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Analytical surveillance of emerging drugs of abuse and drug formulations

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Abstract

Uncontrolled recreational drugs are proliferating in number and variety. Effects of long-term use are unknown, and regulation is problematic, as efforts to control one chemical often lead to several other structural analogs. Advanced analytical instrumentation and methods are continuing to be developed to identify drugs, chemical constituents of products, and drug substances and metabolites in biological fluids. Several mass spectrometry based approaches appear promising, particularly those that involve high resolution chromatographic and mass spectrometric methods that allow unbiased data acquisition and sophisticated data interrogation. Several of these techniques are shown to facilitate both targeted and broad spectrum analysis, which is often of particular benefit when dealing with misleadingly labeled products or assessing a biological matrix for illicit drugs and metabolites. The development and application of novel analytical approaches such as these will help to assess the nature and degree of exposure and risk and, where necessary, inform forensics and facilitate implementation of specific regulation and control measures.

Keywords

Drug abuse; synthetic cannabinoids; mass spectrometry; detection; fractional mass filter; mass defect filter

Introduction

Tobacco, alcohol, and illegal drugs continue to be major contributors to preventable morbidity and mortality across the globe, with cigarettes alone contributing to 1 in 3 deaths. In the United States, the 2010 National Survey on Drug Use and Health revealed that while cigarette smoking has decreased in the last decade, and use of alternative tobacco products and alcohol have remained relatively constant, the use of marijuana has increased significantly (SAMHSA 2011a). Similarly, the 2011 Monitoring the Future survey showed

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that while cigarette and alcohol use among teens were at their lowest points since 1975, teens are still abusing alternate tobacco products, marijuana, and prescription drugs at unacceptably high levels (Johnston et al. 2012). Indeed, daily marijuana use is now at a 30-year peak among high school seniors. Also alarming is the growing industry and global market in designer drugs, and the manufacture and illicit distribution of counterfeit pharmaceutical or dietary supplements/nutraceuticals, where even less is known about the prevalence of use and long-term health consequences. According to the American Association of Poison Control Centers, the number of calls to poison centers about exposure to synthetic marijuana (THC homologs) increased from 2906 in 2010 to 6959 in 2011, and has remained elevated, with 3332 calls through June of 2012 (AAPCC 2012a). The 2011 Monitoring the Future survey indicated that 1 in 9 high school seniors (11.4%) reported using a synthetic cannabinoid in the prior 12 months. “Bath salts” and “plant foods” containing psychoactive cathinone analogs have also been increasing in prevalence and public concern (AAPCC 2012b) and Khat, *Salvia divinorum*, and other natural products are being distributed in several guises. In addition, the Drug Abuse Warning Network, 2009: National Estimates of Drug-Related Emergency Department Visits indicated that between 2004 and 2009, similarly large increases in the involvement of non-illicit drugs (prescription drugs, over-the-counter medications, and supplements [e.g., herbal remedies]) have been observed in all types of drug-related hospital visits (misuse/abuse, suicide attempts, adverse reactions, and accidental ingestions) (SAMHSA 2011b).

Whether targeting naïve consumers or experienced substance abusers seeking to achieve intoxication while evading detection and prosecution, the continuing emergence of designer drugs and alternative forms and formulations of addictive substances has caused considerable public concern. In 2008, the estimated global sales of alcoholic beverages exceeded \$1 trillion, and annual revenues from the global tobacco trade are approaching \$500 billion (Eriksen et al. 2012). The global market in illicit or recreational drugs is of similar magnitude. Bloomberg Business Week reported in 2011 that in just a few years a complex global supply chain, enabled by the Internet, had appeared to produce, package, and ship a multiplying variety of narcotics and “legal” substances, thus taking an increasing share of the market for recreational drugs, which is estimated by Jeffrey A. Miron of Harvard and the Cato Institute to be \$121 billion in North America alone. Faced with these multibillion dollar enterprises, large consumer populations, and considerable health concerns, the U.S. Food and Drug Administration (FDA), Department of Justice Drug Enforcement Agency (DEA), and other regulatory bodies and public health officials must find rational and effective means for bulk chemical and product surveillance, the evaluation of potential chemical exposures and risks to consumer safety, and the implementation and assessment of interdiction and control efforts. The agencies’ work is confounded by the use of misleading labels on some products (e.g., synthetic cannabinoid formulations state “not for human consumption”) and the lack of ingredient labeling requirements for tobacco and certain alcoholic drinks, much less for legally and (post-DEA ban) illegally sold synthetic cannabinoids and other designer drugs. Rather than identify the specific active substances and ingredients, illicit drug manufacturers often claim that their products are legal or do not contain compounds that have been specifically banned by the DEA. It can also be reasonably anticipated that as specific (compound-targeted) analytical methods are developed for the

forensic analysis of samples to detect specific banned substances, manufacturers and users will switch to other analogs or products to evade detection, identification, and prosecution. Thus, analytical challenges exist in ensuring the safety of certain drug products, as well as identifying illicit products and prosecuting their manufacturers, traffickers, and users.

In the present mini-review, specific examples and applications of advanced mass spectrometry (MS) analytical approaches are shown to allow product characterization and identification of chemical constituents. The case of synthetic cannabinoids in herbal products shows application to a current abuse problem, and the case of tobacco products shows how the methods can also be applied systematically and extensively in a well recognized, real world setting of immense public health importance. The intent is to suggest ways in which advanced analytical methods can help to assess degree of exposure and risk and, where necessary, inform forensics and facilitate successful implementation of specific regulation and control measures.

Mass spectrometry based methods

Direct ionization of samples using atmospheric pressure ionization interfaces

Several sampling interfaces have recently been developed for the analysis of volatile and non-volatile analytes as they are ionized directly from solid or liquid samples. Evolving from direct insertion probes (DIP) that operated within the mass spectrometer's vacuum and enabled electron or chemical ionization, these newer approaches typically involve atmospheric pressure ionization interfaces such as direct analysis in real time (DART), desorption electrospray ionization (DESI), laser diode thermal desorption (LDTD), and atmospheric solids analysis probe (ASAP) sources. The obvious advantage of this ionization approach is that, in many instances, no sample preparation is needed for the analysis of the principal chemical constituent(s) in a variety of sample matrices, including solutions or liquid samples, solid powders or crystals, and compressed pills or complex herbal formulations.

In the DART process, positive $M+H$ ions are formed by the reaction of helium metastables with atmospheric water, which yields protonated water clusters, followed by the subsequent proton transfer from these species to the analyte molecule. Fragment ions can also be observed in DART mass spectra, and encouraged by increasing the cone voltage applied; but, contrary to DESI, multiply-charged ions or alkalimetal-cation adducts are never present. Addition of ammonia, methylene chloride, or other "dopant" vapors to the DART gas stream has also been used to form single-charged adducts (e.g., $[M+NH_4]^+$ or $[M+Cl]^+$) of compounds that would not readily form molecular ions or (de)protonated molecules. In contrast to DESI, DART is primarily a small-molecule technique, and generally enables effective ionization of compounds with molecular weights below 1 kDa. These direct ionization interfaces have been successfully used for the analysis of a variety of bulk drugs and drug products, and can also characterize synthetic cannabinoids in herbal formulations as shown in Figure 1. In this example, small samples of each herbal formulation have been directly ionized in air, and several cone voltages have been applied to assist in ionization and/or fragmentation (only 20 and 90 V are shown). At the lower voltage, the predominant ion detected by the AccuToF mass spectrometer during the analysis of the herbal product

Mr. Nice Guy is observed at 342.1864. This is consistent with the presence of a singly charged protonated compound $[M+H]^{1+}$ with the empirical formula $C_{24}H_{24}NO$, or 342.1858 (which is within the mass accuracy/resolution of the AccuToF), such as the aminoalkyl indole JWH-018, one of the synthetic cannabinoid analogs frequently found in herbal formulations and a substance that is controlled in many countries including the United States. The tentative identification of this compound by its close match between monoisotopic mass observed and calculated is further supported by the ions detected at a higher cone voltage, where apparent fragment ions can be readily assigned to structural elements contained within JWH-018-like synthetic cannabinoid analogs. There are additional advantages of this approach beyond ease of sample preparation and speed of analysis, including the relatively high resolution and accurate mass detection afforded by the AccuToF as compared to quadrupole mass filters and ion traps, and the non-destructive nature of the sample ionization. However, disadvantages include the absence of a separation system, such that all ionized analytes from the sample can be present in each MS scan, which can make deconvolution and structural elucidation considerably more difficult. For example, the DART-AccuToF spectrum recorded for the product Meditation reveals ions consistent with the presence of multiple synthetic cannabinoid analogs and their fragment ions. This is clearly useful structural information, but the approach precludes retention time as a possible means of resolving isobaric analytes (other than stereoisomers), and of providing an orthogonal piece of analytical evidence supporting structural assignment, which is often needed in forensic or criminal proceedings. In addition, if the protonated or deprotonated analyte of interest ($[M+H]^{1+}$ or $[M-H]^{1-}$) is not the most prevalent species, further difficulty in interpretation of the spectral information and structural assignment is often encountered. So in this instance the DART-AccuToF data are consistent with the presence of JWH-018 or any geometric or structural isomer with the same empirical formula; and we must also consider other chemicals with any plausible empirical formula that sums within the mass accuracy of the analytical instrument.

Solid-phase microextraction coupled to gas chromatography/mass spectrometry

Solid-phase microextraction headspace autosamplers coupled to gas chromatography/mass spectrometry (SPME-HS-GC/MS) affords another relatively simple sampling approach based on solute adsorption onto a solid phase fiber. It is often used to preconcentrate volatile compounds, such as flavors and fragrances, from the headspace of a material of interest prior to MS, without requiring extraction, derivatization, or solvent evaporation. With this approach, analytes establish equilibria among the sample matrix, the headspace above the sample, and a polymer-coated fused fiber, and then are desorbed from the fiber onto a GC column where they are separated prior to MS analysis. By manipulating the polarity and thickness of the coating on the fiber, matrix temperature, and sampling time, and optimizing several other parameters affecting extraction, an analyst can often obtain highly consistent, quantifiable results from extremely low concentrations of analytes. Automated systems for SPME allow fiber conditioning, sample heating, solvent/standard addition, mixing and agitation, and reading and processing of barcodes. SPME also has application in the field, where on-site sampling can be done and samples can be analyzed later in the laboratory without significant loss of analytes. After desorption onto the gas chromatograph (or elution for liquid chromatography [LC]), standard approaches to separation and detection are

applicable. GC separation coupled to quadrupole mass analysis allows the use of industry standard separation columns and ionization conditions (70 eV) for the characterization of both retention times and nominal mass resolution MS scans. In particular, GC/MS using electron ionization (EI) is widely accepted in terms of admissibility and defensibility in court because of its reproducibility, sensitivity, selectivity, and high degree of consistent fragmentation produced at 70 eV, which facilitates interpretation and spectral searching strategies for chemical identification or structural elucidation. Several reports of the application of SPME qualitative and quantitative analyses of chemical constituents in tobacco products (Clark and Bunch 1997; Yang and Smetena 1998) led to further investigation of its utility in the analysis of novel tobacco products (Snus, E-cigarettes, and dissolvable products) and so-called “Spice” products and herbal formulations suspected of containing synthetic cannabinoids.

Figure 2 shows the total ion chromatograms and mass spectra for SPME analysis of two herbal formulations. In the total ion chromatogram for Mr. Nice Guy, one predominant peak was detected at the retention time of approximately 31.3 min, which is also the retention time of the reference standard JWH-018. The mass spectrum recorded for the analyte peak was also essentially identical to that of JWH-018. As suggested by the DART-AccuToF data previously described, the total ion chromatogram recorded for the SPME analysis of Meditation revealed more than one peak of interest. The GC/MS system was sufficient to resolve the presence of three analytes with mass spectra suggestive of known synthetic cannabinoid structures. The peak at retention time 28.5 min is consistent with the retention time of the JWH-250 reference standard, and can be discriminated chromatographically from the isobaric JWH synthetic cannabinoid analogs with a molecular ion at m/z 335, such as JWH-201 (retention time 29.4 min) and JWH-238 and JWH-302 (28.9 min) (data not shown). Fragment ions present in the mass spectrum are also consistent with its identification as JWH-250. The SPME analysis of Meditation also revealed a small chromatographic peak at 31.3 min, which was found to be consistent in retention time and mass spectral fragmentation with the presence of JWH-018. The third analyte with a mass spectrum suggestive of a synthetic cannabinoid had a retention time of 36.1 min. This analyte's retention time and mass spectrum were essentially the same as those of JWH-081, and could be chromatographically discriminated from isobaric analogs at m/z 371, including JWH-164 (33.6 min), JWH-166 (35.8 min), JWH-267 (32.3 min), JWH-408 (38.4 min), and JWH-197 (31.9 min). It is important to note that the herbal formulations and synthetic cannabinoid reference standards were heated to 200 °C to facilitate volatilization and head space analysis of the synthetic cannabinoids (see (Cox et al. 2012) for full methodological details).

The FDA continues to review and regulate tobacco products as mandated by the Family Smoking Prevention and Tobacco Control Act. The FDA has acted under this authority and banned the use of characterizing flavors, other than menthol, in cigarettes. Still, the tobacco (and alcohol) industry continues to manufacture and distribute certain products without complete ingredient and additive listings. Despite that, and the lack of information about manufacturing and chemical exposure, the complex composition of flavored smokeless products such as Camel Snus becomes readily apparent with the SPME analysis, as shown in Figure 3. Here the method included the addition of saline and gentle heating to 90 °C, an

approach that facilitates the detection of volatile components and prevents the fiber from being overloaded by nicotine. Snus Frost is characterized by large amounts of menthol and carvone, compounds present in mint and used for their cooling sensation, anti-irritation, and analgesic properties mediated through ion channels (transient receptor potential channel M8 [TRPM8] in particular) and kappa-opioid receptor interactions. Snus Mellow, in contrast, shows less menthol and carvone in the total ion chromatogram. Instead, it is characterized by tetramethylpyrazine and ethyl vanillin, which are essentially absent in Frost and impart a cocoa/nutty and vanilla flavor. Interestingly, vanillin is also a TRP channel modulator, showing activity at the TRPV3 receptor subtype, and several of the volatile terpinoid and alkyl alcohol-like flavorants are also TRP channel ligands, such as eugenol, isopulegol, cinnamaldehyde, thymol, hexanol, and octanol (Clapham 2007); Vriens et al. 2008; Komatsu et al. 2012). Thus, these compounds would be anticipated to be active in the mouth and the trigeminal nervous system, and may thereby provide additional cues that become associated with nicotine and act to reinforce nicotine use, properties that are of considerable interest to the FDA and tobacco addiction and cessation researchers.

Ultra-high performance liquid chromatography and quadrupole time-of-flight

Liquid sampling interfaces for MS also are evolving and becoming more frequently described in forensic analysis, often with a focus on taking advantage of the increasing speed and resolution available through the use of small particle sizes (<2 μm particles used in ultra-high performance liquid chromatography, UHPLC) or new stationary phase and column technologies (e.g., fused-core particles for rapid HPLC). In certain circumstances, sample throughput can be maximized by direct injection of liquid samples or extracts without further purification or concentration, a process that is made more robust and cost-effective by the availability of inexpensive sample filters and guard columns. However, most autosampler and liquid sampling technologies can also accommodate and automate a wide variety of sample preparation and clean-up techniques, such as solid phase extraction (or other sorption-based methods), liquid/liquid extraction, and membrane assisted solvent extraction. Considerable effort has been made to further establish the utility of UHPLC-MS for the separation and identification of complex mixtures. In particular, analytical systems coupling UHPLC to high resolution mass analyzers are becoming less expensive and more robust, and are finding increased application in forensic and other analytical areas of interest. The most commonly used and versatile high-resolution mass analyzer designs are the time-of-flight (ToF) analyzer and the orbitrap mass analyzer. These high resolution instruments can provide accurate mass information out to at least a thousandth of an amu and resolving powers of 20,000 to 100,000, such that ions at 300.1394 m/z and 300.0673 m/z would be reported as two separate peaks. A particular advantage of ToF mass analyzers is that they are capable of extremely fast scan times, and are therefore well suited to fast GC and UHPLC separations. Also, the high resolution and mass accuracy provides certain advantages over quadrupoles, ion traps, and q-trap systems, including the ability to apply fractional mass filtering (also referred to as mass defect filtering) and other post data-acquisition processing methods, which further enables targeted and more broad-spectrum screening approaches for detection of designer drugs.

Traditional targeted MS methods typically rely on chromatographic separation (retention time), ionization and fragmentation, library searches, and detection of known masses, or multiple reaction monitoring (MRM) transitions for identification/confirmation, and are therefore often unable to detect or identify newly discovered or unreported designer drugs. However, high-resolution MSe (in which low and high collision energy data are collected nearly simultaneously for every m/z) in conjunction with fractional mass filtering provides an alternative method for nontargeted analysis (Grabenauer et al. 2012). This is possible because most compounds within a family of designer drugs are based on a template core structure (essential pharmacophore), and therefore often have similar intact structures and common fragment ions. Modifications to the core may result in a substantial shift in total mass, with only a minimal shift in fractional mass. Searching for related compounds based on fractional mass is a common strategy for metabolite identification (Zhang et al. 2003) and has proven useful for removing interferences from complex biological matrices (Zhu et al, 2006; Bateman et al., 2007). The technique has been applied in depth to a family of designer drugs composed of indole-derived synthetic cannabinoids closely related to JWH-018 (Grabenauer et al. 2012). In this example, a single fractional mass filter with a 50 mDa window around the exact mass of the prototypical compound JWH-018 encompasses over 80% of all currently published structures in this family. Filtering for precursor ions of common fragment ions enables detection of compounds with fractional masses that fall outside the range of filter parameters. However, application of a fractional mass filter to fragment ions prior to precursor ion searching increases the breadth of analogs that can be detected by this approach. Indeed, Grabenauer et al. have demonstrated that applying such fractional mass filters to a liquid chromatography–mass spectrometry (LC-MS) data set facilitates the detection of designer drug analogs while filtering out matrix elements and other components in unknown samples, as illustrated in Figure 4.

Summary and conclusions

The dearth of objective data about unlabeled or misleadingly labeled products containing drugs of abuse and addictive substances creates challenges for ongoing efforts at exposure and risk assessment, regulation, and control. Furthermore, the chemically diverse nature of designer drugs, and the potential for structural variation, requires that a variety of analytical approaches be used when bulk drug substances or suspected illicit drug users are encountered. Several advantages and disadvantages of the three advanced analytical techniques described here are listed in Table 1.

Whether plant-based (heroin, cocaine, cannabis) or synthetic (designer cannabinoids and cathinones), illicit drugs and drug products are normally complex mixtures which rarely contain the drug alone. Flavors and volatile aromatics may be added, and cutting agents and caffeine are commonly found in stimulants and bath salts-like formulations containing cathinone or phenethylamines. In addition, as a consequence of the nature of the plant-based product, or the crude clandestine laboratory conditions under which they are produced, chemical composition often shows large variability. As further information about the substances and their active ingredients becomes available, and their biological fate is determined, more sophisticated methods for quantitative MS and MS/MS may be developed for research and validated for workplace drug testing urinalysis, where the selected ion or

reaction monitoring methods afford extremely sensitive and selective targeted analyses, such as that required for the detection of LSD metabolites. These quantitative MS assays are often relatively exacting, as is required to meet the Department of Health and Human Services, Substance Abuse and Mental Health Services Administration's Mandatory Guidelines for Federal Workplace Drug Testing Programs, which establish comprehensive standards for all aspects of laboratory drug testing and procedures. However, it is also reasonable to anticipate that when designer drugs constitute the chemical class of interest, structural analogs will be developed to evade detection and law enforcement. Thus, alternative approaches, involving broad spectrum screening or chemical class-specific detection are also needed. Indeed, recent advances in instrumentation and improved methods of sample introduction, ionization, and mass analysis are now capable of extending the application of MS to targeted screening and more proactive, broad-spectrum analytical approaches to surveillance and drug profiling. These approaches may also reveal how products have been manipulated to facilitate the delivery of active ingredients and maintain use, or mask unpleasant characteristics associated with risk perception, or, in the specific case of banned substances, avoid regulation and law enforcement.

Forensic and crime laboratories must be able to assess a wide range of matrices, substances, and formulations, and use more rapid, sensitive, accurate, and comprehensive methods to detect and identify chemical constituents in order to cope with the increasing demand for testing associated with emerging public health concerns and national and international drug laws (ST/NAR/34 2006). When faced with an unknown chemical or drug product, the chemical analysis should strive to detect and identify the characteristic chemical signature of the major, minor, and trace components. Similarly, analysis of a biological fluid for drugs and their metabolites should be as comprehensive as possible, particularly when testing for designer drugs. In addition, the Internet-assisted, global character of drug use and drug trafficking requires the timely exchange of analytical data between laboratories and researchers, health practitioners, and law enforcement authorities at the national, regional, and international levels. Thus, there is also a considerable need for the establishment of centralized data repositories and harmonization of recommended methods for drug testing laboratories, such as those promulgated by the United Nations Office of Drugs and Crime Laboratory and Scientific Section (e.g., see ST/NAR/34 2006).

In this mini review, advanced analytical approaches are shown to provide an effective means for the rapid analysis, detection, and mass spectral identification or confirmation of the chemical constituents of bulk drug substance and herbal formulations. In addition, the MS^E with fractional mass filtering approach facilitates the identification of drugs and their metabolites in complex biological matrices. Once obtained, the mass spectral information can immediately be made available to forensic scientists and the research community through www.forensicdb.org, or other web-based databases of chemical and spectral information. Many of these databases also accept reference spectra from the research community for consideration and inclusion in their spectral libraries. In most instances, library searching of mass spectra and other spectroscopic data in these databases is possible, further facilitating identification. Ultimately, such analytical efforts will help meet the forensic needs of rapidity, throughput, and reliability, and will enable the scientific

community to better address drug abuse, harm reduction, legislation, and the effectiveness of law enforcement.

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Figure 1. DART-AccuToF mass spectra recorded for the synthetic cannabinoid-containing herbal formulations Mr. Nice Guy (top) and Meditation (bottom) recorded at cone voltages of 20V (left) and 90V (right). Analysis of Mr. Nice Guy indicated the presence of a synthetic cannabinoid such as JWH-018. Analysis of Meditation indicated the presence of two synthetic cannabinoids such as JWH-081 and JWH-250.

Figure 3. SPME-GC/MS analysis of the smokeless tobacco products Camel Snus Frost (top) and Camel Snus Mellow (bottom). Major constituents tentatively identified by mass spectral searching are labeled.

Figure 4.

Low collision energy total ion chromatogram for Mr. Nice Guy unfiltered (A) and with a fractional mass filter centered at 0.1859 with a window of ± 50 mDa (B). Extracted ion chromatograms for high collision energy fragment ions at 127.056 m/z (C), 155.051 m/z (D), and 214.124 m/z (E). The peak at 4.36 min is JWH-018 and the peak at 4.86 min is JWH-019. Note that JWH-019 was only detected after fractional mass filtering. Reprinted with permission from Grabenauer M, Krol WL, Wiley JL, Thomas BF. Analysis of Synthetic Cannabinoids Using High-Resolution Mass Spectrometry and Mass Defect Filtering:

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Table 1

Advantages and disadvantages of analytical instrumentation

Analytical Method	Advantages	Disadvantages
DART-TOF	<ul style="list-style-type: none">• Minimal sample preparation• Accurate mass data• In source fragmentation	<ul style="list-style-type: none">• No chromatographic separation• Lack of sensitivity• No mass selected fragmentation
SPME-HS-GC/MS	<ul style="list-style-type: none">• Minimal sample preparation• Onsite fiber sampling• Library searchable EI-MS spectra	<ul style="list-style-type: none">• Possibility of uneven analyte adsorption and overloading• Long GC method run times
UHPLC-MS-QTOF	<ul style="list-style-type: none">• Accurate mass data• Mass selected fragmentation• Post acquisition data processing	<ul style="list-style-type: none">• Expensive equipment costs• Limited library search capabilities