Newly Emerging Drugs of Abuse and Their Detection Methods

An ACLPS Critical Review

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ABSTRACT

Objectives: *Illicit drug abuse has reached an epidemic level in the United States. Drug overdose has become the leading cause of injury-related deaths since 2008 due to the recent surge of opioid overdose by heroin, controlled prescription drugs, and nonmethadone synthetic opioids. Synthetic designer drugs such as synthetic cathinones ("bath salts") and synthetic cannabinoids ("Spice" and "K2") continue to emerge and attract recreational users.*

Methods: *The emergence of new drugs of abuse poses a steep challenge for clinical toxicology laboratories. Limited information about the emerging drugs and their metabolism, "rebranding" of the illicit drugs, and a lack of Food and Drug Administration-approved screening methods for these drugs contribute to this difficulty. Here we review detection methods that can aid in identifying emerging drugs of abuse.*

Results: *One promising approach is the utilization of untargeted drug screening by mass spectrometry. Historically, gas chromatography-mass spectrometry has been the gold standard.*

Conclusions: *Liquid chromatography-tandem mass spectrometry and liquid chromatography-high-resolution mass spectrometry offer improved detection capability of new drugs with simplified sample preparation, making it the new standard.*

Case Descriptions

Case 1 was a 25-year-old woman with medical history of polysubstance abuse who was found unresponsive in her home. She regained consciousness after receiving naloxone intramuscularly and was brought into the emergency department. She admitted to taking "Percocet" and "Molly." The initial urine drug screening immunoassay panel was positive for opiate and oxycodone [Table 1], whereas gas chromatography–mass spectrometry (GC-MS)-based untargeted urine comprehensive drug screening detected oxycodone and ethylone. 3,4-Methylenedioxy-N-methylamphetamine (MDMA or "Molly") was not detected.

Case 2 was a 33-year-old man with medical history of polysubstance abuse who became unresponsive and was brought into the emergency department. He regained consciousness after receiving naloxone intramuscularly. He admitted to taking 10 bags of "heroin" intravenously before losing consciousness. The initial urine drug screening immunoassay panel was negative (Table 1); however, the GC-MS–based untargeted urine comprehensive drug screening detected fentanyl and methylnorfentanyl (a metabolite of 3-methylfentanyl), but not heroin or its metabolites.

In these cases, patients histories appear discordant with laboratory findings, but these are typical scenarios that reflect the current drug abuse epidemic and emerging drugs of abuse. In this article, we will review not only

Table 1
The Results of the Urine Drug Screening Immunoassay Panel in Case 1 and Case 2[a]

Screening Test	Case 1	Case 2
Amphetamine (1,000 ng/mL)	Negative	Negative
Barbiturate (200 ng/mL)	Negative	Negative
Benzodiazepine (200 ng/mL)	Negative	Negative
Buprenorphine (5 ng/mL)	Negative	Negative
Cocaine metabolite (300 ng/mL)	Negative	Negative
Opiate (300 ng/mL)	Unconfirmed positive	Negative
Oxycodone (100 ng/mL)	Unconfirmed positive	Negative
Phencyclidine (25 ng/mL)	Negative	Negative
THC metabolite (100 ng/mL)	Negative	Negative

THC, tetrahydrocannabinol.
[a]Urine specimens from cases 1 and 2 were analyzed by Syva EMIT II Plus Assays (amphetamine barbiturate, benzodiazepine, cocaine metabolite, opiate, phencyclidine, and THC metabolite) (Siemens, Munich, Germany). Urine enzyme immunoassays (buprenorphine and oxycodone) (Immunalysis, Pomona, CA) were analyzed on the VIVA E analyzer (Siemens). The cut-off levels of each assay are provided in parenthesis in the table.

these issues, but also the challenges and limitations of current laboratory testing as it relates to emerging drugs.

Overview of Drug Abuse Epidemic in the US

The abuse of illicit drugs, including newly emerging drugs of abuse, poses a serious threat to public health, not to mention a great challenge to the health care system. In the past several years, the incidence of drug abuse and drug overdose death has rapidly increased, reaching epidemic levels. The number of deaths caused by drug overdose has surpassed that caused by motor vehicles accidents and firearms since 2008, becoming the leading cause of injury death. In 2015, the Centers for Disease Control and Prevention (CDC) reported 52,404 deaths, an 11.4% increase from 2014, as a result of unintentional overdose, with more than 60% (33,091) attributed to opioids. For the first time, deaths from heroin and nonmethadone synthetic opioids including illicitly obtained fentanyl and its analogues, surpassed deaths related to prescription opioids.[1]

Heroin abuse has surged since 2007, with the number of users almost tripled from 161,000 in 2007 to 435,000 in 2014.[2,3]The number of deaths involving heroin overdose increased 248% between 2007 and 2014. This is partly driven by the increasing availability of heroin in the US, and consequently some prescription drug abusers convert to heroin as a cheaper alternative.[2,3]

Abuse of controlled prescription drugs (CPDs) poses another major threat. This is mainly due to the diversion of prescription opioid analgesics, most commonly those containing oxycodone and hydrocodone. The number of deaths attributable to CPDs has outpaced that of cocaine and heroin combined since 2002; and in 2014 alone, the number of drug overdose deaths involving CPD reached 25,760, compared to that of heroin (10,574) and cocaine (5,415).[2,3]

As a strategy to reduce the abuse of CPDs and protect the health and safety of our community, state-run programs called prescription drug monitoring programs have been launched in every US state. Prescription drug monitoring programs collect and maintain information on all filled prescriptions for controlled substances in a searchable database for use by prescribers and providers. This statewide electronic database has been shown to decrease drug diversion and to improve patient safety.[4] Periodic urine drug testing for prescribed pain medications is another way to monitor patient compliance.[4] Currently, many reference toxicology laboratories offer quantitative drug screening results with interpretation (eg, medMATCH at Quest Diagnostics, Secaucus, NJ) to detect consistency or inconsistency of the drug screening results with the patient's prescribed medications, allowing clinicians to easily detect the abuse of CPDs. The combination of these strategies may effectively curtail prescription drug diversion and CPD abuses in the future.

While the above-mentioned "old drugs" still play major roles in the abuse drug market, novel substances such as synthetic designer drugs continue to emerge and attract many recreational users. The most common designer drugs include synthetic cathinones, commonly known as "bath salts," and synthetic cannabinoids, also known as "Spice" and "K2." These newly emerging drugs are easily accessible, causing harmful health consequences and presenting an ongoing challenge for clinical toxicology and forensic laboratories.

According to the 2016 Drug Enforcement Agency (DEA) Emerging Threat Report, 2,679 identifications of psychoactive compounds were made among seized and analyzed drugs by the DEA's laboratory system. Among them, 1,299 identifications (48.4%) fall in opioids, 984 identifications (36.7%) fall in synthetic cannabinoids, and 347 identifications (13.0%) fall in synthetic cathinones. Among the 1,299 opioid identifications, fentanyl is comprised of 877 identifications (67.5% of opioid identifications).[5] These statistics underline the significance of the threat by emerging drugs of abuse.

Overview of Emerging Drugs of Abuse

Synthetic Cathinones ("Bath Salts")

Amphetamine is a potent and prototypical central nervous system stimulant. It is medically used for attention deficit hyperactivity disorder and narcolepsy, but its analogs, such as methamphetamine and MDMA, are abused worldwide. Cathinone, with an extra β-keto group to the amphetamine structure, is an active compound in *Catha edulis* (khat), a flowering plant indigenous to the Horn of Africa and the Arabian peninsula.[6]

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Synthetic cathinones, known as "bath salts" or "legal high," are the new designer stimulant drugs of the 21st century. They emerged around 2007 in the US, and their popularity quickly gained and peaked in 2011. Since then, their use showed a slow decline, with 522 poison control center calls for exposure in 2015, representing a 10% decrease from the 582 calls in 2014.[2,3] These drugs have been "rebranded" or falsely represented as "MDMA" or "Molly"; therefore recreational users may consume synthetic cathinones unknowingly, and the data about the usage of synthetic cathinone may be underrepresented.[2]

More than 100 different synthetic cathinone compounds have appeared on the underground market and sold as "bath salts" or "plant food" and labeled "not for human consumption" in order to circumvent abuse drug regulations. The commonly abused synthetic cathinones include, but are not limited to, ethylone, 3-fluromethcathinone (3-FMC), 4-FMC, methedrone, methylenedioxypyrovalerone (MDPV), methylone, pentylone, and pyrovalerone **Figure 1**. These drugs cause amphetamine-like sympathomimetic effects, including tachycardia and hypertension, as well as psychoactive effects such as euphoria, increased alertness, and violent behavior. Cardiac arrest, rhabdomyolysis, acute kidney failure, and death have been reported following use.[6]

In 2015, there was a dramatic increase in the overdose cases related to a new synthetic cathinone alpha-pyrrolidinopentiophenone [also known as alpha-pyrrolidinovalerophenone (α-PVP) or "Flakka"]; in Florida.[2] "Flakka"-associated psychosis (eg, hyperstimulation, paranoia, hallucinations, and violent aggression) and cardiotoxicity have been reported.[7-11]

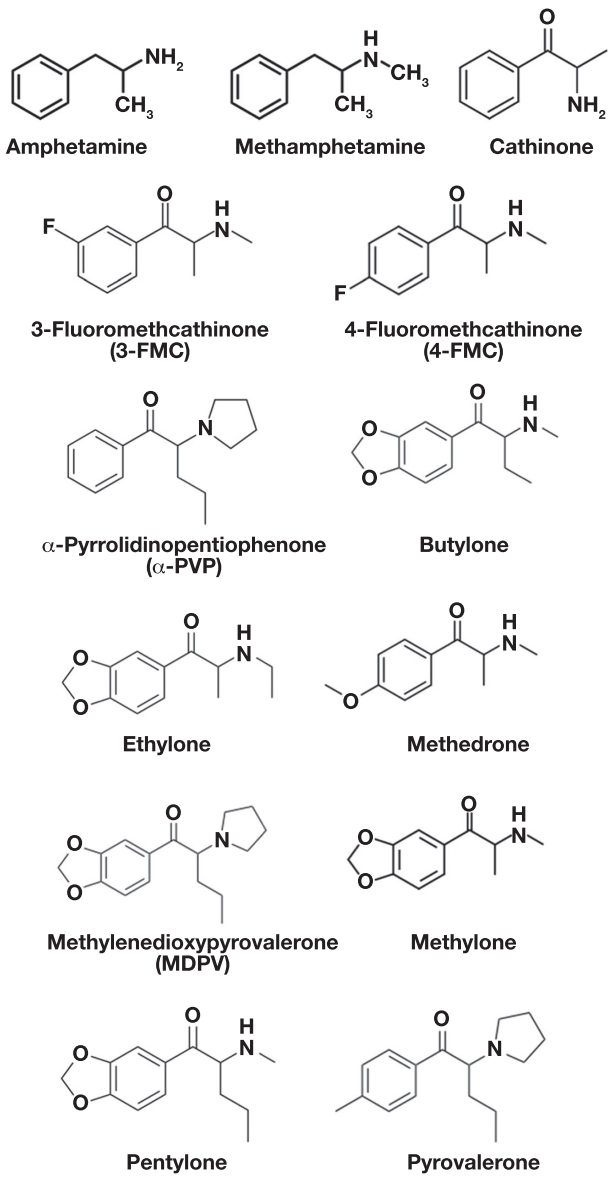

Figure 1 Chemical names and structures of synthetic cathinones.

Synthetic Cannabinoids ("Spice")

Various synthetic cannabinoid receptor agonists were originally developed through pharmacological studies of the receptors, but these compounds have subsequently been produced by illegal laboratories and sold as herbal incense products such as "Spice" or "K2".[12-14] These products started to emerge in the US in 2008 and quickly gained popularity among adolescents and young adults as legal alternatives to marijuana because of their psychoactive effects and elusiveness in routine drug screening.[15] Their availability and usage have also been increasing recently. In 2015 there were 7,779 calls to poison centers across the country regarding synthetic cannabinoid exposure, which is a 111% increase from the 3,682 calls in 2014, and is the highest number of calls ever recorded since these drugs first appeared on the recreational drug market[2,3]

Synthetic cannabinoids are two to 100 times more potent than tetrahydrocannabinol (THC) because both the parental compounds and their metabolites are potent agonists of the THC receptors, as opposed to having partial agonist effect like traditional marijuana.[16] As cannabinoid receptor agonists, they can cause similar psychoactive effects such as elevated mood or relaxation; however, severe acute toxicity including agitation, delirium, psychosis, and death have been reported due to increasingly potent pharmacological effects.[2,15]

Synthetic cannabinoids are not structurally related to the THC or the "classic" cannabinoids. The first generation of "Spice" (eg, JWH-018 and 073, JWH 250, and CP 47,497) was quickly replaced with the next generation (eg, AM-2201, XLR-11, AB-CHMINACA, and AKB48 [APINACA]) **Figure 2**,[17] which has caused outbreaks of severe intoxication or death.[18]

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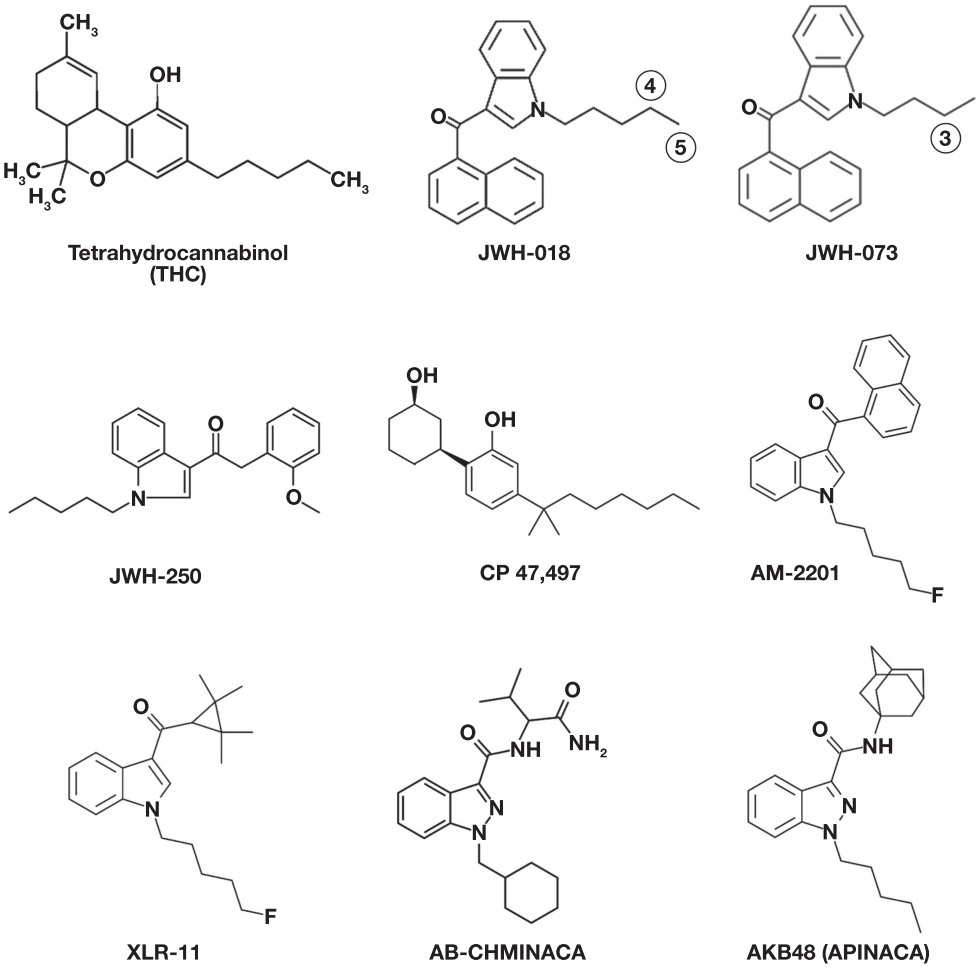

Figure 2 Chemical names and structures of natural and synthetic cannabinoids. Both JWH-018 and JWH-073 are metabolized through hydroxylation at ④ [JWH-018 N-(4-hydroxypentyl) metabolite, ⑤ [JWH-018 N-(5-hydroxypentyl) metabolite], or ③ [JWH-073 N-(3-hydroxypentyl) metabolite].

Opioids

Fentanyl-Tainted Drugs and Fentanyl Analogs

Fentanyl abuse is currently exploding across the US. As a prescription synthetic opioid and a Schedule II controlled substance, fentanyl was originally developed in the 1960s as an analgesic for pain management in cancer patients. Its potency is approximately 80 to 100 times higher than morphine and 25 to 40 times higher than heroin at the μ opioid receptor. This powerful opioid activity has made it an attractive drug of abuse. Fentanyl users experience an intense but temporary feeling of euphoria. Adverse effects include a dangerous reduction in respiration and hypoxemia, which may result in hypotension, fainting, anoxic brain injury, seizures, and death.[19,20] Recent reports have shown a dramatic increase in fentanyl-related deaths since 2010.[21-23]

Fentanyl and its analogs such as acetylfentanyl, butyrylfentanyl, and 3-methylfentanyl **Figure 3** have been used as adulterants in not only heroin but also in cocaine.[3] Unlike fentanyl, its analogs have no licensed medical use but have similar or greater potency at the opioid receptor, leading to life-threatening respiratory depression. Fentanyl and its analogs, sold as heroin or instead of heroin, are widely available and greatly increase the risk of overdose death. According to the 2016 DEA Emerging Threat Report, of the 15 synthetic opioids identified in seized drug specimens, nine were reported for the first time in 2016. Submitted samples included fentanyl and its analogs being sold alone or in combination with heroin.[5] The DEA reported that fentanyl and its analogs were responsible for more than 700 deaths across the US between late 2013 and late 2014.[2] Data from the CDC indicate that the number of deaths attributable to fentanyl and its analogs has increased significantly since that time and is continuing to rise.[1]

AH-7921 and U-47700

Both AH-7921 and its structural isomer U-47700 (Figure 3) were developed as structurally unique synthetic

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opioid analgesics in the mid-1970s but were never subjected to clinical trials.[24-26] AH-7921 is as potent as morphine,[24] but U-47700 is 7.5 times more potent than morphine in animal models.[27] The recreational use of these compounds was first reported in 2012.[28] Since then, several fatalities secondary to overdose of these compounds have been reported.[29-31]

Mitragynine

Mitragynine Figure 4 is the major psychoactive alkaloid of the plant kratom, which is indigenous to Southeast Asia. While illegal in some countries, mitragynine started to emerge in the US as a legal psychoactive product available online. Pharmacological studies have shown that

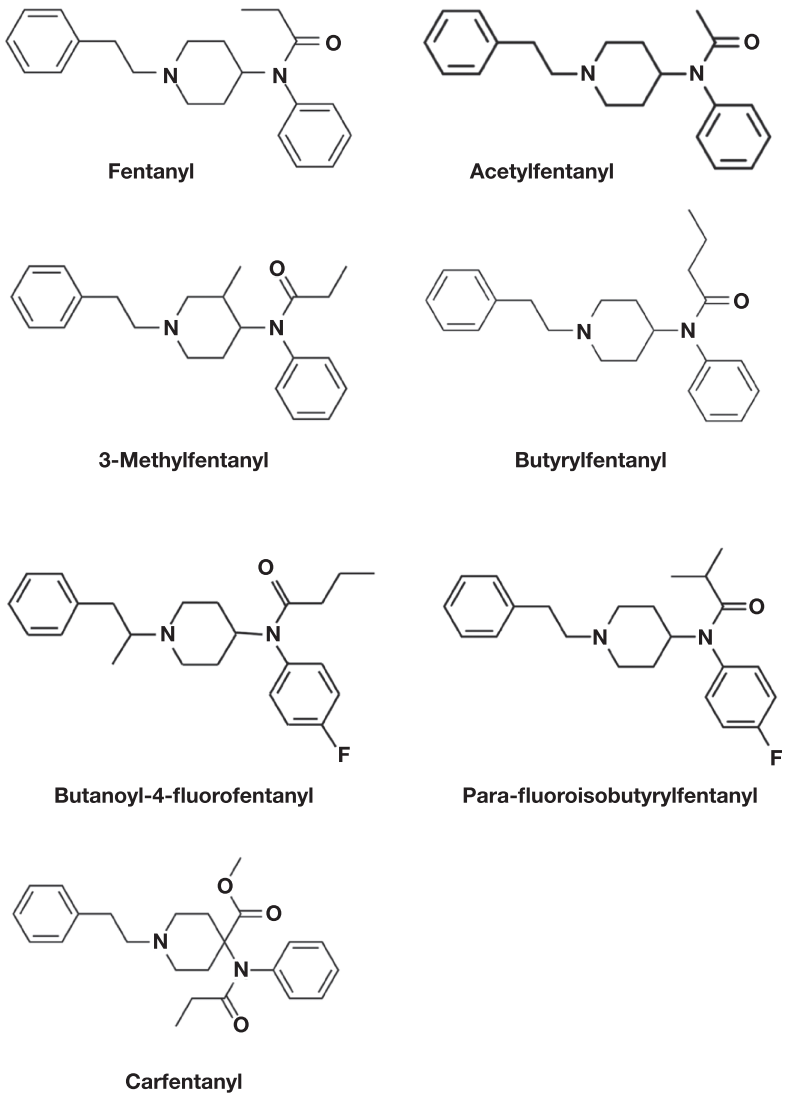

Figure 3 Chemical names and structures of fentanyl and fentanyl analogs.

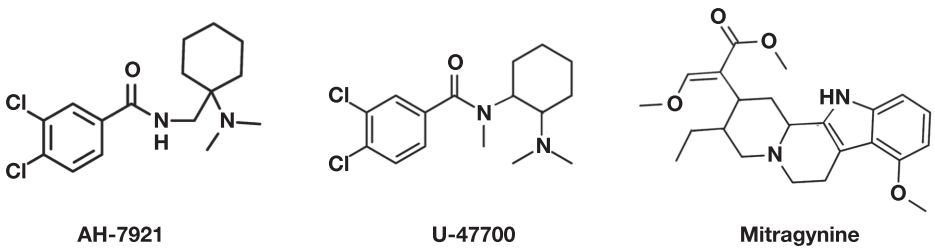

Figure 4 Chemical names and structures of AH-7921, U-47700, and mitragynine.

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mitragynine produces stimulant effects at a low dose, while sedative narcotic effects at a high dose by acting as a selective and full agonist of the μ-subtype opioid receptor. Traditionally, mitragynine has been used in Eastern medicine to treat a variety of ailments.[32] In the US, mitragynine has been used recreationally as well as a means to treat symptoms of opioid withdrawal outside of established medical programs.[33] Toxicity from use can include agitation, as well as sedation. 7-Hydroxymitragynine is a minor constituent of kratom, but it demonstrates potency 46 times higher than mitragynine.[34]

Regulatory Aspects and Laboratory Detection of Emerging Drugs of Abuse

All of the aforementioned compounds except fentanyl have been listed as Schedule I controlled substances (no currently accepted medical use) under the United States Controlled Substances Act. However, as emerging drugs are identified and scheduled, new analogs or derivatives of existing drugs are developed to circumvent regulation more rapidly than law enforcement and regulatory agencies are able to respond. Similarly, laboratory detection capability always lags behind the pace of emergence of new drugs due to the analytical chemistry work required to develop a reliable method of accurate identification. In the next section, we will discuss the laboratory detection of these newly emerging drugs.

Overview of Urine Drug Testing

Drug Testing Methods

Urine is the preferred biological specimen for drug screening because drugs are more concentrated in urine than plasma, which prolongs the drug detection window. Other biological specimens, including blood, saliva, and sweat, can also be used for drug testing.[35] For most clinical and forensic applications, initial testing is conducted with immunoassay panels specific for classes of drugs with similar structures. They are qualitative or semiquantitative tests to evaluate the presence or absence of a substance based on a preestablished cut-off. Definitive identification of a specific drug and/or its metabolite(s) requires more sophisticated tests with mass spectrometry (MS), coupled with either gas or liquid chromatography (GC and LC, respectively).

Immunoassays use antibodies developed to react with epitopes in the target compounds to detect drugs and/or their metabolites. The major advantages of immunoassays include fast turnaround time, simplicity of the assay procedures, wide availability of immunoassay platform (either analyzers and/or point of care), and ability to detect multiple drugs within the same class, whereas the major disadvantage is their limited specificity and sensitivity potentially leading to false positive or negative results. That is why the positive results of antibody-based drug screens are considered "presumptive" or "unconfirmed" positive until the test results are confirmed by more specific MS-based assays. Likewise, the absence of a positive test does not definitively eliminate the potential presence of a drug in the same class or with similar pharmacologic activity.

The classes of drugs commonly covered in the urine drug screening by immunoassay include amphetamines, barbiturates, benzodiazepines, buprenorphine, cannabinoids (THC), cocaine metabolite, methadone, opiates, oxycodone, and phencyclidine assays.[36-38] An immunoassay kit specific to 6-monoacetylmorphine (heroin-specific metabolite) (Immunalysis, Pomona, CA) has been approved recently by the US Food and Drug Administration (FDA) for clinical use. This kit detects heroin use without cross-reactivity to morphine, morphine metabolites, and many common analgesics. These assays are available as kits that can be applied on different automated analyzers. Generally, most of these immunoassays are offered as one immunoassay panel.

GC-MS or LC-MS(-MS)-based assays are analytical techniques regarded as more definitive in identifying specific drugs. In these methods, the mixture of compounds within the specimen is separated first by chromatography and then further interrogated by MS. The direct coupling of MS with GC was first developed in the 1970s, dramatically improving both sensitivity and specificity of the analysis of the mixture of compounds.[39]

GC-MS has long been used as a gold standard method for toxicology testing.[39]As the name suggests, the gaseous phase chromatographic separation takes place in a heated oven. Thus, the analytes must be small and nonpolar in order to be thermostable and volatile. That means any compounds that are nonvolatile and/or unstable at high temperatures cannot be analyzed easily by GC-MS without modification. To overcome this limitation, chemical modification with derivatizing agents such as pentafluoropropionic anhydride (PFPA) are required to mask the polar groups, thereby improving volatility. The sample preparation is, therefore, laborious with multiple steps (extraction, derivatization, clean-up, etc) before running GC-MS-based assays.

LC-MS emerged later as an alternative analytical method for drug screening. LC-MS has the advantage of eliminating the requirement for volatility, thus simplifying the sample preparation as well as improving sensitivity for larger and nonvolatile molecules. LC-MS is now

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commonly equipped with two quadrupole detectors in tandem (LC-MS-MS). The first detector generates precursor (or parent) ions that are in turn selectively allowed to enter the second detector, where further fragmentation occurs and product (or daughter) ions are produced. Generally, LC-MS-MS has higher analytical specificity than GC-MS. With its higher sensitivity and shorter sample preparation process, LC-MS-MS has been used in place of GC-MS in drug screening.[40]

MS-based drug screening can be classified into untargeted or targeted screening. The untargeted drug screening uses the full scan analysis in which the entire mass spectra, including both unfragmented and major fragmented ions, are scanned. Unknown analytes in the specimen are identified by their retention times (comparison of the observed retention time with the ones previously recorded of the known compounds) in the total ion chromatogram and mass spectra (software-assisted library matching of mass spectra of the unknown analytes with preestablished reference mass spectra of the known analytes.) (Please review **Figure 5** for the detailed information of these steps.) It can potentially detect any compounds, as long as their mass spectra are available. This method is especially suited for detection of infrequent or newly emerging drugs of abuse, although compounds might be missed at low concentrations due to reduced sensitivity. With targeted drug screening on the other hand, a selected ion monitoring (in GC-MS) or selected reaction monitoring (in LC-MS-MS) mode is used to monitor only preselected ions (or their ion transition) to detect only preselected compounds of interest. The targeted method attains better sensitivity than the untargeted method and is suitable for the detection of frequently abused drugs and monitoring of prescription compliance.[40,41]

The sensitivity of MS-based drug testing is also influenced by various factors, including sample preparation method (eg, liquid extraction and solid phase extraction), type and size of chromatography columns, and parameter setting (eg, voltage and frequency) and specifics (eg, accuracy, resolution, and scanning speed) of the MS instrument.[41,42] Consequently, the sensitivity of drug testing might be different in each laboratory, even if they utilize a similar methodology.

Challenges for Clinical Toxicology Laboratories

The ongoing emergence of designer drugs is a great challenge for toxicology laboratories in several ways. First, limited information about the chemical structure of these newly emerging drugs makes laboratory detection difficult. The new compounds and their metabolites usually do not cross react with immunoassays that target the existing classes of drugs of abuse. Availability of mass spectrum of the compound is a prerequisite for the detection by MS-based screening assays. Second, a lack of information about the metabolism and pharmacokinetics of these compounds complicates their detection in urine. Third, illicit drugs are often "rebranded" in the underground market (eg, bath salts circulate as "Molly," and fentanyl analogs are sold as "heroin"), making the clinical histories unreliable and the targeted drug screening less useful. Untargeted drug screening is required in these situations but is limited to the existence of a library match for the emerging drugs.

Laboratory Tests for New Emerging Drugs

Immunoassays

Synthetic cathinones have some structural similarities with amphetamine (Figure 1), which could cause cross-reactivity in some of the commercially available amphetamine immunoassay kits such as CEDIA Amphetamine/Ecstasy Drugs of Abuse Assays (ThermoFisher Scientific, Waltham, MA).[43] MDPV has also been reported to cross react on SYNCHRON System(s) Phencyclidine Drugs of Abuse Testing (Beckman Coulter, Brea, CA).[44] The following synthetic cathinones (Ethylone, 3-FMC, 4-FMC, MDP, methedrone, methylone, pyrovalerone, and α-PVP) do not cross-react with Syva EMIT II Plus Amphetamine Assay (Siemens, Munich, Germany) up to 5 μg/mL.[45]

Synthetic cannabinoids are not expected to cross react with THC immunoassays due to structural differences (Figure 2). At present, immunoassays have been developed by several manufactures for rapid detection of some designer drugs. Neogen Corporation (Lexington, KY) launched enzyme-linked immunosorbent assays (ELISA) for synthetic cathinones (bath salts) and synthetic cannabinoids (Spice or K2). Randox Toxicology (Crumlin, UK) offers several (ELISA) kits for synthetic cannabinoids, synthetic cathinones, and mitragynine. Immunalysis (Pomona, CA) has developed three distinct homogeneous enzyme immunoassay K2 Spice kits for the detection of synthetic cannabinoids. But none of these immunoassay kits are approved by the FDA for clinical use as of this writing.

GC-MS Based Assays

Bath Salts.—Previous publications have reported GC-MS identification of synthetic cathinones in biological samples, including MDPV and α-PVP with different detection limits.[46,47] Consistent with these studies, we have also identified a series of synthetic cathinones (Ethylone, ethylpentylone, MDPV, methedrone, methylone, and pentylone) in clinical specimens using GC-MS–based untargeted comprehensive drug screening after liquid-liquid extraction, as exemplified by case 1.

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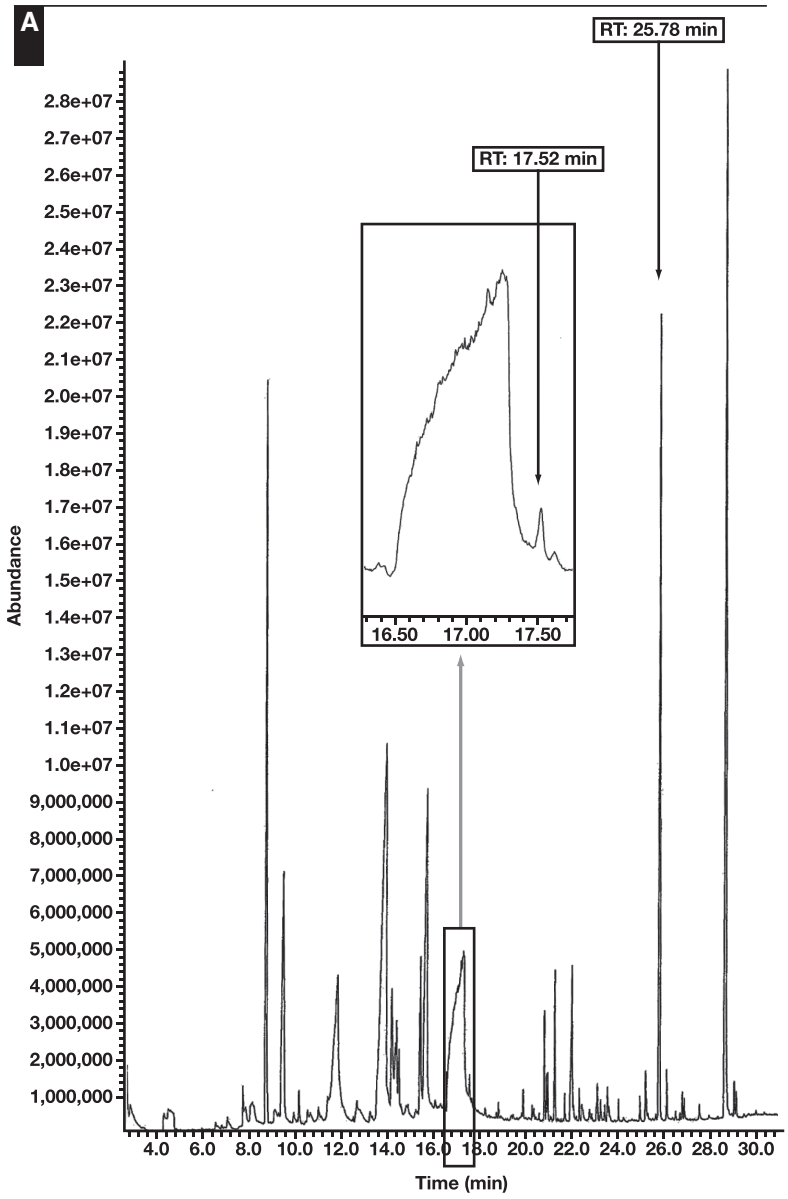

Figure 5 Total ion chromatogram (TIC) (**A**) and software-assisted library matching of mass spectra of the unknown compounds eluted at 17.52 min and 25.78 min (**B**-**E**) in the specimen of Case 2. The urine specimen underwent liquid-liquid extraction with activated charcoal. The extracts were dissolved in methanol and injected into gas chromatography–mass spectrometry (Agilent Technologies 5973 mass spectrometer, Santa Clara, CA) operated in full scan using electron ionization. The mass spectra of the unknown peaks at 17.52 min (shown in the inlet) and 25.78 min in the TIC (**A**) were shown in (**B**) and (**D**), respectively.

Synthetic cathinones are extractable from urine specimen by liquid-liquid extraction with organic solvents and detectable at 500 ng/mL by GC-MS even without derivatization, but PFPA-based derivatization further improves the limit of detection to 50 ng/mL for the synthetic cathinones with secondary amine (Methylone, methedrone, ethylone, 3-FMC, and 4-FMC).[45]

Spice.—Contrary to synthetic cathinones, synthetic cannabinoids are more difficult to detect in clinical specimens using GC-MS. One reason is their rapid and extensive metabolism. For example, JWH-073 and JWH-018, the prototypal synthetic cannabinoids, are quickly metabolized to monohydroxylated or carboxyl metabolites, and the monohydroxylated metabolites are further glucuronidated before urinary excretion, and these compounds are not excreted in a parental form in urine.[48-50] These polar and hydrophilic metabolites, even after glucuronidase treatment, are not only more difficult to extract by liquid-liquid extraction, but also to analyze by GC-MS than parental compounds. Their relatively large molecular size is another factor that makes these compounds

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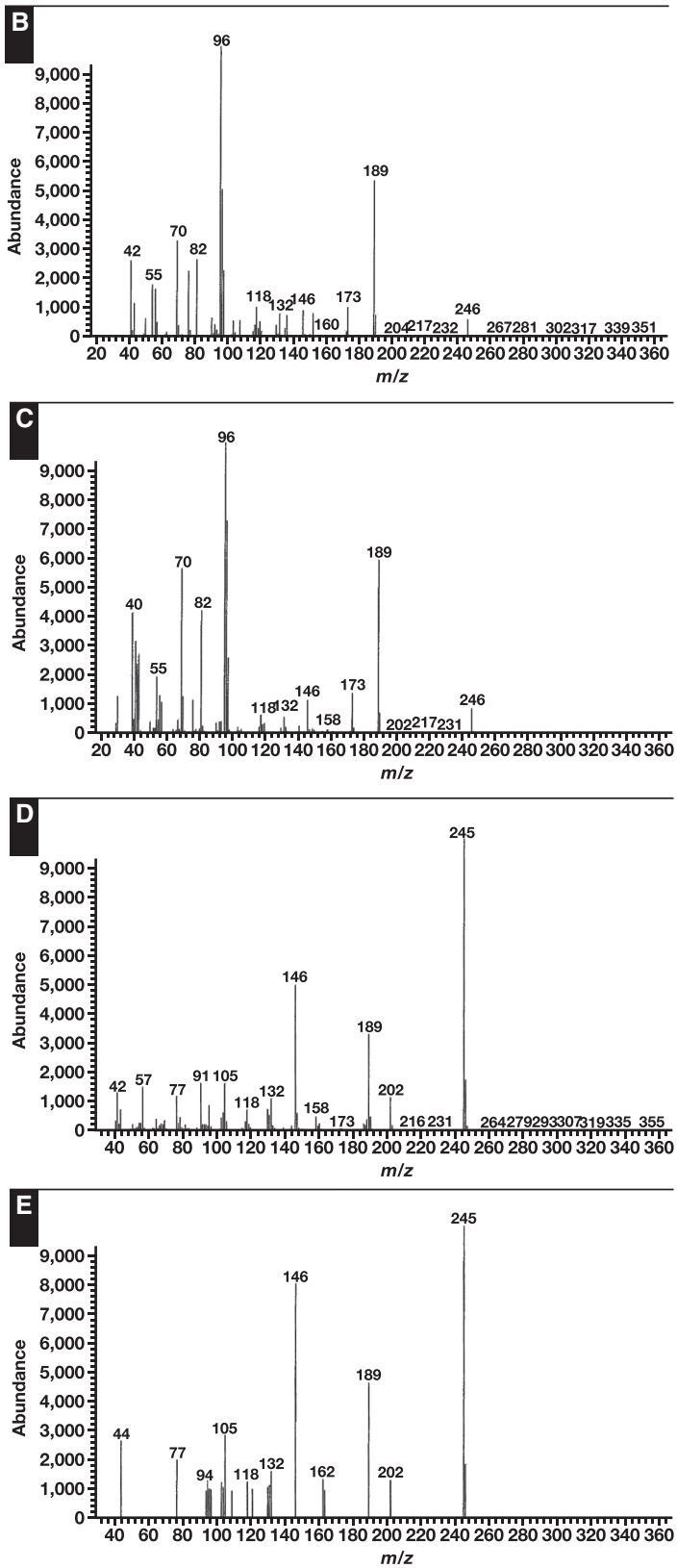

Figure 5 (cont) These mass spectra were identified as methylnorfentanyl, a metabolite of 3-methylfentanyl and fentanyl through software-assisted library matching of mass spectra with the preestablished reference mass spectra of methylnorfentanyl (**C**) and fentanyl (**E**) in the *Mass Spectra of Designer Drugs 2012* (Wiley). The limit of detection of fentanyl analogs spiked in the blank urine is around 100 ng/mL, making this detection system adequate for overdose cases of fentanyl and its analogues. Note that the retention times (RT) of methylnorfentanyl and fentanyl are predicted to be 17.5 min and 26.5 min, respectively, comparable to the actual retention times of these unknown peaks (17.52 min and 25.78 min) in TIC (**A**).

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less compatible with GC-MS. AB-CHMINACA, a newer synthetic cannabinoid with different chemical structure, should be even more difficult to analyze by GC-MS than JWH-073 and JWH-018, presumably because of the presence of more polar groups within the molecule. Due to structural similarity, other newer synthetic cannabinoids such as XLR-11 and AKB48 (APINACA) are expected to be as difficult as AB-CHMINACA to detect by GC-MS (Figure 2). For these reasons, LC-MS(-MS) is preferable for detection of synthetic cannabinoids.[41,51]

Fentanyl Analogs.—The FDA has recently cleared the Immunalysis SEFRIA fentanyl urine immunoassay for qualitative determination of fentanyl in human urine at a cutoff of 1 ng/mL. Fentanyl analogs, acetyl fentanyl and butyryl fentanyl, can be detected in this assay with 100% cross-reactivity. This addition will greatly facilitate the detection of fentanyl and its analogs in the clinical specimens. Fentanyl, as well as multiple fentanyl analogs (acetyl fentanyl, 3-methylfentanyl, butyryl fentanyl, butanoyl-4-fluorofentanyl, and para-fluoroisobutyrylfentanyl) (Figure 3), are detectable by GC-MS–based untargeted comprehensive drug screening, as shown in case 2 (Figure 5). Many of these specimens were collected from patients of self-reported "heroin" overdose cases, but no opiates were detected (see case 2).

Other Synthoid Opioids.—Literature indicates that the synthetic opioids U-47700 and AH-7921 are detectable from urine specimen by GC-MS.[29,52] Consistently, U-47700 has been detected in urine specimens from multiple patients with GC-MS–based untargeted comprehensive drug screening in our laboratory (data not shown).

Mitragynine.—Mitragynine and its metabolites have been detected in human urine specimens using GC-MS with solid phase extraction and derivatization, with a limit of detection of 100 ng/mL.[53] Chemical derivatization is not an absolute requirement for mitragynine detection by GC-MS.

LC-MS-MS and LC-High Resolution-MS Based Assay

GC-MS has been the gold standard for toxicology testing, but GC-MS has a limited utility for detection of polar compounds. This could be problematic for toxicology testing, because drugs are often metabolized in the liver and become more polar before being excreted in urine. Sample preparation such as glucuronidase treatment or derivatization is often needed for GC-MS-based analysis, but detection gains from this processing are often not enough to detect compounds of interest (see the discussion about Spice).

In contrast, LC-MS (-MS) is able to analyze polar compounds with lower limits of detection. This simplifies the sample preparatory procedures for LC-MS-MS, minimizing the burden on technical staff and reducing the turnaround time. LC-MS-MS-based assays have been developed for newly emerging drugs.[40,41] Indeed, LC-MS(-MS)-based testing has been reported for synthetic cathionones,[54,55] synthetic cannabinoids,[49,51] fentanyl analogs,[56,57] U-47700,[56,58] and mitragynine.[59]

LC-MS-MS still requires mass spectral libraries for compound identification; that means newly emerging drugs without mass spectral information cannot be identified by LC-MS-MS. The advent of LC-high-resolution (HR)-MS has provided a solution for this problem. Both LC-time of flight-MS or LC-orbitrap MS are able to resolve molecular mass to 0.001 atomic mass units, compared to the 1 atomic mass unit for conventional MS. This allows for tentative identification of unknown compounds by deducing the molecular formula from accurate mass databases without using mass spectral libraries.[41] This is a very powerful system for toxicology laboratories to detect newly emerging drugs, but the cost of LC-HR-MS system is a major hindrance for standard clinical laboratories, reducing its utility to a specialty instrument held at only a handful of reference laboratories.

Case Summary and Future Trends of Clinical Toxicology Testing of Newly Emerging Drugs of Abuse

These cases represent the current trends of "rebranding" the drugs of abuse. The "Molly" bags obtained by the patient in case 1 likely contained Ethylone, but not MDMA, whereas the "heroin" bags obtained by the patient in case 2 likely contained fentanyl and 3-methylfentanyl, but not heroin (diacetylmorphine). These cases also demonstrate the variability in street nomenclature of drugs from different classes and underscore our inability to rely on historical information to accurately identify community drug trends.

Accurate laboratory analysis and drug identification will be critical in guiding individual medical management as well as gathering epidemiologic data to inform timely public health and law enforcement responses. Immunoassay kits for these emerging drugs have been developed and are commercially available; however, besides the aforementioned FDA-cleared fentanyl immunoassay kit, none of them are FDA-cleared for clinical use. If FDA-cleared immunoassay kits are developed, it should improve the overall detection of these emerging drugs of abuse in the clinical cases that are currently unrecognized.

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GC-MS has been the gold standard in toxicology testing, and GC-MS-based drug screening is still powerful and useful. But GC-MS has limited utility for detection of synthetic cannabinoids (or Spice). With its simpler and easier sample preparation and better detection capability, LC-MS-MS and even newer LC-HR-MS are gaining popularity for toxicology testing. These new technologies will become new gold standards of toxicology testing in the future.

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