MASS SPECTROMETRY ANALYSIS OF DRUGS OF ABUSE: CHALLENGES AND EMERGING STRATEGIES

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Mass spectrometry has been the "gold standard" for drugs of abuse (DoA) analysis for many decades because of the selectivity and sensitivity it affords. Recent progress in all aspects of mass spectrometry has seen significant developments in the field of DoA analysis. Mass spectrometry is particularly well suited to address the rapidly proliferating number of very high potency, novel psychoactive substances that are causing an alarming number of fatalities worldwide. This review surveys advancements in the areas of sample preparation, gas and liquid chromatography-mass spectrometry, as well as the rapidly emerging field of ambient ionization mass spectrometry. We have predominantly targeted literature progress over the past ten years and present our outlook for the future. © 2020 Periodicals, Inc. Mass Spec Rev

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I. INTRODUCTION

Mass spectrometry (MS) is the accepted "gold standard" in the broad field of drug analysis because of its sensitivity, specificity, and flexibility. In the area of toxicology, MS-based drug testing is used in a wide variety of applications such as establishing the cause of death, monitoring prescription drug levels in the blood, and verifying substance identity and levels in impaired driving cases. A literature search with Web of Science for "forensic," "drugs," and "mass spectrometry" from 2010 to present yields the following distribution of publications presented in Figure 1.

While assembling this review, it was immediately apparent that a complete review of all recent aspects of MS-based toxicological drug analysis would be better served by a book (or series), in order to address all facets of this complex field. To narrow the scope, we have chosen to focus upon the use of MS for the analysis of "drugs of abuse" (DoA) in terms of current practices in forensic toxicology and exploring areas of growth aimed at addressing unmet needs. We define DoA (better termed by the less stigmatizing "drugs of misuse," or DoM) as compounds that include illicit substances (e.g., heroin [HER]) as well as those that may be used therapeutically but are also commonly misused for recreational (e.g., cannabinoids) or other (e.g., diversion of prescribed drug) purposes. These can be categorized broadly into the following areas: anti-depressants (e.g., benzodiazepines [BDZs]), stimulants (e.g., amphetamines [AMPs], cocaine [COC]), opioids, hallucinogens, and cannabinoids. We have purposely chosen to exclude alcohol, inhalants, and tobacco from this list to further limit the scope for this review. Although topics addressed in this manuscript necessarily bridge all the disciplines identified above, we will predominantly focus upon the MS analysis of drugs that have important forensic relevance, which we define as providing definitive evidence for legal purposes, whether in living donors or postmortem.

There is a significant overlap between forensic drug testing applications and those from closely related fields, most notably clinical toxicology. Forensic toxicology, for example, measures drugs in the context of death or human performance (e.g., impaired driving, sports doping, workplace testing) in a wide range of sample matrices. Clinical toxicology, by contrast, deals with the impact of drugs in both acute poisoning and long-term monitoring (e.g., substance use disorders) and is largely confined to urine and blood. However, the differences between such fields dissolve with progression down the standard analytical sequence: for example, the methods used for compound identification by high-resolution MS (HRMS) in forensic, clinical, wastewater, and food residue applications are essentially indistinguishable. This review will cover sample preparation considerations, chromatographic methods such as gas and liquid chromatography (LC) separations coupled with

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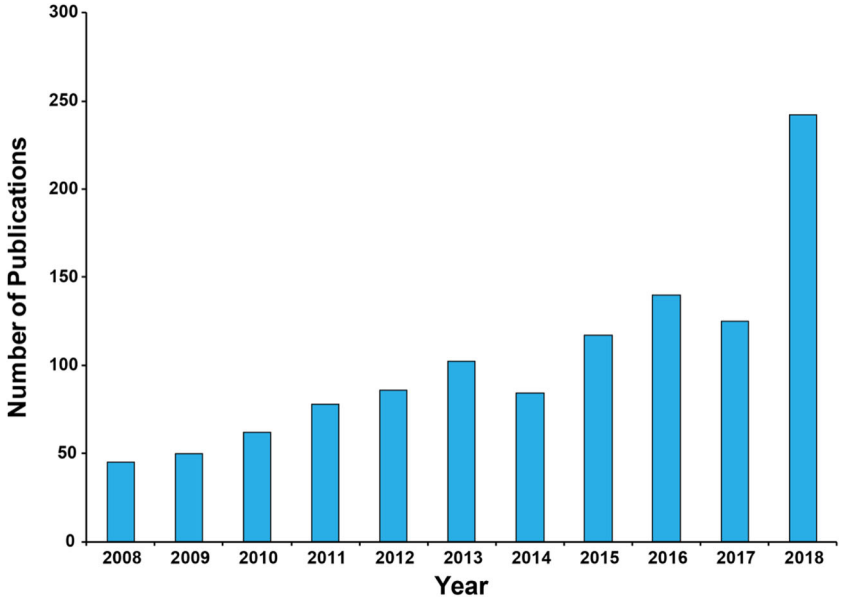

FIGURE 1. Growth of mass spectrometry-based drug testing publications between 2008 and present. [Color figure can be viewed at wileyonlinelibrary.com]

MS, and the growing field of direct MS-based methods including ambient and other ionization strategies. Each analysis strategy inevitably has specific advantages and challenges with respect to the classes of DoA, sample type, and required sample preparation(s). The issue of whether qualitative or quantitative measurements are possible and/or required will also be examined.

II. SAMPLE PREPARATION CONSIDERATIONS

Given the diversity of sample types and analytes involved in MS-based drug measurements, we begin with a synopsis of the steps taken to prepare samples for analysis, including hydrolysis, derivatization (most common for gas chromatography, or GC) and extraction strategies. The emerging field of ambient ionization, where samples are directly measured, frequently obviates most sample derivatization and extraction steps, though several specific cases using solid-phase microextraction (SPME) and other sample preparation methods will be discussed in Section V.

A. Hydrolysis

Sample hydrolysis to liberate free drugs from their metabolized conjugates is generally restricted to urine measurements, though this step is often omitted for "dilute-and-shoot" LC methods (Cao, Kaleta, & Wang et al., 2015; Alcántara-Durán et al., 2018). While drug conjugates are amenable to MS analysis (Papini et al., 2006; Dickerson et al., 2012; Roslawski et al., 2019), they show poor sensitivity and often lack commercially available standards. Hydrolysis is typically conducted with commercial glucuronidase enzyme, a product whose purity and activity has markedly increased. Historically, hydrolysis was achieved with either acid (which hydrolyzed acetylmorphine and compromised BDZs) or fairly crude enzyme preparations of low activity, requiring hours to achieve even partial hydrolysis for the most resistant compounds such as codeine (Malik-Wolf et al., 2014). By contrast, Sitasuwan et al. (2018) reported >80% hydrolysis of codeine glucuronide with different recombinant glucuronidase products in 30 min.

B. Sample Extraction

The classical extraction methods of DoA from various matrices are liquid–liquid extraction (LLE) and solid-phase extraction (SPE) and are still widely reported, despite the fact that they use large volumes of organic solvents (LLE) and are time-consuming. SPE offers the advantage of high preconcentration factors but requires larger sample sizes and a multistep procedure that can reduce analyte recoveries. The concept of "green chemistry," centered on 12 principles, aims to reduce the environmental impact of synthetic chemistry (Anastas & Warner, 1998; Anastas, 1999). Green analytical chemistry, in turn, is based on another 12 principles with the goal of combining good performance with environmental sustainability (Gałuszka, Migaszewski, & Namieśnik, 2013). Microextraction techniques based on different concepts have been developed with the idea of "miniaturization" in mind, and range from novel solid extracting phases to the use of very low volumes of solvents (micro to nanoliters). Novel solid-phase microextraction (SPME) techniques have been thoroughly reviewed, starting from the pioneering work of Arthur and Pawlizsyn, who developed SPME in 1990 (Arthur & Pawliszyn, 1990), explaining advantages and disadvantages, application fields, and matrices of interest (Płotka-Wasylka et al., 2015). In another recent review, different microextraction techniques for the analysis of cannabinoids and their metabolites are described and discussed (Jain & Singh, 2016a), and an overview of the most common microextraction techniques applied to forensic toxicology for the determination of DoA in biological samples highlights the importance of sample preparation before introduction in the suitable instrument (He, 2017). In this

review, we limit our discussion to microextraction and green extraction techniques.

1. Solid-Phase Microextraction

SPME was first presented in 1990, and since then its use has increased steadily in various application fields (Arthur & Pawliszyn, 1990). In its most straightforward configuration, an SPME device consists of a fused silica fiber coated with an extraction phase fit on a syringe-like device (Fig. 2).

The SPME fiber can be immersed directly in a liquid sample (DI-SPME) or exposed to its headspace (HS-SPME), with the extraction of the analytes based on partitioning between the two phases. Nonvolatile, high-molecular-weight (MW) compounds require a membrane-coated fiber for better reproducibility and accuracy. In general, DI-SPME has been used more frequently in LC-based methods, whereas HS-SPME is recommended for the extraction of more volatile and semi-volatile compounds typical in GC-MS measurements. If derivatization is needed, it can also be done directly on the fiber, prior to GC analysis.

DI-SPME-GC-MS has been used for the determination of levamisole and minor COC congeners in hair samples (Fucci et al., 2014), and AMPs in oral fluid (Souza et al., 2011). Various fiber coatings, extraction strategies, and other variables (temperature, stirring, pH, in-tube, automation, etc.) have been presented (Spietelun et al., 2012), demonstrating the versatility of this approach, which is still on the cutting edge, even 30 years after the original prototype was presented. A recent review examines all aspects of SPME in drug analysis and toxicology (Goryński, 2019). Among the most recent approaches, the use of a stainless-steel wire coated with acid-oxidized multi-walled carbon nanotubes has been reported for the HS-SPME of AMP-like stimulants in human urine before GC-Quadrupole (Q)MS analysis (Song et al., 2018). Particularly interesting is the use of microliter-scale tips equipped with biocompatible SPME fibers (polydimethylsiloxane-divinylbenzene; C18; C18-SCX silica) for the GC-QMS determination of AMPs, cathinones, and metabolites in human urine. Optimum derivatization was achieved with pentafluoro-propionic anhydride after extraction (Alsenedi & Morrison, 2018).

HS-SPME was used to extract several AMPs and tetrahydrocannabinol (THC) from Drugwipe®5 sweat screening devices (Securetec, Ottobrunn, Germany). After HS sampling (10 min), the fiber was exposed for 3 min at 90°C in another vial containing a derivatizing agent before final desorption into the GC-MS injector (Gentili et al., 2016). Polydimethylsiloxane coated fibers were used to extract 14 illicit drugs belonging to the groups of phenethylamines, cathinones, a piperidine derivative, a tryptamine derivative, and synthetic cannabinoids in raw liquid, powder, and herbal samples using the direct-heating HS-SPME. Analytes were desorbed from the fiber into a GC-QMS instrument (Fujii et al., 2015). Other examples report the simultaneous determination of 17 compounds belonging to the classes of opioids, and AMPs (Aleksa et al., 2012), as well as opiates in adult and pediatric hair using HS-SPME-GC-QMS (Moller et al., 2010). The use of SPME in the extraction of cannabinoids has been thoroughly surveyed in a recent review article (Jain & Singh, 2016a).

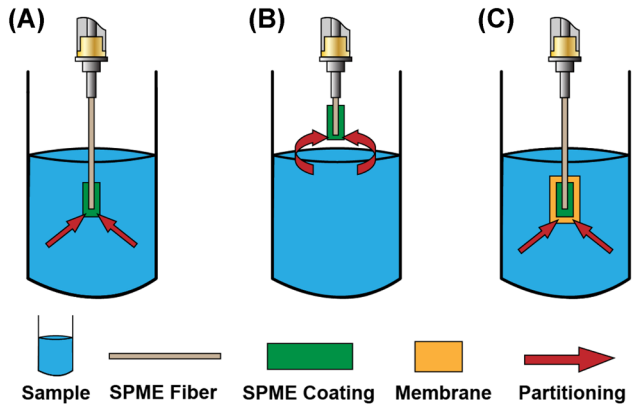

FIGURE 2. Extraction modes in solid-phase microextraction (SPME). (**A**) Direct-immersion SPME, (**B**) headspace SPME, and (**C**) membrane-protected SPME. Not to scale. [Color figure can be viewed at wileyonlinelibrary.com]

2. Microextraction With Packed Sorbent (MEPS)

The technique MEPS is the miniaturization of SPE, in which the sorbent is immobilized within a special removable needle in a syringe-like device (Fig. 3). This was first presented in 2004, and since then, MEPS use has been growing steadily (Abdel-Rehim, 2004).

The selection of sorbents for MEPS is the same as in conventional SPE. Sample loadings are typically between 10 and 250 µL, and the devices can be operated either manually or automatically, as described in a comprehensive review by the inventor (Abdel-Rehim, 2011). A fast method (15 min) based on MEPS-GC-QMS for the simultaneous determination of COC and its metabolites in human urine was recently reported (Jagerdeo & Abdel-Rehim, 2011). Microwave-assisted derivatization using *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide and 5% trimethylchlorosilane in 2 min has also been presented (Rosado et al., 2017). MEPS has been used in combination with micro pulverized extraction and aqueous acetylation (AC) for the determination of AMPs in human hair before GC-triple quadrupole (QqQ)MS analysis. The method presented is faster and more straightforward when compared with previous approaches for hair as a matrix (Miyaguchi et al., 2009).

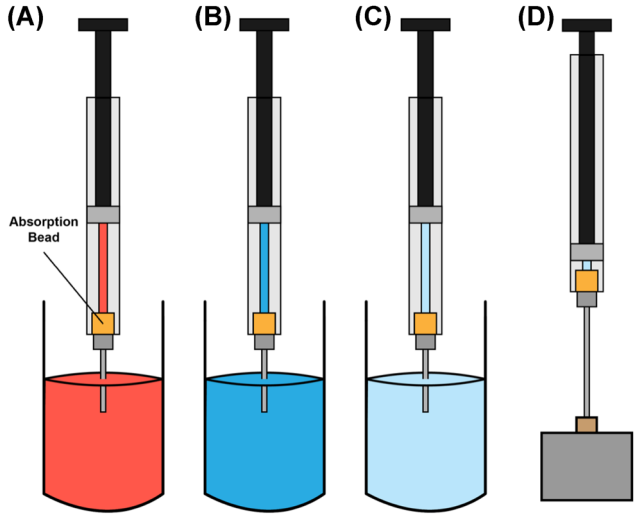

FIGURE 3. Microextraction with packed sorbent workflow. (**A**) Sampling, (**B**) washing, (**C**) elution solvent, and (**D**) injection. Not to scale. [Color figure can be viewed at wileyonlinelibrary.com]

3. Other SPE Approaches

In solid–liquid extraction with low-temperature purification, analytes partition between a solid matrix and water-miscible organic phase at room temperature. When the temperature is lowered to −20°C, the aqueous phase solidifies, and analytes in the supernatant organic phase can be directly introduced into the GC-MS system. This procedure has been demonstrated with GC-QMS analysis of COC in eight postmortem human livers (Magalhães et al., 2013).

Disposable pipette extraction has been used as an alternative to classic SPE in the determination of COC and metabolites, nicotine, and cotinine in meconium followed by GC-QMS. The solid phase is packed at the bottom of a pipette tip, and the operating protocol, though very similar to classic SPE, involves only a few microliters of solvent for analyte extraction (Mozaner Bordin et al., 2013). Another interesting approach consists of the use of molecularly imprinted polymers for the SPE determination of AMP-like stimulants from whole blood (Kumazawa et al., 2012).

4. Liquid-Phase-Based Microextraction Techniques

There are several extraction procedures described under the acronym of LPME, based on liquid-phase extractions with minimal amounts of solvent (e.g., microliters) to extract and preconcentrate drug analytes from different samples. The main LPME techniques fall into three main groups: single-drop microextraction (SDME), dispersive liquid–liquid microextraction (DLLME), and hollow-fiber microextraction (HF-LPME). An exhaustive description of these techniques falls beyond the scope of this review, and only a simple introduction will be given. The principles of operation, advantages, and pitfalls are thoroughly explained in several review articles (Sarafraz-Yazdi & Amiri, 2010; Yamini, Rezazadeh, & Seidi, 2019).

The basic principle of SDME utilizes a drop of organic solvent that is either immersed in an aqueous solution (direct-immersion-SDME) or suspended at the tip of a syringe (headspace extraction-SDME) (Liu & Dasgupta, 1996) (Fig. 4).

High enrichment factors are obtained because the volume of the acceptor phase drop is small (micro- to sub-microliters) and is done using simple equipment. In the case of headspace extraction, only volatile or semi-volatile analytes are sampled, further decreasing any interferences. Since its first introduction, several SDME modes have been presented to boost extraction efficiencies, such as three-phase mode (Ma & Cantwell, 1999), bubble-in-drop (Williams et al., 2011), and continuous-flow microextraction (Liu and Lee, 2000). Recent reviews provide a comprehensive overview of SDME (Jeannot, Przyjazny, & Kokosa, 2010, Tang et al., 2018).

Presented in 2006, DLLME uses a ternary system comprised of an aqueous sample, a water-immiscible extractive solvent, and a dispersive solvent (miscible with both water and the xtractive solvent) (Rezaee et al., 2006) (Fig. 5).

Extractive and dispersive solvents are mixed and transferred into an aqueous sample, forming a cloudy emulsion. Rapid equilibrium and mass transfer of analytes between organic and aqueous phases are achieved. Centrifugation separates the extractive solvent containing the analytes at the bottom of the tube, which is subsequently removed with a syringe. Various strategies such as salt addition or ultrasound assistance can be used to boost recoveries. DLLME has been widely used in DoA analysis because its simplicity, versatility, high preconcentration factor, and extraction recoveries. DLLME has been used for the extraction of various classes of compounds of forensic interest, as reported in a recent review article (Jain & Singh, 2016b).

In the case of polar analytes, silylation is often used for derivatization, requiring anhydrous conditions to avoid silylating agent hydrolysis, since DLLME is mainly conducted in water. A combination of DLLME and injector port silylation (IPS) has been described and validated for the analysis of quinine in urine GC-MS (Jain et al., 2013). A comparison of three extraction procedures, SPE, LLE, and DLLME was performed for the determination of fentanyl (FEN) in urine with GC-QMS. The results obtained for a real sample with the different techniques were in good agreement, demonstrating that the use of DLLME can be successfully utilized in the forensic analysis (Gardner et al., 2014). The technique was also reported for the extraction of free AMP-type stimulants, fenproporex, diethylpropion, and sibutramine in urine before GC-QMS analysis. High recovery percentages (>91%) were obtained for all drugs. No derivatization was needed; the organic phase was withdrawn from the extraction vial and injected directly in the GC-MS system (Cunha, Lopes, & Pereira, 2016). In another study, two microextraction techniques, HF-LPME, and ultrasound-assisted low-density solvent dispersive liquid–liquid microextraction (UA-LDS-DLLME) were applied and compared for the extraction of a variety of DoA in urine and blood samples, followed by

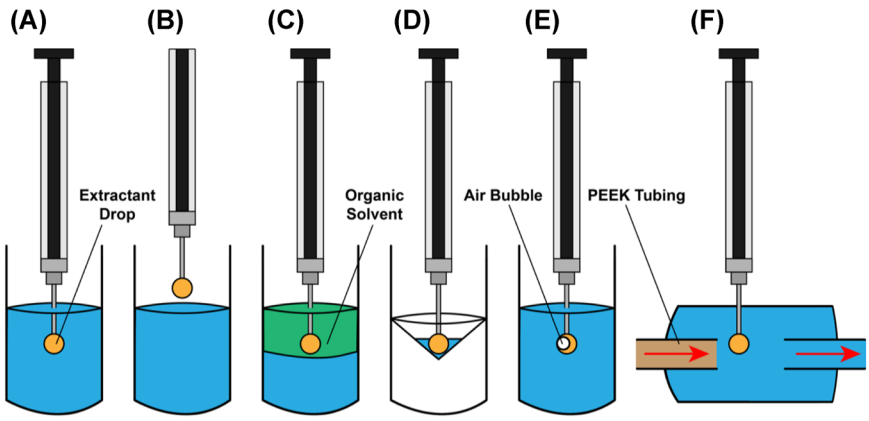

FIGURE 4. Extraction modes in single-drop microextraction (SDME). (**A**) Direct-immersion SDME, (**B**) headspace SDME, (**C**) three-phase SDME, (**D**) drop-to-drop microextraction, (**E**) bubble-in-drop SDME, and (**F**) continuous-flow microextraction. Not to scale. [Color figure can be viewed at wileyonlinelibrary.com]

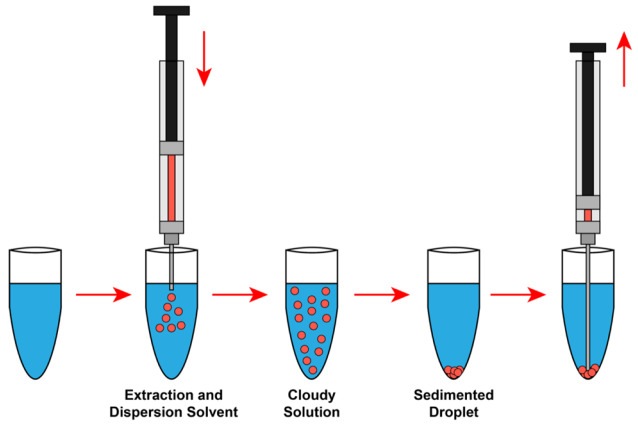

FIGURE 5. Classical dispersive liquid–liquid microextraction workflow. Not to scale. [Color figure can be viewed at wileyonlinelibrary.com]

GC-QMS analysis. UA-LDS-DLME makes use of ultrasound assistance for emulsification, avoiding the need for a dispersive solvent, making the technique even "greener." These procedures are comparable in terms of simplicity, rapidity, and recoveries, albeit with slightly higher recoveries for UA-LDS-DLLME, spanning from 79% to >100%. LDS-DLLME is an excellent strategy to avoid possible matrix effects and is faster than HF-LPME, but both techniques prove excellent for biological samples (Meng et al., 2015; Meng et al., 2017). Another investigation reported the use of UA-DLLME for the extraction of AMP-like drugs from whole blood before GC-QMS analysis (Lin et al., 2017).

The HF-LPME technique was introduced in 1999 for the extraction of methamphetamine (MAMP) as a model compound from aqueous samples (human urine and plasma) (Pedersen-Bjergaard & Rasmussen, 1999) (Fig. 6).

In the classic mode of operation, the organic solvent is trapped in the pores of a porous polypropylene hollow fiber, forming a thin layer on the walls. The internal volume of the HF is filled with a suitable acceptor phase solvent, and then the fiber is dipped into a vial containing the analytes in an aqueous sample (donor phase). The analytes are extracted from the donor to the acceptor phase via the organic solvent immobilized in the HF pores, which has formed a supported liquid membrane. The extracted solution is then removed and analyzed. Depending on the number of phases, two-phase or three-phase HF-LPME can be distinguished: in the two-phase approach, the trapped organic solvent and acceptor phase are the same, and it is generally used for analytes immiscible with water. The three-phase approach is suitable for acidic or basic analytes with ionizable functions; the analytes in the aqueous sample are extracted via the immobilized organic solvent, and then into the aqueous acceptor phase inside the internal volume of the HF. The control of pH is key in this mode: analytes must be kept in their neutral form in the sample (donor) to dissolve into the liquid membrane, and the acceptor phase pH must be adjusted to ensure their subsequent extraction for analysis.

HF-LPME can also be conducted either in static or dynamic modes. Static mode uses vibration or stirring to speed up extraction into the acceptor volume, whereas dynamic mode, uses a syringe pump to repeatedly pull the aqueous sample in and out of the HF for more efficient extraction, requiring optimization and increased instrumental control (Sharifi, Abbasi, & Nosrati, 2016). Full automation has been proposed, where all steps could be performed by a commercial autosampler with dedicated software (Ouyang, Zhao, & Pawliszyn, 2007). Various parameters can be adjusted to enhance extraction efficiency in HF-LPME, such as different fiber materials, organic solvents, pH, use of modifiers, temperature, stirring speed and time, and other variables linked to the instrumentation being used. A three-phase, static-mode approach was successfully used for the determination of AMPs in 50 mg hair samples. After decontamination, hair samples were placed in tubes containing water with NaCl as a modifier, a three-phase HF-LPME using diethyl ether and a 0.1 M HCl solution as the acceptor phase allowed quantitative extraction of the analytes before GC-ion trap (IT)MS analysis (do Nascimento Pantaleão et al., 2012). An HF-LPME method based on two immiscible organic solvents, *n*-dodecane in the pores of the HF and acetonitrile as the acceptor phase, followed by GC-QMS detection was validated for the efficient extraction and determination of COC, ketamine (KET), and lidocaine in human urine. No additional cleanup was needed, and limits of

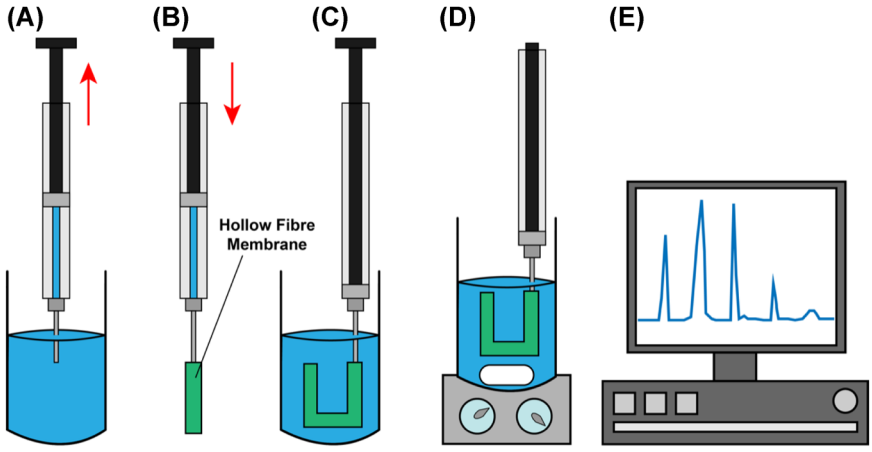

FIGURE 6. Steps of operation with hollow-fiber liquid-phase microextraction (HF-LPME). (**A**) Filling syringe with acceptor phase, (**B**) filling the HF lumen with acceptor phase, (**C**) bending the HF (U shape) and insertion into the aqueous sample, (**D**) agitation, (**E**) instrumental analysis. Not to scale. [Color figure can be viewed at wileyonlinelibrary.com]

detection (LODs) were sufficiently low for toxicological analyses (Yamini et al., 2014). Another article reports the GC-QMS determination of 11 BDZs and their main metabolites using three-phase mode HF-LPME from human urine. The analytes were derivatized using tert-butyldimethylchlorosilane, and/or trifluoroacetic anhydride (de Bairros et al., 2015).

5. Other Liquid-Phase Extraction Approaches

Switchable hydrophilicity solvents (SHS) can switch between two forms: hydrophobic and immiscible in the water when in air, and fully miscible in the water when in an atmosphere of CO_2 (Jessop et al., 2010; Lasarte-Aragonés et al., 2015). These solvents (e.g., *N*,*N*,*N*'-tributylpentanamidine; *N*,*N*-dimethylcyclohexylamine) can be used in microextraction techniques (Lasarte-Aragonés et al., 2015). Liquid–liquid microextraction based on switchable hydrophilicity solvent (SHS-LLME) has been validated for the GC -QMS determination of 11 DoA in the urine of a suspected KET user. The method involves small amounts of solvent (≤500 μL) and is a "green" alternative (Xu et al., 2018).

C. Derivatization

For GC-MS analyses, derivatization is frequently necessary to increase the thermal stability and volatility of polar, thermolabile, and low volatility compounds. It employs a myriad of chemical modifications to achieve this, including AC, trifluoroacetylation (TFA), pentafluoropropionylation (PFP), heptafluorobutyration (HFB), trimethylsilylation (TMS) for basic compounds. Methylation (ME), extractive ME, PFP, TMS, and *tert*-butyldimethylsilylation (TBDMS) are typically used for acidic compounds (Maurer, 2003). As described later in the GC-MS section, negative ion chemical ionization (NICI) in GC-MS is frequently used in trace analysis for its high sensitivity. In the case of compounds without electronegative substituents, a suitable derivatization reaction is employed to add them. Perfluoroacyl and pentafluorobenzyl (PFB) derivatives are typically used, though sometimes they exhibit extensive fragmentation, leading to low molecular ion abundances. Frequently, *O*-(pentafluorobenzyloxycarbonyl)-benzoylchloride (PBBCl) and *O*-(pentafluorobenzyloxycarbonyl)-2,3,4,5-tetrafluorobenzoyl chloride (PBTFBCl) are used as derivatizing agents for primary and secondary amines to achieve high sensitivity in NICI-GC-MS analysis, such as for the measurement of methylphenidate (Leis & Windischhofer, 2010) and AMPs in plasma (Leis & Windischhofer, 2012). In another example, 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH) in urine is considered a valid biomarker for cannabis use, extracting THC-COOH from the matrix via SPE before derivatization. A silylation procedure with *N*,*O*-bis-trimethyl-silyl-trifluoroacetamide (BSTFA) has also been validated and optimized for use with GC-IT tandem MS (MS/MS) (Prata, Emídio, & Dorea, 2012). THC and COC have been extracted from hair samples and analyzed with GC-QMS without derivatization. Hair samples were decontaminated with dichloromethane, enzymatically digested with Proteinase K, then extracted by LLE with pentane. The organic fraction was neutralized, evaporated, then reconstituted with hexane before injection (Breidi et al., 2012).

Injection port silylation has been used for γ-hydroxybutyrate (GHB) GC-MS measurements, where GHB is converted into di-trimethylsilyl derivatives just prior to analysis. Derivatization occurs in the GC injection port, yielding a faster reaction and avoiding derivative degradation that can occur during off-line silylation. IPS has been used to produce GHB and *trans*-4-hydroxycrotonic acid (used as a potential internal standard) derivatives using *N*-methyl-*N*-[tert-butyldimethylsilyl]trifluoroacetimide with tert-butyldimethylchlorosilane (99:1) to determine the concentration of endogenous GHB in urine samples, optimizing injector temperatures to achieve the highest derivatization yields. This method has been applied to toxicological analyses (Elie, Baron, & Birkett, 2012).

The use of monolithic silica spin columns has been proposed for the simultaneous extraction and derivatization of AMPs in human urine. The procedure is very similar to a classic SPE extraction with cartridge preactivation, sample loading, and washing steps, but is done in an apparatus spinning at 5000 rotations per minute (rpm). In the last step, both elution and derivatization with ethyl acetate containing 1% propyl chloroformate occur at the same time, with centrifugation rate determining derivatized analyte elution times. This method is "green," requiring small solvent volumes and no evaporation, reducing sample loss, and is easy to perform (Nakamoto et al., 2010). An "in-vial" derivatization procedure has also been proposed for GHB methylation in urine, plasma, and whole blood that reduces reagent quantities and accommodates small sample volumes (Ingels et al., 2013).

Microwave heating can be exploited to speed up derivatization protocols, reducing them to a few minutes, because higher temperatures can be reached in less time. In an interesting study, three popular derivatization reactions were evaluated, demonstrating the time-saving advantages over conventional heating: AC for morphine (MOR) and codeine (COD), PFP for 6-monoacetylmorphine (6-AM), and TMS for THC. Detection was achieved in these studies using GC-MS, LC-MS and LC-MS/MS (Damm et al., 2009). An orthogonal design was also proposed to improve the performance of microwave-assisted derivatization of AMP and MAMP followed by NICI-GC-MS using 2,3,4,5,6-pentafluorobenzoyl chloride as derivatization reagent (Chung et al., 2009). In another investigation, microwave assistance was satisfactorily used for on-spot derivatization of GHB and gabapentin for their determination in dried blood spots (Sadones et al., 2016). The quantitative TMS of 11 phenylalkyl amines, including AMP and 3,4-methylenedioxiamphetamine (MDA), in urine, using *N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide in several solvents in the presence of suitable catalysts has also been presented, and is highly efficient using microwave heating when compared with other procedures (Molnár et al., 2015).

III. GAS CHROMATOGRAPHY-MASS SPECTROMETRY

A. Introduction, History

In this section, we give an overview of research utilizing GC-MS since 2010 related to the analysis of DoA in different biological matrices.

The list of illicit drugs has increased rapidly because of the emergence of new drug classes (new psychoactive drugs [NPD]). In 2016, the European Union (EU) Directorate-General for Internal Policies published a review of EU drug policy revealing that, in general, cannabis is the most commonly

used drug in the EU, followed by COC, MDMA, and AMPs (European Parliament, 2016). The use of different drugs can vary considerably between countries; however, there is an increasing tendency for the use of NPD. Immunoassays can be used for rapid confirmatory screening tests but are not available for all compounds. In the case of positive immunoassay results, suitable analytical methods and techniques are needed to identify and quantify the increasing number of potential substances, focusing on identification, confirmation, and quantitation. When analytes are unknown, efficient GC, and/or comprehensive, two-dimensional (2D) gas chromatography (GCxGC) separations, coupled with electron ionization (EI) MS are used. This strategy provides fragment rich mass spectra, and remains the reference technique of choice for the analysis of volatile, semi-volatile, low-polarity, low-MW, and non-LC-MS amenable DoA (Saito et al., 2011; Meyer & Maurer, 2012), despite the fact that in many cases, elaborate sample preparation and derivatization steps are required.

B. Ionization Techniques

The ionization technique of choice in GC-MS is EI for the majority of drugs, providing legally defensible identification and reliable qualitative and quantitative analysis at high sensitivity and selectivity. EI is a gas-phase, hard ionization technique that occurs in high temperature and high vacuum environment. Because of this, ion–ion or ion–molecule reactions are unlikely to occur. Consequently, matrix effects are very limited or absent, compared with LC-MS. The ionization process is the result of intramolecular reactions only and is reproducible with wide variety of instruments and conditions, not influenced by external agents that can also be present in the ion source. This results in highly reproducible mass spectra that can be compared with those present in spectral database libraries such as the National Institute of Standards and Technology (NIST), MassBank, and others, for the unequivocal identification of the analyte. Those libraries can be used when confirmation is needed (targeted analyte), but also in the case of untargeted analytes.

In the case of scarce or absent molecular ion formation, and to follow metabolic pathways of new designer drugs, positive-ion chemical ionization (PICI) can be used to obtain information on the MW (Ewald, Fritschi, & Maurer, 2007; Sauer et al., 2008; Sauer et al., 2009). In the analysis of AMPs in urine (Tzing et al., 2006), EI yields a high degree of fragmentation, with interfering low *m/z* ions that limit selectivity and interfere with ion intensities. These authors tested different PICI reagents (methane, methanol, acetonitrile, carbon disulfide, tetrahydrofuran; furan), obtaining the highest selectivity with furan. The metabolism of three designer drugs, mephedrone, butylone, and methylone was also followed in rat and human urine using PICI-GC-MS (Meyer et al., 2010). NICI with GC-MS has been reported in several studies for the determination of cannabinoids in the hair (Kim et al., 2011) as well as BDZs and associated metabolites in whole blood (Gunnar, Ariniemi, & Lillsunde, 2006; Karlonas et al., 2013), using methane as the reagent gas. NICI was also used for the determination of 50 DoA belonging to different classes in oral fluid as an alternative sample matrix (Langel et al., 2011). MDMA and its unconjugated phase I metabolites have also been analyzed with NICI-GC-MS in human urine (Schwaninger et al., 2011). The value of integrated GC-EI-MS and GC-NICI-MS in human hair to give higher sensitivity and complementary results has also been demonstrated for opiates, AMPs, MDMA, KET, and metabolites (Wu et al., 2008).

An innovative interface to obtain enhanced molecular ions, while retaining library matchable typical EI spectra, has been proposed by Amirav (2017). It is called GC-MS with Cold EI, based on EI of vibrationally cold molecules in supersonic molecular beams (SMB). An additional advantage of this interface is that sensitivity is not influenced by the column flow rate; therefore, this can be increased up to 100 mL/min for fast screening without losing sensitivity, and the authors have demonstrated its use for the fast analysis of HER and COC.

A unique approach to obtain soft ionization in GC-MS has also been presented by the Zenobi Group. They use a dielectric barrier discharge ionization (DBDI) source (Mirabelli, Wolf, & Zenobi, 2017), in which an appropriate voltage is applied to an active capillary plasma source connected to the MS inlet. A low-temperature plasma is formed within the device, yielding $[M+H]^+$ ions with almost no fragmentation or adduct formation with either air or nitrogen as plasma gases. This source has been successfully used to measure 14 DoA in a standard mixture (Mirabelli, Wolf, & Zenobi, 2017). The DBDI source is further discussed later in the ambient ionization section.

C. Analyzers

Single-stage, low-resolution quadrupole mass analyzers are the workhorse in DoA analysis because of their simplicity, relatively low-cost, and operation in both in full scan and in selected-ion monitoring (SIM) modes. Most GC-MS publications still utilize this type of analyzer for measurements in many different sample matrices (e.g., urine, blood, hair, nails, oral fluid, etc.), providing LODs suitable for many forensic and toxicological purposes. Superior sensitivity and selectivity can also be achieved with IT, hybrid Q-Time of Flight (Q-TOF), Orbitrap, and triple quadrupole (QqQ) analyzers operating in MS/MS mode. Their use has increased enormously in the last few years in DoA analysis as well as many other diverse areas, particularly when coupled with LC-MS and ambient ionization methods, as will be discussed later.

1. Quadrupole

DoA can be used in criminal activities, often in combination with alcoholic drinks, for so-called drug-facilitated crimes, where the victim is involuntarily incapacitated, leaving them with no memory of the subsequent assault. A wide range of drugs have been used in drug-facilitated crimes, making their identification in biological specimens and beverage/food residues challenging, especially when considering their rapid excretion and/or metabolite formation. GC-EI-QMS has been used for the simultaneous determination of 128 drug-facilitated sexual assault drugs in urine, after SPE extraction (Adamowicz & Kała, 2010). Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) extraction method followed by GC-QMS was used for the determination of eight BDZs in a milk-based alcoholic drink, a complex matrix due to the presence of proteins and fatty acids (Famiglini et al., 2015). Other examples of the detection of BDZs and KET in common alcoholic and nonalcoholic drinks can be found in the literature (Acikkol, Mercan, & Karadayi, 2009; Gautam, Sharratt, & Cole, 2014).

A rapid screening, fast GC-MS method, utilizing a short column (10 m × 0.18 mm i.d., 0.18 μm film thickness) and QMS was developed for THC-COOH, COC, opiates, buprenorphine (BUP), FEN, and metabolites in urine in 6 min (Strano-Rossi et al., 2011b). The same approach was used for the determination of 52 stimulants and narcotics in urine (Strano Rossi, de la Torre, & Botrè, 2010). The same authors also describe the simultaneous determination of several FENs and their metabolites in urine (Strano-Rossi et al., 2011a). Twenty-five DoA in blood and urine were simultaneously determined using a GC-QMS after flash derivatization directly in the matrix and DLLME extraction (Mercieca et al., 2018).

Hair is a complementary matrix to urine and blood for screening DoA, and GC-QMS proved to be a valid and less expensive alternative to LC-MS/MS approaches for this matrix (Orfanidis et al., 2017). In many cases, hair is preferable to fluids because it is easier to collect and store, and provides detectable drug signatures after extended periods from the time of use. A comprehensive review of analytical methods for various classes of DoA in hair offers an overview of pretreatment steps and analytical approaches (Wada et al., 2010). An example is the identification and quantification of DoA in human hair using GC-QMS in SIM mode. Opiates were extracted using a mixed-mode SPE from a small amount of hair samples and semi-quantitatively determined by GC-QMS (Barroso et al., 2010). GC-QMS was used to identify and quantify six designer synthetic cathinones in human urine (Hong et al., 2015).

Sweat represents another noninvasive, simple matrix for DoA screening, and it can be collected by DrugWipe®5A, a sweat drug screening device (Securetec, Ottobrunn, Germany). The device is used to rub the forehead skin, allowing the simultaneous detection of opiates, COC, AMP/MAMP, and cannabinoids. If the result is positive, a second sample is collected and analyzed using HS-SPME followed by GC-MS. This two-tiered approach allows the determination of a wide range of DoA with GC-QMS (Gentili et al., 2016). This HS-SPME GC-QMS strategy has been used for the determination of the same DoA in hair (Merola et al., 2010).

Another matrix that is gaining attention in forensic analysis is fingernail clippings. DoA accumulates inside the nails, remaining stable for a long time. AMP-like drugs and KET can be successfully extracted from nails and determined with GC-QMS (Kim, Shin, & In, 2010). Teeth are another alternative tissue that can be used for drug testing, together with nails, hair, and bones, even in postmortem specimens. Teeth are much more resistant to degradation from temperature and pressure than other tissues and can be used to detect drugs for an extended period of time in postmortem samples. Following the same sample preparation used for hair, cannabinoid compounds were determined in teeth with GC-QMS (Ottaviani et al., 2017). AMP can be determined in oral fluid, an easily collected matrix that is also difficult to adulterate (Choi et al., 2012). Cannabinoids have also been extracted from human breastmilk samples using HS-SPME and quantified using GC-QMS (de Oliveira Silveira et al., 2017). The same authors also investigated LPME as extraction method for COC-like compounds and metabolites from human breastmilk followed by GC-QMS analysis (de Oliveira Silveira et al., 2016). An uncommon specimen for DoA testing is the human placenta, which has been analyzed with GC-MS for DoA and other compounds (Joya et al., 2010). In this work, the human placenta from a voluntary pregnancy interruption was used, but noninvasive tissue collection in a full-term pregnancy is feasible and would be able to provide evidence of in utero exposure.

In emergency toxicology, fast semi-quantification of analyte/s is imperative to make a fast, life-saving decision regarding the most suitable treatment for the patient. A fast LLE, multi-analyte procedure was developed for the quantification of 40 DoA in urine, followed by full scan GC-QMS and one-point calibration (Meyer, Weber, & Maurer, 2014).

2. Ion Traps

One of the potential limitations of ITMS is matrix interference, which can lower sensitivity when working in full scan mode. On the other hand, it offers the possibility to perform tandem MS experiments with less expensive instrumentation. GC-ITMS was used in the determination of trace levels of *N,N'*-dimethyltryptamine in beverages, such as Ayahuasca and Vinho da Jurema, often consumed in religious rituals in South America (Gaujac et al., 2013). Ecstasy (MDMA) and metabolites in plasma and urine were also determined using GC-ITMS (Gomes et al., 2010). The simultaneous quantification of COC-like compounds and opioids in blood, muscle tissue, and water (as a simulation of the vitreous humor) demonstrated the advantages of GC-ITMS/MS to remove matrix interferences while preserving good sensitivity and selectivity (Rees, McLaughlin, & Osselton, 2012). Cannabinoids were extracted from hair samples using HS-SPME and determined using GC-ITMS/MS (Emídio, de Menezes Prata, & Dórea, 2010b). The same research group reports the extraction of the same compounds from human hair samples using HF-LPME followed by GC-ITMS/MS. A factorial design was employed to optimize the operative conditions (Emídio, de Menezes Prata, & Dórea, 2010a). Several barbiturates were also determined in head hair samples with prior alkaline digestion and LPME (Roveri, Paranhos, & Yonamine, 2016). PICI GC-ITMS was also used in the determination of GHB and its precursors, γ-butyrolactone and 1,4 butanediol in dietary supplements, utilizing isotope dilution MS (Rosi, Frediani, & Bartolucci, 2013). Pregabalin has been determined in hair samples using GC-ITMS/MS coupled with ethyl chloroformate derivatization and DLLME (Ianni et al., 2018).

3. Time-of-Flight

To date, a very limited number of publications report the use of TOF in the GC-MS analysis of DoA. This analyzer offers fast scanning and sufficient data points across the peak, and for this reason, it is more advantageous for fast-GC and GCxGC. An example is given by the determination of 35 BDZs in urine, preceded by SPE (Arnhard et al., 2012). In the case of fast separations and GCxGC, high-speed TOF instruments allow the detection of narrow peaks with nominal mass resolving power.

4. Orbitraps

In the growing world of novel psychoactive substance (NPS), there is a mandatory requirement for fast identification and quantification in seized materials. In most cases, neither certified standards nor scientific data are available. HRMS using different analyzers, such as Orbitrap (and TOF) can provide sufficient mass accuracy to resolve isobaric ions for possible identification of untargeted compounds (Hernández et al., 2011). The only drawbacks of such

high-end systems are their higher costs and the need for highly trained personnel. In an interesting study, Frison et al. used a combined approach that included GC-QMS, Orbitrap LC-electrospray ionization (ESI), ESI-MS^n ITMS, and nuclear magnetic resonance (^1H and ^{13}C NMR) for the characterization of 102 seized powders. This multiple approach allowed the identification and the molecular structure characterization of a new designer drug, a KET analog called deschloroketamine (Frison et al., 2016). Recently, Orbitrap technology with GC-HRMS was used to develop a high-throughput screening for 288 DoA and poisons in human blood (Pan et al., 2019).

5. Triple Quadrupole

Many procedures report the use of QqQ analyzers in the GC-MS/MS determination of DoA in various matrices because of their quantitative highly reproducible results, high sensitivity, and specificity via multiple reaction monitoring (MRM). Examples include the determination of AMP-type stimulants in blood and urine (Woźniak et al., 2018), THC-COOH in oral fluid (Barnes, Scheidweiler, & Huestis, 2014) and in human plasma (Rosado et al., 2017), KET and norketamine (NKET) in urine and plasma (Moreno et al., 2015), and GHB in ante- and postmortem whole blood samples (Castro et al., 2016).

D. Two-Dimensional Gas Chromatography

The development of two-dimensional gas chromatography (2D GCxGC; GCxGC) represents an outstanding step forward for GC analysis. This approach was first developed in 1991 (Liu and Phillips, 1991) and has become very popular because of its vastly improved peak capacity, especially for very complex samples, where MS identification can be impaired by matrix composition and co-eluting analytes. There are numerous advantages that 2D GCxGC offers over 1D GC, including superior increases in selectivity, sensitivity, and separation power. Two exhaustive reviews on this topic appeared in 2008 (Mondello et al., 2008) and 2016 (Tranchida et al., 2016), describing the many advantages over 1D GC, and recent advancements made in combination with different MS analyzers. Mitrevski, Wynne, & Marriott (2011) have reviewed 1D and 2D GCxGC methods in drug profiling, highlighting differences and advantages of the two approaches.

To simply describe GCxGC, two columns, generally of different polarities, are connected in series with a transfer system called a modulator. The first column is a conventional GC column that separates the analytes based on their volatility, generating peaks typically 30–60 sec wide. The modulator collects, focuses and injects the eluting peak into the second column in a few short pulses. The second column is a short (1–2 m) microbore column that allows very fast separations, typically a few seconds. The combination of the refocusing process and orthogonal separation increases the number of peaks being resolved, boosting analyte profiling. The chromatographic peaks are very narrow and rapidly eluting, requiring fast acquisition rates.

Low-resolution TOF is by far the most popular because of its high sensitivity, fast scanning speed, and full-spectrum acquisition. For example, residues of opiates, opioids, COC-like compounds, sedatives, and other drugs were quantitatively screened in three hair samples using GCxGC-TOF-MS. Their identification was confirmed using automated library spectra searches (Guthery et al., 2010). GCxGC-TOF-MS was employed for MDMA extracts (Schäffer et al., 2012), HER and COC profiling with pixel-based chemometric processing (Gröger et al., 2008), and compared with GC-QMS in the targeted and nontargeted analysis (Schäffer et al., 2013).

New generation, rapid-scanning QMS can also be coupled with GCxGC. These analyzers offer a less expensive option for qualitative purposes and identification confirmation because of their fast acquisition speeds (compared with older systems), and there are indications that they can allow for quantitative analysis. Kolbrich, Lowe, & Huestis (2008) used a GCxGC-QMS system for the simultaneous quantification of AMPs and metabolites in human plasma prior to derivatization. GCxGC-QMS was also used in a validated method to extract (SPE) and quantify BUP in postmortem blood samples (Nahar, Andrews, & Paterson, 2015). GCxGC was successfully used for the detection of THCOOH in human fingernail clippings and head hair with a detection limit of 10 fg/mg using a QqQ-MS detector (Jones et al., 2013).

IV. LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY

A. Introduction

In this section, we focus primarily on mass spectrometry combined with LC for the analysis of a large number of chemically diverse DoA in biological matrices (rather than seized drugs) and discuss issues related to sample preparation, instrumental techniques of separation and identification. Our aim is to describe selected broad-spectrum screening methods used in daily production work rather than proof-of-concept studies or methods aimed at a specific class of compounds. The area of forensic LC-MS has been reviewed extensively over the years by the Maurer group (Maurer, 1998, 2007; Meyer & Maurer, 2016).

The compatibility of an LC-MS screen with a large and rapidly evolving number of compounds is essential in forensic toxicology, as the samples encountered are typically positive for multiple drugs and metabolites (Roemmelt, Steuer, & Kraemer, 2015). However, "forensic" toxicology, as noted in the Introduction, also includes fields where a limited range of target compounds has been clearly defined by a regulatory agency. The reader is also referred to literature from related fields, most notably wastewater (Hernández et al., 2018), metabolomics (Gika et al., 2019) pesticide residue analysis (Wong et al., 2018), where similar challenges and techniques are encountered. The accepted rule in forensic (vs clinical) toxicology is that initial identification of a drug must be confirmed by a second orthogonal method (Society of Forensic Toxicologists, 2006). As a result, most of the methods described here are confined to a qualitative screening, with the intention of subsequent confirmation being performed with a targeted quantitative assay.

B. Sample Preparation

As per any type of analytical step, the sample preparation method must consider the classic trio of cost, quality and time. A busy clinical laboratory dealing with an overdosed patient may opt for an "dilute-and-shoot" procedure for urine (Eichhorst et al., 2009;

Kong et al., 2017) or solvent precipitation (Lung et al., 2016) for serum, while a forensic lab may choose a more robust and comprehensive method despite the added cost and time (Han et al., 2019). Moreover, both clinical and human performance testing are largely confined to a small number of matrices (i.e., urine, serum, blood), and even dermal absorption samples (Cappiello et al., 2011), while a forensic lab must be prepared to process a wide range of ante- and postmortem sample types (e.g., hair, nails, gastric contents). The specificity of sample preparation may also be dictated by the range of compounds sought, so specific extraction methods are often contraindicated in broad-spectrum testing. The role of automation should also be considered: specifically, whether the gains in productivity are justified by the increased risk of contamination between samples. We will survey these methods in turn, starting with the most basic.

A simple dilution of the sample (typically urine) with a solvent containing internal standards is widely used owing to its ease and speed as well as having the nonspecificity needed for broad-spectrum testing (Deventer et al., 2014). Two drawbacks of this technique are its limitation to unconjugated drugs present at relatively high concentrations, and the potential for significant sample matrix effects. However, the approach is ideal for the analysis of seized synthetic drug powders where dilution, rather than any concentration or matrix removal, is all that is needed (Fiorentin et al., 2019). Preparation of seized biological products for the identification of low-concentration drugs such as synthetic cannabinoids, by contrast, is considerably more involved (Langer et al., 2014). A variant of the "dilute-and-shoot" procedure is protein precipitation, where sample proteins are precipitated with an appropriate mix of solvents, retaining the target compounds in solution. This step is typically only done once, though some have found two cycles to be more effective (Roslawski et al., 2019). Protein precipitation is amenable to a broader range of matrices, including various forms of blood, and has a low compound specificity that is ideal for broad-spectrum screening.

LLE has been the classic technique for GC-MS sample preparation but is diminishing in use with the growing popularity of both automated liquid handlers (to which LLE is poorly suited) and LC-MS, with its acceptance of aqueous samples requiring minimal preparation. LLE is infrequently used in the broad-spectrum analysis (Partridge et al., 2018) and is seen more often in group-specific testing (Caspar et al., 2018). Two interesting versions of LC-MS-aimed LLE have recently appeared in the drug testing literature. The first is the use of ionic liquids, which have attractive features such as an exceptionally low ratio of extracting solvent to sample and no requirement for an evaporation step. Unfortunately, recoveries tend to be modest, matrix effects high and use to date has been restricted to nonpolar drugs, such as anti-depressants (De Boeck et al., 2018) and BDZs (De Boeck et al., 2018). There is also the problem of isolating the (heavier) extracting solvent from the sample-solvent mixture, though the use of magnetic ionic liquids has been suggested as a means of making the process compatible with lab automation (Mafra et al., 2019). The other recent development in LLE is the use of supramolecular solvents, where essentially quantitative recovery with negligible matrix effect has been reported for 11 illicit phenethylamines extracted from oral fluid with a mixture of alkaline hexanol and tetrahydrofuran (Accioni et al., 2019).

SPE has seen extensive use in forensic broad-spectrum applications (Sauvage et al., 2006; Pedersen et al., 2013; Sundström et al., 2013; Palmquist and Swortwood, 2019). This approach typically combines cation exchange with hydrophobic interaction: while this works well for most drugs, there may be significant blind spots for small polar or neutral species such as GHB and alcohol. Some groups (Petersen et al., 2013; Steuer et al., 2019) have addressed this problem by targeting minor metabolites of such species (e.g., glucuronides, carnitines), which have sufficient hydrophobicity to improve both extraction and chromatographic retention, but the incorporation of these drugs into a broad-spectrum assay remains an elusive challenge. On the other hand, inefficient extraction may be desirable for compounds such as gabapentin, where even therapeutic concentrations may be far higher than those of other drugs (Heltsley et al., 2011).

The lengthy commitment of the MS instrument to signal acquisition during a typical chromatographic separation has prompted the exploration of parallel online extraction methods such as turbulent flow chromatography (TFC), to exploit this available time increase throughput. Grant has described a multiplex TFC to process >1000 serum samples per day but pointed out that the greatest gains can only be realized with a minimal acquisition window, that is, a small portion of the LC run (Grant, 2016). TFC has also been used with the goal of reducing manual handling rather than increasing throughput: a single-channel TFC instrument was used to analyze serum, urine, and whole blood for a broad mix of compounds ranging from acetaminophen to diazepam using three extraction columns in series linked to a phenylhexyl separation column (Roche et al., 2016). With plasma samples, TFC combined with protein precipitation offered a slight reduction in matrix effect and improved recovery of lipophilic species such as diazepam while protein precipitation alone provided superior recovery for polar compounds (e.g., opiates) (Helfer et al., 2017).

C. Separation Methods

While chromatographic procedures for drug analysis exist in countless forms, the needs of a forensic broad-spectrum analysis veer toward the nonspecific and so the choice of chromatographic conditions needs to be a compromise between the most polar analytes (e.g., benzylpiperazines, GHB, opiates) and the least (e.g., BDZs, cannabinoids). While earlier workers regularly used C_{18} columns having a solely partition-based mechanism of retention, the current trend is toward a stationary phase with an additional pi-pi component (e.g., biphenyl, phenylhexyl). An alternative approach to polar analytes is to target a minor (but less polar) metabolite rather than the parent compound (see above).

Separation methods need not be confined to chromatography, with much recent interest in ion mobility (with or without LC) to very rapidly provide some separation of species based on the differential migration of ions in an electric field in a manner unrelated to their MW (Lapthorn, Pullen, & Chowdhry, 2013). This is especially pertinent for compound groups prone to isobaric forms, such as cathinones (Joshi et al., 2014) and opiates (Adams et al., 2018). In the latter study, the authors found that while ion mobility did not offer clear separation gains over LC and required the use of an *a priori* library of collisional cross-section values to optimize separation, it provided an independent parameter for compound

identification which, unlike retention time (RT) or fragment ion ratios, was not affected by concentration. Isobaric compounds of similar structures present a problem better suited for chromatography rather than mass spectrometry: yet, it is uncommon for the adequacy of chromatographic separation to be evaluated extensively with a large mix of isobaric compounds having similar RTs (Helfer et al., 2017).

Chromatographic run times vary markedly, often exceeding 30 min (Remane, Wetzel, & Peters, 2014; Alcántara-Durán et al., 2018). While 10–15 min is more typical, even this still translates to a fraction of the throughput achievable on a clinical immunoassay platform and highlights the chronic turnaround time problem facing forensic laboratories (State of California, 2003; Canadian Broadcasting Corporation News, 2018). For example, a forensic lab in Florida (Tiscione et al., 2017) found an average reporting time of 19 days for blood screen requests. Some efficiencies can be gained through parallel processing during sample preparation (e.g., 96-well plates, liquid handlers) but the LC separation remains a bottleneck in that lengthy periods of analysis time are devoted to the processing of a single sample: consequently, there is little opportunity to increase throughput through LC multiplexing as has been reported for single-analyte tests such as 25-OH vitamin D (Netzel et al., 2011).

D. Detection Techniques

A wide variety of MS detection techniques have evolved, each tailored to the needs of the end user. For example, laboratories with a large daily workload of workplace testing samples, minimally experienced staff, an uncompromising client expectation of rapid turnaround time, a predominance of a handful of drugs with no requirements for nontargeted analysis will choose a QqQ system rather than an elaborate TOF with library searching. By contrast, a lab specializing in postmortem work and needing to ensure a comprehensive search will likely prefer to use HRMS for screening with a QqQ for confirmation, though some (Rosano, Ohouo, & Wood, 2019) chose the reverse strategy. While most attention is typically focused on identification of positives, the certainty of negative results is equally important: QqQ may enjoy some advantage here over HRMS, as its extended periods of dedicating the instrument to a specific compound make it less prone to the stochastic issues that can confound HRMS due to brief acquisition times (Berendsen et al., 2017).

Still, there is a trend toward broad-spectrum HRMS screening methods owing to the challenges presented by NPS: specifically, their rapid proliferation and geographically heterogeneous distribution (European Monitoring Centre for Drugs and Drug Addiction, 2019). While targeted QqQ methods remain widely used, they are arguably more dependent than HRMS on the availability of reference standards which hinders their ability to definitively identify this endlessly mutating group of compounds.

Regardless of the type of MS used, the predominant LC-MS ionization interface in forensic LC applications is ESI rather than atmospheric pressure chemical ionization (APCI). The latter is less prone to ion suppression/enhancement but at the cost of significantly lower sensitivity: this is especially important for HRMS methods where sensitivity is typically compromised in order to achieve comprehensive compound identification. APCI has been employed primarily for lipophilic neutral drugs such as cannabinoids (Desrosiers, Scheidweiler, & Huestis, 2015), BDZs (Verplaetse, Cuypers, & Tytgat, 2012), anti-psychotics (Fisher et al., 2013) as well as a broad-spectrum panel (Remane et al., 2010). In a non-forensic application, a combined ESI and APCI source delivered stronger signals with superior signal-to-noise than either mode alone for the analysis of nine BDZs and zolpidem in pharmaceutical products (Galaon et al., 2014).

Mass separation by single-QMS has effectively been replaced by either fragmentation or high-resolution methods. Fragmentation is usually performed in a collision cell and only infrequently within the source itself. The in-source fragmentation technique is limited by its inability to fragment compounds requiring a high collision energy, as well as by a strong dependence on conditions such as source design, gas pressure, and the presence of co-eluting matrix compounds, all of which tend to give poor reproducibility, requiring instrument-specific spectral libraries (Parcher et al., 2018). Nevertheless, one study found fewer false-positive samples with added in-source fragmentation compared with LC-TOF-MS alone: this came at the cost of slightly more false negatives, though exclusively at levels near the lower reporting threshold (de Castro et al., 2012). In another study, the detection of drugs in postmortem blood by UHPLC-MS with in-source fragmentation was found to be only slightly inferior (73.1% sensitivity) compared with LC-MS/MS (74.4%) (Rosano, Wood, & Swift, 2011). By contrast, LC-TOF-MS with in-source fragmentation accurately detected two fragment ions in only 75% of a panel of 200 sports doping drugs, compared with 100% when using LC-QTOF (Domínguez-Romero et al., 2015).

Initial broad-spectrum quadrupole methods were based on collision cells used selected reaction monitoring (SRM) (Bjørk et al., 2010; Bassan, Erdmann, & Krüll, 2011; Vincenti et al., 2013), with data being collected in defined intervals over the course of a chromatographic run. This approach has given way to scheduled MRM (sMRM) where each channel has its own RT window: the duty cycle is reduced, by acquiring at any given time only those compounds within a narrow window of their expected RT, and set to a fixed value to provide consistent data acquisition across all chromatographic peaks. The shorter duty cycle permits the inclusion of additional channels (e.g., qualifier ions or new compounds) or simply more data points across a chromatographic peak. Further specificity was added by data-dependent analysis (DDA), where an initial MS (Mueller et al., 2011) or MS/MS (Sauvage et al., 2006; Dresen et al., 2010) scan is used to trigger a product ion scan based on survey ions exceeding a given threshold: for compounds with inherently rich spectra, this can greatly enhance identification confidence but the gains are limited for compounds with inherently few fragments, such as AMPs. While the dependent scan adds appreciably to the duty cycle, risking a sub-optimal number of data points per chromatographic peak, its acquisition frequency can be limited by a fixed number of survey signal triggers and a post-acquisition exclusion period.

The use of QqQ instruments continues to be widespread because to their modest cost, ease of implementation and the reduced complexity of interpreting MRM data, but there have been few recent advances in their data acquisition. A likely reason is that chromatographic peak width and data acquisition capability (in the form of minimum dwell time) inherently limit the number of MRMs and dependent scans achievable in a duty cycle. In addition, MRM-based QqQ methods require continual

updating with the emergence of new drugs and cannot be used to perform retrospective analyses: these concerns do not apply to MS scan-triggered DDA but its ability to reliably identify new compounds is limited by unit mass resolution.

By contrast, HRMS methods play an increasingly prominent role in the broad-spectrum screening literature, in part through their provision of accurate mass for both precursor and fragment ions, which reduces the number of likely candidates for a given chromatographic peak. Early HRMS methods were based on single-stage mass scanning to identify compounds by accurate precursor ion mass and RT only (Maurer, 1998). While some methods used in-source fragmentation to generate spectra (Power et al., 2012; Domínguez-Romero et al., 2015), their modest and variable effectiveness in generating multiple fragment ions has led to them being largely replaced by collision-induced dissociation methods. These techniques include DDA, as described above, with the triggering event for the dependent scan being a precursor ion selected from the most abundant ions and/or an inclusion list of compounds of interest.

The other broad family of HRMS techniques is data-independent analysis (DIA), where the spectra derived from all precursor ions are acquired with varying degrees of precursor ion selectivity. A significant benefit of DIA over DDA is an improved rate of detection because all precursor ions (and their spectra) are detected: by contrast, DDA may fail to detect compounds if the precursor signal is below threshold, the spectrum is dominated by other species or precursor ion selection is based solely on an inclusion list. In the basic form of DIA, originally named "MS^E" (Plumb et al., 2006), precursor ions are detected by an initial survey scan followed by fragmentation at fixed (Sundström et al., 2013) or multiple (Caspar et al., 2018) collision energies to create spectra for library searching. This allows for retrospective analyses for emerging drugs such as U-47700 (Kriikku et al., 2019) or, interestingly, established drugs (barbiturates) previously thought to be detectable solely by negative mode ionization (Høj et al., 2019). Also, provided that the LC method is suitably universal, there is no need to continually update the MS detection method: only the MS library needs to be kept current.

The specificity of DIA is enhanced in techniques such as Sequential Windowed Acquisition of All Theoretical Fragment Ion Mass Spectra ($SWATH^{TM}$) which, unlike MS^E, fragment the precursor ions in fixed (Scheidweiler, Jarvis, & Huestis, 2015) or variable (Whitman and Lynch, 2019) *m/z* segments to narrow the lineage of each fragment ion scan. The high resolution and scan speed of HRMS is essential, making this approach unsuitable for QqQ instruments. A feature of all HRMS methods is their ability to measure isotopic ratios, or the distribution of isotopologues based on known molecular formula and prevalence of individual isotopes. An important limitation of this identification parameter is that the unavoidably lower intensities of the higher *m/z* ions may increase the rate of falsely rejected identifications: consequently, the use of isotopic ratios for identification is inconsistent in the HRMS screening literature. We will provide some highlights of the forensic screening MS literature since 2010 under the following three indices of performance: sensitivity and specificity of target compound identification, range of target compounds identified, and quantitation.

E. Sensitivity and Specificity of Compound Identification

The identification reliability required of an MS method is dependent on its role in the analytical process. A screening method places a premium on sensitivity rather than specificity and so is less bound to guidelines for definitive identification (Clinical & Laboratory Standards Institute [CLSI], 2014; Society of Forensic Toxicologists, 2006) based on scoring systems for data from the LC (i.e., RT) as well as the MS (e.g., no. of qualifiers and their relative intensities). As a result, the RT tolerance in screening methods for compound identification varies broadly from, for example, 0.15 min (Roman et al., 2013) to 1.5 min (Mueller et al., 2011), depending on the origin of the RT targets. In any case, the RT criterion is less essential with HRMS, as shown by Colby and coworkers who evaluated a graded series of identification workflows for 100 urine samples containing 170 drugs and metabolites analyzed by a DDA-based QTOF method (Colby, Thoren, & Lynch, 2018). They found that a scheme based on purely MS data (accurate mass, isotopic pattern and fragment ions) had a positive predictive value of 82% for correctly predicting structures, compared with a modestly improved 96% when RT was included, concluding that this parameter was not required to identify drugs with a reasonable level of accuracy by HRMS.

The use of a tight RT criterion typically requires that a standard be run concurrently, which becomes impractical when these are unavailable or large in number. To overcome this limitation, Sauvage and coworkers showed that QqQ DDA data could enhance identification confidence over targeted MRM results even when the latter used an RT criterion and qualifier MRMs (Sauvage et al., 2006; Sauvage et al., 2008). Specifically, lysergic acid diethylamide (LSD), atropine, and clomipramine gave false-positive results using an RT criterion compared with purely full scan MS data acquired at three fixed collision energies. This DDA approach was elaborated by the addition of a collision energy spread rather than fixed values, allowing for a significant decrease in the duty cycle, by groups such as Dresen et al. (2010) who used sMRM for 700 compounds (one channel each) to trigger a dependent scan for the two most abundant precursor ions from urine samples. Identification of a broad range of compounds, from morphine glucuronide to THC, was based on purity against a reference library. Still, full reliance on MS data must be applied with caution, especially with incompletely resolved isobaric compounds. Sundström, Pelander, & Ojanperä (2017) found that an MS^E acquisition correctly identified all members in four sets of isobaric amphetamine-type compounds in pure solvent without the use of an RT parameter, while DDA with a predefined list of precursor ions was only able to distinguish compounds in two sets.

Numerous groups have demonstrated the reliability of the nontargeted DIA approach. Rosano et al. (2013) used MS^E to screen for over 950 compounds in blood from 300 forensic cases with a QTOF instrument. Using identification requirements for RT (±0.3 min), precursor mass (±5 ppm) and one fragment mass (±5 ppm), they obtained a detection rate of 99% of 1528 compounds by QTOF compared with 80% for combined LC-MS and LC-MS/MS quadrupole methods. The large majority (73%) of the additional compounds detected by the QTOF were simply metabolites of drugs already identified,

but 21% were revealed as new drugs based on isotope ratio patterns and the presence of ≥2 fragment ions. This approach was further refined by Rosano, Ohouo, & Wood (2019) as a quantitative confirmatory method using the same identification criteria as above. No discrepant results were obtained for 114 urine samples when compared against a targeted 64-compound QqQ-MS/MS method.

The effectiveness of positive mode DDA was evaluated in 50 autopsy samples against a library that included 2500 compounds (including fragment spectra at three collision energies) and an additional 5000 having accurate precursor mass only (Broecker et al., 2011). Several chemical groups were under-represented in the library owing to inadequate ESI signals, the formation of sodium adducts, signal instability or the presence of multiply charged species. In the autopsy samples, they identified 311 compounds (based on accurate mass, library match and isotope pattern) derived from 125 drugs, compared with 178 compounds from 94 drugs determined by LC coupled to a diode array detector and GC-MS. However, low-level compounds such as THC and some BDZs, as well as compounds with a preference for negative mode ionization were either missed or detected only at higher levels.

SWATH^{TM} has also proven to be effective in drug identification but has received limited attention in the literature to date, mainly in the form of a comparison with DDA. The prevailing view is that while DDA provides better quality spectra (on account of its greater precursor selectivity), SWATH^{TM} gives fewer false-negative results due to precursor ions not subjected to MS/MS (Arnhard et al., 2015). This inherent balance between compound identification and the range was shown for DDA (with and without precursor restrictions), SWATH^{TM} and MS^{E} through their ability to detect drug metabolites prepared by incubating 8 test compounds with rat liver microsomes. Of the 227 metabolites ultimately found in the mixture, 5% did not generate MS/MS data by DDA: this figure rose to 29% when the mixture was added to a blank urine matrix, illustrating the role of competitive interference from additional ions triggering MS/MS scans. Both SWATH^{TM} and MS^{E} scored 100% for both sample types, but the overall order was reversed when MS/MS data quality was considered: 9 of the 10 most abundant product ions in the MS/MS spectra of the 8 test compounds recorded by DDA scans of the rat liver microsomes originated from the test compounds themselves, compared with only 6 and 3 for SWATH and MS^{E}, respectively (Zhu, Chen, & Subramanian, 2014).

The merits of DDA versus DIA have been evaluated by numerous groups (Zhu, Chen, & Subramanian, 2014; Arnhard et al., 2015; Sundström, Pelander, & Ojanperä, 2017; Whitman and Lynch, 2019). In one example using urine samples, a list of 200 compounds was examined in a DDA experiment to select precursor ions by mass, RT and signal strength, followed by fragmentation at compound-specific collision energy for up to three consecutive scans (Sundström, Pelander, & Ojanperä, 2017). A 5 Da precursor selection window helped capture a wider range of isotopes to enhance information in the acquired spectra. Identification was based on precursor mass (±25 mDa), RT (±0.3 min), and spectral purity (>80%, a combination of fit and reverse fit). In the DIA arm, *m/z* 50–700 precursor ions were fragmented at a fixed collision energy in every second scan. Compounds were identified by precursor mass (2.5 mDa), RT (0.2 min), and minimum abundances for precursor and qualifier ions. Using these criteria for the limit of identification (LOI) of drugs spiked into urine from six volunteers, DIA achieved a consistently lower LOI than DDA. The authors pointed out that the fixed collision energy value used to create spectra libraries may impair the spectral quality of compounds with optimal values lying outside the usual range. Both techniques were applied to 50 authentic samples, where DIA made 266 identifications for 46 different substances; the results for DDA were 225 and 42, respectively, the lower values likely being due to inherently higher LOI and interference from co-eluting compounds.

In a study more pertinent to forensic screening, SWATH^{TM} was compared with DDA for drug screening in the blood (Roemmelt et al., 2014). The SWATH^{TM} survey scan was set to *m/z* 100–700 with 20 Da sections submitted to an *m/z* 100–650 dependent scan with a collision energy ramp of 35 ± 15. Settings for DDA were similar, with selection criteria of 10 most significant ions and a half-peak width exclusion time. Identification was based on accurate mass, isotope ratio (albeit with large tolerance) and purity score against a library of 534 compounds. The authors observed a concentration dependence for fragment ions with SWATH^{TM} but noted the phenomenon was not confined to this technique. The fragment ion spectra could also be distorted by the presence of deuterated internal standards, especially if these shared common ions with target precursors. DDA was found to deliver better quality spectra, but this difference was considered insignificant when compared with the inferior results obtained by MS^{E}. The screening sensitivity of the two techniques was evaluated using flow injection (vs column chromatography) of mixtures of 6, 16, and 20 analytes. Both methods were similar at $n = 6$ analytes, but DDA detected only 13/16 and 15/20 analytes in the respective mixtures, even at a setting of 20 experiments. SWATH, by contrast, identified 6/6, 15/16, and 20/20 of analytes present. The sensitivity of DDA can also be compromised by ion suppression due to co-eluting analytes and an inappropriately wide isotope exclusion window, which may result in the rejection of ions from compounds with similar masses. Pointing out the infrequent specification of the precursor window in DDA papers, intended to avoid needless triggering of dependent scans by the $[M + H]^+$ peak, the authors commented on the risk of false-negative results when a compound's precursor mass lies within the exclusion window of a dominant neighbor. While such precursors would clearly not be missed with SWATH^{TM}, the presence of multiple precursor ions may limit the usefulness of SWATH if paired with a DDA-acquired library typically acquired with a precursor window of 1 Da.

Two important parameters for method robustness are addressed infrequently but deserve mention. The first is spectral reproducibility: for example, an assessment of how reliably compounds in a test mixture are identified at three levels over several weeks (Helfer et al., 2015). The second is the number of fragments required for a library match: this may be as low as 1 or is often simply not specified at all, illustrating the risk of reliance on a simple purity score (Ott et al., 2017).

F. Range of Compounds Identified

Most LC-MS methods described here were intended for screening purposes only. In a forensic environment, samples screening positive would require a targeted confirmatory

analysis. The compound range of this second assay is of lesser concern as the target is already suspected: the main challenge is the initial identification of candidate drugs for further evaluation. While this range of method-compatible compounds is largely defined by physicochemical properties such as lipophilicity and preference for forming cationic versus anionic precursor ions, most LC-MS papers choose instead to describe the compound range in terms of the total number of targets. The main factors dictating an LC-MS method's ability to detect drugs are listed in Table 1.

A method's detection range will unavoidably affect turnaround time. Recognizing this limitation of a typical LC-MS screening method in detecting the wide range of drugs encountered in a clinical setting, McMillin et al. (2015) opted for a hybrid immunoassay-MS strategy where drug screening of specific groups was assigned to immunoassays where conventional LC-MS added significantly to reporting time while exhibiting poor detection (e.g., barbiturates, THC-COOH) or providing no added information.

In a forensic lab, though, there is lower urgency to deliver rapid results, and a greater need to maximize the range of detectable compounds. These requirements were balanced with a DIA (MS^E) method which incorporated an exceptionally broad group of 467 compounds ranging from GHB to THC-COOH glucuronide in ante- and postmortem blood using identification by accurate mass, RT, isotope pattern and presence of qualifier ions against an in-house library (Telving, Hasselstrøm, & Andreasen, 2016). Interestingly, this list included drugs typically analyzed in negative ionization mode, such as phenobarbital and salicylate, as their toxic levels are typically high enough to permit detection in positive mode. GHB was prone to marked matrix interference, but not at a level that would impair recognition of a true positive case. The method was shown to be robust with respect to RT, response, mass error and resolving power over 6 months.

A positive ionization DDA method of similar scope was used for whole blood samples extracted with butyl chloride to screen 320 compounds, quantitate 39, and search for unknowns in a 15-min chromatographic run (Partridge et al., 2018). The compounds ranged from the less polar opioids (e.g., codeine) to methadone and synthetic cannabinoids but did not include GHB or THC-COOH. MS/MS scans were prioritized (or excluded: e.g., internal standards, known interferences) from an inclusion list of about 450 masses followed by the two most abundant precursor ions. Compounds in the inclusion list ranged from established drugs with available reference materials to presumptive metabolites and tentatively identified NPS. Criteria for a positive screen were precursor mass ±10 mDa and RT ±0.5 min, with presumptive positives submitted for manual review against more rigorous standards for identification (mass ±2 mDa, RT 2%, MS/MS consistent with contemporaneous reference standard). Storage of centroid (vs continuous) data using a relatively high signal threshold allowed individual sample files to be kept to a reasonable 30 Mb size limit. This is an important consideration for a production assay (of which this paper was an uncommon example), as any LC-MS service will struggle if required to process a large number of samples daily with continuum data file sizes of 500 Mb or more. Lastly, synthetic cannabinoids were found to be particularly prone to sample carryover, providing a cautionary note about directly reporting LC-MS results without a second confirmatory test.

The compound range issue has also been approached through the use of a publicly shared MS database to maximize the size of the reference library available for the analysis of NPS in hair (Fabresse et al., 2019). This matrix also has the advantage of acting as a potential reservoir for novel parent drugs, an especially important feature for synthetic cannabinoids where metabolism is both extensive and uncertain. An atypical feature of the DDA method was the use of two exclusion lists (to reduce interference from endogenous substances) and no inclusion list (to avoid selection bias). A similar public library strategy for HRMS has been promoted by others (Mardal et al., 2019).

It is worth pointing out that most methods described as "nontargeted" still rely on a mass spectral library of a fixed number of known compounds. Consequently, the determination of truly "unknown unknowns" must be achieved by some other means, such as *in silico* fragmentation to predict RT and spectra. The latter approach was illustrated for blood samples

TABLE 1. Limitations of compound detection range.

Issue	Example	References
Adulteration	Substituted human performance sample	Goggin et al. (2017), Kluge et al. (2018)
Stability issues with sample collection, storage, preparation	Ethanol, cathinones	Adamowicz & Malczyk (2019)
Drug present in the sample as metabolite only	Synthetic cannabinoids	Wohlfarth et al. (2013)
Incompatibility with LC conditions	GHB	Ingels et al. (2013)
Incompatibility with source polarity	Barbiturates	Høj et al. (2019)
Data not acquired	Absent from inclusion list (DDA) or MRM list (QqQ); interfering substance (DDA)	
Very low concentrations	LSD	Dolder, Liechti, & Rentsch (2015)
Unavailability of standards or reference spectra	NPS	

DDA, data-dependent analysis; GHB, γ-hydroxybutyrate; LC, liquid chromatography; LSD, lysergic acid diethylamide; NPS, novel psychoactive substances; QqQ, triple quadrupole.

with the supplementation of an in-house MS library with *in silico* fragmentation using MS^E (Mollerup et al., 2017). Compounds were first tentatively identified with a targeted approach against an in-house library of 1457 compounds by using precursor *m/z*, RT, fragment ions, and isotopic pattern. Those peaks not identified were then filtered from further consideration based on RT, low response, common endogenous and exogenous (e.g., dietary) compounds, recurring masses, co-elution with a dominant target, and adducts alternate to a protonated pseudo-molecular ion. This reduced the number of spectra to be reviewed by three orders of magnitude while retaining 73% of true positives identified by a targeted screen. The molecular formula was then determined based on accurate mass and isotope pattern and compared against several large chemical structure libraries. Lastly, the resultant candidate structures were *in silico* fragment-matched using a combinatorial fragmentation approach to the observed spectra. Several limitations were noted. First, the threshold intensity filter reduced the effectiveness of identification by the isotopic pattern in the subsequent step, though the authors noted that this could be partially avoided by making the threshold mass-dependent, as low-MW compounds such as MAMP can be reliably identified by accurate mass alone. Second, a few compounds do not reliably form protonated pseudo-molecular ions. Lastly, frequently occurring but illicit compounds may be incorrectly excluded, though the authors pointed out that this would not be an issue in practice, as the sequence would have been run only after a targeted search, which should have detected these compounds. The result of the nontargeted screen applied to 44 driving under influence of drugs (DUID) samples was the discovery of 3 drugs and 14 metabolites not identified by the targeted approach. The sensitivity of the nontargeted workflow alone was shown using samples spiked with 11 novel BDZs where the LOI for 9 drugs was within an order of magnitude of that obtained by the targeted method.

In the absence of commercially available standards, investigators have used incubations of parent drug with human liver microsomes to obtain LC-MS properties of predicted metabolites of NPS such as synthetic cannabinoids (Presley, Logan, & Jansen-Varnum, 2019) or the novel FEN analog, cyclopropyl-fentanyl (Cutler & Hudson, 2019). An alternate approach used metabolite targets for 45 anti-depressants through dosing of parent drugs to rats and identifying metabolites in urine (Wissenbach et al., 2011). This approach not only increases sensitivity and specificity of drug detection, but also provided evidence that the subject had consumed the drug in question.

The consumption of a drug can also be inferred by searching for its metabolites with a mass defect filter (MDF) (Zhang et al., 2009). MDF was included in the DDA study mentioned above (Zhu, Chen, & Subramanian, 2014), where MS/MS spectra were acquired for precursor ions having *m/z* ±50 mDa and mass defect ±40 mDa compared with the parent drug's respective values. They found that while only 95% and 96% of drug-related material precursor ions generated MS/MS spectra when triggered by the most prominent ions and the MDF, respectively, the MS spectra quality was significantly superior to those obtained by SWATH and MS^E.

While efforts to increase the range of detectable compounds are commendable, it is worth pointing out that a small number of drugs likely account for most of the compounds identified. One forensic lab commented that in their experience 20 drugs were responsible for up to 80% of fatal poisonings (Ojanpera et al., 2005). Considering the turnaround time pressures mentioned earlier, each laboratory needs to determine its own point of diminishing returns with respect to the range of detected compounds. Similarly, the sample selection criteria for drug screening should be carefully considered: in a group of 576 misdemeanor and felony DUID cases, it was found that the drug testing results, even when positive, rarely added value in supporting a DUID charge (Tiscione et al., 2017).

G. Quantitation

A broad-spectrum quantitative LC-MS method for forensic work is beset by issues not faced by a purely qualitative assay. These begin with the challenge of preparing calibration mixtures containing dozens of compounds at suitable levels in compatible solvents and culminate in ensuring the acceptability of a potentially vast number of calibration curves and quality control results. These problems are obviously not unique to forensic LC-MS, as they also confront workers in fields such as doping control and residue analysis. However, some of these challenges can be avoided by limiting quantitation to a subset of all targets (Bidny et al., 2017; Partridge et al., 2018), using stored calibration curves (Roemmelt, Steuer, & Kraemer, 2015) or simply using the method as a qualitative screen only, which appears to be the route taken most often.

A novel standard addition-type approach that has yet to see widespread use is the use of threshold accurate calibration (Rosano, Ohouo, & Wood, 2017), where each urine sample is analyzed separately both with and without known amounts of added target compound. As a result, each sample serves as its own calibrator, which allowed the avoidance of internal standards and the acceptance of potentially marked matrix effects through the use of simple dilution rather than a more elaborate extraction.

A distinctive challenge of forensic drug testing, whether in biological matrices or seized samples, is that the very wide range of concentrations routinely encountered creates the risk of carryover in the LC-MS. The problem is magnified by the prevalence of high-concentration samples in a large analytical batch, whose size discourages inter-sample blanks or repeat analyses with diluted samples. Moreover, quantitation over several orders of magnitude may exceed the ability of the MS instrument, a problem which has been addressed by monitoring the target compound's pseudo-molecular ion and a minor isotopologue (i.e., $(M+H+2)^+$) at low and high concentrations, respectively (Liu, Lam, & Dasgupta, 2011). This approach has been used to quantitate MAMP across a concentration range of 50–200,000 ng/mL (Miller et al., 2017).

There is limited forensic toxicology literature on quantitation by DDA and DIA broad-spectrum methods. Using SWATH acquisition, results for 76 internal standard-matched compounds obtained by daily external multi-point calibration were compared with those from weekly external calibration, daily external one-point calibration and internal calibration (i.e., using a compound's response factor vs its internal standard) (Elmiger et al., 2017). Accuracy for three levels of QC material spanning a range from toxic to lethal blood concentration was found to be similar for all

levels except the lowest. The authors concluded that external calibration in either format met the requirements for accurate quantitation while one-point internal calibration was the best choice for rapid screening. They found no issues with selectivity but did not comment on whether the internal standards interfered with target compound spectra, especially if the two shared common fragments.

V. AMBIENT IONIZATION MASS SPECTROMETRY

A. Introduction

Ambient ionization MS methods are those which operate under ambient (open air) conditions and require little to no sample preparation for the formation of ions from samples in their native environment, outside of the MS. Classical methods of ion generation require the introduction of the analyte either directly to high vacuum or contained in solution from which ions can be extracted into or generated in the gas phase. Atmospheric pressure ionization techniques that create ions from dilute sample solutions are not truly considered ambient (e.g., ESI, APCI, APPI, AP-MALDI) and will not thoroughly be discussed here. Ambient techniques tweak the desorption properties of these well-established techniques for true ambient analysis; however, these techniques often heavily rely on these processes for ionization. As such, the reader is directed to discussions of ionization mechanism for ESI (Kebarle & Tang, 1993), APCI (Horning et al., 1973; McEwen & Larsen, 2009), and atmospheric pressure photoionization (APPI) (Kauppila et al., 2002) processes, as they are relevant to the discussion below.

The introduction of the ambient mass spectrometry techniques such as desorption electrospray ionization (DESI) in 2004 and direct analysis in real time (DART) in 2005 have seen significant growth in the literature and uptake from the clinical and forensic communities. Commercial systems for both sources have been developed and have seen broad use in a wide variety of applications. The success and simplicity of these approaches have spun off an ever-growing body of related ambient ionization techniques, with more than 80 techniques now described in the literature. Given that a discussion of all these techniques is infeasible, this review will focus on ambient methods that have appeared in the literature for multiple (>5) demonstrations of drug analysis. This section presents individual sections for the ambient ionization techniques discussed, which are outlined in Table 2 along with desorption/ionization characteristics. Short fundamental descriptions of the technique are presented, but it is not germane to this review to describe the nuances of each of the techniques in full. The reader is directed to a number of thorough reviews of ambient ionization that have been reported in the recent literature, including reviews on general aspects of ambient ionization (Harris, Galhena, & Fernandez, 2011; Huang et al., 2011; Javanshad & Venter, 2017), plasma-based ambient techniques (Shelley & Hieftje, 2010; Ding & Duan, 2015; Smoluch, Mielczarek, & Silberring, 2016), forensic analysis (Green et al., 2010; Correa et al., 2016), clinical applications (Ferreira et al., 2016), applications in high-throughput screening (Li et al., 2013), and ambient ionization from the perspective of green analytical chemistry (Molina-Díaz et al., 2019).

TABLE 2. Desorption/ionization conditions for ambient ionization mass spectrometry techniques.

Technique	Name	Year	Desorption	Ionization
DESI	Desorption electrospray ionization	2004 (Takats et al., 2004)	Liquid extraction	Electrospray
DART	Direct analysis in real time	2005 (Cody, Laramée, & Durst, 2005)	Plasma desorption	Corona discharge
ELDI	Electrospray-assisted laser desorption/ionization	2005 (Shiea et al., 2005)	Laser ablation	Electrospray
ASAP	Atmospheric solids analysis probe	2005 (McEwen, McKay, & Larsen, 2005)	Thermal desorption	Corona discharge
EASI	Easy ambient sonic-spray ionization	2006 (Haddad, Sparrapan, & Eberlin, 2006)	Liquid extraction	Sonic spray
FAPA	Flowing atmospheric-pressure afterglow	2006 (Andrade et al., 2006)	Plasma desorption	Corona discharge
DBDI	Dielectric barrier discharge ionization	2007 (Na et al., 2007)	Plasma desorption	Dielectric barrier discharge
DAPPI	Desorption atmospheric pressure photoionization	2007 (Haapala et al., 2007)	Plasma desorption	Photoionization
LAESI	Laser ablation electrospray ionization	2007 (Nemes & Vertes, 2007)	Laser ablation	Electrospray
DSA	Direct sample analysis	2007 (Chen et al., 2007)	Thermal desorption	Corona discharge
LTP	Low-temperature plasma	2008 (Harper et al., 2008)	Plasma desorption	Dielectric barrier discharge
PSI	Paper spray ionization	2010 (Liu et al., 2010)	Liquid extraction	Electrospray
CBS	Coated blade spray	2014 (Gómez-Ríos & Pawliszyn, 2014)	Liquid extraction	Electrospray

B. Liquid/Spray-Based Techniques

The majority of publications involving drug analysis with the liquid/spray-based techniques have utilized DESI and paper spray mass spectrometry (PS-MS), with significant literature contributions from easy ambient sonic-spray ionization (EASI) and coated blade spray (CBS), and more minor contributions from thermal-desorption electrospray ionization (TD-ESI) and solid-substrate ESI techniques. All of these techniques produce ions from evaporating aerosol droplets, in a manner similar to that observed in electrospray as described by Kebarle & Tang (1993) (note that EASI does not require applied high voltage). A generalized instrumental schematic for each of the techniques is presented in Figure 7, but note that other geometries and designs have been implemented as well.

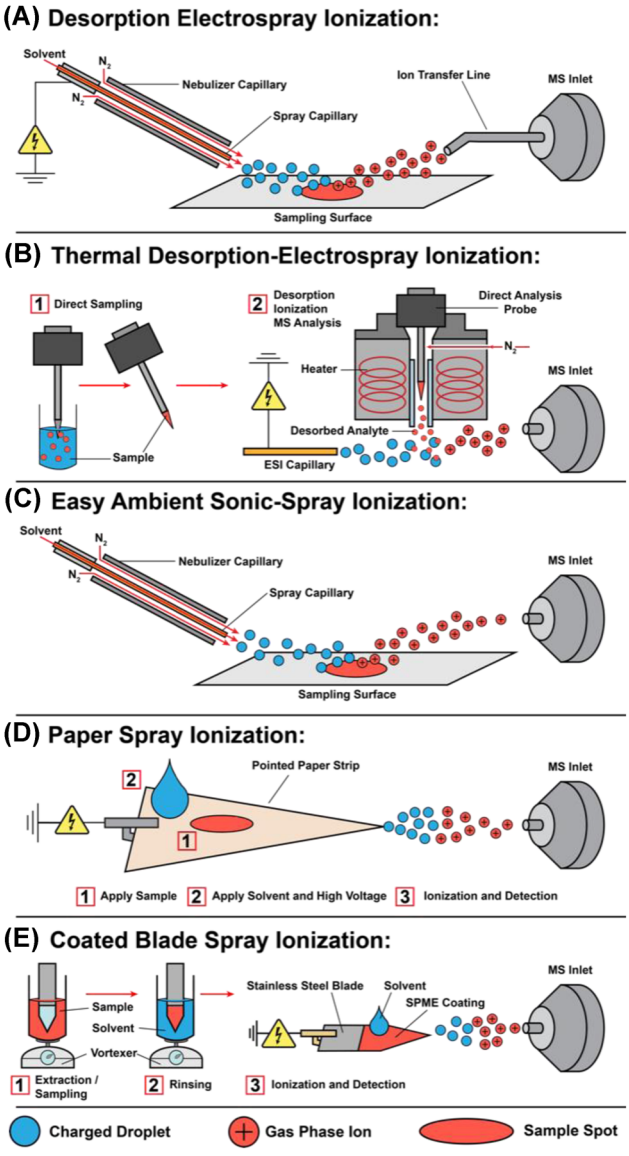

FIGURE 7. Generalized instrumental schematics for (**A**) desorption electrospray ionization (DESI), (**B**) thermal-desorption electrospray ionization, (**C**) easy ambient sonic-spray ionization (EASI), (**D**) paper spray ionization (PS), and (**E**) coated blade spray ionization (CBS). Not to scale. [Color figure can be viewed at wileyonlinelibrary.com]

1. Desorption Electrospray Ionization

DESI was the first ambient ionization technique described in the literature, paving the way for the development of a large variety of related ambient ionization techniques that appeared in the ensuing decade (Takats et al., 2004). DESI is achieved by spraying solvent from an ESI probe onto a surface to be analyzed. The charged droplets desorb analytes from the surface, and the ionization of analytes occurs in an electrospray-like fashion, with evidence for both a heterogeneous charge-transfer and a droplet pick-up ionization mechanism described in the literature (Takats, Wiseman, & Cooks, 2005). Many applications of DESI involve chemical imaging of biological tissues, which is one of the unique strengths of this method; however, imaging studies in drug analysis are most often directed toward therapeutic drug monitoring and are not thoroughly discussed here (Wiseman et al., 2008; Lamont et al., 2018). The reader is directed to two DESI reviews in the literature (Ifa et al., 2010; Morelato et al., 2013). The success of DESI has led to the development of commercialized ion sources.

Initially, DESI was demonstrated for a variety of rapid qualitative screening applications, including the detection of AMP, opiates, cannabinoids and BDZs from untreated, and urine extract samples (Kauppila et al., 2007). Although Kauppila et al. reported no matrix effects in their analysis of urine, a more thorough study into matrix effects using opioids as a model compound by Suni et al. (2011) found that sensitivity was decreased by 20–160-fold for urine. In another forensic screening application, DESI was used for the rapid determination of BDZs in alcoholic beverages without any sample pretreatment (D'Aloise & Chen, 2012). DESI was also demonstrated for the noninvasive qualitative detection of COC and metabolites from fingerprints, using only a small area of the fingerprint, allowing for high-throughput, replicate analysis (Bailey et al., 2015). DESI has been used for the qualitative analysis of illicit COC samples from three seizure events by Australian police, and preliminary geographical origin determination was demonstrated (Stojanovska et al., 2015). In an indirect screening method, Bianchi et al. (2019) tested a variety of sample substrates coupled to DESI for increased signal and stability in the screening of NPS in oral fluid samples in both positive and negative ionization modes. The use of these sample substrates allowed for improved lower limits of quantification (LOQs) and method validation was demonstrated, however, some isomers were unable to be differentiated. While the use of sample substrates improved sensitivity and precision in this study, the full analysis time of 15 min required was significantly slower than direct DESI screening applications.

Further development of DESI involving coupling with SPME or other extraction procedures allowed for sensitive quantitative analysis. The direct DESI-MS/MS screening and quantitative measurement of DoA on SPME fibers for the analysis of raw urine were achieved by Kennedy et al. (2010) in good agreement with conventional immunoassay, GC-MS, and LC-MS/MS results. Thunig et al. (2012) employed hollow-fiber liquid-phase microextraction (LPME) with DESI to measure 4 DoA from urine samples. LPME significantly reduced the matrix effects observed in direct DESI urine analysis, allowing for a LOQ for diphenhydramine of 140 ng/mL to be achieved.

Since DESI is an ambient method that lacks chromatographic separation, it must rely on MS/MS or HRAM data for selectivity, though in many cases this is not sufficient for structural isomer differentiation. Ion mobility has been used with other ambient techniques to increase selectivity and the use

of DESI couple to an ion mobility TOF-MS has been demonstrated by Roscioli et al. (2014). The analysis of drug tablets and creams was achieved, and though there was no differentiation of structural isomers, IMS coupling with DESI demonstrated increased selectivity and sensitivity.

Ambient sampling, portable mass spectrometers have significant potential for *in situ* forensic or clinical analyses, and DESI is well suited for these systems, evidenced by the first-ever coupling of an ambient technique to a handheld IT-MS (Keil et al., 2007). They used the handheld MS system to analyze COC on a variety of surfaces, including currency. Further applications of DESI with portable MS include the use of an IT-MS for the rapid screening of synthetic cathinones and AMP in forensic samples with low or sub ng/mL LOD values (Vircks & Mulligan, 2012). Their ruggedized CIT-MS has also been used to sample positive and negative controls, as well as forensic drug powders for 32 different DoA (O'Leary et al., 2015). Structural confirmation and identity were achieved using MS/MS and automated library searching using established reference libraries. In a further development of this portable MS, a flexible source platform was developed, allowing for ambient sources (including PSI, DESI, swab touch spray, and APCI) to be rapidly interchanged to target specific applications (Fedick et al., 2018). Anticipating an incorporation of this system into forensic practice and routine analysis, Mulligan et al. performed comprehensive analytical validation studies using common DoAs and a variety of NPS to demonstrate the throughput, selectivity, accuracy, precision, robustness, and ruggedness of the portable MS system featuring interchangeable sources (Lawton et al., 2017). The use of portable DESI-MS systems, and especially a system featuring interchangeable sources has demonstrated strong potential for use in *in situ* forensic applications as well as high-throughput screening.

2. Thermal-Desorption Electrospray Ionization

A major application in forensics involves the remote sampling of large and/or immovable objects (e.g., luggage, *in situ* analysis), presenting challenges for traditional forensic techniques. Given that drug concentrations are often high in these types of applications, eliminating sample carryover or interference can be more important than sensitivity. When sampling is done using probes, switching between sampling probes can be cumbersome with significant carryover, otherwise significant sample preparation is required for sample introduction. In TD-ESI, a removable metal probe can be used to sample immovable, remote surfaces or other solids and liquids and then be inserted into a standard ESI source (following simple modification), with typical analysis times of 10 sec (Huang et al., 2013). The probe is quickly cleaned with a gas torch post analysis, eliminating sample carryover. Heated nitrogen gas acts to desorb thermally stable analytes from the probe and transfer them to the ESI plume, where ions are subsequently generated through the interaction of the desorbed analytes with charged solvent species. Sampling, desorption, and ionization are separate events in space and time.

In the first demonstration of TD-ESI with the metal sampling probe, MDMA and codeine standards were deposited on different surfaces (e.g., business card, wooden desk at ~50 ng/cm^2) and sampled with a fine metal probe for their successful qualitative detection (Huang et al., 2013). TD-ESI was further applied to the rapid identification of drugs in drained gastric lavage fluid and whole blood from drug overdose patients (Lee et al., 2017). In this study, the entire analytical process was completed in <30 sec without sample pretreatment, characterizing flunitrazepam, LSD and MDMA in drained gastric lavage fluid with reported LODs of 500, 500, and 1 ppb, respectively. Prepared whole blood samples from overdose patients were analyzed for KET, COC, AMP, and NKET with reported LODS of 1–10 ppb. Stability was demonstrated through replicate drug measurements in both dilute gastric juice and whole blood. Calibrations indicated that TD-ESI may be suitable for quantitation of DoA in gastric lavage fluid and extracted whole blood.

The versatility of the TD-ESI source was highlighted in the analysis of 30 DoA directly from tablets, soft drinks, and drug powders without sample preparation, as well as in drug-laced cigarettes and instant coffee, following a quick methanol extraction (Chiang et al., 2018). TD-ESI, in this case, was applied as a rapid (30 sec per analysis) qualitative prescreening tool using MS/MS data that could be directly compared with ESI-MS/MS libraries. Active ingredients in seized drug materials were detected at less than 2 mg/g of the total sample weight, and consecutive analysis of tablets using an acupuncture needle as the sampling probe did not show cross contamination or interference. Acceptable precision was demonstrated for all compounds, and LODs were determined as 1–60 ng/g in a tobacco/coffee matrix.

Detection of illicit drugs using TD-ESI/MS was further applied to a variety of food and drink matrices of forensic interest as well as stamps (Cheng et al., 2019). Many of these sample matrix components are either nonvolatile or will thermally degrade in the source, thus minimizing matrix effects and interferences for the analytes using TD-ESI/MS. Green tea, whole fat milk, and fruit and vegetable juice were spiked with BDZs, AMPs, and NPS, yielding LODs of 100 ppb with direct analysis. Similarly, direct analysis of instant coffee and matcha powders for KET, AMP, *para*-methoxyamphetamine (PMMA) and LSD gave LODs of 100–1000 ppb. Direct analysis of KET, MDMA, 5-MeO-AMT, and LSD on postage stamps returned LODs of 1.3–6.5 pg/mm^2. DoA have also been found in gummy candies, a challenging matrix. A gummy bear was prepared using gelatin and grape juice spiked with BDZs and a gelatin and green tea mixture used as an outer coating such that the drugs were contained only within the gummy bear, and not on the surface. Sampling of the outer layer detected only caffeine and other green tea components, while sampling the inner part successfully detected the presence of BDZs.

TD-ESI/MS has been demonstrated in the literature as a rapid screening technique that is amenable to quantitative analysis. The source can easily be interchanged for conventional ESI and the sampling probe is removable and can be brought to the sampling site, enabling a wide range of sampling options and has been demonstrated for the direct analysis of an impressive number of matrices with minor matrix effects observed. Preliminary test indicates TD-ESI could be used for quantitative work, though this has not been thoroughly demonstrated.

3. Easy Ambient Sonic-Spray Ionization

EASI was originally reported as "desorption sonic-spray ionization (DeSSI)" in 2006 (Haddad, Sparrapan, &

Eberlin, 2006). EASI operates at ambient pressures and, uniquely, uses no high voltages at the spray capillary, heating, auxiliary gases, or corona discharges. A supersonic nebulizing gas, often nitrogen, coaxial to the spray capillary, leads to the formation of aerosol droplets and the subsequent production of gaseous ions. Although the formation of ions with EASI is well supported through various applications, a detailed investigation into the underlying mechanism of ionization in sonic methods has not been reported. EASI-MS was recently reviewed by the inventors of the technique (Teunissen et al., 2017). It should be noted that sonic-spray ionization (SSI) at atmospheric pressures was originally reported in 1994 as an ionization interface in CE-MS and LC-MS systems (Hirabayashi, Sakairi, & Koizumi, 1994) and further developed as a novel ion source in 2003 (Takats et al., 2003). Uniquely, EASI-MS combines the ionization concept of SSI with the desorptive concept of DESI; in EASI-MS the supersonic spray is used to both desorb and ionize the analytes of interest. Given the absence of an applied high-voltage and overall simplicity of the ion source design/implementation, the technique shows the potential for on-site testing for forensic applications by minimally trained personnel. (Teunissen et al., 2017).

The first demonstration of EASI-MS compared its performance with DESI for the analysis of diazepam and therapeutic drugs in tablets. EASI demonstrated similar sensitivities, had mass spectra with less abundant solvent cluster ions, and enabled increased sample matrix penetration, resulting in longer lasting signals relative to DESI (Haddad, Sparrapan, & Eberlin, 2006). EASI-MS was successfully demonstrated in both positive and negative ionization modes, generating representative mass spectra a few seconds after exposing the drug tablet to the supersonic spray. It was noted that operating conditions (gas flows, angles, etc.) greatly affect signal intensities and stability; under optimized conditions, EASI showed signal variations around 20%.

EASI-MS is a logical pairing with thin layer chromatography (TLC), which is commonly used for initial forensic screening. Developed TLC plates can be rapidly tested with EASI-MS, confirming or identifying resolved compound identities in 10 sec/spot with increased specificity, providing more defensible results. Several publications demonstrating EASI-MS coupled with TLC have appeared, including the analysis of ecstasy tablets (Sabino et al., 2010) and COC and crack COC sample measurements (Sabino et al., 2011). Seized ecstasy drug tablets were subjected to TLC for the separation of five AMPs, KET, and the common cutting agents caffeine and lidocaine (Sabino et al., 2010). Primarily $[M + H]^+$ ions were observed for all of the drugs with the exception of MDEA which was also produced water and sodium adducts. These studies demonstrated detection limits in the low μg range. EASI-MS has been applied to the analysis of NPS such as *meta*-chlorophenylpiperazine (m-CPP) in seized ecstasy tablets using QMS in parallel with other techniques to provide a complete chemical profile of the tablets, since EASI-MS was unable to differentiate the ortho and para isomers from m-CPP (Romão et al., 2011). The analysis of seized blotter papers suspected to contain LSD has been demonstrated using EASI-MS (Romão et al., 2012). Identification of LSD in forensics is often done using the Ehrlich spot test, a nonspecific colorimetric analysis. This lack of specificity presents a major problem as an increasing number of structurally similar NPS appear in the drug stream that could yield false negatives using the colorimetric test. Authorities identified the presence of one such NPS, 9,10-dihydro-LSD, in several of the blotters and EASI-MS analysis was applied, however, it was found that the lack of MS/MS required the use of EASI-FT-ICR in order to avoid false positives. Additionally, EASI-MS was applied to TLC plate measurements. EASI-MS and TLC-EASI-MS are presented as screening techniques where sensitivity for LSD and 9,10-dihydro-LSD was found to be comparable to LC-UV.

In an effort to further simplify EASI-MS, the self-pumping of solvents or liquid samples by utilizing the Venturi effect was demonstrated, eliminating the need for mechanical pumping, further lowering both the cost and complexity of the method (Santos et al., 2011). Venturi-EASI (V-EASI) operates by inserting a thin fused silica capillary into a liquid sample, using the nebulizing gas to aspirate the sample and perform ionization. Compared with direct infusion electrospray, V-EASI gives a lower (two to three times) absolute signal intensity but superior signal-to-noise ratios, with less ionization suppression and adduct/dimer formation.

At the current stage of development, EASI-MS and V-EASI-MS are most suited to initial qualitative screening techniques, although preliminary quantitative demonstrations have been presented. Applications of EASI-MS have primarily employed single quadrupole mass analyzers and lack structural confirmation, though can be coupled to MS^n or HRMS systems. However, it is more likely that due to the simplicity of EASI, that applications will be directed toward portable MS systems for *in situ* analysis of DoA. Indeed a V-EASI source coupled to a portable MS featuring canned air and disposable parts has been reported as "one of the easiest and cheapest ways to make ions" (Schwab et al., 2012).

4. Paper Spray Ionization

PS-MS, first introduced in 2010 (Liu et al., 2010) is a rapid ambient ionization technique that is garnering considerable attention in the recent literature for a wide range of applications, including in forensic and clinical analysis. In PS-MS, small amounts of unprepared samples (ca. 10 μL) are directly spotted on pointed paper strips (acting as a porous solid-substrate), which are then positioned in front of (*ca.* 5 mm away) the MS inlet. Solvent and high voltage (ca. 3–5 kV) are then applied: as solvent wicks to the tip of the paper it transfers analytes with it, and ionization occurs in a manner similar to that observed for electrospray/nanospray (Espy et al., 2012), with the notable difference that no pneumatic assistance is needed. Alternatively, solid surfaces can be swiped with the paper strips prior to measurement, as demonstrated for the qualitative MS/MS analysis of small amounts of HER and COC from surfaces (Wang et al., 2010).

Paper properties (e.g., pore size, thickness, flow rate, and type) can have significant impacts on the analysis. Many different papers, including filter, glass fiber, and chromatography papers have been characterized for use in PS-MS (Liu et al., 2010) as well as a series of papers made from natural fibers, thin synthetic fibers, a microarray membrane, and various nanofibers (Lai et al., 2015), silica-coated papers (Zhang et al., 2011), polymers (Dulay & Zare, 2017), and molecularly imprinted polymer substrates (Tavares et al., 2018). A thorough investigation on the impact of paper properties on matrix effects in the analysis of

FEN and synthetic cannabinoids found that there is a trade-off between analyte recovery and ionization efficiency and that no one paper is optimal (Bills et al., 2018). Solvent choice was also shown to be more impactful on PS-MS results than paper properties. Ionization suppression and recovery in direct measurements of whole blood, plasma and urine, have been investigated using alprazolam, benzoylecgonine (BEG), methadone, and MOR, among others; ionization suppression is generally highest in urine and lowest in blood, whereas recovery was lowest in blood and highest in urine (Vega et al., 2016). Imperfections in the production of the paper points, particularly the angle and quality of the tip has been shown to significantly impact the analytical performance of PSI (Yang et al., 2012; Vandergrift et al., 2018). Improvements in reproducibility can be achieved using commercially available paper spray cartridges, which are simpler to handle and whose tip quality is more consistent, allowing high-throughput, automated analysis. Espy et al. (2014) demonstrated the use of these PS cartridges for the quantitative analysis of AMPs, MOR, COC, and Δ9-THC in whole blood and achieved improved sample to sample variations of 1–5% RSD with <10 μL of blood and inter-assay accuracies and precisions 87–117.9% and 1.3–16.5% (respectively, excluding morphine). A bespoke PS cartridge with an integrated SPE step that improved detection limits in the analysis of plasma samples has been demonstrated (Zhang & Manicke, 2015). Recently, a commercial PS system utilizing a multiplexed sampling plate containing 24 individual paper spray tips that can be coupled to an autosampler and has demonstrated its use for the detection of controlled substances in the blood (Ren et al., 2019).

There is a considerable need in forensic and clinical analysis for rapid screening methods to reduce sample backlogs: given the ease and speed of sampling, PS-MS is especially well suited for these applications. PS-MS has been demonstrated to rapidly screen for 4-chloroAMP in human saliva (Jhang et al., 2012) as well as a variety of other chloro and fluoroAMPs in saliva with improved LODs compared with AP-MALDI or ELDI (Lee et al., 2012). NPS are often sold in the form of blotter papers, which can be cut into triangles and directly measured for DoA by PS-MS (Carvalho et al., 2016). PS-MS has also been applied to the identification of a variety of synthetic cannabinoids and cathinones and other drugs (41 total) from 42 different powder or plant materials, and notably, an e-cigarette liquid found to contain AB-CHMINACA and PB-22 using high-resolution accurate mass (HRAM) MS and MS/MS with an analysis time of <2 min; this technique allows for both targeted and nontargeted analysis (Kennedy et al., 2016). PS with HR-MS/MS was further developed as a targeted semi-quantitative MS/MS drug screening methodology for over 130 drugs and drug metabolites in the positive-ion mode as well as for a set of barbiturates and structural analogs in the negative ion mode, demonstrating potential for acidic drug analysis (McKenna et al., 2018). The authors report good qualitative agreement with LC-MS/MS and a true positive rate of 92% and a true negative rate of over 98% when applied to postmortem blood samples. While HRMS systems (such as Q-TOF and Q-Orbitrap methods) offer higher selectivity by combining exact mass with MS/MS, they require more frequent calibrations and are expensive, making them inaccessible to many forensic laboratories. The use of QqQ-MS systems for PS offers a cheaper, more robust, and thus more accessible alternative. PS-QqQ-MS has been used for rapid, direct, and quantitative drug and metabolite screening at low ng/mL levels in postmortem blood samples (Jett, Skaggs, & Manicke, 2017). In a direct comparison of PS-HR-MS/MS and LC-HR-MS/MS for comprehensive urine drug testing using 103 authentic human urine samples, it was found that the direct PS method offered comparable screening power to the LC method, which included urine precipitation, conjugate cleavage, and liquid extraction (Michely, Meyer, & Maurer, 2017). A thorough review of PS-MS for the analysis of different biofluids has also been reported (Manicke, Bills, & Zhang, 2016).

Aside from the direct analysis of biofluids, PS-MS has been used for several other interesting forensic applications. Costa et al. (2017) demonstrate the analysis of DoA from fingerprint samples while retaining ridge detail by using a silver nitrate solution. Paper spray lacks a chromatographic separation, and frequently employs MS/MS or HRAM for additional selectivity, though these couplings are often still unable to differentiate structural isomers. A coupling of PS-MS and surface-enhanced Raman spectroscopy (SERS), using paper SERS substrates which can be inkjet printed, demonstrates the increased selectivity and confirmation gained from the use of SERS through the differentiation of the isomers MOR and hydromorphone (Fedick et al., 2017). Selectivity can also be enhanced by utilizing ion mobility spectrometry. A coupling of PS to high-field asymmetric waveform ion mobility demonstrated the separation of morphine, hydromorphone, and norcodeine, which cannot be differentiated using MS/MS alone (Manicke & Belford, 2015). Another logical pairing to increase selectivity in PS application is TLC, since PS-MS can be achieved directly from TLC papers used for drug analysis (De Carvalho et al., 2016). Santos et al. (2017) used TLC papers with Dragendorff reagent (revealing agent) to positively identify COC, lidocaine, and levamisole in ten street crack samples. PS-MS was then applied to revealed spots for quantification, and the authors reported no significant difference between PS-MS and GC-FID results. Recently, PS-MS has been presented as a promising analytical tool for harm-reduction drug checking, with the potential to prevent accidental overdoses (Vandergrift & Gill, 2019).

Among the ambient methods, PS-MS is among the most applicable for quantitative analysis because of the ease of internal standard introduction (either prespotted onto the paper prior to sample introduction or mixed into the sample). Quantitative analysis with PS-MS has been investigated for therapeutic drugs in dried blood spots (Manicke et al., 2011), the determination of eight AMPs in whole blood, with reported detection limits between 15 and 50 ng/mL (Teunissen et al., 2017), the semi-quantitative measurement of FEN and norfentanyl in methanol, urine, and an analgesic slurry (Vandergrift et al., 2018), and quantitative analysis of COC and several opiates in prepared dried blood spots, with reported LOQs of 0.5–16 ng/mL and a linear range that encompasses the entire therapeutic range (Su et al., 2013). Quantitative analysis of substance use disorder patient urine samples for high-throughput analysis of FEN analogs and other NPS in urine was achieved using HRMS and MS/MS with commercial PS cartridges (Kennedy et al., 2018).

The robustness and simplicity of PS-MS make it especially well suited for *in situ* applications with miniature or portable mass spectrometers. A review of PS for portable mass spectrometry has recently been published (Silva et al., 2019). Notable demonstrations of PS coupled to miniature MS systems include the analysis of synthetic cannabinoids using an IT (Ma et al., 2015), and a FLIR cylindrical IT-MS with automated MS/MS library searching (O'Leary et al., 2015). The FLIR system

analyzed 25 positive controls, 4 negative controls, and 3 authentic powdered drug samples. For the positive control samples, 68 of 69 MS/MS spectra collected produce relative average match probabilities high enough for "true positive" identification, no negative control samples resulted in false positives, and all of the powdered drug evidence samples were correctly identified using the automated, commercially available "Wiley Registry of Tandem Mass Spectral Data, MSforID" library. These results indicate that field-portable MS systems can be used *in situ* for drug analysis and the automated library searching feature eliminates the need for user interpretation of spectra and can allow for use by nontechnical operators.

PS-MS has been demonstrated for a wide range of forensic and clinical applications, and given the rapidity, simplicity, low-cost of implementation, and demonstrated use with portable MS systems, it has the potential to reduce crime and clinical lab backlogs, and be implemented in roadside testing, point-of-care, or other *in situ* applications.

5. Coated Blade Spray

The lack of sample preparation for many ambient methods makes them attractive, but there are instances where sample preparation should not be overlooked, as it can greatly reduce ion suppression, matrix effects, and improve limits of detection. CBS shares many characteristics observed in PSI, but incorporates an extraction and preconcentration sample preparation step by using an SPME coated, pointed stainless-steel blade. This has the potential to greatly improve analytical performance and can be tailored to the analysis of specific matrices or analytes. A thorough review of SPME materials and applications has been reported recently (Reyes-Garcés et al., 2017).

CBS was first reported by Pawliszyn et al. in 2014 (Gómez-Ríos & Pawliszyn, 2014), and reports that an entire analysis (analyte extraction, rinsing, desorption/ionization, peak integration, and quantitation) can be completed in less than 3 min with LOQs in the low pg/mL range, considerably lower than many of the other ambient techniques. CBS can be considered a solid-substrate ESI technique, using a thin stainless-steel blade as the substrate (Gómez-Ríos & Pawliszyn, 2014; Gómez-Ríos, Tascon, & Pawliszyn, 2018). Prior to sampling, the blade is "preconditioned" by vortex agitation in a solution of methanol/water. Sampling/extraction occurs by immersing the preconditioned blade in a vial containing the sample matrix or by spotting a small volume of biofluid directly onto the SPME coated blade. This is followed by a quick (ca., 10 sec) rinse in water with vortex agitation, shown to be critical for reducing ionization suppression (Gómez-Ríos & Pawliszyn, 2014). A small volume ($\leq$20 μL) of desorption solvent (typically organic) is directly applied to the coated blade and high voltage (ca., 3.5 kV) applied to generate gas-phase ions.

Similar to other solid-substrate ESI based methods, CBS signal intensity and duration are affected by the desorption solvent choice and volume, the wetting time, and the voltage applied, as well as by the amount of analyte extracted from the matrix by the SPME coating (Gómez-Ríos & Pawliszyn, 2014). Analyte extraction from the sample solution or biofluid droplet is governed by the kinetics of analyte partitioning, though short, pre-equilibrium extractions are often used (e.g., 10 sec). The SPME coating (extraction phase) can either be a polymer or polymeric particles attached to the substrate (steel blade) using a chemical binder. The first demonstration of CBS used the biocompatible polymer C_{18}-polyacrylonitrile (C_{18}-PAN) for the effective extraction of hydrophobic small-molecule drugs including COC and diazepam from plasma and urine samples (Gómez-Ríos & Pawliszyn, 2014) with low pg/mL LOQs. Additionally, a variety of AMPs, opioids, and COC were quantified in phosphate-buffered saline with similar LOQs.

A desirable feature of CBS is the robustness and reproducibility of the sampling devices and technique. Other solid-substrate ESI methods (e.g., PS-MS, wooden tip ESI, etc.) use porous nonconductive substrates with poorly defined tips, leading to the generation of multiple ESI events at the tip, reducing the efficiency of ion transfer into the MS. Stainless-steel blades can be reliably machined to produce sharp, well-defined tips, which increase the ionization efficiency (Gómez-Ríos, Tascon, & Pawliszyn, 2018). In a continuation of complex sample analysis with CBS, diazepam and COC were quantitatively analyzed in phosphate-buffered saline and methadone and oxycodone in urine, and the use of a 10 μL sample directly applied to the coated blade (with various coatings) was investigated (Piri-Moghadam et al., 2016).

A validated (with respect to linearity, precision, accuracy and LOQ) quantitative analysis of drugs (including AMPs, COC, opioids, and BDZs) using CBS for biofluid spots (10 μL—plasma and whole blood) has been presented in the literature as a new approach for sensitive rapid screening (Gómez-Ríos et al., 2017). The method required 7 min total analysis time, offering a balance between improved analytical performance from sample preparation and high-throughput analysis. The sample preparation step allows for low LOQ values; LOQs were <10 ng/mL in blood spots and <5 ng/mL in plasma spots. Stability studies showed that most compounds were stable on the coating even at room temperature for up to 7 days, with longer stability noted at lower temperatures. In an extension of this quantitative work, a high-throughput methodology was developed in which 96 CBS extractions were performed simultaneously on spiked urine and plasma samples allowing the total analysis time to be reduced to 55 s per sample, while maintaining excellent analytical performance and low LOQ for a variety of illicit and commonly abused drugs (Tascon et al., 2017).

A recent review of CBS is presented in the literature (Gómez-Ríos, Tascon, & Pawliszyn, 2018). While the technique is slightly more complicated than PS or other related methods, CBS presents a balance between sample preparation and rapid analysis. The analytical performance achieved with CBS is superior to the other solid-substrate techniques in many cases due to the extraction of samples—concentrating analytes while leaving behind matrix species, including enzymes, which can convert or degrade analytes, making it especially well suited for the analysis of biofluids. The ability to interface the technique with 96-well-plates and robotics can bring the analysis time down to 55 s per sample. The inventors of the technique speculate that future applications may improve selectivity by using smart coating materials, on-coating derivatization, determination of total drug concentration in urine through enzymatic hydrolysis prior to extraction, as well as improvements in automation allowing for total analysis times of 10–15 sec (Gómez-Ríos, Tascon, & Pawliszyn, 2018).

6. Other Solid-Substrate Electrospray Ionization Techniques

Throughout the development of ambient mass spectrometry, many applications involving ESI from noncapillary solid substrates such as leaves and other plant material, bone,

toothpicks, bamboo, fabrics, sponges, pipette-tips, medical swabs, metal needles, copper wires, nanostructured tungsten oxide, among others, have been developed; an overview of solid -substrate ESI has been presented by Hu, So, & Yao (2013). These substrates simplify sampling loading and avoid clogging issues prevalent in classical ESI methods. Note that PS-MS and CBS-MS are also considered solid-substrate ESI techniques, but have been discussed in separate sections due to the wealth of literature on these methods. Of the solid-substrate ESI techniques, several applications for the analysis of DoA have been shown, including the use of wooden tips (Hu et al., 2011, 2016; So et al., 2013; Ng et al., 2019), bamboo pen nibs (Chen et al., 2013), C18 pipette-tips (Wang et al., 2014), and medical swabs (Pirro et al., 2015; Morato et al., 2019). The technique is remarkably simple, the analyst need only apply sample, solvent, and high voltage to the substrate and position the substrate in front of the MS inlet to induce electrospray-like ionization.

Wooden tips have been used in several examples for DoA analysis. Benefits of the technique include its simplicity, low cost and ease of coupling with nano-ESI ion sources. Liquid samples are loaded by pipetting them onto the tip of a device or dipping the tip into solution, and solid samples can be scraped from crevasses, corners, and small openings, a unique advantage for forensic applications. The hydrophilic and porous properties of the wooden tips increase the duration of the signal. A sharper tip generates a much higher quality signal, and thus wooden tips are often sharpened prior to analysis. The first demonstration of the technique for DoA analysis analyzed KET collected "from a tiny crack on a concrete floor" (Hu et al., 2011). The tip was first prewetted to allow the powder to adhere, then 5 μL of solvent was applied to facilitate the spray, and data was collected over 20 sec. The technique was further applied to the prescreening analysis of KET and NKET in urine and oral fluid samples with minimal sample preparation (dilution with methanol) (So et al., 2013). So, Ng et al. found that diluted urine and oral fluid aliquots of 2 μL were sufficient for the quantitative MS/MS detection of KET and NKET with LODs of 20 and 50 ng/mL in oral fluid and urine, respectively. In another application using wooden tip-ESI, 144 herbal dietary supplements were tested for 33 common adulterating drugs, detecting mainly BDZs (Hu et al., 2016). The development of a sampling system incorporating a moving stage with 20 wooden tips attached in this study allowed for high-throughput analysis (ca. 15 sec per sample). The presented method is a qualitative prescreening method as no internal standard was used. The quantitative capabilities of wooden tip-ESI was explored by analyzing a variety of common DoA in urine and oral fluid in a continuation of So, Ng et al.'s (2019) previous work with KET and NKET. Most analytes demonstrated acceptable detection limits that meet internationally established cutoff values, but THC and THC-COOH performed very poorly in these analyses. Overall, wooden tips as a solid substrate have been demonstrated for the quantitative analyses of many DoA in a variety of different matrices, require very small sample loadings, exhibit no carryover, and are extremely simple and economical to use.

In another solid-substrate ESI application, commercial C18 pipette-tips (conventional pipette-tips with a C18 resin acting as an extraction phase) were used to purify and enrich KET and NKET in urine to generate much lower detection limits (0.3 and 0.8 ng/mL, respectively) than observed previously in wooden tip-ESI (Wang et al., 2014). The method also gave superior analytical performance based on several other measures including linearity, precision, and accuracy. One drawback is the necessary sample preparation step that required 2–3 min for a total analysis time of ca. 5 min per sample.

Medical swabs are ubiquitously used to noninvasively sample biological fluids for a variety of clinical tests and in forensic toxicology. Given that they are already approved for medical use, they represent an opportunity to use as a solid-substrate for MS analysis in a wide range of forensic and clinical applications. Pirro et al. (2015) demonstrated their proof -of-concept use for qualitative DoA detection in oral fluid, analyzing 14 common DoA, with the intended application of point-of-care drug testing or *in situ* forensic applications. Using a LIT-MS, MS^2 and MS^3 experiments were performed in a total analysis time of 4 min (following a 15-min drying step after sampling) to provide acceptable detection limits and identification, except for BUP and THC, presumably due to poor ionization characteristics. Quantitative analysis or *in vivo* sampling was not achieved in this study, and sample volumes could only be estimated (ca. 40 μL). However, medical swabs have been developed for the volumetric sampling and demonstrate the potential for quantitative sampling. Morato et al. (2019) have demonstrated the use of volumetric absorptive microsampling swabs as substrates for the quantitative analysis of 30 common DoA using 10 μL samples of oral fluid, optimal for forensic or toxicological applications given that small volumes are often all that can be collected. The authors made considerable effort to fully validate the MS/MS method, which yields results within 2 min (following a 15-min drying step) with satisfactory analytical performance. LODs for the compounds ranged from 0.08 to 4.86 ng/mL except for AMP, MAMP, and mephedrone, and no carryover effects were observed. For quantitative *in vivo* sampling, an internal standard mixture must be spotted onto the swab following sampling, resulting in a slight overall reduction in analytical performance. The quantitative performance is still acceptable given that only three drugs (6-AM, AMP, and MAMP) gave LOD values above cutoff values established by DRUID.

Medical swabs for the analysis of DoA in oral fluid are a promising choice given their already established and extensive use in the medical field as noninvasive sampling devices. The swabs are cheap, can volumetrically sample small amounts (10 μL), and have been validated for quantitative analysis. The method requires no sample preparation (except for a drying step), and the promising applications presented thus far indicate the potential for this technique to be used in roadside drug testing, point-of-care, or other forensic and clinical *in situ* applications.

C. Plasma-Based Techniques

Plasma-based ambient ionization techniques operate via similar principles as APCI, in that energetic/reactive species generated by some form of plasma are subsequently used to ionize analytes. Table 3 summarizes a variety of these approaches. Figure 8 illustrates generalized schematics for plasma-based ambient ionization techniques. The reader is directed to two recent reviews on plasma-based ambient ionization mass spectrometry techniques (Ding & Duan, 2015; Smoluch, Mielczarek, & Silberring, 2016).

TABLE 3. Plasma-based ambient techniques.

Technique	Sampling principles	Plasma
Direct analysis in real time (DART)	Metastable neutrals (e.g., N_2, He) are generated in a confined plasma and passed over a sample outside of the source with heat for desorption/ionization.	Corona or glow discharge
Atmospheric solids analysis probe (ASAP)	A probe is used to introduce the sample into a heated desolvation gas stream (from and APCI or ESI probe), thermally desorbed analytes are ionized by a corona discharge.	Corona discharge
Flowing atmospheric-pressure afterglow (FAPA)	The plasma discharge is physically and electrically isolated from the sample. The sample is introduced into the flowing afterglow and analytes are desorbed/ionized.	Corona or glow discharge
Direct sample analysis (DSA)	A probe is used as a solids sampling device, or liquids samples are deposited onto a mesh. A corona discharge is generated by an APCI source with N_2 and the produced reagent ions are directed to the probe or mesh to facilitate desorption/ionization.	Corona discharge
Dielectric barrier discharge ionization (DBDI)	A plasma is generated within the source and passed over a sample for desorption/ionization.	Dielectric barrier discharge
Low temperature (LTP)	An alternating current electric field generates a low-temperature plasma that extends from the source and directly interacts with the sample to effect desorption/ionization.	Dielectric barrier discharge

APCI, atmospheric pressure chemical ionization; ESI, electrospray ionization.

1. Direct Analysis in Real Time

Since its introduction in 2005 (Cody, Laramée, & Durst, 2005), DART has become an established technique for rapid mass spectral analysis in a wide range of forensic and clinical sampling applications. It can analyze solid, liquid, and gas samples; a critical review of the technique is presented by Gross (2014) as well as a review discussing forensic and security applications (Pavlovich, Musselman, & Hall, 2018). If the technique were to be named from its desorption/ionization characteristics, then perhaps a name like thermal-desorption penning ionization-induced APCI may have been chosen. The ionization mechanism has been investigated for both positive-ion (Song et al., 2009) and negative ion modes (Song et al., 2009). In DART, metastable neutrals (e.g., He, N_2) generated in a confined plasma source are used to effect analyte ionization by directing them onto a surface to be measured. In comparison with other ambient methods, DART particularly excels at the analysis of surfaces (Grange and Sovocool, 2011). In the initial demonstration of DART, it was applied to the analysis of DoA on business cards, pharmaceutical tablets, concrete, cocktail glasses, plastics, leaves, currency, airline boarding passes, and human skin, among many others, and was able to detect GHB spiked into gin (at 10 ppm) within seconds, a compound that is particularly challenging to analyze with LC-MS (Cody, Laramée, & Durst, 2005). Coupling of DART to a QqQ-MS was used to investigate the direct quantification of drugs in biological matrices (Yu et al., 2008). The authors investigated matrix effects from plasma samples and found them to be analyte-specific. Reproducibility, sensitivity, linearity, bias, matrix effects, and a direct comparison to LC-MS/MS found the technique to be generally adequate for bioanalytical or forensic applications, though noted limitations include poor selectivity in some cases and "harder" ionization that fragments more labile compounds, such as glucuronides, within the source. DART has been demonstrated for a number of forensic and clinical screening applications, including the analysis of synthetic cannabinoids from commercial herbal products (Dunham, Hooker, & Hyde, 2012; Musah et al., 2012; Lesiak et al., 2014; Habala et al., 2016), the direct detection of THC from hair samples (Duvivier et al., 2014), the rapid (0.5 min) screening of NPS with confirmation by LC-MS (Nie et al., 2016), detection of synthetic cathinones and metabolites in urine with SPME-DART coupling (LaPointe et al., 2015), as well as the untargeted analysis of DoA in hair (Duvivier et al., 2016), among others. In 2009, a validation of the DART source coupled to an accurate mass TOF-MS for use in forensic screening of DoA was achieved, allowing the Virginia Department of Forensic Science to incorporate DART for the qualitative screening of solid forms of DoA (Steiner & Larson, 2009).

Though DART is primarily used as a screening or qualitative analysis technique, a few quantitative applications have been demonstrated. The quantification of DoA in biological matrices was achieved in both *in vivo* and *in vitro* applications, demonstrating comparable results to LC-MS/MS and potential as an effective tool for high-throughput, real-time bioanalysis (Yu et al., 2008). A coupling of DART to SPME devices made from PEEK mesh was demonstrated for DoA quantitation in oral fluid and urine with satisfactory LOQs (ca. 0.5 ng/mL), linearity, and accuracy over the evaluated range (0.5–200 ng/mL) (Vasiljevic, Gómez-Ríos Gn, & Pawliszyn, 2017).

DART, like other ambient techniques, has reduced selectivity when compared with chromatographic methods. To address this, Musah et al. (2014) utilized in-source CID and HRMS for a variety of NPS, as did Lesiak et al. (2013) who demonstrated differentiation of closely related isobaric synthetic cathinones. Advanced statistical treatments of data generated

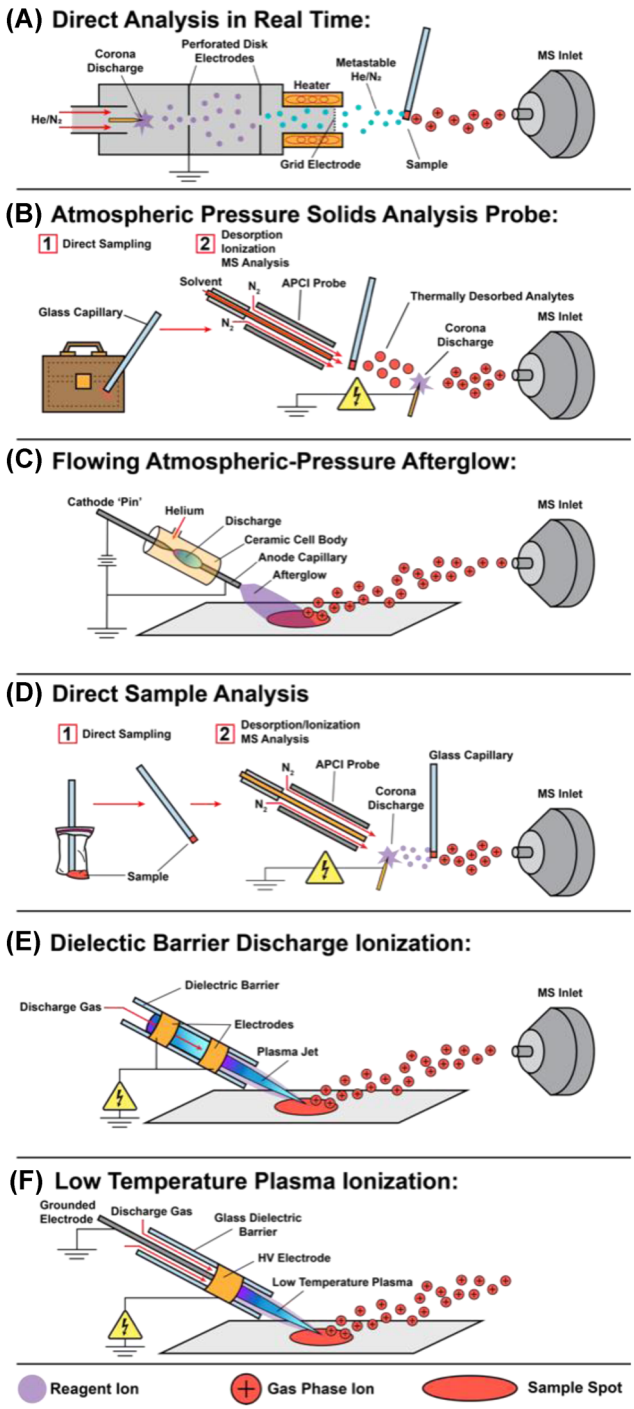

FIGURE 8. Generalized instrumental schematics for (**A**) direct analysis in real time (DART), (**B**) atmospheric solids analysis probe (ASAP), (**C**) flowing atmospheric-pressure afterglow (FAPA), (**D**) direct sample analysis (DSA), (**E**) dielectric barrier discharge ionization (DBDI), (**F**) low-temperature plasma (LTP). Not to scale. [Color figure can be viewed at wileyonlinelibrary.com]

from high-resolution DART measurements have been used to identify HER sources (Cui et al., 2019), and identify NPS from neutral loss spectra (Fowble, Shepard, & Musah, 2018).

Portable MS applications with DART are particularly attractive given the simplicity of the source and its commercial availability. DART with portable MS has been demonstrated for the identification of powdered drug samples, tablets, and herbal samples (Brown et al., 2016) as well as for DoA in biofluids by coupling SPME to a portable single quadrupole system (Gómez-Ríos et al., 2017). In summary, DART-based methods can rapidly desorb and ionize analytes, particularly from solids or solid surfaces, providing real-time information regarding DoA, and the technique has been commercialized for portable applications.

2. *Atmospheric Pressure Solids Analysis Probe (ASAP)*

The ASAP allows for the direct analysis of liquid or solid samples in seconds and can be accomplished using any commercial instrument with an ESI or APCI source by creating a port to insert the sample probe into the heated desolvation gas stream. The technique, first described in 2005, uses the heated nitrogen desolvation gas from an ESI or APCI source to thermally desorb analytes from a sampling probe, with subsequent ionization of the vapor occurring via the APCI corona discharge (McEwen, McKay, & Larsen, 2005). The probe used to introduce sample must be free of volatile components that may interfere with analysis, and glass melting point capillary tubes are most often employed as sampling probes. The MS/MS detection of COC from several U.S. 1-dollar bills has been demonstrated by directly introducing them into the heated desolvation gas stream. The range of compounds amenable ASAP is limited to compounds with some polar character, that can be thermally desorbed and do not thermally degrade, as well as those that can be ionized by APCI. Although the ASAP method has not been extensively demonstrated for the analysis of DoA, there are a few select applications of note.

Jagerdeo et al. (2015) demonstrated the direct, rapid analysis of black tar HER and impurities (including codeine, morphine, noscapine, papaverine, and 6-AM), crack COC, alprazolam from urine, as well as a rodenticide which has increasingly been reported to be detected in seized drug samples as an adulterant. A capillary tube was rubbed on solid samples or submerged into liquid samples and allowed to dry, then transferred to the source into the path of the hot nitrogen gas supplied by an APCI probe. The ASAP source, in this case, was coupled to a linear ion trap MS system to facilitate full scan, MS^n, and rapid polarity switching. Quantitative work was not thoroughly investigated, though a calibration of alprazolam in urine was demonstrated to suggest quantitation could be achieved. ASAP-MS/MS has been used for the direct analysis of raw urine samples for the qualitative trace detection of AMPs (Crevelin et al., 2016). The LOD for the compounds was determined to be 0.002 to 0.4 ng/mL with acceptable precision. No sample carryover or matrix suppression effects were observed.

ASAP coupled with HRMS (LTQ-Orbitrap) has also been used for the direct analysis of black tar HER (and impurities) and "Spice" packets containing 10 different synthetic cannabinoids and a synthetic cathinone in positive APCI mode, comparing it to laser diode thermal desorption (LDTD) (Jagerdeo & Wriston, 2017). The signal for the analysis of black tar HER using ASAP lasted for more than 8 min, which allowed for a full scan and six MS/MS experiments to be completed. Optimization allowed the total MS analysis time to be reduced to <1 min.

Given that ASAP can be easily interfaced with any commercial mass spectrometer, often requires no sample preparation, uses disposable, inexpensive glass capillaries for sampling that eliminate carryover, exhibits lessened matrix

effects due to APCI ionization, can sustain a signal for minutes, and allows for rapid analysis and high throughput, it is a very attractive candidate in rapid, routine analysis of forensic samples for both targeted and nontargeted analysis, provided that the analytes are amenable to the method.

3. Flowing Atmospheric-Pressure Afterglow (FAPA)

FAPA is a plasma-based source traditionally used for elemental analysis. It has appeared in the literature in recent years for the analysis of organic compounds at atmospheric pressure with soft ionization. In FAPA, analytes are directly desorbed and ionized from a sample of any phase (solid, liquid, gas) with minimal sample preparation; desorption is presumably due to the temperature of the helium gas (>200°C) and the presence of excited species. The exact ionization mechanism is unclear, but occurs either directly by Penning ionization or through interaction with reagent ions such as protonated water clusters (Shelley, Wiley, & Hieftje, 2011). FAPA was originally termed helium atmospheric-pressure glow discharge and was first reported in a paper that thoroughly outlines the design and behavior of the ionization source (Andrade et al., 2006). In an improved geometry over the original pin-to-plate design, a pin-to-capillary geometry significantly reduced background noise and improved analytical performance in both positive and negative ionization modes. It has been used for the detection of MAMP in tap water, with a demonstrated LOD of 0.7 ng/mL, and applied to untreated urine analysis with minimal sensitivity losses (Shelley, Wiley, & Hieftje, 2011). This new FAPA design allowed its sensitive and quantitative use for DoA detection, with less matrix effects than other plasma-based sources. It has been used for the rapid direct analysis of methcathinone from crude reaction mixtures without sample preparation (Smoluch et al., 2012) with an estimated LOD of 10 ng. FAPA is not very effective at analyzing compounds >400 Da. The analysis of the designer drugs JWH-122, 4BMC, pentedrone, 3,4-DNNC, and ethcathinone has been demonstrated using two FAPA sampling methods (methanolic aerosol from nebulizer or heated crucible for thermal desorption) (Smoluch, Mielczarek, & Silberring, 2016). The variety of forms in which these NPSs are distributed, including being deposited on various sorbents or biomasses and as trace components amongst other adulterants and additives can cause challenges with traditional methods. FAPA-MS is a direct technique that appears capable of performing direct analyses in the already extensive, and ever-increasing number of NPS sample matrices. The method demonstrates sensitivity that is comparable with direct inlet probe EI analysis since ca. 5 ng of material can be detected. The methanolic aerosol method was investigated for quantitative use with 10 μL injections of an ethcathinone solution, demonstrating acceptable quantitative or semi-quantitative performance, though notably not as sensitive as some of the other plasma-based techniques.

FAPA has been interfaced with an electrochemical flow cell to identify electrochemically produced drug metabolites, especially important to the field of predictive toxicology for the analysis of NPS whose metabolic pathways are unknown or poorly understood (Smoluch et al., 2014). The authors report much higher LODs than previously established by other demonstrations of FAPA (e.g., 2.5 μg/mL MAMP) but note that the goal was to demonstrate the utility of the coupling of the electrochemical cell to a FAPA source for use in predictive toxicology and presented the first coupling of a flowing liquid system to FAPA. The "halo-shaped" FAPA or h-FAPA improves the reproducibility of sample introduction (Pfeuffer et al., 2013). It uses concentric tubular electrodes to form a halo -shaped discharge, and allows sample introduction (solution, vapor or aerosol) through an inner capillary, improving the interaction between sample and plasma to enhance both desorption and ionization, while retaining surface sampling capabilities. Proof-of-concept DoA testing demonstrated superior sensitivity and precision over earlier FAPA designs for COC and metabolites.

FAPA is a direct, rapid method of analysis with potential for use in a wide range of forensic applications given that no or minimal sample preparation is needed, and can analyze solid, liquid, or gaseous samples. New source geometries have significantly improved its analytical performance. The technique is not nearly as well represented in the literature as other plasma-based sources like DART and DBDI, and though some quantitative measurements have been demonstrated for drugs, there is still work to be done to establish FAPA as a viable source for forensic and clinical applications for DoA.

4. Direct Sample Analysis (DSA)

DSA is an ambient technique that has been commercialized by PerkinElmer, originally termed surface desorption APCI in 2007. The technique combines the features of DESI and APCI; a corona discharge is generated by an APCI source with nitrogen gas and the produced primary ions (protonated water clusters) are directed to a liquid or solid sample absorbed onto a surface (or powder) to desorb and ionize analytes of interest (Chen et al., 2007). A high-throughput sample introduction system interfaced with the DSA-MS can transport up to 24 samples per second with a duty cycle as low as 10 ms per sample. In this initial demonstration, pharmaceutical tablets and powders were directly analyzed in 1 min. Much lower gas pressures (6.89 kPA) are used in the DSA source than used in a typical DESI source (~2 MPa), suggesting a more facile coupling to miniature mass spectrometers, obviating the need for compressed gas tanks.

A commercial DSA-TOF-MS system has been demonstrated for the rapid analysis of 369 DoA from seized pills, vials, powders, and urine samples (Daugherty & Crowe, 2014). Analyte identity is confirmed using the exact monoisotopic masses of precursor and fragment ions and isotope ratios to identify analytes through comparison with a system database containing all US Schedule 1–5 drugs. Sample introduction is achieved using disposable mesh for liquid samples (5 μL) or glass capillaries for solid samples with minimal or no sample preparation. The source can easily be switched for a traditional LC source in minutes, if desired. The DSA-TOF-MS system was applied to the analysis of an emerging class of variable phenethylamines known as "NBOMes" (which have similar properties to LSD and are variants of the 2C-X series) from blotter papers (Botch-Jones et al., 2016). The analysis time for designer drugs on blotter papers was successfully reduced to ca. 15 sec using the direct DSA-TOF-MS method, though it should be noted that quantitation of NBOMes was not accomplished, and the constitutional isomers 25T4-NBOMe and 25T7-NBOMe could not be differentiated. McGonigal et al. (2017) have also

demonstrated the use of the AxION DSA-TOF-MS system for the detection of 26 synthetic phenethylamine street drugs (representing all commercially available compounds at the time) including some of the NBOMes previously mentioned as well as several 2C-X analogs of mescaline. Methodologies for the rapid screening of opioids in seized street drug samples have also been developed (Moore et al., 2019). A qualitative method for the determination of 18 compounds (opiates, FEN analogs, and synthetic opioids) with in-source CID was used for structural confirmation. Matrix interferences were found to be minimal, even when samples were prepared as 90% adulterants, maintaining good detection levels and mass accuracy for all analytes. In this study, 81 seized drug samples were analyzed both by traditional GC-MS and by DSA-TOF-MS, the results agreed in 80 of 81 cases. In an interesting case, a sample suspected to contain HER was analyzed by GC-MS and no controlled substance was identified, but DSA-TOF-MS with in-source CID was able to qualitatively identify furanyl FEN from its characteristic fragments and the precursor ion mass.

The PerkinElmer AxION DSA-TOF-MS system represents yet another alternative that can be used to rapidly screen forensic liquid and solid samples. Methods for DoA with this technique have largely been qualitative and focus on HRAM combined with in-source CID for the identification of compounds. DSA-TOF-MS has been successfully demonstrated for the identification of true unknowns and NPS where no reference standards or libraries exist.

5. *Dielectric Barrier Discharge Ionization (DBDI)*

The DBDI source (a glow discharge device), is comprised of a copper sheet electrode and a discharge electrode, with an insulating glass slide in between that acts as both a dielectric barrier and sample plate (Na et al., 2007). The DBDI source has been employed for several applications involving the direct and rapid solvent-free analysis of DoA, discussed further in a 2015 review, which also discusses a variety of different source geometries (Tang et al., 2015). Advantages of DBDI include a mild discharge (soft ionization), small size, stable operation at atmospheric pressure, low power consumption, simplicity, and nonthermal characteristics, making it especially well suited to portable or miniature mass spectrometers. Kumano et al. (2013) utilized a low-pressure DBDI source to develop a prototype portable LIT-MS capable of discontinuous sample gas introduction and a vacuum headspace technique. This prototype system was found to be sensitive enough for the qualitative MS/MS detection of 0.1 ppm MAMP, 1 ppm AMP, 1 ppm MDMA, and 10 ppm COC in an aqueous K_2CO_3 sample headspace, with the assumed sensitivity differences due to proton affinity and vapor pressures. The portability and size of the DBDI-MS limit the sensitivity that can be achieved, though the source was found to be >50 times more sensitive than an APCI source on the same MS. Another example of a low-pressure DBDI source being coupled to a miniature MS was used to analyze drugs (caffeine, COC, and MOR) and explosives using air as a carrier gas to further simplify the method (Usmanov et al., 2016). All analytes produced $[M + H]^+$ ions. Habib et al. (2018) demonstrated the trace-level analysis of AMPs in water and urine samples with LODs in the pg/mL range. Validation studies indicate that the method is a viable alternative to routine analytical work done by conventional GC-MS or LC-MS methods for the rapid detection of amine-based drug compounds in urine for doping tests or in forensic laboratories.

Synthetic cannabinoids are a class of NPS that are often contained within botanical matrices, hampering their analysis with traditional methods without extensive sample preparation and routine screening tests often do not incorporate these types of compounds. DBDI-MS offers a direct and rapid analysis strategy for these compounds present in herbal matrices, and the use of a sample heater to assist in sample introduction has greatly increased the analytical performance of DBDI for these compounds allowing the technique to partially replace GC-MS methods for fast screening (Smoluch et al., 2015).

In one of the most sensitive direct couplings of SPME with MS reported in the literature at the time, Mirabelli, Wolf, & Zenobi (2016) reported an SPME-DBDI-MS methodology capable of sub pg/mL DoA detection limits that employ thermal desorption of SPME fibers followed by DBDI ionization. At the time of the publication, all direct couplings of ambient MS ionization techniques to SPME relied on the simultaneous desorption/ionization of analytes directly from the SPME device, apart from solvent desorption techniques which limit the enrichment potential of SPME. Even when a relatively short, 5 min extraction time was used, LOD of 0.3 pg/mL were achieved for diazepam and COC. Quality control experiments demonstrated that no carryover effect was observed over the entire concentration range analyzed. The remarkable sensitivity and precision demonstrated in this SPME-DBDI coupling with thermal desorption open the possibility for forensic and clinical applications requiring very low-level analysis. For example, this coupling was used to rapidly screen drugs in beverages (vodka, wine, and cola) and biological fluids (urine and blood plasma) using ultrasound as an agitation method to affect the rapid extraction of analytes using thin-film microextraction from matrix, and thermal desorption to introduce gas-phase analytes to the DBDI source (Mirabelli et al., 2019). The authors note that the biocompatibility of the thin-film microextraction devices used in the study may have future applications in noninvasive oral fluid or sweat sampling with rapid, trace-level analysis.

A simplified, cost-effective DBDI source was constructed and applied to the analysis of a variety of drugs including pharmaceutical compounds (AMP and scopolamine), synthetic cannabinoids, psychostimulants, methoxyphenidine, and COC (Furter & Hauser, 2018). The qualitative analysis of the drug standards was demonstrated using 20 μL samples, as well as the direct analysis of pharmaceutical tablets, and 5F-APINACA from "Funky Buddha" leaves (marshmallow plant). The entire DBDI circuitry can be purchased as a preassembled module from internet vendors for ca. 20 euros.

The DBDI source appears to hold significant promise as an inexpensive ambient ionization strategy for DoA measurements. Its small size, amenability for portable instrument applications and low cost should see future developments as the technique matures.

6. *Low-Temperature Plasma (LTP)*

Similar to the DBDI source, the LTP source, first demonstrated in 2008, uses an alternating current electric field to induce a dielectric barrier discharge for solvent-free, low-temperature desorption and ionization of compounds in the solid, liquid or

gas phase with a specially designed electrode configuration (Harper et al., 2008). The difference from DBDI is that the counter electrode is incorporated within the source, yielding a more direct platform for surface sampling (Harper et al., 2008). The technique was initially demonstrated for a wide variety of DoA applications with little to no sample preparation, including the direct analysis of COC on human skin, antihistamines from pharmaceutical drug tablets, caffeine, and other compounds from urine, as well as the direct analysis of aqueous solutions, with reported LODs as low as 1 ppb. Although the relatively poor spatial resolution it affords does not lend itself well to high -resolution chemical imaging, the tolerance to the sample position and angles used in the desorption setup are promising for the analysis of large surface areas such as the rapid screening of luggage for DoA. The LTP shares many characteristics with other plasma-based ambient desorption ionization sources with a few notable differences: the probe design allows for plasma species to be extracted from the discharging area by the discharge gas flow and electric field. A low temperature "torch" (ca. 30°C) extends from the glass tube of the source that directly interacts with a sample or surface. Mass spectra exhibit similar but gentler ionization than ESI. The use of air as a discharge gas further increases source simplicity and its applicability for portable MS systems.

The use of LTP-MS for the direct analysis of DoA in biological matrices was demonstrated for 14 drugs from the opiate, stimulant, euphoriant, and sedative drug classes which were qualitatively analyzed in urine, saliva, and hair extracts, with quantitative measurement, demonstrated using BEG in urine (Jackson et al., 2010). Detection limits in urine can be improved by dilution, which greatly reduces matrix effects. Although thermal assistance is not required for desorption/ ionization of most compounds with the LTP, detection limits can be improved with assistive sample heating.

The LTP source has also been applied to the direct, quantitative analysis of 11 DoA in saliva including phenethylamines, synthetic cannabinoids, synthetics cathinones, piperazines, and KETs (Wang et al., 2018). The higher MW compounds (e.g., synthetic cannabinoids) performed poorly without thermal assistance and thus a sample temperature of 200°C was required for sensitive analysis of all compounds, and LODs for the compounds in the study ranged from 3.0 to 15.2 ng/mL, comparable with immunoassays, but with lower sensitivity than LC-MS/MS methods. Arrays of multiple LTP probes (1, 7, and 19 probes) have been demonstrated to increase the sampling area and reduce LODs for select DoA applications (Dalgleish et al., 2013). The authors demonstrated the online chemical derivatization of mephedrone, MDMA, and methylone using trifluoroacetic anhydride increased selectivity, though it was noted that sensitivity decreased in these specific examples. The increased surface area of the arrays makes them ideal candidates for the rapid screening of larger surfaces such as suitcases as well as for integration with portable mass spectrometers.

Integration of LTP probes into portable mass spectrometer systems is a logical pairing given the absence of solvent and other waste products, the low power requirements, low cost, small size, and low gas flows used. Wiley, Shelley, & Cooks (2013) have demonstrated the use of a handheld LTP probe for "point-and-shoot" analysis that weighs ca. 0.9 kg. This source was able to sustain a plasma continuously for 2 hr using a small 7.4V Li-polymer battery and small helium or air cylinder, and was able to detect 1 μg of MAMP from a human finger two hours after its deposition using MS/MS. Despite the smaller size, lower power requirements and gas flows, analytical performance was not degraded using the handheld LTP source compared with a conventional, large-scale source.

LTP-MS has been demonstrated for both qualitative and quantitative measurements of DoA from a variety of classes. At the current stage of development, LTP ionization shows the most promise for applications involving surface sampling, rapid screening, semi-quantitative screening, and most notably for integration with portable MS systems, given its small size and cost, that it operates without solvent, generates little waste, and has low power and gas flow requirements.

VI. OTHER TECHNIQUES

For completeness, this section presents the ambient ionization technique desorption atmospheric pressure photoionization (DAPPI), as well as two laser-based methods, matrix-assisted laser desorption ionization (MALDI) and surface-assisted laser desorption ionization (SALDI). The general instrumental schematics for these methods are illustrated in Figure 9.

A. Desorption Atmospheric Pressure Photoionization

DAPPI utilizes a heated nebulizer microchip to direct a jet of hot, vaporized solvent toward a sample on a surface for thermal desorption; desorbed analytes are directly ionized using a photoionization lamp, or indirectly via gas-phase interactions with dopant (solvent) molecules (Haapala et al., 2007). In the

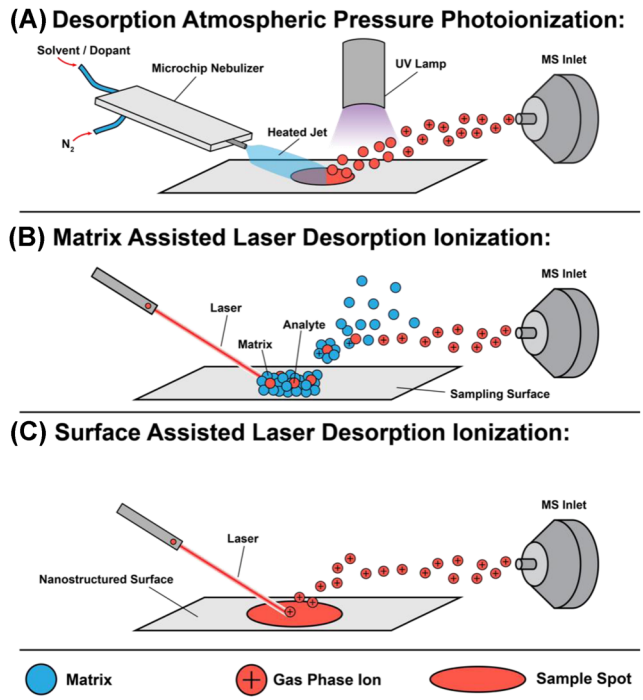

FIGURE 9. Generalized instrumental schematics for (**A**) desorption atmospheric pressure photoionization, (**B**) matrix-assisted laser desorption ionization (MALDI), and (**C**) surface-assisted laser desorption ionization (SALDI). Not to scale. [Color figure can be viewed at wileyonlinelibrary.com]

first demonstration of the technique, MDMA was used as a test compound, reporting a LOD of 56 fmol using an ion trap MS. A comparison between DESI and DAPPI demonstrated the superior analytical performance of DAPPI for less to nonpolar polar compounds. In addition to dried sample spots on sample plates, DAPPI has been demonstrated for the direct qualitative analysis of drug tablets. It is noted that no significant signals were observed using pure methanol as solvent, and that the presence of a dopant solvent (toluene or acetone) was required for an efficient ionization of target analytes. Toluene forms both molecular ions (via charge exchange) and protonated molecular ions (via proton transfer) while acetone only forms protonated molecular ions. This phenomenon has been exploited for specific DoA applications, discussed below.

DAPPI was demonstrated for the qualitative analysis of DoA in tablets, blotter paper, plant resin and bloom using an ion trap MS (Kauppila et al., 2008). MDMA and AMP were identified in confiscated ecstasy tablets and the DAPPI spectra produced were similar to those shown in previous measurements of these tablets with DESI. Similarly, phenazepam and BUP were directly detected from tablets via MS/MS, and DAPPI analysis of blotter papers qualitatively confirmed the presence of LSD and bromobenzodifuranylisopropylamine (bromo-dragonFLY, ABDF). DAPPI was further applied to the analysis of cannabis products (marijuana and hashish), generating strong THC or cannabidiol (CBD) signals, although the isomers could not be differentiated without MS^2 characterization.

Confiscated powdered drug samples have also been qualitatively analyzed using DAPPI by dissolving them in solvent and spotting them on sample plates, which were dried before measurements (low ng amounts of drug deposited) (Luosujärvi et al., 2009). The confiscated powders were shown to contain AMP, MDMA, MAMP, HER, and COC by DAPPI and DESI, confirmed by GC/MS. For two samples confirmed to contain MAMP, DAPPI-MS using toluene dopant gave positive results for one sample, whereas with acetone both were positive, highlighting the importance of DAPPI dopant solvent choice. Though sensitivity can be lower in select applications using toluene, a distinct advantage of using toluene is that the production of molecular ions allows for matching of produced MS/MS spectra to extensive EI mass spectral libraries.

Highlighting the versatility of the DAPPI source for a wide variety of matrices, the technique was further applied to the direct analysis of DoA in herbal products and designer drugs in tablets and powders with a quadrupole ion trap MS (Kauppila et al., 2011). Dried *Psilocybe* mushrooms contained psilocin and psilocybin, as confirmed with GC/MS, however psilocybin could not be detected using DAPPI, which was likely due to poor thermal desorption or thermal degradation of the compound. Opium bricks were directly analyzed, and qualitative detection of several opiates was achieved. Qualitative analysis of Spice samples demonstrated detection of several synthetic cannabinoids and NPS. Seized drug tablets were analyzed using DAPPI-MS/MS for qualitative detection of 3-fluoroMAMP, m-CPP, AMP, and BDZs. A white drug powder was sampled using double-sided tape with the excess powder shaken off, and directly analyzed with DAPPI for the detection of MDPV and methylone.

In a direct comparison, DAPPI was shown to be more tolerant to matrix effects from urine than its predecessor DESI, with observed decreases in sensitivity for the direct analysis of BDZs and opioids of ~2–15-fold for DAPPI and ~20–160-fold for DESI; the two were shown to have comparable sensitivities in neat solvent (Suni et al., 2011). However, DAPPI-MS for the analysis of DoA from urine exhibits poorer analytical performance than established LC-MS and GC-MS methodologies.

The product ion spectra of the protonated molecular ions of the isomers THC and CBD are remarkably similar, presenting a selectivity challenge to many of the other ambient ionization techniques. Kauppila et al. (2013) showed that the direct analysis of cannabis samples with DAPPI-MS/MS allows for the differentiation of THC and CBD through the use of toluene as a solvent, which produces molecular ions (rather than acetone which produces protonated molecular ions) with clearly differentiable product ion spectra. Protonated molecular ions of THC and CBD generate nearly identical product ion spectra that cannot be differentiated by other ambient methods that produce $[M + H]^+$ ions. DAPPI-MS may be a valuable tool in reducing forensic laboratory backlog since seized cannabis samples could be quickly analyzed, and only those cannabis samples containing very small amounts of THC would require lengthier GC-MS or GC-FID analysis.

Recently, in another ambient photoionization approach, direct coupling of SPME and capillary APPI (cAPPI) with a confined ionization region yielding sub-ppt detection for a wide range of polar and nonpolar compounds has been reported (Mirabelli & Zenobi, 2018). The authors use the novel cAPPI source to directly interface SPME to MS for the analysis of MDMA, KET, lidocaine, and FEN, among other compounds, and use the more conventional GC-cAPPI-MS to evaluate the performance of the cAPPI source. The average LOD determined for the DoA in aqueous solution in this study was 30 pg/mL, representing a significant improvement over other APPI-based methods, primarily due to the preconcentration of the analyte using a 2-min extraction of the sample with an SPME fiber.

DAPPI has been shown to be a sensitive, effective tool for both screening and quantitative analyses of DoA. One of the unique features of DAPPI (and cAPPI) compared with other ambient ionization techniques is that using the proper solvent, molecular ions can be generated which may assist in the differentiation of isomers with tandem mass spectrometry, notably in the case of THC and CBD above, wherein the two cannot be differentiated using techniques that generate protonated molecular ions. DAPPI can also be used for the analysis of nonpolar compounds, a distinct advantage over many of the other ambient ionization techniques, extending the range of analytes amenable to analysis and making DAPPI a more universal ion source.

B. Laser Ablation-Based Techniques

Although not considered ambient ionization mass spectrometry strategies, because laser desorption-based techniques are relatively simple and allow direct analysis, we have chosen to include a short section on these methods and their use for drug testing. The two main laser desorption strategies that have emerged in the literature as promising drug measurement strategies are MALDI and matrix-free laser desorption ionization variants best described as surface-assisted laser desorption ionization (SALDI) methods. Both of these methods have been described elsewhere (van Kampen et al., 2011; Guinan et al., 2015a).

In MALDI methods, typically a high ratio of matrix to sample is deposited on a sample plate, or directly on a sample surface, which is then introduced to the high vacuum of the MS. The matrix is a compound containing a chromophore(s) that absorbs strongly at the wavelength of the desorption laser. During the desorption step, the matrix acts to assist in both the desorption and ionization of analytes. The related method, SALDI specifically engineered surfaces such as graphenes, silica, or nanostructures are used as a substrate platform to affect laser desorption ionization, obviating the need for an added matrix. Both techniques are soft ionization strategies, producing intact (or protonated) molecular ions, and are relatively simple to use.

1. Matrix-Assisted Laser Desorption Ionization

In the recent MALDI-MS literature related to drug testing, there has been significant emphasis exploiting the method to provide spatially resolved measurements. The micron-sized focal point of the desorption laser can be positioned (or moved) over different sample regions, providing profiling or imaging of analyte concentrations, ideal for detecting or imaging drugs in hair or fingerprints, useful for forensic, clinical and enforcement purposes. As MALDI-MS hair analysis examples, Vogliardi et al. (2010) validated a fast screening method for COC in hair, and there are numerous examples of the use of spatially resolved measurements along the length of the hair shaft to determine the time of use. Other examples for the analysis of hair using MALDI-MS include the detection of COC (Musshoff, Arrey, & Strupat, 2013; Flinders et al., 2015), MAMP (Miki et al., 2011), KET (Shen et al., 2014), zolpidem (Shima et al., 2015), and synthetic cannabinoid isomers (Kernalléguen et al., 2018). In a recent publication, Flinders et al. (2017) presented optimized sample preparation and instrumental parameters for DoA by MALDI-MS/MS imaging.

The analysis of drugs in fingerprints is another area where MALDI-MS is seeing development. Examples include the detection of a variety of drugs present in developed, cyanoacrylate lifted latent fingerprints (Sundar & Rowell, 2014), the mapping of illicit drugs in fingermarks (Groeneveld et al., 2015), and real crime scene fingerprints (Bradshaw, Denison, & Francese, 2017). These applications are predominantly forensic in nature and illustrate the potential usefulness of this direct analytical strategy.

2. Surface-Assisted Laser Desorption Ionization

The obvious advantage of SALDI based methods is the elimination of the requirement of matrix addition prior to measurement. A variety of different surfaces have been employed as sampling substrates. Silicon-based materials are common, and in early work, the Kraj group demonstrated the detection of MDMA synthesis impurities by spotting small samples on porous silicon sample plates, followed by laser desorption (Kraj et al., 2006). SALDI with porous silicon has been used to detect illicit drugs in saliva (Guinan et al., 2012), in fingerprint sweat (Della Vedova, 2015), and an interesting application to monitor methadone compliance by testing saliva, urine or plasma (Guinan et al., 2017). Nanostructured materials have been seeing increasing use for SALDI drug detection, including silicon nanopillar arrays (Alhmoud et al., 2014), nanoporous silicon microparticles (Guinan et al., 2015b), mesoporous germanium (Abdelmaksoud, Guinan, & Voelcker, 2017), and recently Ag nanoparticles/ZnO nanorods (Du et al., 2019).

VII. CONCLUSIONS AND FUTURE OUTLOOK

With the rapid growth of NPS, it is likely that HRMS instruments will become increasingly prevalent as forensic screening tools for both GC and LC modes of separation, as well as for direct, ambient ionization analysis strategies. While much attention has been dedicated to the analytical performance of these methods, their modest rate of production has gone largely unmentioned, and so it is unsurprising that forensic toxicology turnaround times are lengthy to the point where the outcome of a legal case may be compromised. To fully realize the potential of HRMS in forensic toxicology, significant reductions in both instrumental analysis and operator review times will need to be made with, ideally, the latter being eliminated entirely. Looking forward, we boldly suggest the following goal for chromatographic methods: fully automated screening and semi-quantitation of 100 blood samples daily for GHB, THC, LSD, secobarbital, an isobaric pair of compounds such as crotonyl- and cyclopropyl-fentanyl, and a previously unreported synthetic cannabinoid. When such an assay is in widespread production use, the full potential of chromatography-based MS for forensic toxicology testing may be considered to be within reach.

Turnaround time and accurate mass, by contrast, are less of an issue with ambient ionization methods, as they are intended primarily for rapid screening in the field (ideally) or in the laboratory. As with the chromatographic methods, the challenge of ambient ionization will be their implementation for routine production by minimally trained staff, as well as the immediate translation of MS data into an easily understood format. Just as the MS itself has evolved from a complex room-sized instrument with limited applications into a compact box suitable for answering a myriad of analytical questions, we expect no less of an evolution with its means of sample introduction. The future is very promising for the continued evolution of mass spectrometry-based strategies for the analysis of DoA. Over the next decade, the rapid proliferation of refined analytical systems and strategies occurring now will undoubtedly yield powerful solutions to the complex analytical challenges posed in forensic as well as clinical applications.

ABBREVIATIONS

AC	acetylation
AMP	amphetamines
APCI	atmospheric pressure chemical ionization
APPI	atmospheric pressure photoionization
ASAP	atmospheric pressure solids analysis probe
BDZ	benzodiazepines
BEG	benzoylecgonine
BSTFA	*N*,*O*-bis-trimethyl- silyl-trifluoroacetamide
BUP	buprenorphine
CBD	cannabidiol
CBS	coated blade spray
COC	cocaine
COD	codeine
DAPPI	desorption atmospheric pressure photoionization

DART direct analysis in real time
DBDI dielectric barrier discharge ionization
DDA data-dependent analysis
DESI desorption electrospray ionization
DeSSI desorption sonic-spray ionization
DI-SPME direct-immersion solid-phase microextraction
DIA data-independent analysis
DLLME dispersive liquid–liquid microextraction
DoA drugs of abuse
DoM drugs of Misuse
DSA direct sample analysis
DUID driving under influence of drugs
EASI easy ambient sonic-spray ionization
EI electron ionization
ESI electrospray ionization
FAPA flowing atmospheric-pressure afterglow
FEN fentanyl
GC gas chromatography
GCxGC; 2D-GCxGC two-dimensional gas chromatography
GHB γ-hydroxybutyrate
HER heroin
HF-LPME hollow-fiber liquid-phase microextraction
HFB heptafluorobutyration
HR-MS high-resolution mass spectrometry
HRAM high-resolution accurate mass
HS-SPME headspace solid-phase microextraction
IPS injector port silylation
IT ion trap
KET ketamine
LC liquid chromatography
LDTD laser diode thermal desorption
LLE liquid–liquid extraction
LOD limit of detection
LOI limit of identification
LOQ limit of quantification
LPME liquid-phase-based microextraction
LSD lysergic acid diethylamide
LTP low-temperature plasma
m-CPP *meta*-chlorophenylpiperazine
MALDI matrix-assisted laser desorption ionization
MAMP methamphetamine
MDA 3,4-methylenedioxiamphetamine
MDF mass defect filter
MDMA 3,4-methylenedioxyamphetamine
ME methylation
MEPS microextraction with packed sorbent
MOR morphine
MRM multiple reaction monitoring
MS mass spectrometry/mass spectrometer
MS/MS tandem mass spectrometry
MW molecular weight
NICI negative ion chemical ionization
NIST National Institute of Standards and Technology
NKET norketamine
NMR nuclear magnetic resonance
NPD new psychoactive drugs
NPS novel psychoactive substances
PBBCI *O*-(pentafluorobenzyloxycarbonyl)-benzoylchloride
PBTFBCI *O*-(pentafluorobenzyloxycarbonyl)-2,3,4,5-tetrafluorobenzoyl chloride
PFB pentafluorobenzyl
PFP pentafluoropropionylation
PICI positive-ion chemical ionization
PMMA *para*-methoxyamphetamine
PS-MS paper spray mass spectrometry
Q quadrupole
QqQ triple quadrupole
QuEChERS quick, easy, cheap, effective, rugged and safe
SALDI surface-assisted laser desorption ionization
SAMSHA substance abuse and mental health services administration
SDME single-drop microextraction
SERS surface-enhanced Raman spectroscopy
SHS switchable hydrophilicity solvents
SIM selected-ion monitoring
SMB supersonic molecular beams
SPE solid-phase extraction
SPME solid-phase microextraction
SRM selected reaction monitoring
SWATH sequential windowed acquisition of all theoretical fragment ion mass spectra
TD-ESI thermal-desorption electrospray ionization
TFA trifluoroacetylation
TFC turbulent flow chromatography
THC tetrahydrocannabinol
THC-COOH 11-nor-9-carboxy-Δ9-tetrahydrocannabinol
TLC thin layer chromatography
TMS trimethylsilylation
TOF time-of-flight
UA-LDS ultrasound-assisted low-density solvent
V-EASI venturi easy ambient sonic-spray ionization

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