Waters™

Delivering an Open-Access Drug Early Warning System for Novel Psychoactive Substances

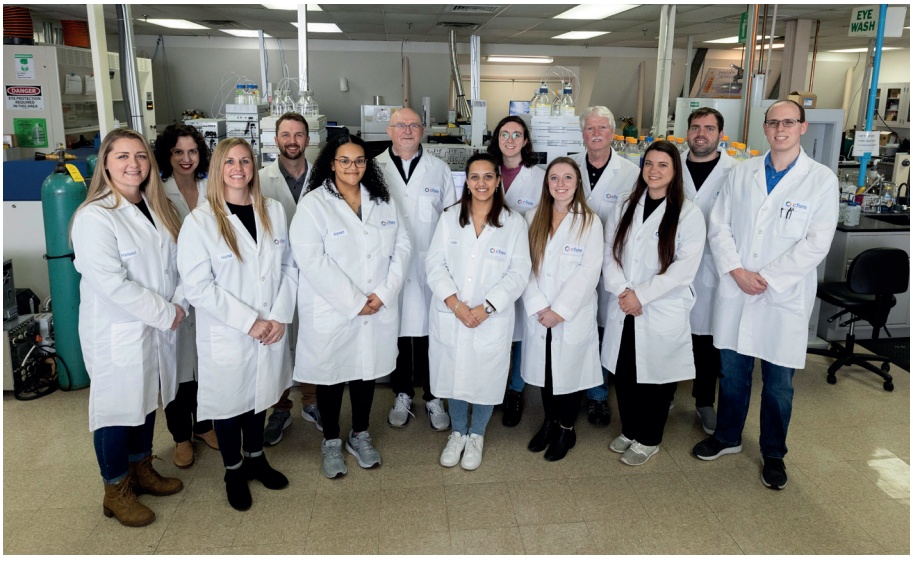
The Center for Forensic Science Research and Education's evidence-based approach to identifying emerging drugs delivers high impact data for real-time action with help from Waters analytical instrumentation and customer support services.

NOVEL PSYCHOACTIVE SUBSTANCE IDENTIFICATION AT THE CFSRE

The Center for Forensic Science Research and Education (CFSRE) is a non-profit, state-of-the-art forensic laboratory located in Willow Grove, PA, working in collaboration with forensic science, public health, emergency medicine and criminal justice agencies to rapidly identify emerging drugs known as novel psychoactive substances (NPS), which are associated with intoxications and adverse events. The CFSRE also conducts research, development, and new technology assessments, and delivers educational and training services for the forensic science community and beyond.

The CFSRE umbrella entity (the Fredric Rieders Family Foundation) was founded by Dr. Fredric Rieders, who also established the privately-owned NMS Labs located in Horsham, PA. The CFSRE's educational programs include short-term placement for visiting scholars and researchers; partnerships to deliver STEM programming for local high school students; and continuing professional development for forensic scientists, law enforcement, and legal professionals.

CFSRE's NPS Discovery program serves as an open-access drug early warning system and flagship program for the identification and characterization of new and emerging synthetic drugs appearing in the United States. The CFSRE's data and results are consolidated into reports and resources to allow for the rapid dissemination of information to colleagues and affected communities, providing the foundation for real-time action by public health and safety stakeholders.



The Center for Forensic Science Research and Education (CFSRE) is a non-profit, state-of-the-art forensic laboratory working to rapidly identify emerging NPS.

WORKING WITH WATERS

Melissa Fogarty, CFSRE Laboratory Manager, has worked with Waters™ since she started at the CFSRE on a grant-funded project over 10 years ago, testing out Waters QTof mass spectrometers and beta testing new Waters software. In addition to method development and validation of NPS, Melissa is responsible for the maintenance of analytical instrumentation and laboratory operations, so she works closely with the laboratory's equipment vendors. Melissa explains how the CFSRE team's work with Waters has evolved:

"NPS identification is challenging, and I appreciate how Waters personnel are there when we need them. They check in with us all the time. Waters support engineers collaborate with us on how to get something to work better or fix tweaks. We also work with Waters by talking to people about how we use their systems. For example, I visited a Pittsburgh hospital with some Waters personnel to share our experience working with the Waters QTof instruments. I think it's relatable to hear the perspective of another scientist instead of a salesperson."

Dr. Alex J. Krotulski, CFSRE Associate Director and Program Manager for NPS Discovery, explains the unique environment of NPS identification:

"NPS first emerged in the recreational drug supply a little over a decade ago, and they have become a common phenomenon in forensic samples. We see different waves of new drugs constantly emerging and disappearing. Nothing is ever consistent, except for some substances that experience sustained proliferation in different drug supplies over time. It's really a very dynamic and, at times, volatile drug market. I often say if you think you know what you know about NPS based on information from three months ago, you probably don't know much about the market. It really changes that quickly."

CFSRE's NPS Discovery program is doing vital research on new drugs that other laboratories often don't have the time or resources to complete.

In their efforts to advance research, education, and outreach in the forensic sciences, the CFSRE has relied on Waters liquid chromatography coupled with quadrupole time of flight (QTof) and tandem mass spectrometry (LC-MS/MS) instrumentation, along with the support of Waters customer service and applications personnel to navigate the ever-shifting challenges in identifying NPS.

CHALLENGES OF NPS

The term "NPS" encompasses a broad class of recreational drugs that have emerged as cheaper alternatives to prescription or illicit drugs. These substances are often produced in clandestine labs without standard quality or regulatory protocols, making them difficult to recognize and comprehend in terms of their chemical structure, composition, and adverse effects. NPS pose great health risks to people who use drugs, including poisoning, mental health disorders, severe damage to the nervous system, need for emergency medical care, and even mortality.

The swift turnover rate is a contributing factor to the challenge of promptly identifying NPS. Once research on the substance is complete, it usually either disappears from circulation or is reformulated to evade legal restrictions. Method development and validation are required for each new drug because every substance has a different chemical structure requiring a new test. Also, establishing the limit of detection for each NPS in a targeted test panel can be arduous and ineffective due to regular modification. Since NPS are found in several forms (*e.g.*, pills, liquid, powders, capsules, gels, sprays, crystals, *etc.*), sample preparation can also be difficult.

Yet, despite these challenges, forecasting trends and monitoring NPS trade and prevalence is vital for better preparedness in terms of education and training for health care professionals and policymakers. As a result, forensic toxicology laboratories like the CFSRE must enhance their testing capabilities and expand their current testing panels to efficiently identify and quantify these substances.

"Our NPS Discovery program alone identifies 30–40 new substances annually in the United States. Depending on the drug class, the life cycle of an NPS is 6–9 months. So, we need to be quick. We don't have the ability to develop new assays for every drug we think may emerge, so we must work smarter. Generally, once we see a new substance, it'll be 2–4 weeks before we can determine whether that drug will be present in other sample populations. Once we make that decision, we have an expedited process to develop and validate an assay within a week. If we know the drug has isomers, or we suspect additional isomers will emerge, it can take longer than a week. But because of our high-resolution MS (HRMS) screening methods, we're able to still sequester cases in that time period when the confirmation method is not yet validated, and then continue testing cases after it is."

DR. ALEX J. KROTULSKI
CFSRE Associate Director and Program Manager for NPS Discovery

CFSRE's NPS Discovery program is doing vital research on these new drugs that other laboratories often don't have the time or resources to complete. As a result, the CFSRE team is filling a huge gap in the identification and knowledge about NPS that serves the entire field.

"We do the legwork for other laboratories that don't have time to do the legwork. Then we take that research and push it out via NPS Discovery, published manuscripts, and presentations such as 'Lunch and Learns' so people can take that knowledge and use it in their own laboratories, without having to use their resources doing something that we can help them with."

MELISSA FOGARTY
CFSRE Laboratory Manager

The CFSRE's ability to do this research is funded from several different sources. Alex describes the variety in their program's stakeholders:

"Our primary funding comes from the National Institute of Justice, which is a program under the Department of Justice here in the United States. We have grants and contracts for our clinical work from the National Institutes of Health and the National Institute of Drug Abuse. We also have different partnerships on the public health side funded ultimately by the Centers for Disease Control and Prevention (CDC) through different public health agencies. We generally have about 20-30 various grants and projects going on at a given time."

IMPORTANCE OF HIGH-RESOLUTION MASS SPECTROMETRY

With about 30 employees and 5,000 sqft. of laboratory space, the CFSRE works with advanced analytical instruments from various vendors for the identification of NPS, including several high-resolution mass spectrometers, LC systems coupled with tandem quadrupole mass spectrometers, and gas chromatograph coupled mass spectrometry (GC-MS) systems. The laboratory analyzes about 1,000 samples/month and has acquired their state-of-the-art instrumentation through the generosity of the Fredric Rieders Family Foundation, as well as partnerships with NMS Labs, Waters and other vendors.

Melissa describes the role of this technology in the NPS Discovery program:

"Our agenda really focuses on isomeric species of NPS and method development and validation on our Waters instrumentation. We focus on the unknowns. Our high-resolution mass spectrometers are critical to our ability to figure out these unknown structures based on the fragments, structural elucidation, and molecular formula. We have a very good collaboration with our vendor, Cayman Chemical, who makes the structure once we figure out what it is. Using all these advanced tools in our laboratory helps us identify these substances quickly and get the information out to the public. We'll also go back and analyze old data if someone else made the first identification of an NPS to see if we had missed it in the past."

Melissa Fogarty, CFSRE Laboratory Manager, started her 10-year relationship with Waters by testing Waters instruments and beta testing software.

One of the advantages of HRMS data is that it can be analyzed retrospectively as new information on NPS emerges. With the Waters HRMS method all the data on all compounds is collected all the time using data independent acquisition. Known compounds are identified by comparing sample data with library data. Unidentified components can be further investigated by powerful discovery workflows using elemental formulae derivation and in-silico fragmentation. Alex explains these benefits:

"High-resolution MS has been a game changer for us. Now we can maintain these comprehensive library screening methods, which allow us to look for more than 1,000 drugs and metabolites in different samples. High-resolution MS also allows us to do unknown identification and structural elucidation."

However, identifying new substances is just the beginning. After Melissa completes the method development and validation process, the NPS Discovery team uses cutting-edge MS technology from Waters for the next steps. Alex explains:

"Eventually, we'll use the method for testing samples, which are primarily blood, but also urine and other biological matrices. We use high-resolution MS to figure out how often we're seeing a substance, then we switch to our triple quad systems to confirm and quantify the drug. Over time, the Waters triple quads have become increasingly sensitive, which helps meet our needs. For example, we're now seeing opioids more potent than fentanyl that require us to look at sub-nanogram per milliliter concentrations. It's good that we've been able to stay on the frontline of that capability with Waters. It's a very cyclic process. After that, sometimes the drug goes away, and we start the process over for a new NPS. It's very non-routine. While sometimes we have lulls, often we have high-priority cases where we need to get results to our forensic and public health partners quickly. There's no time to pat ourselves on the back, though. It's time to move on to the next drug and the next case."

The CFSRE constantly manages lab capacity with careful planning, prioritization, and allocation to ensure that all instruments are used effectively.

For NPS or unexpected compounds for which very limited information is available, the team uses Waters UNIFI Scientific Information System's powerful investigative tools to aid identification. UNIFI highlights any components that have not been identified using the scientific library for further investigation using powerful discovery tools. The elemental composition of the component or components under investigation is automatically calculated and searched against on-line libraries and databases using both precursor and fragment ion data.

"The elemental composition feature in UNIFI that converts the accurate mass data to a formula is one of the best tools we have. It does a great job. It works well with our Waters QTof mass spectrometers to identify unknown peaks, and sets us in the right direction when we are trying to solve complex questions regarding unknown drug molecules."

DR. ALEX J. KROTULSKI
CFSRE Associate Director and Program Manager for NPS Discovery

Like any non-profit, the CFSRE constantly needs to manage lab capacity and equipment to make the most of limited resources. Melissa is responsible for laboratory management, which requires careful planning, prioritization, and allocation to ensure that the instruments are used effectively and efficiently. She also manages regular maintenance and repair to minimize downtime and disruptions to experiments and research.

"We do have schedules on all the instruments, but if something critical comes through, it does take priority. We take those needs into consideration when upgrading equipment. We were functioning for a long time on our single Waters Xevo™ TQ-S Micro. If anything happened to it or it needed maintenance, we were down. We acquired another one in December 2022 to help with those bottlenecks."

MELISSA FOGARTY
CFSRE Laboratory Manager

NATIONAL AND INTERNATIONAL COLLABORATIONS

NPS identification is a naturally collaborative field, and the CFSRE works with many other toxicology labs, medical examiner and coroner offices, crime laboratories, and clinical partners on projects.

"We focus on the esoteric things that other labs aren't testing for yet. Each collaborator has different questions, and they come to us for our chemistry and toxicology expertise. We test all types of toxicology samples, including postmortem samples associated with death investigation, and we also deal with law enforcement on impaired driving cases. From medical examiners, we sometimes get drug materials that are found and recovered from bodies because they're using that information as they try to determine the cause and manner of death."

DR. ALEX J. KROTULSKI
CFSRE Associate Director and Program Manager for NPS Discovery

The CFSRE also holds both a national and global role in NPS research and method development. The NPS Discovery program collaborates with other programs in countries around the world, including the United Nations Office of Drugs and Crime, the European Monitoring Center for Drugs and Drug Addiction, as well as collaborators in Asia, Oceania, and South America.

"Every country and region are a little bit different. The United States certainly has its opioid problem. Europe doesn't generally have the prevalence and the proliferation of novel opioids, but they do see some of them from time to time. Europe sees more synthetic cannabinoids and some types of cathinones. Generally, it depends on the population or area. We often share information back and forth with our European partners because everyone around the world has an interest in what we find – and the drugs often come from a common source, so we are all linked in one way or another."

DR. ALEX J. KROTULSKI
CFSRE Associate Director and Program Manager for NPS Discovery

The CFSRE also works closely with NMS Labs. Melissa explains how the two organizations support each other:

"NMS Labs was Dr. Rieders' for-profit laboratory. They perform routine tests like DUID testing and postmortem testing. We help when they hit a wall or find something new. They are bound by more red tape because they have numerous accreditations across clinical and forensic practice. The setup of our laboratory and its focus on rapid results means we could get results out to a collaborator in a week or so, whereas NMS needs to spend more time making sure they check all the boxes for accreditation before they can offer a test to their clients. Both of our labs and both of our approaches have their benefits and limitations, and in this scenario, we are increasing focus on getting quick and accurate results to support public health and safety the best we can. Also, our NPS Discovery program gets discarded and deidentified samples from NMS, and we recheck them with our larger library to see if there's anything else in the sample – we think of this as a new type of drug surveillance."

The CFSRE has relied on Waters instrumentation to navigate the ever-shifting challenges in identifying NPS.

NEXT STEPS

The rapid pace of technological change can make it challenging for forensic toxicology laboratories to keep up with new equipment, software, and techniques. Keeping abreast of new developments and incorporating them into laboratory workflows requires ongoing investment and training. The CFSRE has plans to expand its capabilities, as well as its size, when it moves to a new building in 2024. Melissa describes the impact the new laboratory will have on the center's research:

"Right now, we're at max capacity in the lab. We will have a lot more room in the new laboratory for more instrumentation and more personnel. As we grow and get larger grants and contracts, we are going to keep putting the latest and greatest technology into our laboratory to make sure that we can continue staying out on the cutting edge and providing the best resources we can."

Melissa is particularly interested in adding new analytical tools to the laboratory's resources, and she hopes to start testing the Waters SELECT SERIES™ Cyclic IMS™, which combines novel cyclic ion mobility separation with state-of-the-art high performance, time-of-flight MS.

"Ion mobility could help us with isomeric species, which are the most challenging compounds. It can take a lot of time to pull those apart and get into the nitty gritty of the fragmentation patterns. We can achieve that with our current instrumentation, but it takes longer. I'm eager to see how the Waters SELECT SERIES Cyclic IMS could help us speed up that process."

MELISSA FOGARTY
CFSRE Laboratory Manager

Most of all, the upcoming laboratory expansion and investments in new analytical technology provides new possibilities for the CFSRE when it comes to pushing the boundaries of NPS identification. Alex explains:

"We've been fortunate through our great collaborations to remain at the forefront of forensic toxicology analysis. That's evident in our ability to push the envelope as it relates to high-resolution MS. Now we're often used as a resource by other labs trying to understand how to implement high-resolution MS in their testing workflows, or with questions about data processing or the legal process. We feel like we've really contributed to moving this field forward, particularly when it comes to the technology advances and increases in sensitivity and specificity. We love what we do – we're out in front and helping other labs all the time."

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