

Front & Center

Detecting Novel Psychoactive Substances in Forensic Samples: Challenges and Opportunities for Analytical Tools

The availability and use of novel psychoactive substances (NPS) has increased rapidly in the last decade, although the market may now be stabilizing to some extent. With new substances surfacing at an alarming rate in recent years, national and international early-warning systems have been established across the globe to help authorities stem the tide of illicit drugs on the black market. LCGC recently spoke with Craig McKenzie, PhD, a senior lecturer in forensic chemistry and co-director of the newly established Centre of Excellence in New Psychoactive Substances Research at the University of Dundee, to delve deeper into the unique analytical challenges and threats posed by NPS.

What Are NPS?

The United Nations defines NPS as “substances of abuse, either in a pure form or a preparation, that are not controlled by the 1961 Single Convention on Narcotic Drugs or the 1971 Convention on Psychotropic Substances, but which may pose a public health threat.” Many of these so-called “new” substances were actually first synthesized many years ago, often as a result of academic studies or as pharmaceutical drug candidates and these are now appearing on the illicit drugs market, although many are also previously unknown.

These emerging drugs (also known colloquially as “designer drugs,” “internet drugs,” “bath salts,” and “legal highs”) pose health risks and create similar



mind-altering effects as traditional illicit substances such as cannabis, cocaine and heroin. They have different chemical structures, sometimes differing only in single atoms or minor configurational changes, in an attempt to evade law enforcement. For legal purposes, their structures need to be compared to certified reference standards to confirm their identity, making analytical work extremely challenging.

As NPS and precursor materials for NPS are banned and seized by authorities, newer redesigned NPS quickly emerge, creating a difficult-to-break and fast-moving cycle where improved analytical methods for identifying NPS and their metabolites in both biological and seized drug samples are desperately needed.

The Needle in the Haystack Is Also a Moving Target

Identifying unknown targets is one of the most complicated analytical challenges for forensic laboratories attempting to detect NPS in seized drugs and biological

samples. Says McKenzie, “If you don’t know what you’re looking for, how do you find it?” In biological samples we also often need to understand the formation of metabolites of the parent drugs and their detection in urine or blood samples.

Whereas traditional controlled drugs are well characterized, the same cannot be said for many NPS. The structure of a newly detected NPS frequently does not match any reference standards held by the laboratory, or those reference standards may not be commercially available. Often analysts comb through secondary databases and libraries searching for appropriate comparisons—if any exist at all.

To determine the molecular structures of such unknown NPS, high resolution mass spectrometry (HRMS) and nuclear magnetic resonance (NMR) spectroscopy are becoming increasingly important tools for forensic laboratories with NMR being applied to seized powder samples and high resolution mass spectrometry being applied to the analysis of biological

matrices as a screening tool. If a new substance is detected it can be purchased as a certified reference material if available or, if not available, synthesized in-house if possible, to confirm the NMR and HRMS findings.

The standard analytical approach varies by sample type, too. To analyze a powder sample, thin layer chromatography followed by gas chromatography-mass spectrometry (GC/MS) is the typical approach if compounds are contained within mass-spectral libraries. For blood or urine samples, the standard technique has been targeted MS/MS. Although this method can confirm the presence of a known compound and quantify it, it does not reveal what else (i.e., unknown targets and metabolites) might be present in the sample.

HRMS instruments, such as Quadrupole Time-of-Flight (QToF), offer many benefits to forensic laboratories. Since these instruments can be used for non-targeted acquisitions, a lot of information is collected that may be useful after the time of the initial analysis. With HRMS, one can retrospectively investigate data from a sample, for example, as information on new substances or metabolites becomes available.

With HRMS instruments, analysts can also derive the elemental composition from accurate mass measurements. "In our work, we usually don't have reference standards for the metabolites. But knowing the structure of the parent drug and with high resolution accurate mass data on molecular and fragment ions from the QToF, allows us to work out which elements are likely to be present and therefore predict chemical structures of drug metabolites. That's one of the greatest benefits of high-resolution MS."

Another major challenge is the rapid rate of change. To evade law enforcements, those that illegally design and synthesize NPS frequently change their chemistry. As an NPS becomes internationally controlled (banned), chemists will change the structure simply by adding, for example, a fluorine or methyl group. "And suddenly, the forensic chemists need to make a whole set of new reference stan-

dards again and carry out new metabolite identification studies," says McKenzie. This is where the ability to retrospectively analyze HRMS data is a valuable tool for forensic scientists.

A related problem for forensic laboratories is that some NPS are extremely potent at low concentrations. Fentanyl analogues, for example, which are often, in some jurisdictions, combined with other substances such as heroin, represent only a very low percentage of the mixture, yet are very active in the body. Extremely sensitive methods are required to detect them in biological fluids. Says McKenzie, "Analysts need methods that can also look for a needle in a haystack. Sometimes you have less than 1% of a fentanyl analogue within heroin, but it is still very active. It can be challenging to detect and of course concentrations within body fluids will be extremely low."

An Informatics Intervention

MS informatics is becoming vitally important to the identification of NPS, according to McKenzie. The sheer volume of data collected by high resolution mass spectrometer instruments requires the use of sophisticated techniques to filter out the noise. Informatics automates the otherwise time-consuming process of sifting through massive amounts of data, for example, manually checking a data set against reference libraries.

When screening compounds, NPS that are already well known and for which standards have been made available and which are included in targeted screens can be identified fairly easily using triple quadrupole MS/MS systems and can also be accurately quantified. However, informatics which allows for much faster and automated screening to find NPS for which limited information is available is needed. For example, the ability to look for theoretical fragment ions using mol. files is very valuable to forensic toxicology laboratories. In addition the ability to look for characteristic fragments and neutral losses that may be indicative of a class of NPS is a valuable tool. This becomes extremely important when looking for

drugs like fentanyl analogues or synthetic cannabinoids. Hundreds of synthetic cannabinoids and many different fentanyl analogues are being identified in casework and there are many more theoretically possible structures which may come onto the illegal drugs market in future. Compounds that meet predetermined criteria can be noted as being present in the sample with high confidence. Others may need much more investigation and interpretation. Informatics, such as Waters UNIFI Scientific Information System, helps analysts using high-resolution MS to extract relevant information from large amounts of data and to perform structural elucidation with more confidence.

The Future

To deal with the challenge of finding the unknown, McKenzie says the work of the team at the University of Dundee involves horizon scanning, which monitors internet forums where users are talking about new drugs. "We are looking for emerging substances that may not have gained a foothold yet," he explains. "We look at patents, just as illegal drug manufacturers are doing. We try to predict what drugs might appear next in the illicit market, and then we obtain or prepare these potential targets. At that point, we have enough material to fully characterize the drug analytically using a wider range of analytical techniques from ATR-FTIR to UPLC-QToF and 2D-NMR."

The Dundee team and their collaborators also try to determine a substance's unique metabolites and biomarkers and plan to prepare metabolite standards to assist in future analyses and pharmacokinetics studies. McKenzie and his colleagues believe that as the worldwide community is trying to keep pace with emerging NPS, information sharing and pooling of resources is essential. Also critical is "understanding what technological and other tools are needed now and what will be needed in the future. To explore this they are working in partnership with companies like Waters Corporation to help develop new technologies and new informatics tools."