# The Evolving Science of Drug Detection

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Science is continuously looking for ways to explain what is happening in the natural world. It is an evolutionary process where theories evolve into fact or are disproven by new research findings produced by new analytical methods and technology. Even a cursory review of the scientific literature shows almost a weekly variety of newly developed instrumentation or analytical procedures to answer important questions in fields such as physics, chemistry, and genomics.

This evolutionary process also affects forensic toxicology, the study of the effect of toxins on living organisms and its application to medicolegal investigations. Forensic toxicologists, like those at the Civil Aerospace Medical Institute (CAMI) in Oklahoma City, OK, are called upon to analyze biological samples for the presence of drugs or poisons and then interpret their findings in legal settings. New drugs, both licit and illicit, hit the streets of the United States every week. Pharmaceutical companies develop the newest antihistamine or pain reliever. The older players of the illicit drug world like cocaine and methamphetamine are still prevalent in significant numbers. Recent years have seen the emergence of compounds like synthetic cannabinoids, bath salts, and, more recently, the dangerous fentanyl and its analogs.[9,11] With the continuing legalization of both medical and recreational marijuana, research efforts are focused on better understanding the pharmacology and analysis of cannabinoids such as delta-9-tetrahydrocannabinol (THC), the major psychoactive component of the cannabis plant.[8,12]

Before any conclusions can be reached about the distribution of a drug in the body or the meaning of the presence of a drug metabolite, there must be a method of detection. In fact, for drug-positive medicolegal cases, like those CAMI deals with in aviation accident investigations, there must be a positive screening method followed by a more specific confirmatory test. Each of these methods must be able to detect nanogram (1 billionth of a gram) concentrations of the compounds of interest in a variety of specimen types.[4] The techniques must be accurate, precise, and repeatable, not an easy task when one considers that the specimens to be tested are complex matrices like decomposing fluids and tissues from the body of a deceased accident victim.

Forensic toxicology laboratories, like that found at CAMI, must be equipped with the latest technologies that are able to detect minute quantities of a drug or metabolite. In addition, new analytical methods to separate the drugs of interest from the sample matrices are required to keep up with ever-changing trends in drug use. This article will discuss two drug issues that have arisen in recent years and the technologies that have emerged to assist toxicologists with their mission of detecting these compounds, providing accurate results and comprehensive interpretative reports for their clients.

### Two Recent Drug Trends

Synthetic cannabinoids (SC) are a group of compounds that elicits effects similar to the primary psychoactive component of the cannabis plant, THC.[3] Known by street names such as "K2," "Black Mamba," and "24K Monkey," they have been used in the United States since approximately 2010. These chemicals were originally developed as research tools to investigate the endogenous cannabinoid system in humans and to explore their potential for therapeutic applications.[10] They eventually reached the streets, however, and are abused for their marijuana-like effects. As a result of having a stronger affinity for the cannabinoid receptors, the SCs are 2–100 times more potent than natural THC. Many of the cannabinoid-like (cannabimimetic) effects, therefore, are more intense and may result in serious adverse events, including cardiovascular events, neuropsychiatric disorders, seizures, and even death.[6,7,16]

Another significant drug problem has been the use and abuse of fentanyl, a potent, fast-acting synthetic narcotic analgesic marketed as an adjunct to surgical anesthesia since 1963.[2] It easily crosses into the brain from the blood, where it causes significant central nervous system effects through binding with opiate receptors (50–100 times more potent than morphine). It is used to treat severe, surgical, or chronic pain and is available

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legally in oral, sublingual, injectable, and transdermal forms. The abuse of fentanyl and its more potent analogs (together known as "fentanyls") has increased significantly in recent years due primarily to illicitly manufactured forms. In just 2 years, from 2013–2015, synthetic opioid overdose deaths tripled, with fentanyl and its analogs playing a major role in the crisis.[11] In 2016, carfentanil, an analog of fentanyl 5000 times more potent than heroin, killed 25 people within a 30-d period in Northeast Ohio.[14]

In the case of both synthetic cannabinoids and fentanyl, there are analytical challenges for the toxicology laboratory. The doses administered are quite low, requiring highly sensitive methodologies to detect them in biological fluids and tissues. There are a number of SCs and fentanyl-related drugs that are being abused. Thus, for efficiency, comprehensive methods are needed to detect a variety of these compounds in a single test. Lastly, each of these drug classes feature short half-lives; that is, the parent drug is metabolized and excreted rapidly. The preferred methods, therefore, should be able to detect the ingested drug as well as the by-products of metabolism (metabolites) to confirm the use of the SCs or fentanyl.

### Developing Methods of Detection

Immunoassays, as the term implies, exploit the immune response of antibodies to chemical antigens for detecting drugs or metabolites in biological samples. The first immunoassays were developed in the 1950s to evaluate the human antibody response to bovine insulin used in the treatment of diabetics.[13] Over the years, immunoassays have developed into an integral part of analytical toxicology, detecting drugs or their metabolites in nanogram concentrations. Enzyme mediated immunoassay technique and Enzyme linked immunosorbent assay are the most common systems in use today. The tests are relatively inexpensive and can test a large volume of samples quickly due to the availability of automated instrumentation. However, most immunoassays are relatively nonspecific, meaning the antibodies not only detect the target analyte, but they also "cross-react" with structurally similar compounds present in a sample. As a result, a drug, the drug's metabolites, and even nondrug related chemicals may a produce a positive result. Immunoassays, therefore, are routinely used for initial screening, to be followed by more specific testing that will confirm the identify of, and if necessary, quantify the specific analytes of interest.

There is no one immunoassay, however, that detects all SCs and fentanyls. For example, despite some similarity in chemical structure and activity to THC, screening with routine immunoassays designed to detect marijuana use have been unsuccessful in detecting SCs. A number of new immunoassays have now been developed that are able to find specific SCs and their metabolites.[1,9] As with the SCs, it was discovered that the fentanyls are not detected by the more routine opiate immunoassays and thus require specialized tests to be developed. Today, specific immunoassays have been developed for fentanyl and its analogs.

A more specific analytical approach uses chromatography coupled with mass spectrometry.[15] Chromatography is a process by which a complex mixture of compounds is separated as they flow along a hollow tube, or "column." The compounds reach the detector (the mass spectrometer) at a recorded time and then are shattered, producing a compound-specific spectral pattern of ion fragments. Thus, the compounds can be identified by the recorded time and their fragment pattern when compared with known standards. A number of methods have now been developed using gas chromatography/mass spectrometry (GC/MS) or liquid chromatography/mass spectrometry (LC/MS) for both the SCs and the fentanyls. Liquid chromatography/mass spectrometry is rapidly becoming the gold standard for the identification and quantitation of these and other drugs. More recently, methods using multiple MS systems (e.g., LC/MS/MS) have been developed to achieve even lower limits of detection.[5,14] Some issues for toxicology laboratories are that these methods can be time consuming, expensive, and require a greater level of expertise. They are necessary, however, to detect the small concentrations of drugs and metabolites in biological samples.

### Conclusion

As can be seen by the two examples of SCs and fentanyl, the analysis of drugs in today's forensic toxicology laboratories requires not only the most sensitive instrumentation but also a highly trained staff to operate them. The investment of personnel and instrumentation funding is worth it, however. Drug development and research, drug treatment programs, workplace drug testing, and forensic investigations all benefit from the ability of the toxicology laboratory to analyze complex specimens for complex chemicals. In the case of aviation safety, it is imperative that this be the case, as the flying public depends on it.

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