such as the desire for an increase in favorable effects or a decrease in adverse effects, the evasion of laws based on new drug legislature or scheduling actions, or simply drug user curiosity. These factors lead to the emergence of new NPS on a weekly to monthly basis. This can be very challenging for analytical chemists and forensic scientists who are trying to remain up-to-date with scopes of testing and other associated information (e.g. concentrations, combinations, metabolism).

The history of specific NPS differs based on the origin of their discovery. Some NPS were previously synthesized and studied by pharmaceutical companies or academic researchers, resulting in the availability of peer-reviewed literature or patent filing that can serve as road maps for their synthesis in clandestine (or more sophisticated) laboratories. When studying these substances in the past, often in the 60s, 70s, or 80s, information about activity and potency may have been generated and published – this is

desirable for those intending to produce, sell, or use the substance since they know it will create an effect, whether desirable or, unknowingly, undesirable.

NPS that do not have a historical record are often modified based on the chemical structure of previously described or prevalent substances and, in turn, their activity or potency is assumed based on those comparisons. However, there are truly no accurate ways to evaluate the toxicity of a new synthetic substance without performing experimental studies, either *in vitro* or *in vivo*. The risks associated with NPS use that lead to morbidity and mortality consider all of these factors.

Emerging NPS can be more potent and more toxic compared to the last generation of the substance, leading to an increased risk of drug overdose or death. In addition, emerging NPS can have different effects on the body that are uncharacterized or unstudied, which can complicate aspects of interpretation, whether by scientists, medical professionals, law enforcement, etc. Based on their effects on the body, NPS are often detected among forensic investigations (i.e. postmortem/death, driving under the influence of drugs (DUID)) and clinical investigations (i.e. non-fatal overdoses, emergency department admissions, poison center calls).

The majority of these investigations will include testing of biological samples to confirm the presence of an intoxicating agent (e.g. NPS), however, the issue becomes "is this NPS in the scope of testing". It has become crucial for laboratories to maintain testing protocols that allow for the detection and discovery of NPS. Resolution of these investigations is often contingent on the identification and confirmation of the substance. If NPS and specifically, the newest and emerging NPS, are not incorporated into testing workflows, results could be reported as "negative." This can lead to inaccurate or under reporting, which can have downstream effects such as a lack of connection between an impairment and the presence of a drug, inconsistent autopsy findings in comparison with toxicology testing, public health reporting of drug use or death statistics.

Q: What NPS emerging or recurring trends has your laboratory observed over the years?

A: The emergence of NPS in the United States began around 2008. Since then, the landscape of NPS has evolved differently based on specific classes. Typically, NPS are subdivided into categories including opioid, cannabinoid, benzodiazepine, stimulant, and hallucinogen.

Fentanyl (a drug patented under pharmaceutical development and widely used among current medical practices) was the first major player to take over the NPS opioid landscape. Prior to this time, other fentanyl analogues had emerged – causing considerable numbers of deaths in areas nationally and internationally – but these are largely considered isolated incidences prior to fentanyl's emergence under the current NPS era. Once fentanyl took over as the dominant NPS opioid, clandestine chemists began looking for ways to increase overall output or impact. This ultimately led to the emergence (or re-emergence) of fentanyl analogues. These drugs were largely simple modifications of the basic fentanyl scaffold, substituting or adding atoms or functional groups. This process had differing effects on activity, potency, and overall toxicity. Several fentanyl analogues proliferated nationally, resulting in hundreds to thousands of deaths, which can be accounted for among the rise in opioid deaths during what is currently considered to be an opioid

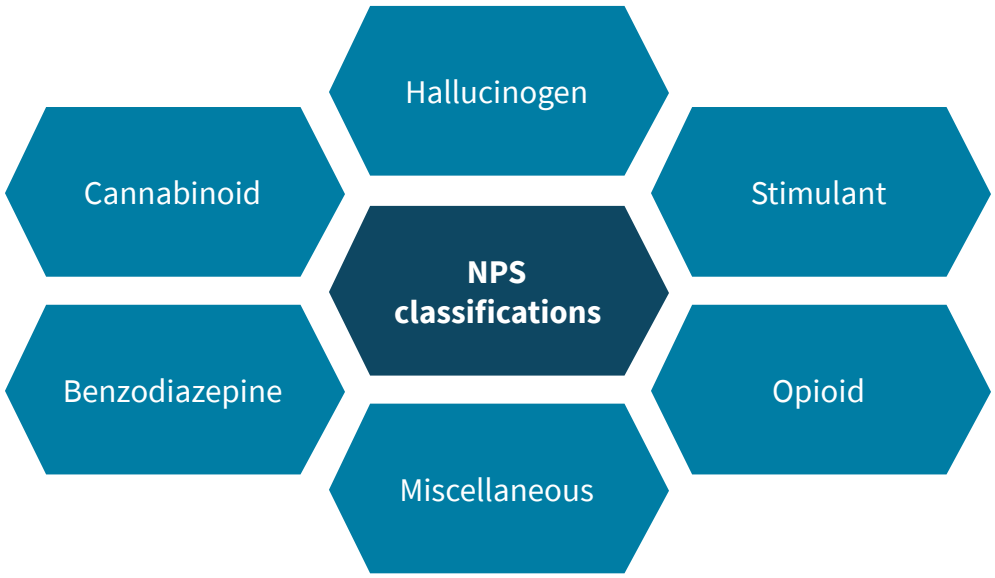


Figure 1: *A diagram showing the seven different NPS classifications*

epidemic. Key players at this time were furanylfentanyl, 3-methylfentanyl, and carfentanil (notorious due to its reported relative potency). During this time, other NPS opioids were also present and prevalent, notably U-47700, a non-fentanyl derived substance (which was also patented by a pharmaceutical company during drug development).

Due to the staggering number of fentanyl analogue deaths, scientists, in collaboration with law enforcement, devised a plan for core structure scheduling of the fentanyl class. Beginning in 2016, this meant that fentanyl analogues were all Schedule I substances, the highest ranking within drug scheduling. As intended, this legislative action resulted in the sharp decline in the number of positive testsfor these substances. Now, in 2020, fentanyl analogues are rare occurrences among the NPS landscape, replaced by new NPS opioids which look structurally different. Fentanyl continues to dominate in this space, but new and emergent NPS opioids continue to appear on at least a monthly basis. This shift has created new challenges for scientists, as many of the new NPS opioids have limited or no available pharmacological data available (where it was previously assumed that the fentanyl analogues retained activity and had similar/increased potency). The current NPS opioid landscape continues to be quite dynamic.

The NPS synthetic cannabinoid landscape largely started with the emergence of new substances that were pirated from academic research and pharmaceutical drug discovery. The most notable substance was JWH-018. The synthetic cannabinoids class historically is the most chemically diverse and analytically challenging – this can somewhat be imagined by the nomenclature used for these substances. Turnover among the trends within this class are often referred to as “generations”, which is a term linked originally to structural representations. Synthetic cannabinoid positivity, like many of the classes, is directly linked to scheduling actions – as a substance is scheduled, a new substance emerges. Through this process, certain structural features have remained or become common, providing insight into preferential synthetic pathways or patterns of use. The most common drugs among this class recently are 5F-MDMB-PINACA (5F-ADB), 5F-MDMB-PICA, 4F-MDMB-BINACA, and MDMB-4en-PINACA.

With respect to NPS benzodiazepines, this class is typically comprised of the fewest structural variations. These substances retained the fused benzene (or other aromatic) ring and diazepine ring, with or without the addition of the triazole ring. Common variations include the addition of halogens (e.g. fluorine, chlorine, bromine). Many of these substances were developed for medicinal purposes, so literature regarding their activity and potency may be available. One challenge among this class is the different uses of NPS benzodiazepines internationally – some of these substances can be prescribed in one country and be emerging or abused in another country. There does not appear to be an overall trend with respect to the next substance to emerge – like other classes, this is usually related to drug scheduling or user preference or availability.

Depending on location, NPS stimulants can be the most commonly encountered NPS class, and this class has seen many new synthetic variants over the years. NPS stimulants are mostly developed to mimic the effects and/or structure of amphetamine, MDMA, and cathinone at their core. To complicate matters, there are several NPS stimulant subclassifications, of which the most commonly encountered substances belong to the beta-keto-methylenedioxyamphetamine category. The first substance from this category was methylone (the beta-keto version of MDMA). Since methylone, several homologues have emerged, including ethylone and butylone, and the series continues over several analogues with elongated carbon tails and amine substitutions. While the variations here seem endless, there is a limit to chain length that dictates effects. Other common NPS stimulants belong to amphetamine and beta-keto-amphetamine categories, including compounds like fluoro-amphetamine and mephedrone, respectively. Trends among this class continue to see the emergence of new substances that are structurally related but differ based on simple function group variations (i.e. adding a methyl group, adding a halogen).

NPS hallucinogens are the least commonly encountered class, and, like other classes, the most commonly encountered substances are often structure related to traditional hallucinogen (e.g. ketamine, PCP, LSD, tryptamine). The rate of turnover among this class can be rapid, but with very few positives – a certain challenge for analytical chemists. Trends among NPS hallucinogens also vary geographically (i.e. East vs. West coast).

Q: How can mass spectrometry be used to detect designer drugs and NPS, and what are its advantages over other screening approaches?

A: Mass spectrometry (MS) is one of the most useful analytical tools for detecting small molecules, such as drugs and NPS. MS allows for the detection of mass characteristics for both intact (or precursor) molecules and their fragments, which can serve as a chemical fingerprint for the identification or structural elucidation purposes. Paired with chromatographic separation, MS has become the gold standard for drug detection in forensic chemistry and forensic toxicology. Increased sensitivity and good specificity have allowed MS to become the go-to analytical technique over others. Due to the ability to separate species among the mass filters, mass spectrometers allow for the analysis of complex sample matrices (i.e. drugs in blood samples, or drugs in a powder that has been cut or diluted with other drugs) – of course, chromatography helps the notion or need for separation. All of these factors together make MS an accurate, reliable, and preferred means for drug identification.

Q: Can you talk us through some of the challenges associated with the various methods for screening and detecting NPS and designer drugs?

A: Like other analytical platforms, mass spectrometers come in many shape and sizes, often due to their capabilities and internal hardware (i.e. mass filters). Mass filters make a mass spectrometer unique, differentiating their abilities to generate specific information among their close relatives. For example, mass spectrometers with quadrupole mass filter only allow for nominal mass measurements, and as such, these instruments are often used for comparative purposes (i.e. library searching, confirmation, quantitation, etc.). Some structural information can be gained by the use of quadrupoles alone, however, better and more accurate structural information is acquired via the use of high resolution mass spectrometry (HRMS) mass analyzers, such as time-of-fight (TOF) or orbitrap. TOF MS generates accurate mass measures which can be compared to the theoretical exact mass of a compound, and within certain constraints, a scientist can determine the chemical formula of a detected species. This information becomes extremely useful when discovering new synthetic drugs, but also has great utility for screening purposes. TOF analyzers placed in parallel with quadrupole analyzers allows for the generation of accurate mass fragment data, which can be used for more reliable structural elucidation (another great benefit).

Quadrupole time-of-flight (QTOF) MS is an expanding field in drug detection and has proved to be the most valuable tool for drug discovery and the most accurate tool for drug screening or identification. However, QTOF systems are very complex platforms and there is no standard method of operation. Due to the variability among mass analyzer operation and vender configurations, QTOF systems can be operated in numerous manners, which can be referred to as acquisition modes. These acquisition modes define how the mass analyzers function, and more specifically how the quadrupole is operated. Examples include MS^2, MS^e (or MS^{ALL}), and MS/MS^{ALL}. MS/MS^{ALL} (or SWATH® Acquisition, as referred to by SCIEX) is the middle of the road option between MS^2 (or information dependent acquisition [IDA], a targeted acquisition approach) and MS^e (or data independent acquisition [DIA]), a non-targeted acquisition approach. SWATH Acquisition is a DIA, non-targeted approach. SWATH Acquisition combines the powers of accuracy and specificity to provide a complete picture of the drugs within a sample while alleviating any of the worry that pertinent information will not be collected. In short, SWATH Acquisition utilizes the quadrupole as a segmented mass filter, meaning it allows only a range of masses to pass through Q1 at a given time (MS^2 allows only one mass to pass at a given time, MS^e allows all masses to pass at a given time). This results in higher specificity among fragment ions produced (compared to MS^e), and increased accuracy when conducting tasks such as structural elucidation.

Some of the most impactful challenges associated with these acquisition modes and NPS detection involve the ability to distinguish isobaric species and to accurately perform structural elucidation. SWATH Acquisition alleviates some of the challenges presented with respect to structural

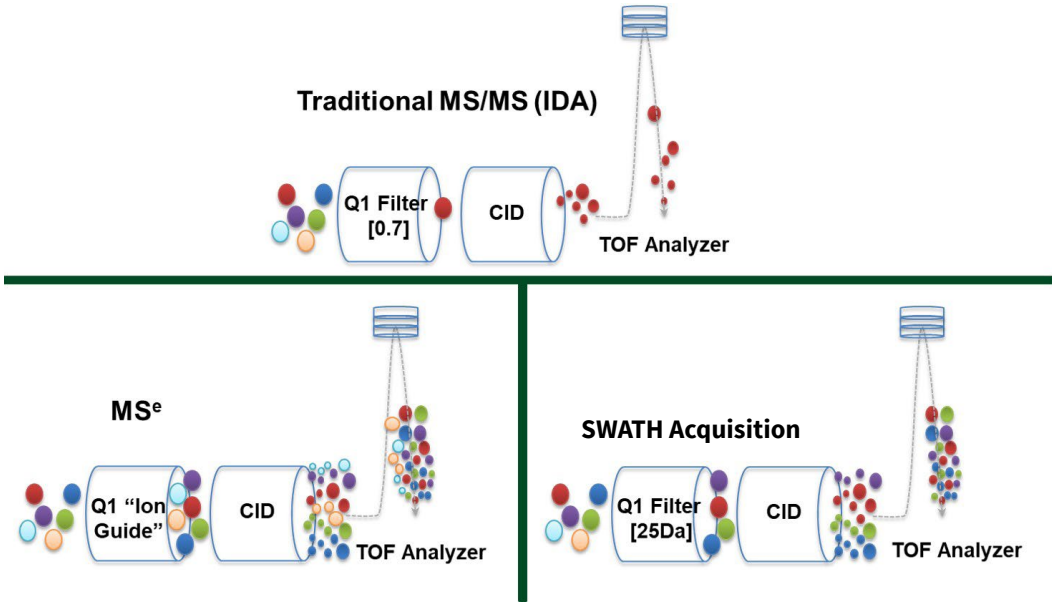


Figure 2: *A schematic view of a traditional QTOF MS depiciting the different acqusition modes: MS2, MSe and SWATH Acquisition. The function of the quadrupole (Q1) dictates what masses make it through to the colliusion cell (CID) and TOF analyzer.*

elucidation because there is certainty that the fragment (or MSMS) data will be available for review. In addition, formula finder searching can be performed on accurate mass fragment data (like those of the precursor ion, or TOF MS data), which allows the scientist to determine the formula of a given fragment. Structural elucidation is a difficult science and requires specific expertise; however, acquisition using the technique described positively impacts the interpretation. Isobaric species, and specifically positional isomers, are a great challenge among all aspects of forensic chemistry and forensic toxicology. Accurate determination of structural isomers is extremely important, especially when the isomer pair can have differing potency or toxicity. The use of HRMS, and specially QTOF MS, can assist with distinguishing isomers, from a mass spectrometer standpoint alone. Like traditional GC-EI-MS data processing, QTOF-MS fragment ion spectra can be compared to a library generated from the analysis of standard reference materials. This links back to the notion that the instruments acquire chemical fingerprints for drugs. This is an added benefit to using accurate mass fragment data to distinguish isobaric species, increasing confidence. However, it should still be noted that certain isomers (specifically several fentanyl analogues) cannot be distinguished by MS methods alone – this remains a great challenge analytically.

Q: What strategies have your lab been using for NPS early identification and discovery? What tools do you have in place to streamline the process?

A: Early on in our program, our laboratory developed and validated two LC-QTOF-MS methods for the detection and discovery of NPS. Both of these methods employ SWATH Acquisition and we have had a lot of success using these methods. We have made it a priority to maintain up-to-date libraries, often incorporating the newest reference standards to become available. This has led to our library database growing to more than 800 compounds, all of which we can accurately identify (this means they include fragment spectra – this is not just a suspect screen).

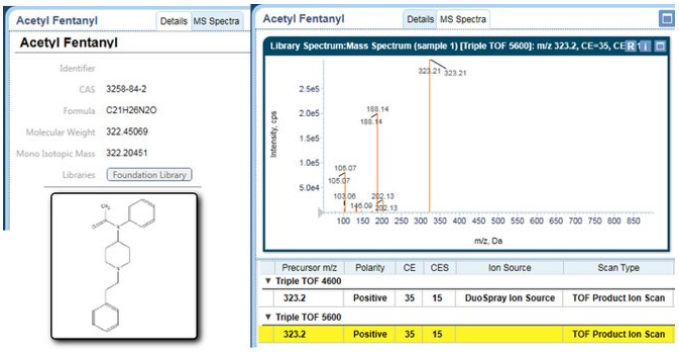


Figure 3: *The library view of a QTOF-MS fragment spectra compared to a library generated from the analysis of standard reference materials.*

While the upfront work to get these methods off the ground was no small task, this is not where the work ends. In order to develop an accurate and timely workflow for the discovery of NPS, a laboratory needs to identify which sample populations they will begin

Figure 4: *Data processing with MasterView™ Software for TOF MS and MSMS data*

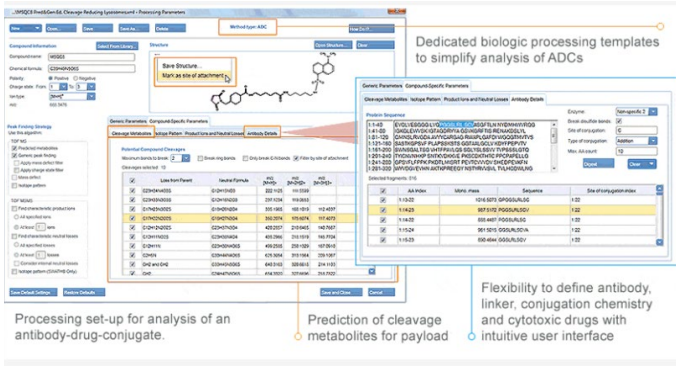


Figure 5: *MetabolitePilot™ Software which has structural drawing features, can be used to piece together a tentative structure of an unknown compound*

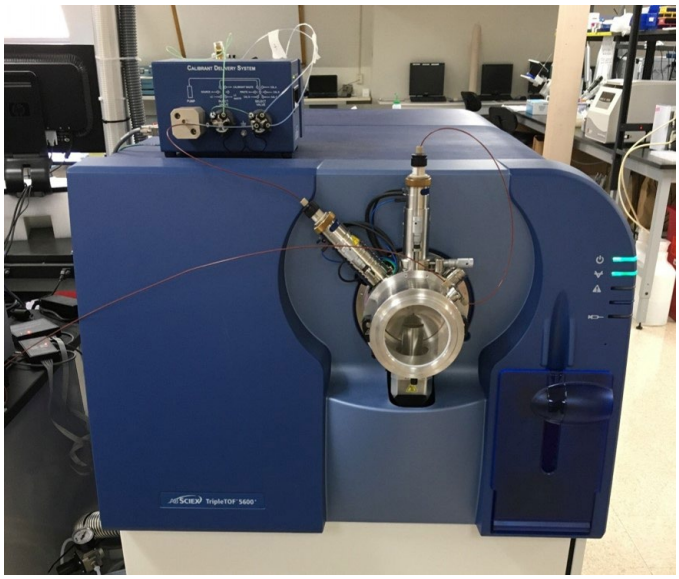


Figure 6: *A front view of the SCIEX TripleTOF® 5600+ LC-MS/MS System used for NPS identifications.*

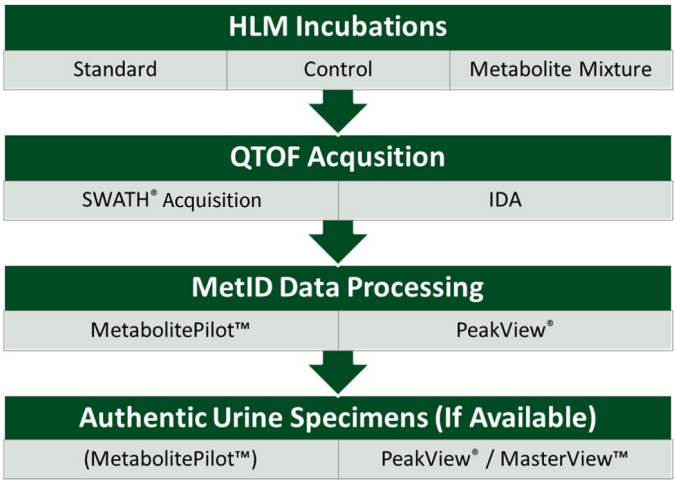


Figure 7: *The customized workflow used for metabolite identifications for new and emerging NPS. Experiments begin with HLM incubations and lead to analysis of authentic urine samples, if available.*

to test or monitor. We began implementing our SWATH Acquisition methods for the detection of emerging synthetic drugs among seized drug materials and toxicology samples. We created partnerships with federal laboratories to test powders entering the country through the mail. We work with state and local partners to test seized street level samples and/or toxicology samples. And finally, for our largest population, we partner with a forensic toxicology laboratory to receive and test discarded sample vial extracts from authentic forensic casework where NPS use is suspected. Through all these avenues, and paired with our non-targeted SWATH Acquisition methods, we are positioned to detect and characterize NPS at their first incidence, or as close as possible to their first incidence, among the drug supply.

For identification purposes, we use SCIEX PeakView® Software and MasterView™ Software to process data and view TOF MS and MSMS data, comparing acquired mass spectra with those that are expected or within the library database. For true unknown identifications of NPS, we use SCIEX PeakView Software and MetabolitePilot™ Software (which has great structural drawing features) to piece together a tentative structure, based on our expertise and what we have seen before with other drugs or NPS.

Q: Can you expand on the work your laboratory has done over the past couple years (more specifically with the work around NPS Discovery) for NPS early identification and discovery?

A: Our laboratory has broken NPS identification and discovery into three main areas surveillance, monitoring, and response.

Under our surveillance initiatives (as described above), we spend a lot of time and effort to discover new NPS as they emerge within the drug supply or as they emerge with death investigation casework. This process can be the most time

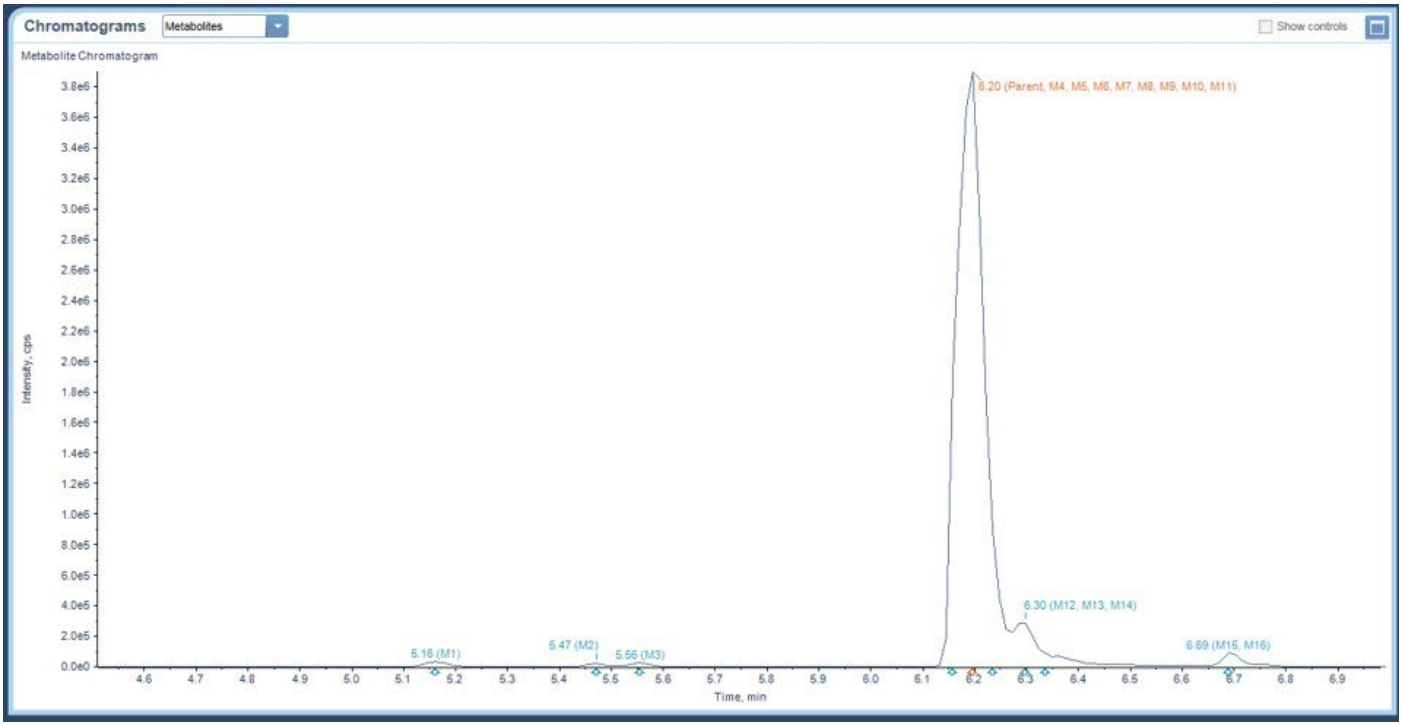


Figure 8: *An example of a MetID chromatogram showing the presence of the parent compound (6.20 min), primary metabolite (6.20 mins, closely eluting), and other minor metabolites (5.16-5.55 mins).*

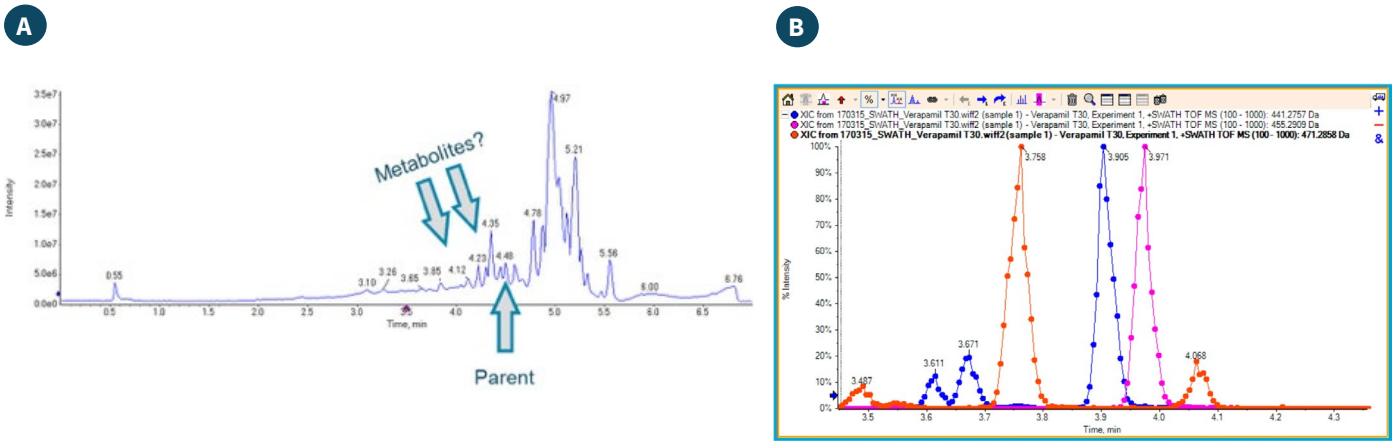


Figure 9: *A) An example of a MetID chromatogram, which allows the parent compound and its associated metabolites to be distinguished. B) Accurate metabolite ID at UHPLC timescales with ultra-fast acquisition capabilities without sacrificing resolution.*

and labor intensive, but it is the initiator for the rest of the work we do – we cannot initiate work with a certain NPS if we do not know that drug exists or if we do not have a good understanding of how to detect it.

Once a new NPS is discovered through our surveillance, we begin monitoring for this substance in all of our other populations, including additional seized drug materials, forensic toxicology samples, and clinical nonfatal overdose samples. This monitoring allows us to determine what substances are most prevalent and are having the greatest impact on the drug market. In reality, not every new NPS we discover will be identified in a toxicology case or will go on to become the next "most popular" substance. With that in mind, it is important for our laboratory to determine what the most prevalent substances are, so we can do further work with these substances to create the best opportunity for scientific impact.

There is often not enough time and resources to study all aspects of all emergent NPS, so we must pick and choose which substances are the most important to study. This leads to our response efforts, which entail work related to confirmation, quantitation, and metabolism. Once we see a notable increase in NPS prevalence among a certain population, we move to create confirmatory methods for those substances so we can get a better idea of the drug's characteristics (and also we must develop confirmatory methods to report our findings among forensic casework).

The confirmatory methods are often quantitative in nature, so we are able to gather information about how much drug was in a person's system when the incident occurred (e.g. overdose, death, accident, etc.). This can help us understand the potency or toxicity of a drug, from a toxicological viewpoint, depending on the information we receive from a case history, autopsy report, and other drugs present. Another important aspect of our response involves metabolite identifications (MetID) and discovery. From a forensic toxicology perspective, it can be vastly important to study metabolism, as the results can help prolong detection windows, help further understand toxicity or effects, and help determine what the most appropriate biomarker is for future method development. For example, synthetic cannabinoids metabolize extensively in the body, typically resulting in little to no parent compound excreted in the urine. This means scientists must perform MetID studies to determine what biomarker to look for in urine samples associated with synthetic cannabinoid use – this initial uncertainty can make this drug class very challenging. Discovery of active metabolites can also be extremely important (think, for example, of heroin → 6-MAM → morphine). MetID studies can help shed light in this area, which can in turn assist with toxicologist's interpretations and/or future analytical method design.

Q: New NPS and designer drugs emerge often into the market, posing a risk to public health. How do you disseminate information to other laboratories and agencies to ensure people have access to the most up-to-date information? In that regard, what approaches is your laboratory taking in terms of sharing the information and intelligence you are gathering on NPS?

A: Our motto has always been simple – rapid and far-spread information sharing to all interested stakeholders. Or in other words, our work is an "open book." It is not beneficial to our colleagues at large if we generate certain information or make certain discoveries and do not share the information as rapidly and widely as possible.

In this space, we have worked hard to create vast networks of stakeholders to whom the information is disseminated.

Our distribution list includes many federal, state, and local agencies, as well as numerous international agencies, with public health, public safety, and scientific interests. Our distribution list is open and easy to join (npsdiscovery@cfsre.org), and we welcome any individuals who have an interest in the information we are distributing.

Our initial dissemination strategy involves direct communication to stakeholders via email, where individuals get a firsthand look at our newest discoveries or trending data. These reports and emails are then secondarily distributed by the recipients to other colleagues or organizations where our information is posted to websites, social media platforms, etc. Dissemination at scientific meetings, conferences, and gatherings is also an integral part of our strategy, as these forums often allow for Q&A or feedback from other colleagues and jurisdictions. In addition, all of the information we generate for NPS is archived on our website (www.npsdiscovery.org) where individuals can access any reports free of charge, including additional access to resources such as recent publications, presentations, and an electronic GC-EI-MS library database.

Dr Alex J. Krotulski,
Research Scientist
Center for Forensic Science
Research and Education (CFSRE)

Streamlining forensic laboratory informatics for NPS screening and quantification

Pierre Negri, PhD
Global Technical Marketing Lead, Forensics, SCIEX

Pierre works with global key opinion leaders in criminal and forensic toxicology research areas to develop and implement new mass spectrometry methods and address customer and market needs.

There has been a significant increase in the number of novel psychoactive substances, worldwide. Yet little is known about the characterization of these substances, and analysis is limited by traditional screening techniques.

This webinar describes the emergence of LC-MS/MS as a powerful and comprehensive technique toxicology screening applications, and presents the solutions available from SCIEX that detect and identify these compounds.

From this webinar you will learn how:

- SCIEX instruments in combination with novel and intuitive informatic solutions provide a streamlined and comprehensive solution for the detection of these novel psychoactive substances
- Data processing in typical workflows is performed on a set of real samples, and how SCIEX OS Software is used to streamline generation of results with a high level of confidence.

WATCH NOW

SCIEX
The Power of Precision

Techniques and solutions for forensic drug screening: an interview with timothy fassette

An interview with Timothy Fassette: Senior Forensic Toxicologist at Henderson Forensic Laboratory, Henderson, Nevada, USA

Drug markets are constantly evolving. This together with the need for forensic scientists to identify unprecedented and ever-increasing numbers of novel psychoactive substances (NPS) presents a significant challenge.

Timothty Fassette, is a Senior Forensic Toxicologist at the Henderson Forensic Laboratory in Henderson, Nevada,

Figure 1: *This unassuming building is the Henderson Forensic Laboratory.*

where he oversees the training of the Laboratory's scientists, analyzes samples sent for DUID (driving under the influence of drugs) analysis, runs method validation on new analytical techniques and directs the quality control and quality assurance program.

In this interview, Timothy shares his insight into some of the challenges, techniques and solutions for forensic drug screening in his laboratory.

Q: What types of case sample do you receive in your laboratory? What are the biggest challenges you face with the caseload you process in your laboratory?

A: Our toxicology section receives whole blood samples for DUI and DUI-drug cases for the city of Henderson and a few other surrounding agencies. These samples are analyzed to detect and give a quantitative concentration of any ethanol and other impairing drugs that a driver may have been under the influence of at the time of their arrest. The biggest challenge that our lab faces now, in reference to the samples we analyze in the lab, is the ever-changing nature of what we are looking for. As anyone that has been in this field long enough can tell you, you are constantly chasing your tail when it comes to testing new and emerging drugs. It seems that just as you start to see one

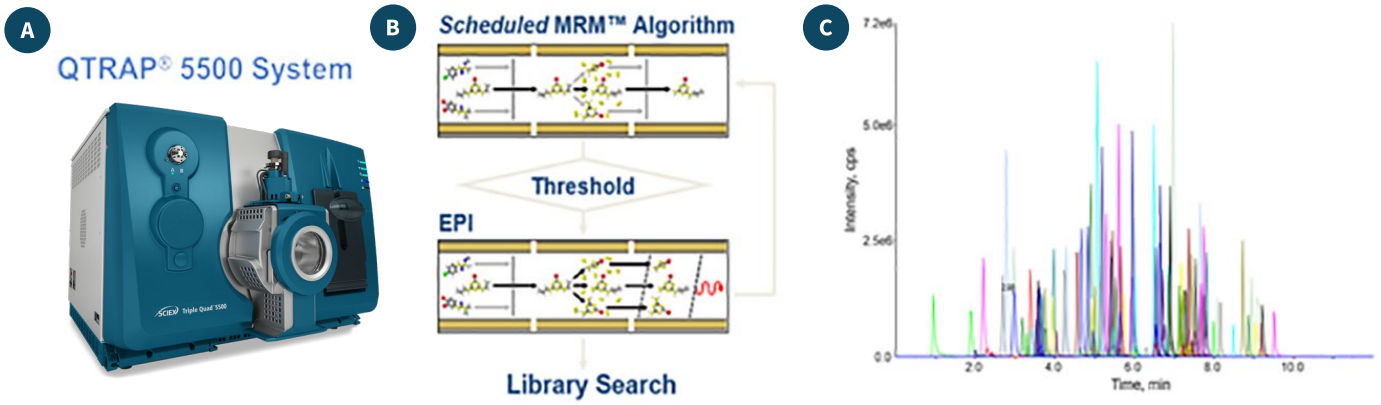


Figure 2: Workflow used for Targeted Screening. *Using the QTRAP® 5500 LC-MS/MS System (a), a targeted method was set up using the Scheduled MRM™ Algorithm (b) to detect the 110 target compounds. Once detected the instrument will automatically switch to ion trap mode and collect full scan MS/MS (c) on each analyte for ID confirmation. This targeted method included MRMs for 12 Opiates, 15 Benzodiazepines, 17 stimulants, 2 OTC-Depressants, 17 Synthetic Canthinones, 35 Rx Depressants, and 13 THC/Synthetic Cannabinoids. Total Ion Chromatogram (TIC) for the MRM survey scan is shown on right.*

and attain the ability to test for it, it is gone and replaced by something else that requires a different extraction and analytical technique. It can be very frustrating at times.

Q: What techniques are used in your lab for NPS detection?

A: We currently use our QTRAP® 5500 LC-MS/MS System for most of our NPS detection. Our drug screen starts with targeted multiple reaction monitoring (MRM) selection of certain ions in Q1, fragmentation in Q2 and the linear ion trap being utilized in Q3 to attain a full MS/MS comparison and library matching. This allows us to distinguish between closely eluting analytes with a few, similar ions that in standard LC-MS/MS analysis would lose selectivity due to only scanning for two or three ions at a time. We have a few in-house confirmation techniques for the NPS drugs that we see on a somewhat routine basis utilizing standard LC-MS/MS triple quad analysis with MRM acquisition, fragmentation and selective mass filtering of two to three ions. Any NPS drug that we routinely screen for — but do not have an in-house test for — are sent out to third party labs for confirmation and quantitation only after we have identified them in the linear ion trap drug screen.

Q: How successful are these techniques at identifying NPS compounds?

A: The techniques are very successful in identifying NPS drugs in our whole blood samples. It allows us to specifically select out ions that may be clumped in a mass of other analytes and extract them out, fragment the ion and then use the MS/MS library comparison to identify each individual analyte through specific mass fragmentation patterns. This is important in differentiating a number of NPS drugs that elute around the same time, with similar ion masses which recently we have seen in our assessment of a number of fentanyl analogues that we have been analyzing in the lab.

Q: Can you expand on the driving under the influence of drugs (DUID) screening method you have developed and how your QTRAP instrument enables you to perform both screening and quantitative analysis in one, comprehensive workflow?

A: The DUID drug screening method that we employ uses a quick and robust extraction method coupled with our MRM, linear ion trap analysis, and MS/MS library searching technique. This allows us to individually identify over 100 drugs in a 10-minute long method on our QTRAP 5500 System. The extraction utilizes a rapid technique for all of our drugs of interest using the Quechers extraction products. Even though the Quechers products are relatively new to the forensic science field, they have been used in many other fields such as environmental and pharmaceutical chemistry for years. Many extraction methods used for identifying drugs in whole blood DUID samples are specifically optimized for certain classes of drugs. While it is not perfect, this extraction technique is able to readily extract drugs from many different drug classes in a single extraction and does not require a long, drawn out extraction technique. For the instrumental analysis we use the QTRAP (linear ion trap) detection system on the instrument. We run a targeted drug screen using Q1 as a mass selective filter, Q2 as the collision cell for fragmentation and Q3 as the linear ion trap to attain a full scan MS/MS analysis (enhanced product ion scan) on the detected drugs. Then, MS/MS library searching is used for the confirmation of detected compounds in the linear ion trap and only those compounds with a library match of 60% or greater will appear on the final report. For our lab, the combination of a thorough and detailed analytical method coupled with a quick and easy extraction method allowed us to significantly decrease our costs and analysis time while increasing the amount of drugs we could readily identify and the set the specific concentration of each drug we have in the drug screen. For our confirmation method

we are able to use the same QTRAP 5500 System instrument due to the fact that the instrument is a triple quadrupole linear ion trap hybrid mass spectrometer and we use a different extraction technique and analytical method (linear ion trap vs selective mass filtering) for our drug screen and quantitation methods. This falls within the guidelines of using different analytical methods for your drug screening and drug confirmation methods set forth by the society of forensic toxicologists and our laboratory accreditation body.

Q: There are applications for forensic compound screening that use a comprehensive library to obtain retention times and MS/MS spectra, and subsequently perform targeted identification of compounds of interest in DUID samples. What are your thoughts on this type of approach?

A: It is a great approach and very similar to the one we use. We found that the targeted drug screening method — using the MRM data dependent ion survey scans followed by the information dependent acquisition data and enhanced product ion experiments — proved to be a fast, selective and sensitive acquisition method. It allowed us to identify over 100 different drugs in a single analysis.

Q: Speaking of the ion trap DUID drug screening method you have developed; can you expand on the statistics you pulled from the retrospective analysis?

A: Our retrospective analysis that we presented at the Society of Forensic Toxicologists (SOFT) annual meeting in 2019 reported on the extensive DUID data that we have attained over the past two years. Prior to switching to this new method, we only tested our DUI samples for drugs if the blood alcohol concentration (BAC) was below 0.084 g%. After implementing this new method, we tested all DUI samples for drugs regardless of the BAC. We were able to report on the amount of cases above the old threshold of drug testing in our lab and show that approximately 65% of the cases that would not have been tested for drugs under the old testing thresholds actually had drugs in their system. As previously mentioned, this robust drug screening method allowed us to test for many drugs, so we were able to analyze drug trends that we have seen over the past few years and add a number of new and emerging drugs that are not routinely screened for in most parts of the country. This included several synthetic cathinones, synthetic cannabinoids, tryptamines, piperazines, and novel benzodiazepines. This drug screening method did not make us beholden to our drug testing vendors to come out with new testing kits — as was the case previously when our drugs screening was done via enzyme-linked immunosorbent assay (ELISA). With this new technique, once we were able to attain a certified reference standard and optimize that standard on our QTRAP 5500 System, we could perform a method validation following specified validation standards and add the new NPS drug to our routine drug screen.

Q: How often does this lead to prosecution?

A: From this same retrospective analysis, we found that in the last two years there has been a decrease of approximately 31% in the number of cases that were plead down from DUI's. This is mainly due to the extra drug data being provided in these DUID reports. Instead of pleading down a DUI case with the only results being a 0.09 g% of ethanol, they are now prosecuting these cases because there may also be THC, alprazolam, hydrocodone, etc in the driver's blood at the time of the crash.

Q: What efforts do you think will be necessary to combat the flux of NPS and in what capacity do you think your laboratory will contribute to this end?

A: In order to combat this influx of NPS drugs you have to stay innovative and flexible. You cannot just rest on the old adage of "this is how we have always done it around here". You need to talk to your colleagues at other labs in your area and see what they are seeing in their impaired driving cases. You need to talk to your drug analysis section and see what drugs they are seeing on the streets and what NPS drugs officers are finding on individuals that they arrest. Finally, you need to attend professional conferences and see what else is being seen in other parts of the country — and also how these labs are testing for NPS drugs. It all comes down to wanting to stay ahead of the curve and innovative in your analysis; there is no try, you either want to do it or you don't. As far as our lab goes, we will always try to stay in front of this as much as we can, and will continue to work with other labs to address this issue and also be a resource for labs that want to learn how to begin testing for these NPS drugs in DUID casework.

Timothty Fassette,
Senior Forensic Toxicologist
Henderson Forensic Laboratory

Identifying the right technique for your needs

Deciding what technique is best to suit your needs can be very difficult. In forensics, the technique required depends on the type of sample you are working with, and whether the drug you are looking for is known or not.

For a quick overview – please follow the flowchart to determine what application note will best suit your needs. For full details of our application notes, please refer to the compendium below.

Is it a biological sample?

Yes

Is it a blood sample?

Yes

Is it...

post mortem blood?

Streamlining unknown screening for postmortem analysis

for driving under the influence of drugs (DUID) testing?

Single injection targeted screening workflow for duid testing

Advancing forensic duid screening with mass spectrometry

for drug screening?

Quantitative analysis of fentanyl and analogues in human whole blood using the QTRAP® 4500 LC-MS/MS System

Rapid screening of 65 common drugs and metabolites in urine and blood using high-resolution mass spectrometry

Rapid identification and quantification of novel psychoactive substances in human whole blood using SWATH® Acquisition

Pioneering tool to characterize emerging fentanyl analogues

No

Is it a urine sample?

Yes

High sensitivity and dynamic range for 93-compound forensic panel analysis in urine

Rapid screening of 65 common drugs and metabolites in urine and blood using high-resolution mass spectrometry

Quantification of major metabolites of K2 in human urine

Using MS/MS^{ALL} with SWATH Acquisition for forensic designer drug analysis with SCIEX X500R QTOF System and SCIEX OS Software

Analysis of kratom's main psychoactive components: mitragynine and 7-hydroxymitragynine

No

Is it a hair sample?

Yes

Detection of fentanyl analogs and novel synthetic opioids in hair

Multi-panel detection of drugs and drug metabolites in hair samples using a comprehensive extraction method

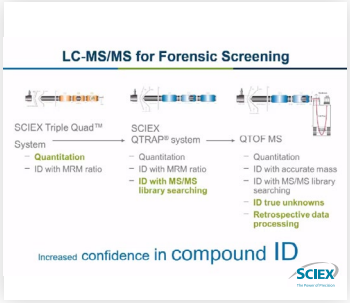
No

Is it a powdered sample?

Yes

SWATH® Acquisition enables the ultra-fast and accurate determination of novel synthetic opioids

Webinar

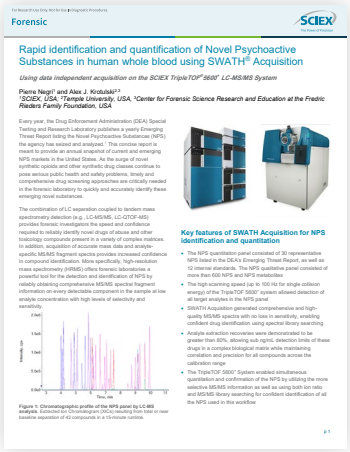


Streamlining forensic laboratory informatics for NPS screening and quantitation

With the sharp rise in the number of novel psychoactive substances (NPS) entering the market, forensics laboratories must have the best tools available to analyze them. LC-MS/MS is a highly sensitive and specific approach, that enables forensic toxicology laboratories to detect and identify, therapeutics and illicit drugs, as well as their metabolites.

From this webinar you will learn more about:

- Challenges for NPS screening
- LC/MS workflows for rapid identification and quantification of NPS
- How SCIEX OS Software for NPS detection is streamlining data processing



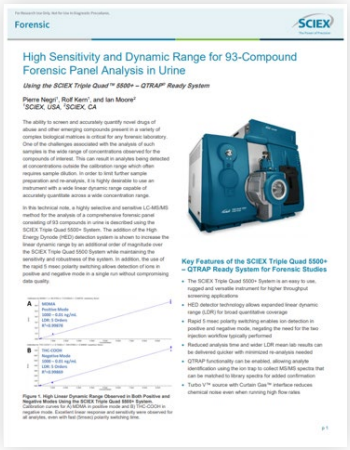
Read technical note

Rapid identification and quantification of novel psychoactive substances in human whole blood using SWATH® Acquisition

Novel psychoactive substances (NPS) pose significant risks to public health and safety, therefore timely and comprehensive drug screening approaches are vital in the forensic laboratory. Building on the ability of liquid chromatography (LC) combined with tandem mass spectrometry detection (LC-MS/MS, LC-QTOF-MS) to accurately identify novel drugs in complex matrices, SCIEX have developed a comprehensive drug screening workflow for the analysis of NPS from whole human blood samples.

From this technical note you will discover:

- The key features of SWATH Acquisition for NPS identification and quantification
- How SWATH Acquisition is combined with SCIEX OS Software to create a comprehensive NPS screening workflow



Read technical note

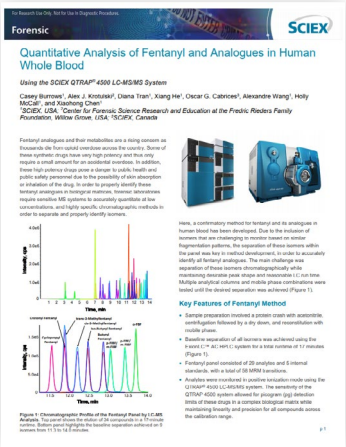
High sensitivity and dynamic range for 93-compound forensic panel analysis in urine

One of the challenges associated with NPS analysis is the range of concentrations observed. If the concentration of NPS analytes fall outside of the calibration range, the sample will need to be diluted so that accurate measurements can be made.

The SCIEX Triple Quad™ 5500+ LC-MS/MS System – QTRAP® Ready is a highly selective and sensitive method with a wide linear dynamic range. It enables quantitation across a wide concentration range, reducing unnecessary sample preparation and re-analysis.

From this technical note you will learn:

- The key features of this method for forensic studies
- The benefits of combining it with the High Energy Dynode (HED) detection system



Read technical note

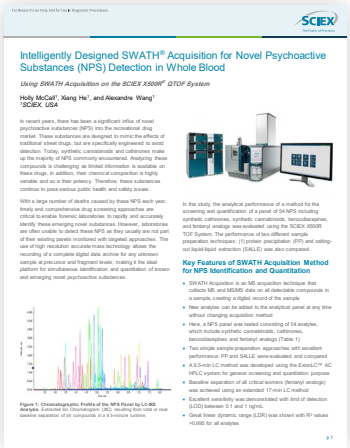
Quantitative analysis of fentanyl and analogues in human whole blood

The potency of fentanyl analogues and their metabolites mean that only a small amount is required to cause an accidental overdose. As the opioid crisis continues to pose a significant threat, it is therefore vital that forensic laboratories can accurately identify these substances in biological matrices.

To achieve this, mass spectrometry (MS) systems and highly specific chromatographic methods are required to quantitate these opioids at low concentrations and separate isomers before identification, respectively.

From this technical note you will discover:

- The key features of the fentanyl method
- Why combining the QTRAP® 4500 LC-MS/MS System and the ExionLC™ AC System are beneficial for fentanyl analysis



Read technical note

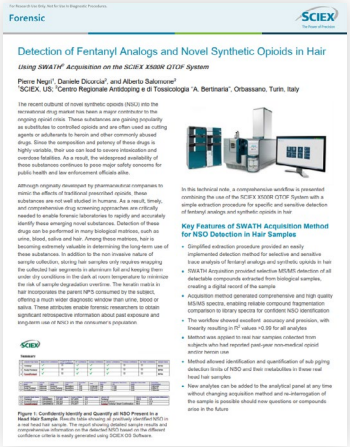
Intelligently designed SWATH® Acquisition for novel psychoactive substances (nps) detection in whole blood

Novel psychoactive substances (NPS) have different chemical compositions and potencies compared to traditional street drugs. This makes detection and analysis challenging. High-resolution accurate mass spectrometry (HRMS) creates a complete digital data archive for unknown samples at precursor and fragment levels, making it an ideal platform for simultaneous identification and quantitation of known and emerging NPS.

SWATH Acquisition is an MS acquisition technique that collects MS and MS/MS data on all detectable compounds in a sample.

From this technical note you will learn more about:

- The key features of SWATH Acquisition for NPS identification and quantitation
- A study evaluating the analytical performance of the SCIEX X500R QTOF System for NPS screening



Read technical note

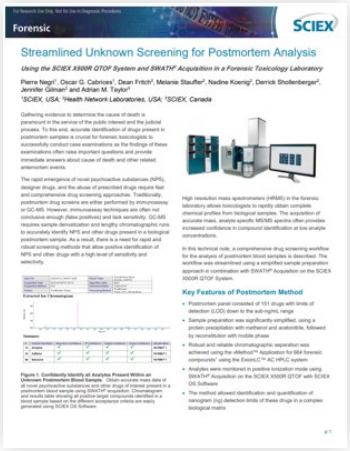
Detection of fentanyl analogs and novel synthetic opioids in hair

The variability in the composition and potency of novel synthetic opioids (NSO) compared to traditional opioids can result in severe intoxication and overdose fatalities. NSO are detected in many different biological matrices, however, hair is a particularly valuable sample used to detect long-term use.

The development of comprehensive screening methods will provide law enforcement agencies and health professionals with a clearer picture of long-term use drug use, their evolution in the consumer market and consumption trends in the specific populations.

From this technical note you will discover:

- The features of the SCIEX X500R QTOF System
- The benefits of combining it with a simple extraction procedure



Read technical note

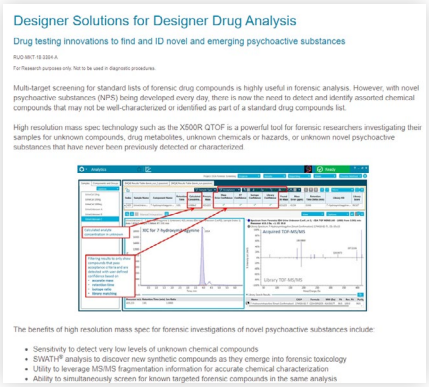
Streamlined unknown screening for postmortem analysis

Accurate identification of drugs in postmortem samples enables forensic toxicologists to successfully determine the cause of death and it is beneficial for public interest and the judicial process. Traditional methods for post-mortem drug screening include immunoassays and gas chromatography mass spectrometry (GC-MS), however, their limitations have led to a search for more rapid and robust screening methods with higher levels of sensitivity and selectivity.

High-resolution mass spectrometry (HRMS) is a technique that can rapidly obtain complete chemical profiles from biological samples with increased confidence at low analyte concentrations.

From this technical note you will uncover:

- The key features of the postmortem method
- The benefits of SWATH Acquisition with the SCIEX X500R QTOF System for screening in postmortem analysis



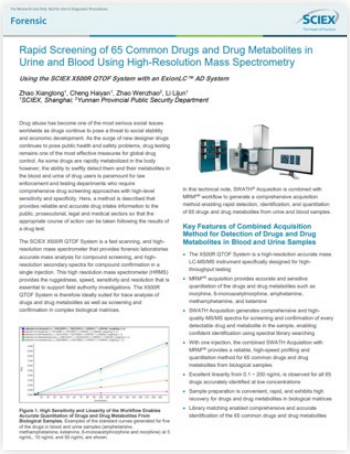
Discover More

Designer solutions for designer drug analysis

High-resolution mass spec technology such as the X500R QTOF System is a powerful tool for forensic researchers investigating their samples for unknown compounds, drug metabolites, unknown chemicals or hazards, or unknown novel psychoactive substances that have never been previously detected or characterized.

From this resource you will discover:

- The benefits of HRMS for forensic investigations of NPS
- Links to useful resources, educational content, products and services



Read technical note

Rapid screening of 65 common drugs and drug metabolites in urine and blood using high-resolution mass spectrometry

Drug abuse is one of the most serious social issues worldwide, as it continues to threaten social stability and economic development. Drug testing remains a highly effective measure of global drug control. However, the rapid metabolism of drugs in the body limits the ability to detect them and their metabolites with high sensitivity and selectivity.

The SCIEX X500R QTOF System is a fast scanning, high-resolution mass spectrometer that provides reliable and accurate drug intake information to support field authority investigations.

From this technical note you will learn more about:

- The key features and benefits of the combined acquisition method for drug and drug metabolite detection in blood and urine samples


Forensic

Multi-panel detection of drugs and drug metabolites in hair samples using a comprehensive extraction method

Using Scheduled MRM™ Algorithm on the SCIEX QTRAP® 6500+ LC-MS/MS System

Pierre Negri[1], Samuele Scuratti[2], and Valentina Longo[3]
[1]SCIEX, US, [2]SCIEX, Italy, [3]*Laboratorio di Chimica Clinica Settore Farmaco-Tossicologia APSS, Trento, Italy*

Read technical note

Multi-panel detection of drugs and drug metabolites in hair samples using a comprehensive extraction method

Although urine and blood testing are the most common forms of drug testing, hair analysis has gained considerable attention over the years as a method enabling the determination of recent past drug use as well as the long term drug use through segmental analysis.

The combination of an easily implemented sample extraction procedure with the sensitivity of the SCIEX QTRAP® 6500+ LC-MS/MS System has enabled accurate identification and sensitive quantification of a wide range of chemically-diverse analytes.

From this technical note you will learn more about:

- The benefits of using this comprehensive workflow for the detection of drugs and their metabolites in hair samples

Access now

Forensics resouce hub

Stay up to date on the science with SCIEX !

Novel psychoactive substances: staying ahead of the curve

Screening, identifying, and quantifying with mass spectrometry

SCIEX
The Power of Precision

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

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