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Complete List of Authors:	Brown, Hilary; Naval Air Warfare Center Weapons Division, Chemistry Division McDaniel, Trevor; Illinois State University, Chemistry Fedick, Patrick; Naval Air Warfare Center Weapons Division, Chemistry Division Mulligan, Christopher; Illinois State University, Chemistry

The Current Role of Mass Spectrometry in Forensics and Future Prospects

Hilary M. Brown,[1] Trevor J. McDaniel,[2] Patrick W. Fedick,[1]* Christopher C. Mulligan[2]*

[1] Chemistry Division, Research Department, Naval Air Warfare Center, Weapons Division (NAWCWD), United States Navy Naval Air Systems Command (NAVAIR), China Lake, California 93555, USA
[2] Department of Chemistry, Illinois State University, Normal, Illinois 61790, USA

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* Correspondence to: Christopher C. Mulligan (cmullig@ilstu.edu) and Patrick W. Fedick (patrick.w.fedick@navy.mil)

* Address reprint requests to Dr. Christopher C. Mulligan, Department of Chemistry, Illinois State University, Normal, IL, 61790-4160. Telephone: (309) 438-2464. Email: cmullig@ilstu.edu

Abstract

Mass spectrometry (MS) techniques are highly prevalent in crime laboratories, particularly those coupled to chromatographic separations like gas chromatography (GC) and liquid chromatography (LC). These methods are considered "gold standard" analytical techniques for forensic analysis and have been extensively validated for producing prosecutorial evidentiary data. However, factors such as growing evidence backlogs and problematic evidence types (e.g., novel psychoactive substance (NPS) classes) have exposed limitations of these stalwart techniques. This critical review serves to delineate the current role of MS methods across the broad sub-disciplines of forensic science, providing insight on how governmental steering committees guide their implementation. Novel, developing techniques that seek to broaden applicability and enhance performance will also be highlighted, from unique modifications to traditional hyphenated MS methods to the newer "ambient" MS techniques that show promise for forensic analysis, but need further validation before incorporation into routine forensic workflows. This review also expounds on how recent improvements to MS instrumental design, scan modes, and data processing could cause a paradigm shift in how the future forensic practitioner collects and processes target evidence.

Introduction

Active crime laboratories are traditionally rigid concerning the forensic analytical techniques they employ, relying on proven, universally-implemented methods and stringent standard operating procedures (SOPs). These laboratories are typically slow to adopt emerging technologies into their routine workflows due to the lack of validation and historical data available.[1] It is not arbitrary that the forensic community at times seems immutable, but their reliance on established techniques is a necessity to the criminal justice system. Criminal investigations, prosecution, and the formulation of a jury verdict all implemented – and are therefore impacted – by established, dependable techniques.[2] The incorporation of unsubstantiated, refutable techniques could result in the lack of a conviction for a guilty party, or worse, an innocent individual being convicted of a crime for which they did not commit. While crime labs remain steadfast in the methods they employ, much of the future of forensic analysis is being developed in academic laboratories and private chemical industry. While a majority of newer methods will not be utilized in case work for many years after their seminal report, if ever, the continued need for higher performance, higher throughput techniques could result in emerging technologies and advanced instrumentation working their way into routine evidence processing.[3]

While innovation is a driving force for the adoption of new methodologies, secondary factors also motivate change, such as the backlog of forensic evidence, budgetary concerns, chain of custody issues, and new and emerging contraband types.[4] Many forensic laboratories are underfunded and overburdened with caseloads, two factors that stifle innovation, as diminished time and resources deemphasize to development of new techniques. Academia, by nature, is constantly innovative. However, the same validation standards do not apply, and novel research often employs prototypical methods and/or is performed on home-built instrumentation,

preventing the timely incorporation of these techniques in crime laboratories. Similarly, much of forensic research in the academic setting is proof-of-principle, demonstrating a new technique's potential, but not against a wide array of authentic, practical situations arising in forensic investigations. Synergistic activities between forensic practitioners, academic research, and industry could therefore result in innovative, streamlined approaches: research and development by academia, optimization and commercialization by industry, and vanguard advisement/rearguard validation by practitioners to produce a lab-adaptable methodology.

As emerging techniques continue to advance, there are several organizations that oversee guidelines for their acceptance. The Organization of Scientific Area Committees for Forensic Science (OSAC),[5, 6] a National Institute of Standards and Technology (NIST) affiliated body, and steering committees comprised of international forensic science practitioners and academics, such as the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG)[7] or Toxicology (SWGTOX),[8] all seek to provide guidelines and minimum standards for forensic analysis methodologies. SWGDRUG, which specifically focuses on criteria for analyzing seized drugs in a forensic setting, categorizes instrumental methods based on their discriminating power (reproduced in **Figure 1**).[7] Since their seminal recommendations, mass spectrometry (MS) has been classified as a "Category A" analytical technique, indicating the capability to provide the highest level of selectivity through the structural information contained in collected spectra; it should be noted that this traditionally applies to MS in the form of gas chromatography-mass spectrometry (GC-MS), or more specifically, EI-MS of chromatographically-separated analytes. Further criterion apply to "confirmatory" (i.e., positive identification) techniques compared to "presumptive" techniques (i.e., probable identification, or "screening"), where a multi-tiered testing strategy is required to abate false positives. Per SWGDRUG, a Category A technique still

requires corroboration with an additional method, but faster, cost-effective, yet lower discerning techniques from Categories B or C can be employed; this has led to the prevalence of MS, particularly GC-MS, for forensic analysis.[7] For instance, a commonly employed protocol is using immunoassays (Category C)[9] to screen for classes of drugs and, if positive, confirmatory analysis is performed using GC-MS (Category A).[10] SWGDRUG guidelines are also followed to help validate alternative methods for controlled substances in the public laboratory system, such as those of the Virginia Department of Forensic Sciences (DFS).[11]

OSAC coordinates a multitude of scientific area committees (SACs) and sub-committees (SC) tasked with developing standard guidelines for diverse forensic evidence types;[5] the organizational structure of said OSAC committees is represented in **Figure 2**. OSAC maintains a web-accessible registry of validated standards for each evidence area, and several of the approved OSAC registry standards incorporate MS, including fire and explosion investigations,[12] materials (trace) evidence for tape[13] and glass,[14] and seized drugs.[15] OSAC also seeks to integrate extraneous standards under its organizational umbrella. For example, many of their current documents regarding fire and explosives analysis are based on historical references from the Technical Working Group for Fire and Explosives (TWGFEX)[16] and other ASTM baseline documents, which are being merged and considered by standards developing organizations (SDO) for OSAC Registry approval.[17]

As seen in SWGDRUG and OSAC registry standards, MS is one of the stalwart techniques in the forensic community due to its inherent selectivity and sensitivity.[7] Hyphenated techniques, such as GC-MS or liquid chromatography – mass spectrometry (LC-MS), are considered the "gold standard" methods for many forensic analyses. These two techniques represent the core of many forensic laboratory protocols due to their reliability, reproducibility, robustness, transferability,

and universality across lab systems.[18, 19] While these techniques have long been implemented in forensic analyses, there have been improvements along the way, as well as the emergence of alternative or synergetic MS usage modes. Non-chromatographic MS methods, such as laser-based techniques like matrix assisted laser desorption ionization (MALDI)[20] and laser ablation-inductively-coupled plasma (LA-ICP),[21] have gained popularity for specific forensic analyses. Ambient ionization-mass spectrometry, or "ambient mass spectrometry," is an emerging research area shown to have wide applicability across the field of forensics.[22] The intrinsic benefits of ambient MS match well with the demands of forensic science, that being rapid, high throughput analysis, reduced sample preparative constraints, simplistic operation (in some cases), and the capability of on-site analysis (when coupled with portable instrumentation),[23] with new ion sources and applications continually being reported.[22, 24] However, even though the seminal ambient ionization techniques, desorption electrospray ionization (DESI)[25] and direct analysis in real time (DART),[26] were reported over 15 years ago, they have only recently been validated for forensic casework,[27, 28] stemming from the slow commercialization of robust, reliable ionization sources that continues to postpone general acceptance by the forensic community for casework.[24]

Herein, this review seeks to provide clarity on the role that MS serves in the forensic science discipline, as well as the future capabilities that novel MS-based methods could afford to the future forensic practitioner. Both traditional applications and new advancements of well-known, hyphenated MS techniques are detailed, as well as promising separation-based methods that seek to offer higher performance (e.g., GCxGC-MS, CE-MS, etc.). Modern, laser-based methods working their way into lab protocols are discussed, as well as emerging techniques like ambient MS that show promise and broad applicability, but need further validation before incorporation into routine forensic workflows. The influence of MS instrumentation development

is also considered, such as the impact that high resolution mass spectrometry (HRMS), sophisticated MS scan modes, and portable MS systems can have on the forensic community, [29-34]

Separation Techniques

Current forensic chemical analyses predominantly utilize separation techniques coupled to mass spectrometry for confirmatory analysis. As discussed, gas chromatography-mass spectrometry (GC-MS) is the "gold standard" for analytical forensic analysis[9, 35] with liquid chromatography-mass spectrometry (LC-MS) a close second.[9] A majority of casework involving controlled substances,[11] toxicology,[29, 36] and fire debris analysis[37] is processed via GC-MS. However, a major deficiency to separation techniques is the relatively low sample throughput. Not including sample preparation, typical run times have reached 10-15 minutes, occasionally exceeding 30 minutes, which contributes to the slow turnaround times most forensic labs are facing. [35, 38] Regardless, hyphenated MS still dominates in forensic labs due to the presence of well-established and validated methods, as well as the commercial availability of broad spectral databases.[24] Recent efforts to improve and optimize these techniques are described below, from novel coupling strategies to integrating multiple degrees of separation.

Gas Chromatography - Mass Spectrometry. GC-MS mostly utilizes electron impact (EI) ionization to produce highly reproducible mass spectra.[38] Using GC retention times and EI-MS spectral matching, compounds can be identified with a high degree of confidence.[39] NIST, Wiley, MassBank, and others provide spectral libraries that are expandable, with high quality, reproducible reference spectra for comparison.[38] As GC is combined with higher performance mass analyzers, such as time of flight (TOF) or orbitrap high resolution MS (HRMS) systems, these spectral libraries have improved overall match accuracies due to exact mass measurements.[38,

[40] GC-MS is typically employed for low molecular weight, volatile, non-polar, and thermally-stable compounds,[9, 29] but disparate compounds (e.g., cannabinoids) can require derivatization to improve volatility or separation via GC-MS analysis, adding to the overall time and cost required for sample preparation.[9] While chemical ionization (CI) sources employed on GC-MS systems have shown proficiency towards forensic analytes,[41] the lack of reproducibility and reference databases has hindered their broad usage.

The time required for chromatographic separation can often be substantial, necessitating improvements that yield shorter analysis times without causing coelution. Fast GC-MS methods can achieve swifter separations and higher throughput by using shorter, narrow columns, higher carrier gas volumes, and faster oven temperature ramp rates.[42] Davidson and Jackson compared fast GC-MS to traditional GC-MS during method development for the analysis of 2,5-dimethoxy-N-(N-methoxybenzyl)phenethylamine (NBOMe) isomers. NBOMe compounds are synthetic phenylethylamine derivatives that are a newer class of novel psychoactive substances (NPS). Separation of isomers was achieved using both the 12-minute traditional method and the developed 6-minute fast method, with no significant loss in separation efficiency.[42] Improving the throughput of GC-MS workflows is seen as a sensible mitigation strategy for the current evidence backlog.

Two-dimensional gas chromatography-mass spectrometry (GCxGC-MS) couples two GC columns in series to improve separation of compounds known to coelute under normal GC assays.[43] This method allows for an increased peak capacity and is especially useful for complex mixtures such as oil-based lubricants,[43] ignitable liquids from fire debris[44] and burnt remains,[45] and human decomposition odor.[46, 47] GCxGC-MS is powerful enough to show slight differences in brands of gasoline (shown in **Figure 3**), providing distinctive markers that may be used to distinguish the source of a gasoline sample,[44] yielding critical intelligence to arson investigations.

Two recent reviews discuss the potential and analytical development of GCxGC-MS in forensics.[48, 49]

Dubois *et al.* developed a headspace solid-phase microextraction GCxGC high resolution time of flight mass spectrometry (HS-SPME-GCxGC-HRTOF-MS) method to analyze decomposition odor from soil and adipocere at a death scene.[47] Previously, one dimensional GC-MS analysis of postmortem odor was admitted as evidence in court as part of *The State of Florida vs. Casey Marie Anthony*.[50] This was the first attempt at using this type of chemical evidence in testimony, but many scientists in the community believed that the method was not sufficiently validated nor generally accepted for use in criminal prosecution.[51] With this newer iteration, multi-dimensional separation coupled with HRMS improves the confidence of volatile organic compound (VOC) detection and speciation. Diverse samples were collected and tested, from around and under the body and adipocere regions, leading to the determination that a body had previously decomposed in a certain location where they sampled, and if it was in late stage decomposition. Such an analysis can provide valuable information to missing persons and buried body investigations, but the authors recommend caution in court admission until routine protocols and overall reliability are established, as required by laboratory accreditation boards.[47]

Combined strategies are also popular and of interest towards improving GC analysis. These methods are more experimental but can provide complimentary, technique-specific results for evidence identification. For instance, Tarifa and Almirall coupled GC-MS with laser induced breakdown spectroscopy (LIBS) to characterize organic and inorganic compounds in gunshot residue (GSR).[52] Samples were collected by swabbing the hands of shooters and non-shooters, with said swabs then being stored in glass vials. Capillary microextraction of volatiles (CMV) headspace sampling was used to collect organic GSR components stemming from common

propellants and subsequently analyzed by GC-MS. The sample swabs were then extracted for inorganic GSR components and analyzed by LIBS. Current GSR analysis relies on SEM-EDX for elemental composition, specifically looking for lead, barium, and antimony.[53, 54] This method combines GC-MS and LIBS to provide both organic and elemental composition, therefore, reducing the risk of false positives.

Liquid Chromatography - Mass Spectrometry. LC-MS is capable of analyzing a wider range of forensic compounds, including polar and less volatile analytes that would require derivatization for GC-MS, ultimately simplifying sample preparation.[9, 29, 55] LC-MS emerged as an alternative to immunoassay drug screening,[9] allowing for better selectivity and sensitivity.[55] Typical immunoassay screening provides only the class of drug from an unknown sample, requiring confirmation with additional analytical techniques.[9, 56] LC-MS/MS can provide better limits of detection and selectivity compared to immunoassay screening, with developed methods for drugs with known MS/MS transitions.[29, 57] However, with new and emerging drugs, such as synthetic cathinones and cannabinoids, immunoassay screening can produce false negative results for contraband that does not fit into standard drug classes, leading to targeted LC-MS/MS screening methods being established.[9, 56] LC analysis coupled to high resolution MS (HRMS) allows for an untargeted screening approach, identifying compounds based on accurate mass.[57] High resolution instruments are powerful, but for most publicly-funded, state crime labs, the cost is highly prohibitive.[9]

Reidy *et al.* developed a LC-MS/MS screening method for 52 drugs and metabolites in urine using a preparatory enzymatic hydrolysis. This method was compared to traditional ELISA immunoassay screening,[56] and limits of detection (LODs) obtained were equal or lower to the

ELISA method. This LC method was able to detect analytes in 20 samples that had concentrations below the ELISA detection limit, as well as extraneous compounds not originally included in the ELISA panel used, with high reliability; there were 4 false positives attributed to the ELISA method. Financially, it was determined that the seven-panel ELISA method costs ~$14.50 per sample, whereas the new LC method could effectively screen for 52 analytes for ~$4.60/sample. The LC method required a ~50% increase in analysis time, with the ELISA and LC methods taking ~4.5 hrs. and 6.75 hrs. for 20 samples and controls, respectively, but provided overall gains in selectivity, sensitivity, and reliability. LC-MS/MS screening methods have been developed for common drug classes in human serum, urine and post-mortem blood,[58] and the benefits of coupling LC methods with HRMS has been reported.[59, 60] For instance, García-Reyes and co-workers reported a dilute-and-shoot LC-HRMS method for quantifying multi-class drugs of abuse and doping agents in urine. Of note, this simplistic sample treatment scheme, which only included direct urine sample dilution, showed little matrix effects, allowing the quantitation of over 80 analytes with detection limits below 5 ppb, lower than minimum limits established by the World Doping Agency.[61]

Electrospray ionization (ESI) is commonly employed on LC-MS systems, which typically creates molecular ions (e.g. protonated, deprotonated, alkali metal adducts, etc.), and minimal fragmentation is observed.[55, 62] LC-MS spectra produced via ESI processes exhibit higher levels of inter and intra-instrument variability, making it more difficult to produce universal databases for spectral matching[63] compared to the stable and reproducible EI spectra collected on GC-MS systems. LC-MS also requires solvent delivery pumps, high volumes of solvent, and a vacuum interface to help desolvate ions as they enter the MS, making these systems bulkier and less amenable to field analysis.[62, 64] Moini *et al.* have combined LC separation with EI-MS on a system

capable of performing field analysis and identifying compounds based on spectral matching.[62] NanoLC was used in order to reduce flow rates, solvent consumption, and desolvate droplets faster. Fentanyl and target derivatives were analyzed using the newly developed LC-EI-MS system and compared to LC-ESI-MS and GC-EI-MS. Of interest, the LC-EI-MS method showed high congruency in regards to both chromatographic data when compared to traditional LC-ESI-MS methods and obtained mass spectra compared to GC-EI-MS (**Figure 4**). [62] There are strategic advantages of this coupling, as LC separation is well suited for polar, less volatile compounds, and reproducible EI fragmentation spectra can be matched with commercially-available spectral databases to identify potential contraband.[65] This is an interesting step towards portable LC-MS systems, particularly in regards to the general acceptance of EI-MS for forensic drug confirmation.[66]

Capillary Electrophoresis-Mass Spectrometry and Microfluidics. Capillary electrophoresis (CE) is an electrokinetic separation technique that utilizes a strong electric field to separate compounds.[67, 68] CE is ideal for portability and on-site analysis because it has minimal sample and solvent volume requirements, produces minimal waste, and separation can be obtained in ~1 min. using ultrafast CE. SWGDRUG includes CE as a Category B technique, however, when coupled with MS for detection, discerning power can be potentially increased to that of Category A methods used for confirmatory analysis.[69] CE-MS has been used for the separation of chiral amphetamines from seized samples[70] and controlled substance isomers,[71] and isomer separation within a minute has been reported with a portable, battery powered CE device.[72]

Recently, Vinueza and co-workers reported the novel use of automated, microfluidic-based extraction coupled with Q-TOF-MS that allowed rapid characterization of dye compounds found in textile fibers collected as transferable trace evidence at crime scenes,[73] showing higher

specificity than prior art microscopic and spectroscopic examinations. The overall method, including both extraction and MS-based identification, could be conducted in as little as 12 min., consuming microliters of organic extraction solvent for reduced consumables cost. Of note, multi-component dye characterization from single fibers with a minimum diameter of ~10 μm was demonstrated.

Laser Techniques

Matrix-Assisted Laser Desorption Ionization. Matrix-assisted laser desorption ionization (MALDI) is an ionization technique commonly employed for large biomolecular targets (e.g., biopolymers, proteins, etc.) and mass spectrometric imaging (MSI). In forensic-related work, MALDI has most notably been used for imaging fingerprints.[74] Here, a matrix is applied on top of the sample containing latent fingerprints to aid in the ionization process. As the sample is rastered, mass spectra are collected at each "pixel" where the laser is fired,[75] providing informative images of chemical information. Recent notable forensic applications of MALDI include imaging fingerprints after visualization,[74, 76, 77] latent fingerprints on banknotes,[78] determining the age of a fingerprint,[79] monitoring cocaine and metabolites in hair,[80] and using protein markers to detect bodily fluids in aged stains.[81] MALDI-MSI forensic applications not only allow fingerprint visualization, but also the determination of additional contraband residues present. A detailed review by Francese *et al.* expounds on the potential of MALDI fingerprint imaging.[82]

Fingerprint powders or cyanoacrylate fuming are commonly used for fingerprint visualization.[76, 79] Hinners and Lee demonstrated that carbon-based fingerprint powder, which is typically used in forensics, can be used not only to visualize fingerprints, but also as an effective matrix for MALDI-MSI. It was previously reported that carbon-based MALDI matrices caused

high background interference. However, in this study, the authors were able to readily distinguish between sample-related signatures and background carbon clusters using high resolution mass spectrometry (HRMS). The fingerprint powder matrix could be used for MSI in both positive and negative ion modes, and it exhibited similar, if not better, performance when compared to traditional matrices.[79] Lee *et al.* performed a related study, using MALDI-MSI to image fingerprints after cyanoacrylate fuming, another common technique used for latent fingerprint visualization. Spectral intensity for sample-related compounds was unchanged during MSI, even after fuming.[76] Since cyanoacrylate fuming and carbon-based fingerprint powders are readily used in the forensic community, integrating these newer MALDI-MSI methods into routine case work could be fairly streamlined. Both imaging methods are performed after the fingerprint evidence is collected and analyzed by accepted techniques, so there is little chance evidence is compromised.[83]

MALDI-MSI can also be used to visualize illicit substances and their metabolites in fingerprints as a means to determine drug use. Groeneveld *et al.* determined the LOD of several drugs of abuse and their metabolites on fingerprints, ranging between 0.1-10 ng/μL. The authors showed that prior visualization techniques did not affect the ability to detect the drug analytes of interest, and MALDI fingerprint images were still able to be obtained to produce complimentary chemical information.[84]

Bradshaw *et al.* applied MALDI-MSI to fingerprint evidence from four high profile cases,[77] lifted from a textured light frame after TiO_2 powder was applied (Print 1), an electrical plug socket after visualization with aluminum powder at a seized cannabis farm (Print 2), a drug packet visualized by cyanoacrylate fuming followed by BY40 dye stain (Print 3), and a window frame after carbon black powder was applied (Print 4). After MALDI-MSI analysis, cocaine was found in Prints 2 and 4, which added additional factors and intelligence to the respective cases.

Specifically, Print 4 was included as evidence for a harassment case, and finding cocaine within the fingerprint supported the collected police interrogation, where the suspect confessed to the crime and cocaine abuse was confirmed by alternate drug testing. (**Figure 5**) Useful ridge detail was unable to be seen from Print 1 following TiO_2 powder visualization and MALDI analysis, attributed to the texture of the surface where the print was located and possible thermal degradation from lamp operation. Ion suppression was observed during analysis of Print 3 due to the BY40 dye. Applying this emerging method to authentic evidence illuminates the advantages and potential disadvantages of the technique.[77] Ideally, fingerprint evidence is found on relatively flat surfaces with prominent ridge details, as both traditional visualization and MALDI analysis can provide useful images for fingerprint matching and secondary chemical information, respectively.[85] In most cases, however, fingerprints are often partial, smudged, or found on complex surfaces. Knowing this, researchers can continue to improve MALDI for varying surface types or post-BY40 application by hindering ion suppression as they seek future method validation.

Laser Ablation Inductively Coupled Plasma Mass Spectrometry. Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) is a commercially-available technique that allows for direct elemental and isotopic analysis from condensed or solid materials. The employed laser ablates controlled areas of sample into an aerosol that then travels into a plasma chamber, where both atomization and ionization occurs.[86] This technique has been used to analyze glass, paint, ink, soil, tape, and paper evidence.[87] Specifically, LA-ICP-MS is considered the "gold standard" for glass analysis. LA-ICP-MS is commonly employed for comparative analysis between evidence found at a crime scene to materials found on or used by a suspect or from a secondary location.[87] Recent efforts have investigated the match criteria for glass evidence,[88, 89]

coupling LA-ICP-MS with spectroscopic techniques for ink [90] and tape analysis,[91-93] and imaging trace elements in post-mortem tissue samples from electrocution and gunshot cases.[94] The flexibility of laser ablation of non-standard evidence types coupled with elemental differentiation continue to produce interesting approaches to forensic intelligence gathering.[95]

Ambient Ionization Mass Spectrometry

Ambient ionization-MS (AI-MS) has been demonstrated toward the rapid analysis of forensic compounds of interest with minimal to no sample preparation, making it appealing for use by non-technical operatiors.[10, 24, 96] A primary goal of applying ambient MS to forensic science is to decrease processing time by foregoing lengthy preparative steps and chromatographic separations. For comparison, hyphenated MS runs are on the minute to hour timescale (not including any sample necessary preparation (e.g., filtration, extraction, etc.)), whereas several direct sampling, ambient MS techniques can produce MS spectra in the matter of a few seconds in an on-demand fashion.[22] The intrinsically shorter analysis times could increase the throughput of evidence processing, making it an intriguing strategy for reducing backlogs in forensic labs.[38] However, the removal of the separation step commonly necessitates multiple stages of MS analysis (MS/MS or MS^n) and/or simplistic preparatory strategies to achieve high specificity and sensitivity from highly complex sample matrices.[22, 97, 98]

Ambient MS ion sources are often simplistic in design and operation, stemming from a rich history of creating said sources using common laboratory consumables and equipment. The field of ambient ionization originated with the seminal reports of desorption electrospray ionization (DESI)[25] and direct analysis in real time (DART),[26] followed by numerous sources that employ ionization mechanisms similar to that of traditional ESI or atmospheric pressure chemical

ionization (APCI).[22] ESI-related sources, such as DESI and paper spray ionization (PSI), are spray-based ionization techniques that utilize solvents to rapidly extract and transfer analytes via charged microdroplets to the atmospheric pressure inlet system of a compatible MS instrument.[64] APCI-like devices use an energetic source like corona discharge to produce primary reagent ions that go on to ionize analyte molecules present.[99] Of the following ambient MS sources discussed, DESI, DART, and PSI are commercially-available and have been thoroughly applied to forensic applications. Other emerging ambient MS methods are presented that are still in the basic research or development stages, but hold high promise toward forensic evidence processing, highlighting recent developments, novel applications, and validation studies necessary for consideration in forensic workflows.

Desorption Electrospray Ionization. DESI, developed by Cooks and co-workers in 2004,[25] primarily employs ESI-like processes for ambient ionization. A spray of charged solvent droplets is directed towards a sample of interest (e.g., solid material, surface residue, etc.), where analyte present is extracted. The primary, incoming droplets then produce secondary droplets containing analyte after surface impact, which are desorbed and detected via MS.[24] DESI has been used for a variety of forensic applications, including illicit drugs, toxicology, explosives, fingermarks, inks and forged documents, gunshot residue (GSR), and chemical warfare agents (CWAs).[24, 96, 100] DESI was first commercialized by Prosolia in 2005, but was recently acquired by Waters in 2018 as part of their MS imaging product line. DESI has proven much more expeditious compared to separation techniques, as compatible samples can be analyzed within seconds after entering the DESI spray region of the source. Sample pretreatment is not required, but care must be taken with complex matrices that are soluble in the solvent systems employed to minimize carryover events. High throughput DESI analysis has been demonstrated, including pharmaceutical screening of up

to 10^4 reactions in an hour,[101] an intriguing attribute for agencies that require high volume evidence processing. Employed spray solvent systems commonly use methanol and water, but can be changed in order to facilitate better solubility, desorption, or ionization of the analyte of interest. Certain solvents can also be chosen to perform online derivatization of analytes, if strategic or necessary.[102]

One disadvantage of DESI is difficulty with quantitation due to positioning sensitivity and matrix effects,[102] leading groups to examine simplistic preparative methods like extraction techniques. Ifa *et al.* recently demonstrated a coupled approach by performing a QuEChERS extraction of chocolate edibles, followed by thin layer chromatography (TLC) separation of extracts, and DESI ionization off the TLC plate for THC analysis. QuEChERS, coined from the attributes of being a quick, easy, cheap, effective, rugged, and safe method, efficiently extracted cannabinoids like THC from the complex chocolate matrix. The extract was spotted onto a TLC plate and allowed to elute, and then DESI line scans were produced via rastering across the developed spots, successfully quantifying the level of THC in chocolate edibles.[103]

DESI can also be used for imaging applications of specific interest to forensics, particularly for fingerprint evidence.[104, 105] Zare and Zhou used DESI imaging and machine learning to glean personal information from latent fingerprints.[106] MS imaging of fingerprints not only yields complimentary ridge detail and spatial patterns for identification, but provides chemical maps of endogenous and exogenous compounds. With machine learning, endogenous compounds can be grouped together to help determine the gender, ethnicity, or age of the person whose fingerprint was analyzed (**Figure 6**). DESI images of 194 fingerprints were processed via the machine learning model, producing accuracies for anticipated gender, ethnicity, and age of 89.2%, 82.4%, and 84.3%, respectively. These accuracies are notable for this proof-of-concept technique, and

further improvement to the model and method could produce a broadly useful tool for latent fingerprint evidence processing.

High resolution MS (HRMS) instruments have also helped to improve the scope of DESI analysis. With high resolution and mass accuracy, compounds of interest can be detected even in complex matrices, separating out some isobaric and interfering species.[107] Bianchi *et al*. developed a method to analyze oral fluid for new psychoactive substances using DESI-HRMS.[108] Van Helmond *et al*. coupled DESI-HRMS with imaging capabilities to classify and image condom lubricants in cyanoacrylate fumed fingerprints from sexual assault evidence.[109] DESI has also been used to detect and image compounds in thermochromic ink from erasable pens. Ifa *et al.* identified characteristic compounds in both the visible and invisible state of the ink, potentially useful in forgery cases.[110]

Direct Analysis in Real Time. Direct analysis in real time (DART), developed by Cody and Laramée in 2005, generated excited-state gas species via glow discharge that ionize target analytes via ion-molecule reactions, akin to the APCI ionization mechanism.[24, 111] Similar to other ambient ionization sources, DART provides rapid sample screening and little to no sample pretreatment. Forensic applications of DART are wide-ranging, including illicit drugs,[112-114] toxicology,[112] explosives,[115] CWAs,[112] ignitable liquids,[116] GSR,[117, 118] paint analysis,[119] and inks.[120, 121] The DART system has been commercialized by JEOL USA (AccuTOF-DART-MS, Peabody, MA), and when employing variable attachment and/or modification strategies, dopant-assisted Argon DART,[122] O_2^- attachment for non-polar compounds,[123, 124] pyrolysis DART,[119, 125] thermal desorption of analytes,[126, 127] can also be performed.

Unlike a majority of reported ambient MS methods, DART has established a presence in the forensic sector. Large, well-established forensic labs, such as the FBI Laboratory, Virginia Dept. of Forensic Science (DFS),[128] Harris County Institute of Forensic Sciences,[129] and Alabama Dept. of Forensic Sciences[130] have implemented AccuTOF-DART-MS for rapid presumptive screening of drug evidence. The Virginia DFS has utilized this instrumental platform as a screening technique for over 10 years,[27, 111] including the validation of a AccuTOF-DART-MS drug screening method, which was subsequently incorporated into the drug analysis scheme at Virginia DFS, reported in 2009 by Steiner and Larson.[27] An important step towards broad implementation was the creation of the NIST DART Forensics Library,[131] an open-access DART-HRMS spectral library which (to date) includes 3,217 positive ion spectra from 828 forensic analytes provided by Bob Steiner at the Virginia DFS. Progressive labs are examining this new technology, but realize that validation efforts are required to comply with SWGDRUG guidelines, and individual labs need to perform cost-benefit comparisons to justify the allocation of staffing and financial resources.

DART-MS screening methods have been thoroughly reported for emerging drugs. Alabama DFS has developed a DART-MS/MS method for methadone, a synthetic opioid, in urine.[132] Initial screening is performed using DART-TOF, followed by confirmation via DART-MS/MS on a triple quadrupole-linear ion trap (Q-TRAP) MS. Both screening and confirmation can be done in as little as 5 min. compared to 3-5 days for traditional immunoassay screening and GC-MS confirmation. Methadone LOD via this method was 250 ng/mL, similar to the traditional immunoassay cutoff at 300 ng/mL, with positive identification rates of 87% and 91% for DART-TOF and DART-MS/MS, respectively. For newer drugs, DART has been a viable screening option

when immunoassay screening kits are not available. Moore *et al*. reported an identification method for newer synthetic cannabinoids using DART-TOF screening and LC-QTOF for confirmation.[133]

Other interesting reports have used DART-MS to analyze stains on fabric, rodenticide adulterant in drug mixtures, and identify insect life stages to help determine time since death. DART-HRMS was used in a violent crime case where three suspects broke into a home and attacked the residents.[134] The residents were eating chocolate ice cream, and the evidence collected included a ceramic shard and one of the suspect's pants, both containing brown stains. DART-HRMS was applied directly to these brown stains, as well as to a sample of the chocolate ice cream, as a means to link potential suspects to the crime scene. DART-HRMS, as well as complimentary LC-MS, confirmed the evidentiary stains to be chocolate ice cream, adding to the prosecution's case. Sisco and Robinson used thermal desorption DART-MS to detect rodenticide adulterants in drug mixtures.[135] Reports of non-controlled, toxic compounds being added to street drug samples have increased, particularly rodenticides, which have been found in cocaine, heroin, methamphetamine, leading to FDA and CDC warnings in 2018. The DART-MS method was able to identify the rodenticides individually and in the presence of drugs. This rapid and sensitive technique could prove useful in drug tampering cases, as well as public health awareness.

Musah *et al*. have used DART-MS and artificial neural networks to identify life stages and species of carrion flies (**Figure 7**).[136] When insects are collected from a scene, they are typically stored in an ethanol solution. The Musah group performed DART-HRMS from the ethanolic solution, revealing unique, diagnostic chemical signatures for each species and life stage. The artificial neural network was developed and trained with a known dataset and was then able to distinguish larvae, pupae, and adult with 100%, 96%, and 93% accuracy, respectively. Classifying species analytically using DART-MS provides data regarding insect speciation, which is perhaps

substantial enough for admissibility in court. Another interesting coupling of DART-MS with advanced data analysis/processing includes Shelley's report of automatic analyte ion recognition and background signal removal via cross-correlation analysis.[137] Here, the use of time-domain profiles provided benefits typical of chromatographic separations (such as a reduction in mass spectral complexity up to 98%) but with the rapidity afforded to ambient MS methods.

Paper Spray Ionization. PSI, developed by the Cooks, Ouyang and co-workers in 2010,[138, 139] utilizes triangular paper substrates as the ionization source, but also the sampling apparatus, when employed for sample swabbing. When compatible spray solvent is applied to the substrate, it wicks through the paper, eluting analytes to the paper egress. Application of high voltage then produces an ESI-like process from the paper for MS analysis. PSI is marked by its highly simplistic design and ease of use for non-scientists,[28] and recent reviews show its potential for forensic investigation.[24, 140-143] Current literature has shown PSI for the analysis of inks and documents,[144-146] drugs of abuse,[146] chemical warfare agent (CWA) simulants in soil,[147] air,[148] and in blood and urine,[149] authentic CWAs in the ambient atmosphere,[150] protein toxin simulants from surfaces,[151] and explosives.[152, 153] The following discussion highlights notable papers that seek to increase the robustness of PSI and validate its use for forensic analysis.

Commercialized PSI sources, including the Velox 360 System formerly from Prosolia, Inc. (Indianapolis, IN) and the more current VeriSpray source from ThermoFisher Scientific (San Jose, CA), provide a plug-and-play solution for benchtop MS systems, allowing forensic laboratories to implement said methods for real time sample screening and method validation. The Velox system uses 3D-printed cartridges to hold the paper substrate, and up to 40 samples can be batch analyzed via autosampler. This cartridge design has been shown to be more reproducible and robust compared to hand-cut paper substrates.[154, 155] The Thermo VeriSpray source includes sampling

plates with 24 individual paper spray tips, and up to 10 plates can be processed via autosampler for the analysis of 240 discrete samples.[38] Ren *et al*. have developed a method for detecting controlled substances in blood using the VeriSpray source coupled with triple quadrupole MS.[156] Six drugs of abuse were detected and quantified in under 2 min., with obtained LODs in the ng/mL range.

Much of the current PSI literature successfully employs traditional, cellulose-based paper substrates (e.g., Whatman filter papers, etc.). However, intuitive substrate modifications have been reported that improve analysis and assist in the sampling and preservation of analytes. Glaros and coworkers developed a PSI-MS method to detect CWA simulants. [148, 149] Follow-up experiments involving authentic CWAs using standard paper substrates were problematic, leading the group to explore integrated metal-organic frameworks (MOFs) on fiberglass substrates to increase adsorption during sampling and desorption of CWAs during PSI analysis.[157] MOFs with pores similar in size to G-series CWAs were used to modify the fiberglass substrate, including UiO-66, UiO-67, and HKUST-1. MOF substrates improved overall signal from other designs, but also increased the lifetime of the agent after collection for up to 1 hr. (seen in **Figures 8A and 8B**), compared to 5 and 15 minutes from untreated paper and fiber-glass, respectively. Online derivatization can also be used to help improve CWA analysis times. Mach *et al*. used 2-[(dimethylamino)methyl]phenol (2-DMAMP) as a complexation dopant with G-series CWAs during PSI-MS. [150] The generated complex has a lower volatility, allowing capture and retainment of these CWAs onto paper substrates. The dopant is added to the paper and dried prior to analysis, and the complexation occurs in near real-time, so additional preparation is not required. Verbeck *et al*. compared polyolefin silica-based paper (i.e., Teslin,® PPG Industries Ohio, Inc.) to traditional cellulose paper for drug analysis.[141] Teslin substrates demonstrated improved signal-to-

noise and LOD over filter paper substrates, utilizing only 1 µL of sample. PSI-MS signal intensity collected from the Teslin substrate also decreased at a slower rate, allowing for longer analysis times and expanded MS^n investigation of unknown analytes. Manicke and Bills demonstrated the use of sesame seed oil to preserve and concentrate cannabinoids from urine and oral fluid samples on paper substrates for PSI-MS analysis (seen in **Figures 8C and 8D**).[158] Cannabinoids, such as THC, have proven challenging with PSI-MS analysis, as they can decompose in dried sample spots and often require non-standard spray solvent systems. THC was preserved on oil-treated paper for up to 27 days at room temperature, and collected LODs were in the ng/mL range. Oil is simply added to the employed filter paper and dried prior to sample deposition.

PSI-MS has been demonstrated for drug toxicological screening and quantitation, marked by fast analysis times and minimal sample preparation. Van Asten *et al.* developed a quantitative method for amphetamines in dried blood spots using the commercialized Velox source,[154] validated using SWGTOX guidelines[8] to show applicability to forensic science. Samples at biologically-relevant concentrations were analyzed and quantified simultaneously in 1.3 minutes. Multiple amphetamine fragment ions collected during MS/MS analysis were used for confirmation and quantitation. Validation categories included accuracy, precision, and reliability (e.g., presence of false-positive candidates, probability of erroneous matches via database searching). Manicke *et al.* developed a screening method for drugs in blood using PSI coupled to a triple quadrupole mass spectrometer.[159] Analysis of 134 drugs and metabolites was performed in approximately 90 sec. from spiked blood samples. A similar drug screening method using PSI coupled to HRMS/MS was also reported.[160] Over 130 drugs and target metabolites were analyzed in a single, 2.5 min. run. All drug concentrations were screened at toxicologically-relevant concentrations, and when

cross-checked with standard LC-MS/MS data, the PSI-HRMS/MS method exhibited a 92% true positive rate and a 98% true negative rate.

Atmospheric Solids Analysis Probe. The atmospheric solids analysis probe (ASAP) can be created by inserting a sampling apparatus into the heated desolvation gas from commercial ESI or APCI sources.[38] ASAP was first described in 2005, where analytes were thermally desorbed from the sampling probe by the heated nitrogen gas and ionized via corona discharge in an APCI source.[161] Jagerdeo and Federal Bureau of Investigation (FBI) personnel demonstrated ASAP-MS for the analysis of forensic samples.[162] This setup was used to analyze rodenticide samples, black tar heroin and associated impurities, and crack cocaine. The authors emphasized the simplicity of the technique, as it was easily coupled to a commercial ESI-MS system. Jagerdeo and Wriston coupled ASAP with HRMS to analyze "spice" packets for synthetic cannbinoids and cathniones.[163] Moraes *et al*. also demonstrated an ASAP-MS/MS technique to detect amphetamines in urine,[164] with LODs for the 5 amphetamine compounds analyzed ranging from 0.002 ng/mL to 0.4 ng/mL.

Direct Sample Analysis. Direct sample analysis (DSA), first described in 2007, combines features of both DESI and APCI.[165] A corona discharge is used to create primary ions, namely protonated water clusters, that are directed towards a positioned sample, and analytes of interest are desorbed and ionized via secondary processes.[38] PerkinElmer has developed a commercial DSA source coupled to TOF-MS and validated a method for 369 drugs of abuse.[166] It has been noted that DSA uses lower gas pressures than typical DESI analysis, reducing the overall consumables load.

Maker *et al.* utilized the commercial DSA-TOF to screen for potentially adulterated and contaminated herbal medicines, using both analytical standards and alternative medicines purchased from local shops.[167] Of the purchased medicines, all labelled ingredients were

confirmed as present using this technique, and no adulterated samples were found. The authors stressed that this did not necessarily prove that these samples were not adulterated, but did demonstrate the fast screening of real medicinals. Dorman *et al.* utilized DSA-TOF to analyze synthetic phenylethylamines in blotter paper paraphernalia from drug evidence provided by the Patton Township (PA) Police Department, confirming the presence of 25B- and 25C NBoMe.[168] Nguyen and Moini examined writing inks using DSA-TOF, comparing performance to separation techniques including GC and nanoLC-MS. DSA was able to identify ink components from all 80 ink samples that were tested (representative data is found in **Figure 9**),[169] and it had comparable performance to LC methods; it was noted that certain compounds were only detected by DSA or LC-MS. Botch-Jones and co-workers demonstrated rapid and effective identification of fentanyl and its cognizant analogs using a commercial DSA-TOF system.[170] Authentic evidentiary seizures from the State of Maine Health and Environmental Testing Laboratory were investigated in this work, with a majority of DSA-TOF results (80 out of 81 samples) agreeing with prior GC-MS analyses, showing promise in forensic evidence screening.

Dielectric Barrier Discharge Ionization. Dielectric barrier discharge ionization (DBDI), reported in 2007 by Zhang *et al*., utilizes a low-power, non-thermal plasma to desorb and ionize surface-bound or liquid-phases analytes.[171] Zenobi *et al.* used DBDI-MS to analyze eight drugs in complex matrices via thin film microextraction (TFME) and thermal desorption,[172] including urine, blood plasma, wine, soft drinks, and vodka. LODs ranged from 3-100 pg/mL in urine, 10-30 pg/mL in vodka, and 30-300 pg/mL in plasma, which are lower than the typical concentrations seen in drug intoxication casework (ng/mL). Zenobi *et al.* also analyzed CWAs using DBDI-MS with detection limits in the ppt range (1.4-58.4 ppt).[173] A DBDI source was used by Bradley and

coworkers to analyze explosives,[174] and Hayen *et al.* quantified TATP and DADP explosives from surfaces[175] Gilbert-López and co-workers reported the novel coupling of LC and DBDI for multi-class explosives found in water and soil matrices,[176] an example of coupling fast, ambient MS methods with separation techniques. With LC-DBDI-TOF-MS, sensitivity gains over more traditional LC-APCI-TOF-MS were observed for the nitroaromatic/nitramine explosives examined. Kindred ion sources of DBDI include active capillary plasma[177] and low temperature plasma probe.[178-180] An ambient microwave plasma coupled to MS was also demonstrated for the analysis of both elemental and organic analysis, potentially useful in explosive/radionuclide mixtures or inorganic/organic GSR mixtures.[181]

Portable and Field-Deployable Techniques

Various branches of military have long since employed portable GC-MS instruments for explosives and CWAs detection.[182-184] GC-MS and LC-MS instruments are also present in the Army's deployable laboratories, like the 2007 CBRNE Analytical and Remediation Activity (CARA) program.[184] Companies such as FLIR Systems, PerkinElmer, Inficon, MassTech, 908 Devices, and Smiths Detection offer commercial, portable GC-MS and MS instruments with inlet systems compatible with ionization sources operating at atmospheric pressure.[183, 185] Inficon, Smiths Detection, and FLIR Systems instruments have been ruggedized and tested to meet military standards. These instruments are often designed to be used by non-scientists and military,[183] but there have been recent reports of deployment for forensic investigation.[35]

Field Demonstrations and Validation of Portable Mass Spectrometers. Several research groups have been demonstrating the use of ambient ionization techniques coupled to portable MS systems.[23, 34] Much of the early and continued academic work has come out of the Cooks group at

Purdue University, where portable and handheld ion trap MS systems were demonstrated for on-site and in-situ detection, including the first coupling of a fieldable system to DESI-MS[186] and one model designed into a wearable backpack.[185, 187] Recently, Zenobi *et al.* coupled a DBDI source to a handheld MS (Mini 10.5, Aston Labs) for the analysis of CWAs.[188] Pawliszyn *et al.* coupled DART with a commercial, portable, quadruple MS (ACQUITY QDa, Waters) to analyze sample extracts.[189] SPME fibers were used to extract drugs of abuse from saliva and then directly introduced into the gas stream of the DART source, where the analytes were thermal desorbed and ionized. Evans-Nguyen and coworkers coupled DART to a ruggedized, portable MS (MT Explorer 50, MassTech) for field analysis of common and designer drugs through cooperation with the Osceola County (FL) Sheriff's Office undercover drug unit and Pinellas County (FL) Crime Laboratory.[190] Real case samples included cocaine, cannabis, Xanax, opiates, black tar heroin, several types of "bath salts," and plant material suspected of containing synthetic cannabinoids, with representative data seen in **Figure 10**. Practitioners expressed interest in the capability of this portable DART-MS system towards "bath salts" and "molly" evidence, since currently-available, colorimetric field tests were unreliable and/or unavailable.

McCullough *et al.* have recently developed a prototype ASAP source to couple with the Waters qDa portable quadrupole mass detector for bulk drug seizure analysis.[191] Typically, this system utilizes nitrogen gas, but for these on-site investigations, a diaphragm pump was used to operate using ambient air. The authors created an onboard spectral library with drug standards and cutting agents using increasing cone voltages (15-70V) to induce in-source fragmentation; this is a common practice when traditional MS/MS is unavailable. This ASAP-MS setup was used to screen 50 representative drug samples from Eurofins Forensic Services (EFS), including heroin, cocaine, ketamine, benzodiazepines, synthetic cannabinoids, cannabis, MDMA, and opium, with

49 out of 50 samples correctly identified and fully matching the results obtained prior by the EFS. Mulligan *et al.* performed DESI and PSI analysis of drug samples on a ruggedized, portable ion trap MS (FLIR AI-MS 1.2, FLIR Systems, Inc.), comparing the obtained MS/MS results to the commercially-available Wiley Registry of Tandem Mass Spectral Data (MSforID).[192] All 32 drug standards were correctly identified using the library, as well as authentic forensic evidence provided by Bloomington (IL) Police Vice Squad and State Police agencies.

Portable MS instruments were also used to monitor the clandestine syntheses of desomorphine and methamphetamine. Hall *et al.* detected desomorphine, a semi-synthetic opioid known as *krokodil*, and its precursor codeine using DESI and PSI on a portable MS.[193] Desomorphine and codeine were sampled from relevant surfaces commonly used for storage, transport and production, yielding LODs ranging from 0.5-200 ng and 0.90-350 ng, respectively. Overall, PSI was shown to have better sensitivity for this application. O'Leary *et al.* used DESI, PSI, and APCI sources coupled to a portable MS to monitor two synthetic routes for methamphetamine production in real time.[194] Evidence analyzed included bulk powder precursor and product, reaction intermediate slurries, gaseous headspace from solvents used for extraction and drying, and residues from utilized glassware, containers, and filtration media. A vehicle-mounted, portable MS instrument was used to detect atmospheric effluent from clandestine methamphetamine labs.[195] Verbeck *et al.* used a membrane inlet mass spectrometer (MIMS) to continuously sample ambient air while in motion around a location containing a mock clandestine methamphetamine operation. Precursors and reaction products were able to be detected via MIMS, and when coupled to GPS coordinates and wind diffusion models, the location of the clandestine operation could be discerned. (**Figure 11**)

Several proof-of-concept, portable MS systems coupled with ambient MS ion sources have been reported, but few have been extensively validated for use in actual forensic scenarios. Lawton *et al*. reported a systematic validation of the FLIR Systems AI-MS 1.2 portable CIT-MS with "plug and play"-style, interchangeable, ambient ionization sources.[28] Following SWGDRUG recommendations, they examined selectivity, accuracy/precision, robustness, ruggedness, and detection limits. To provide flexibility for on-site analysis, a positioning rail was mounted to the front of the instrument that allows hot-swapping of ionization sources and quick repositioning. Available sources included ESI, PSI, DESI, paper cone spray ionization (PCSI), and APCI. It was shown that each of the 5 ionization sources could be used to run discrete samples in ~ 6 minutes, even when considering the time necessary for source swapping; this experiment is depicted in **Figure 12**. Detection rates of ~98% and false positive rates of ~ 0.17% were determined, and the efficacy of differing operator classes was also investigated, ranging from experienced analytical chemists to recent high school and police academy graduates – even with untrained users, detection rates were at least 97.9%. The examination of non-technical users as part of this work is interesting, as it simulates future field practitioners. This MS system was described in further detail by Fedick *et al*., where part lists and design considerations were detailed.[196] The mounting system and 4 different ionization modules could be constructed for less than $2,000, providing a low-cost testbed for forensic practitioners to investigate on both portable and commercial MS systems. Other ambient ionization sources could likely be amended to fit this modular setup, as well.

Novel Scan Modes on Portable Mass Spectrometers. The progression of fieldable mass spectrometry goes further than the coupling of novel ionization sources and refinements to electrical and vacuum systems. Portable mass spectrometers have predominately employed single quadrupole or ion trap mass analyzers,[185] leading researchers to investigate novel operational

strategies to harness additional chemical information with the goal of differentiating isomers, identifying difficult compounds, and classifying emerging synthetic analogs with their molecular precursors.[197-203] Multi-generational collision induced dissociation is one such method in which multiple stages of MS/MS are produced in a single scan.[197] This advanced fragmentation technique yields a similar level of structural information for all targets observed in the base MS spectra, without the need for sequential MS^n scans of each precursor ion of interest. This can enable the rapid differentiation of isomeric compounds in a simplistic manner, not relying on the operator to determine which fragmentation spectra should be generated. Multi-generational CID is also more effective for collecting broad structural information from samples yielding very brief ion signal durations, which has been observed during trace drug residue screening via ambient MS.[28]

While most MS/MS experiments are performed by product ion scans, where the target parent ion is isolated and fragmented, neutral loss and precursor ion scans can also be used.[204] These two MS/MS methods, commonly known as survey scans, are easily implemented on triple quadrupole MS systems, wherein the first and third quadrupoles mass select particular precursor and product ions, while an intermediate RF-only quadrupole serves as a collision cell for fragmentation. As typical mass spectral databases rely on product scans of known standards being continually added, these survey scans could enable law enforcement officers and forensic agencies to determine if a field-encountered unknown has similar structural features to other known drugs, even when a direct match is not obtained. This is important to combat the proliferation of new synthetic drugs and novel drug contaminants found in collected evidence but not yet appended to standard spectral databases. Due to the lack of field-portable, triple quadrupole MS devices, novel methods of manipulating ion traps to "act" like triple quadrupoles have been developed using RF voltage scans combined with AC frequency scans.[198, 199] Similar methods performed on portable

MS systems have been compared to commercial, benchtop instruments, and in certain cases, the fieldable method can actually outperform their lab-scale counterparts.[200] These novel scan methods have been paired with ambient MS techniques to identify drugs of abuse, explosives, and chemical warfare simulants.[201] Additionally, the combination of these scanning methods, where it is possible to acquire mass-to-charge information as precursors while simultaneously acquiring product ion spectra (coined as 2D MS/MS),[202] has been demonstrated on a portable MS for CWA analysis, providing additional information in a time saving manner.[203]

Legality of Portable Mass Spectrometers. The use of portable instruments in the field by law enforcements has legal ramifications, and the need to ensure that data collected as evidence is used in lawful and ethical ways arises. Mulligan *et al.* investigated the use of portable MS systems in practical and theoretical scenarios that could occur during traffic control stops.[205] Applications included detecting trace-level analytes on a variety of surfaces from the car, in latent fingerprints, and emerging evidence types (e.g., drug-spiked electronic cigarette, or E-cig, fluids). Here, PSI paper substrates were used to swab areas from a vehicle that would likely have latent fingerprints, including glass, radio knobs, steering wheels, gear shifts, door handles, seat belts, and license/registration materials. After swabbing, the paper substrate was directly analyzed via PSI-MS. Under current U.S. search and seizure law, law enforcement personnel are able to search your vehicle during a traffic stop without a warrant if there is probable cause, an exception to the 4th Amendment. As drug detection canines can be used to alert officers of contraband in a vehicle, authors postulated whether PSI-MS could be used to swab exterior car door handles or driver's licenses and then analyzed for contraband traces to establish probable cause searching.[206] If used as evidence in court, on-site PSI-MS analysis or any other novel MS method would be scrutinized,

showing the need for comprehensive validation studies so that the Daubert[207] and Frye[208] requirements of court admissibility are met.

Emerging Technologies for Forensic MS

As a majority of forensic evidence is borne in the field, much of the emerging MS research in forensics seeks to perform necessary screening and, preferably, confirmation at the native location, leading to approaches that integrate and mimic current evidence collection strategies. For instance, swab applicators are commonly employed to collect evidence from a suspect's hands or mouth, and one emerging technique streamlined for this application is swab touch spray ionization (STSI). Comparable to PSI, STSI implements swabs with conductive handles to which high voltage and solvent are applied after collection, forming an ESI-like Taylor cone from the swab head, where ionization occurs.[209] STSI has been used to swab a subject's hands for GSR traces after firearm discharge,[210] for the detection of explosives from various surfaces,[211] and for qualitative and quantitative detection of drugs of abuse in oral fluid.[212, 213] Paper cone spray ionization (PCSI), is a 3D variant on PSI that has been demonstrated in forensic applications requiring bulk sample analysis.[196, 214, 215] More recently, filter cone spray ionization (FSCI) was reported,[215] which utilized filter paper crafted into a pyramidal shape to analyze bulk drug evidence with little to no carryover events. **Figure 13** depicts FCSI-MS applied to various types of authentic synthetic cannabinoid and abused pharma tablet evidence. Spray solvent is added to the conical reservoir holding the sample of interest, and when high voltage is applied, extracted analytes flow to the tip where they undergo ESI-like ionization. This method removes rigorous preparative steps, as the bulk solid can be simply added into the cavity of the cone, and after solvent is added, spectra are rapidly obtained and can last up to 8 min. Fatigante *et al.* used this technique to analyze drug

evidence from authentic drug casework, prescription and counterfeit drugs, and veterinary toxicology samples, as well as applying FCSI-MS to trace evidence vacuuming.[216]

Combining multiple analytical techniques into one evidentiary analysis has emerged as a strategy to satisfy SWGDRUG recommendations requiring the implementation of two, independent examinations of seized drug evidence. In 2011, Steiner and Howlett validated a TLC AccuTOF-DART method for forensic drug analysis.[217] This method included 3 SWGDRUG techniques for identification: pharmaceutical identifiers (Category B), TLC (Category B), and DART-MS (purported as Category A). Abonamah, Eckenrode and Moini reported a fieldable nanoLC method coupled with EI-MS for highly reproducible confirmation of fentanyl and associated analogues.[62] More recently, PSI-MS (purported as Category A) has been combined with Raman spectroscopy (Category A) for the analysis of drugs, explosives, and CWA simulants from a single substrate.[218] Commercial paper substrates printed with silver nanoparticles were used, allowing surface enhanced Raman spectroscopy (SERS) to be employed prior to PSI-MS analysis (**Figure 14**). A follow-up paper by Fedick *et al.* coupled a handheld, portable Raman with a miniature MS for the analysis of fentanyls;[219] this further demonstrated the utility of this technique for on-site detection and confirmation. Burr and co-workers reported a portable SERS-PSI-MS method incorporating the FLIR Systems AI-MS 1.2, utilizing novel gold nanoparticle substrates and 3D-printed sampling cartridges to confirm the identity of drug traces, including difficult isomeric combinations.[220] Of note, validation studies involving this integrated SERS-PSI-MS system achieved a 99.8% detection rate with no false positives for trace drug residues as part of a large, blinded reliability study, an important step towards future court admissibility.

Conclusions

The future of forensic MS analysis seems to have diverged into two paths of equal importance and potential impact, the pursuit of higher performance, broadly applied methods for use in the laboratory setting and the development of field-based, rapid techniques simplified for the practitioner. In both approaches, higher evidentiary throughput is seen as critical in meeting the processing demand and reducing the sample backlog seen in most crime labs.[10, 23] Several strategies have emerged from the current scientific literature and the public laboratory system, including advanced separations (e.g., GCxGC-MS, CE-MS, etc.), the pursuit of complementary chemical intelligence (e.g., MALDI-MS and DESI-MS for exogenous compounds in fingerprints), modern approaches to data processing (e.g., machine learning, artificial neural networks, etc.) and MS scan modes (e.g., 2D MS/MS), rapid screening techniques for more targeted, secondary confirmation (e.g., DART-MS pre-screening of drug evidence), portable MS devices, and strategic coupling of techniques (e.g., SERS-PSI-MS).[220]

Regardless of the direction, the important role of gatekeepers and steering committees like OSAC, SWGDRUG and SWGTOX in establishing minimum standards for establishing analytical validity of new techniques cannot be understated. It is then prudent that researchers consider these criteria, along with the underlying legal ramifications, when developing novel MS techniques in forensic and justice applications to ensure future court admissibility. Comprehensive validation of novel MS methods is frequently overlooked in academia during the pursuit of higher performance and broader applicability, but it is imperative in order to facilitate any acceptance into public forensic lab workflows, acceptance as part of expert testimony,[220] and withstand critical scrutiny during cross-examination in order to potentially discredit the technique.[207, 208] Faster acceptance of novel techniques and state-of-the-art instrumentation could be aided by immersing

future forensic practitioners during education and training exercises, a trend observed in the chemical education literature.[221-226]

Moreover, public forensic laboratories typically have limited resources, leading to strict, budget-oriented approaches to resource management.[35] With limited funding, the expansion to novel, costly MS instrumental techniques is difficult, but the recent adoption of AccuTOF-DART-MS and HRMS strategies in select labs suggests that fiscally-viable routes to inclusion can be found. As resource constraints for law enforcement and forensic science increase, so does the responsibility of researchers to provide information regarding cost-effectiveness to assist in resource allocation decision-making; this is strongly asserted in a recent National Institute of Justice (NIJ) Research In Brief publication highlighting the benefits of such endeavors for criminal justice programs.[227] Fiscal-impact analyses, like those recently reported for field implementation of portable PSI-MS systems,[228] and comparable cost-benefit analyses,[229] which consider not only governmental expenditures and savings, but also perceived societal benefits, could prove useful in advising policy and decision makers regarding the financial viability of novel methodologies.

Conflicts of Interest

There are no conflicts to declare.

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Biographies

Hilary M. Brown received her B.S. in Forensic Science from The University of Tampa in 2014. In 2019, she received her Ph.D. in Analytical Chemistry from Purdue University under the supervision of Prof. Julia Laskin. She is currently an NRC postdoctoral fellow at the Naval Air Warfare Center Weapons Division at China Lake, CA. Her current research involves optimizing reaction acceleration conditions for the accelerated product formation of energetic materials and using ambient ionization techniques for environmental analysis of contaminants in soil.

Trevor J. McDaniel graduated from Illinois State University in 2019 with a B.S. in Chemistry. He is currently working towards his Master's Degree in Chemistry under the supervision of Prof.Christopher Mulligan, where his thesis research focuses on assessing pharmaceutical impurities via MS, validating portable instrumentation for on-site drug confirmation, and 3D printing methodologies for rapid prototyping in the analytical sciences. Trevor has career interests in forensic science and design development.

Patrick W. Fedick is a research chemist at the Naval Air Warfare Center Weapons Division in China Lake, CA. Dr. Fedick's research group focuses on the development of mass spectrometry techniques for the accelerated product formation of novel energetic materials, on-line process monitoring for energetic material development, and for the analysis of emerging environmental contaminants in complex matrices. His group concurrently concentrates on the development and application of novel ionization sources to forensic and environmental problems.

Christopher C. Mulligan is a Professor of Analytical Chemistry at Illinois State University, where his research group is focused on applying and adapting portable mass spectrometric (MS) devices for use in areas of societal need. Through his research, Prof. Mulligan seeks to demonstrate the impact and practicality of portable MS systems featuring ambient ionization methods for use in forensic evidence screening, crime scene investigation, and the law enforcement/first response communities.

FIGURE CAPTIONS

Figure 1. SWGDRUG categories of analytical techniques based on their discriminating power. Category A techniques have the highest discriminating power followed by Category B and then C. The number of confirmatory tests required for analysis varies by the categories the analytical methods fall within. Mass spectrometry, in for the form of EI-MS of chromatographically-separated analytes, is regarded as a "Category A" technique.[7]

Figure 2. NIST OSAC organizational structure showing the 5 scientific area committees divided into 25 discipline-specific subcommittees. Mass spectrometry plays a major role specifically in the second scientific area committee, Chemistry/Instrumental Analysis, however it is not exclusive to that committee. Figure recreated from NIST.[6]

Figure 3. GC×GC-TOFMS TIC contour plots of two brands of gasoline (**A**) Shell (**B**) BP. White circles highlight differences between the samples. (Reproduced as part of open access, Sampat *et al.* 2018, MDPI).[44]

Figure 4. (**A**) Comparison of nLC-EI-MS, nLC-ESI-MS, and GC-MS chromatograms. Peaks correspond to heroin (A), acetyl fentanyl (B), fentanyl (C), carfentanil (D), and butyryl fentanyl (E). Inset shows mass spectra of peak B. (**B**) Picture of the nLC-EI-MS system in the field. (**C**) Comparison of isocratic separation chromatograms in the laboratory and in the field. (Reproduced with permission, Abonamah *et al.* 2019, Elsevier).[62]

Figure 5. MALDI-MSI analysis of Print 4, lifted from an interior window frame. (**A**) Optical image of the print after enhancement with carbon black powder. (**B**) MALDI-MSI image of the cocaine fragment at *m/z* 182.2. (**C**) MALDI-MSI image of protonated cocaine *m/z* 304.2 (Reproduced as part of open access, Bradshaw *et al.* 2017, RSC).[77]

Figure 6. (**A**) DESI-MSI negative ion mode image of *m/z* 253. (**B**) Resulting fingerprint classification using the pretrained model. Blue pixels were classified as Chinese male and red pixels were classified as Indian female. These predictions were correct. (Reproduced with permission, Zhou *et al.* 2017, ACS).[106]

Figure 7. (**A**) Fly larvae and DART-HRMS analysis from ethanolic suspensions. (**B**) Fly pupae and DART-HRMS analysis from ethanol suspensions. (**C**) Adult flies and DART-HRMS analysis from ethanol suspensions. Life stages of seven blow fly species: (1) C. vicina; (2) P. regina; (3) L. sericata; (4) L. coeruleiviridis; (5) C. rufifacies; (6) Phoridae spp.; and (7) not included. (Reproduced with permission, Beyramysoltan *et al.* 2018, ACS).[136]

Figure 8. Modifications to paper substrate to improve PSI-MS analysis. (**A**) Paper substrate modified with MOFs to improve analysis of GB (sarin) CWA. (**B**) Retention curves over time of G-series CWAs. (**C**) Sesame oil added to paper substrate to preserve and preconcentrate THC. (**D**) Comparison of different oils used to preserve analytes. Shown in percent analyte remaining after 24 hr. vs. 1 hr. in urine. (Reproduced with permission, Dhummakrupt *et al.* 2018, ACS).[157] (Reproduced with permission, Bills *et al.* 2020, ACS).[158]

Figure 9. (**A**) Ink samples used for analysis. (**B**) DSA spectrum of degradation peaks of Crystal Violet and Michler's Ketone. (**C**) Samples aligned via mesh grid in the DSA sample holder. (Reproduced with permission, Nguyen *et al.* 2018, Elsevier).[169]

Figure 10. (**A**) DART source coupled to the MassTech MT Explorer 50. (**B**) Mass spectra from evidence samples: black tar heroin (top), 4-bromomethcathinone (middle), and 4-methylethcathinone (bottom). (Reproduced with permission, Brown *et al.* 2016, Elsevier).[190]

Figure 11. (**A**) Portable MIMS system replacing the front passenger seat in vehicle. (**B**) Baseline MS data before starting reaction, mapped around lab location. (**C**) MS data obtained from displacement of dibenzylketone, a common impurity, during mock manufacture. (Reproduced with permission, Mach *et al.* 2015, ACS). [195]

Figure 12. (**A**) TIC of entire run (6 minutes), showing 5 discrete source and sample combinations with time required to switch source seen by the signal return to baseline (**B**) APCI-MS data collected for Coleman Fuel. (**C**) DESI-MS data collected from MDMA residue. (**D**) PSI-MS data collected from swabbed 25I-NBOMe residue. (**E**) PCIS-MS data collected from an amphetamine tablet. (**F**) ESI-MS data collected from a cocaine extract. (Reproduced with permission, Lawton *et al.* 2017, ACS).[28]

Figure 13. Synthetic marijuana seizures collected in Central Illinois, including (**A**) XLR-11, (**B**) 5F-ADB, (**C**) AB-Fubinaca, (**D**) AMB-Fubinaca, and (**E**) FUB-144, with corresponding FCSI-MS and MS/MS spectra seen in (**F**)−(**I**), respectively. The majority of seizures contained one predominant synthetic cannabinoid, however, a few contained multiple illicit chemicals. (Reproduced with permission, Fatigante *et al.* 2020, ACS)[216]

Figure 14. (**A**) Depiction of pSERS-MS setup using handheld Raman and miniature MS; nanoparticles are printed on paper substrate to allow for SERS detection, followed by PSI-MS. (**B**) Raman spectra for morphine and hydromorphone (isobars). (**C**) CID spectra for morphine and hydromorphone, *m/z* 286 isolated and fragmented. (Reproduced with permission, Fedick *et al.* 2017, ACS).[218] (Reproduced with permission, Fedick *et al.* 2020, ACS).[219]

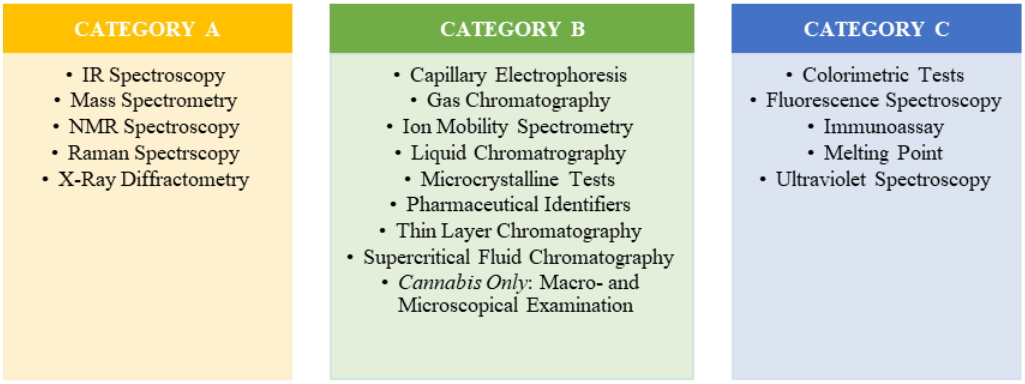
CATEGORY A
• IR Spectroscopy
• Mass Spectrometry
• NMR Spectroscopy
• Raman Spectrscopy
• X-Ray Diffractometry
CATEGORY B
• Capillary Electrophoresis
• Gas Chromatography
• Ion Mobility Spectrometry
• Liquid Chromatrography
• Microcrystalline Tests
• Pharmaceutical Identifiers
• Thin Layer Chromatography
• Supercritical Fluid Chromatography
• *Cannabis Only*: Macro- and Microscopical Examination
CATEGORY C
• Colorimetric Tests
• Fluorescence Spectroscopy
• Immunoassay
• Melting Point
• Ultraviolet Spectroscopy

Figure 1

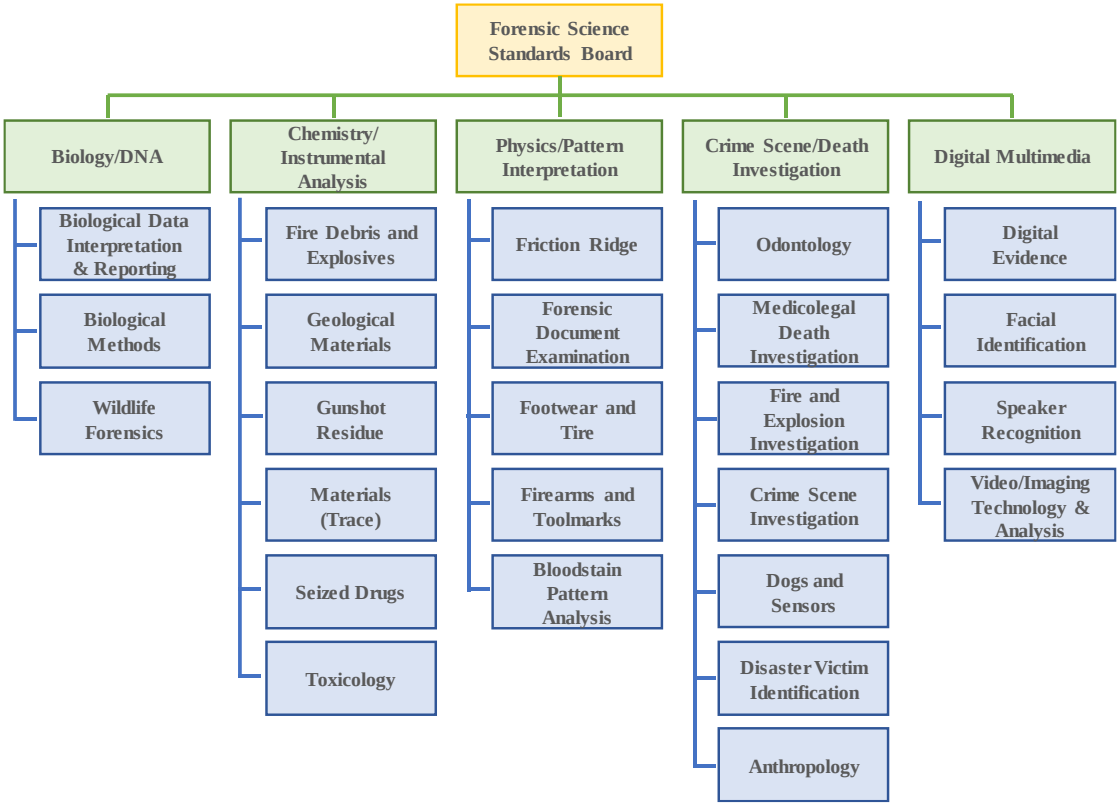
Forensic Science Standards Board
Biology/DNA
Chemistry/ Instrumental Analysis
Physics/Pattern Interpretation
Crime Scene/Death Investigation
Digital Multimedia
Biological Data Interpretation & Reporting
Biological Methods
Wildlife Forensics
Fire Debris and Explosives
Geological Materials
Gunshot Residue
Materials (Trace)
Seized Drugs
Toxicology
Friction Ridge
Forensic Document Examination
Footwear and Tire
Firearms and Toolmarks
Bloodstain Pattern Analysis
Odontology
Medicolegal Death Investigation
Fire and Explosion Investigation
Crime Scene Investigation
Dogs and Sensors
Disaster Victim Identification
Anthropology
Digital Evidence
Facial Identification
Speaker Recognition
Video/Imaging Technology & Analysis

Figure 2

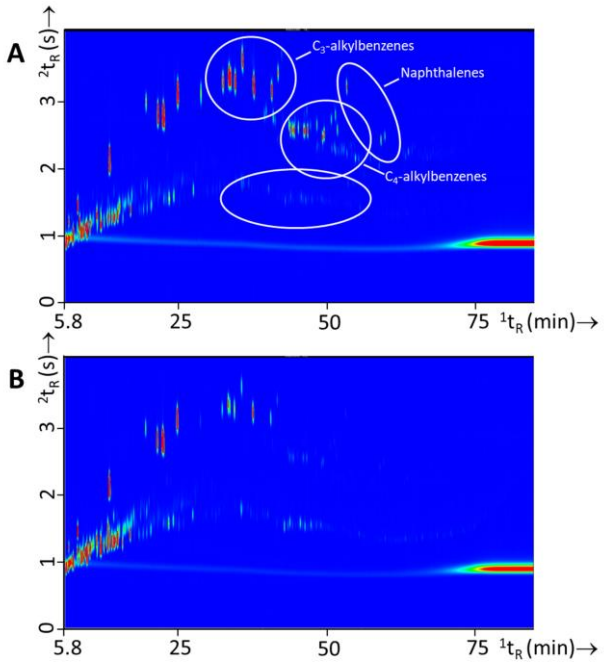
A
B
^2t_R (s)→
^1t_R (min)→
C3-alkylbenzenes
Naphthalenes
C4-alkylbenzenes
0
1
2
3
5.8
25
50
75

Figure 3

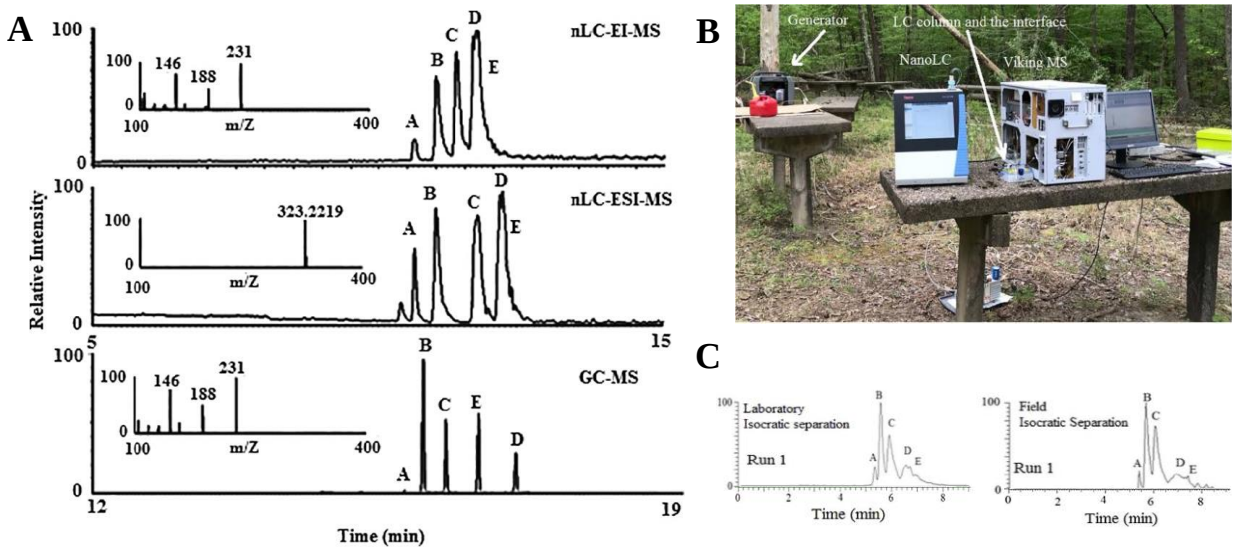
A
nLC-EI-MS
nLC-ESI-MS
GC-MS
323.2219
Relative Intensity
Time (min)
B
Generator
LC column and the interface
NanoLC
Viking MS
C
Laboratory
Isocratic separation
Run 1
Field
Isocratic Separation
Run 1
Time (min)
Time (min)

Figure 4

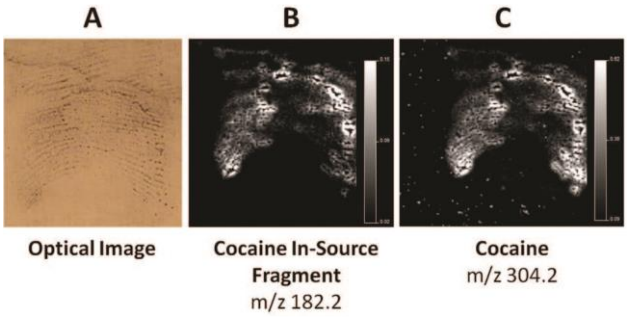
A
B
C
Optical Image
Cocaine In-Source Fragment m/z 182.2
Cocaine m/z 304.2

Figure 5

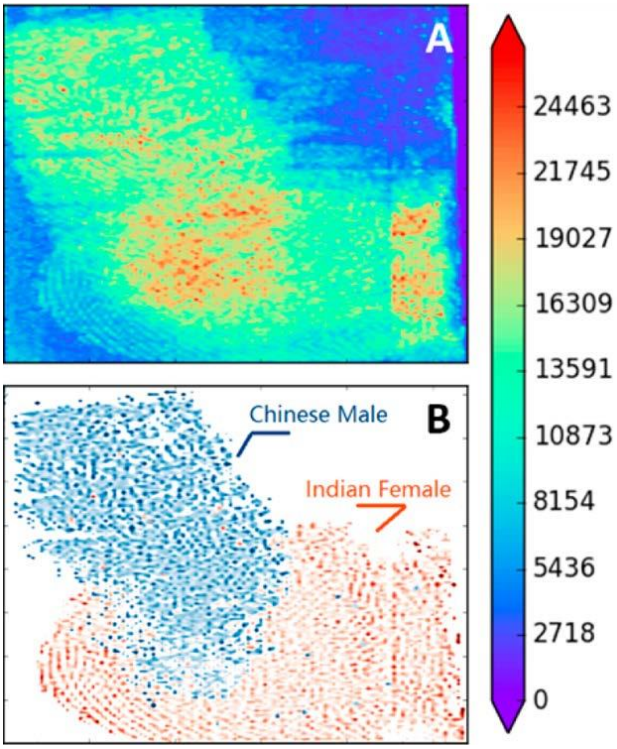
A
B
Chinese Male
Indian Female
24463
21745
19027
16309
13591
10873
8154
5436
2718
0

Figure 6

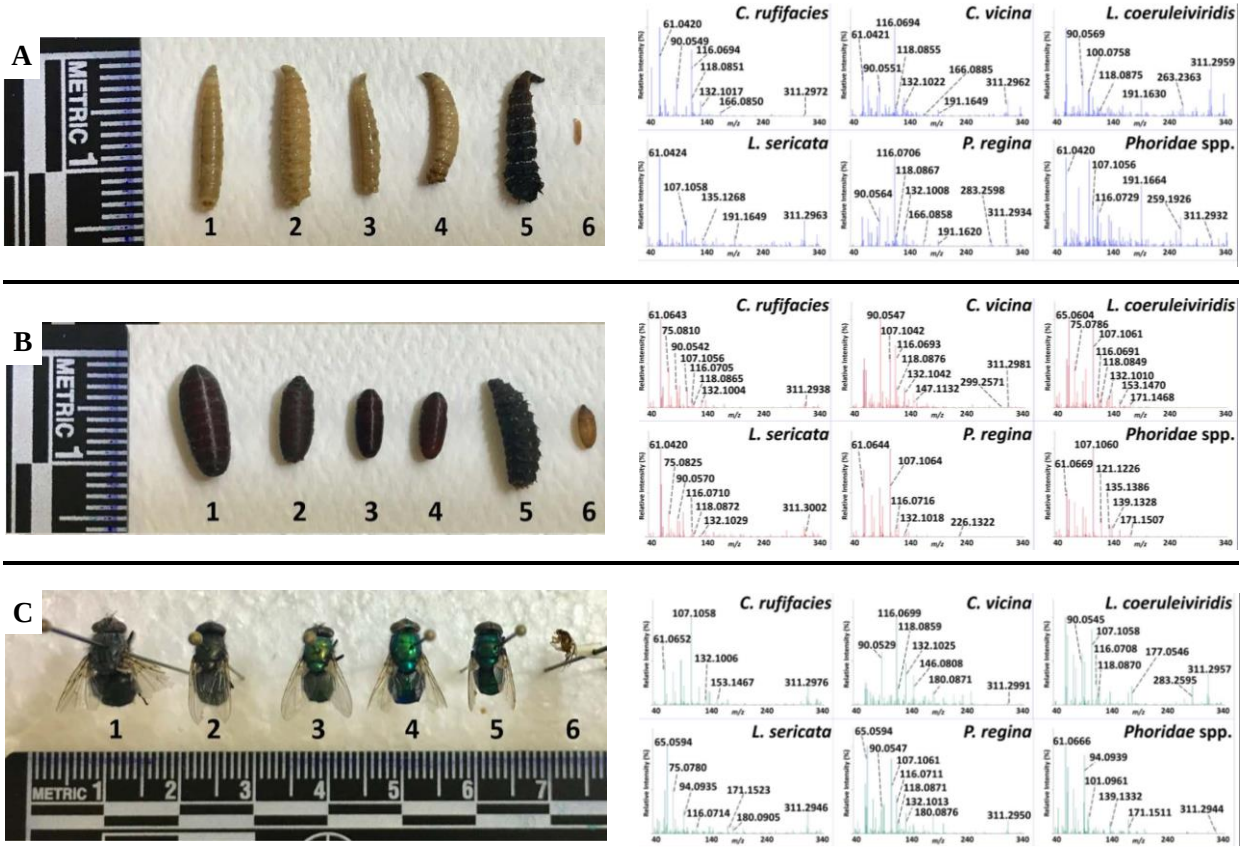
A
METRIC 1
1 2 3 4 5 6
C. rufifacies
61.0420
90.0549
116.0694
118.0851
132.1017
166.0850
311.2972
C. vicina
116.0694
61.0421
118.0855
90.0551
166.0885
132.1022
311.2962
191.1649
L. coeruleiviridis
90.0569
100.0758
311.2959
118.0875
263.2363
191.1630
L. sericata
61.0424
107.1058
135.1268
191.1649
311.2963
P. regina
116.0706
118.0867
90.0564
132.1008
283.2598
166.0858
311.2934
191.1620
Phoridae spp.
61.0420
107.1056
191.1664
116.0729
259.1926
311.2932
B
METRIC 1
1 2 3 4 5 6
C. rufifacies
61.0643
75.0810
90.0542
107.1056
116.0705
118.0865
132.1004
311.2938
C. vicina
90.0547
107.1042
116.0693
118.0876
132.1042
147.1132
299.2571
311.2981
L. coeruleiviridis
65.0604
75.0786
107.1061
116.0691
118.0849
132.1010
153.1470
171.1468
L. sericata
61.0420
75.0825
90.0570
116.0710
118.0872
132.1029
311.3002
P. regina
61.0644
107.1064
116.0716
132.1018
226.1322
Phoridae spp.
107.1060
61.0669
121.1226
135.1386
139.1328
171.1507
C
1 2 3 4 5 6
METRIC 1 2 3 4 5 6 7
C. rufifacies
107.1058
61.0652
132.1006
153.1467
311.2976
C. vicina
116.0699
118.0859
90.0529
132.1025
146.0808
180.0871
311.2991
L. coeruleiviridis
90.0545
107.1058
116.0708
177.0546
118.0870
311.2957
283.2595
L. sericata
65.0594
75.0780
94.0935
171.1523
116.0714
180.0905
311.2946
P. regina
65.0594
90.0547
107.1061
116.0711
118.0871
132.1013
180.0876
311.2950
Phoridae spp.
61.0666
94.0939
101.0961
139.1332
171.1511
311.2944

Figure 7

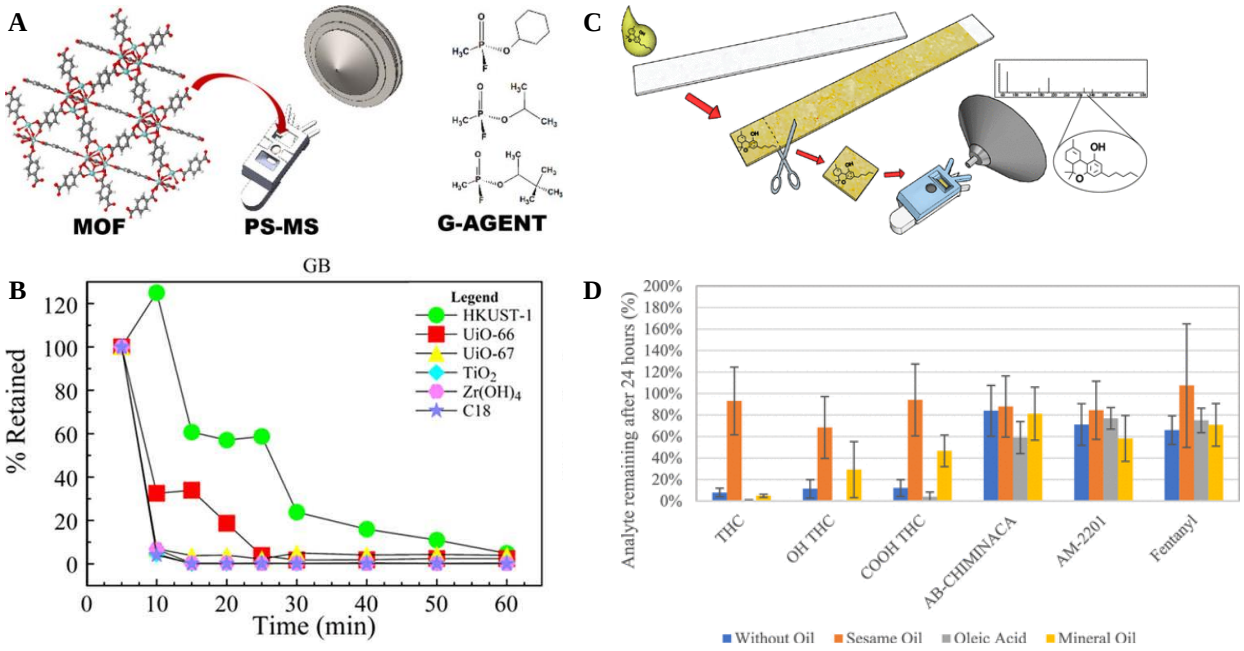
A
MOF
PS-MS
G-AGENT
C
OH
B
GB
% Retained
Time (min)
Legend
HKUST-1
UiO-66
UiO-67
TiO2
Zr(OH)4
C18
D
Analyte remaining after 24 hours (%)
THC
OH THC
COOH THC
AB-CHIMINACA
AM-2201
Fentanyl
Without Oil
Sesame Oil
Oleic Acid
Mineral Oil

Figure 8

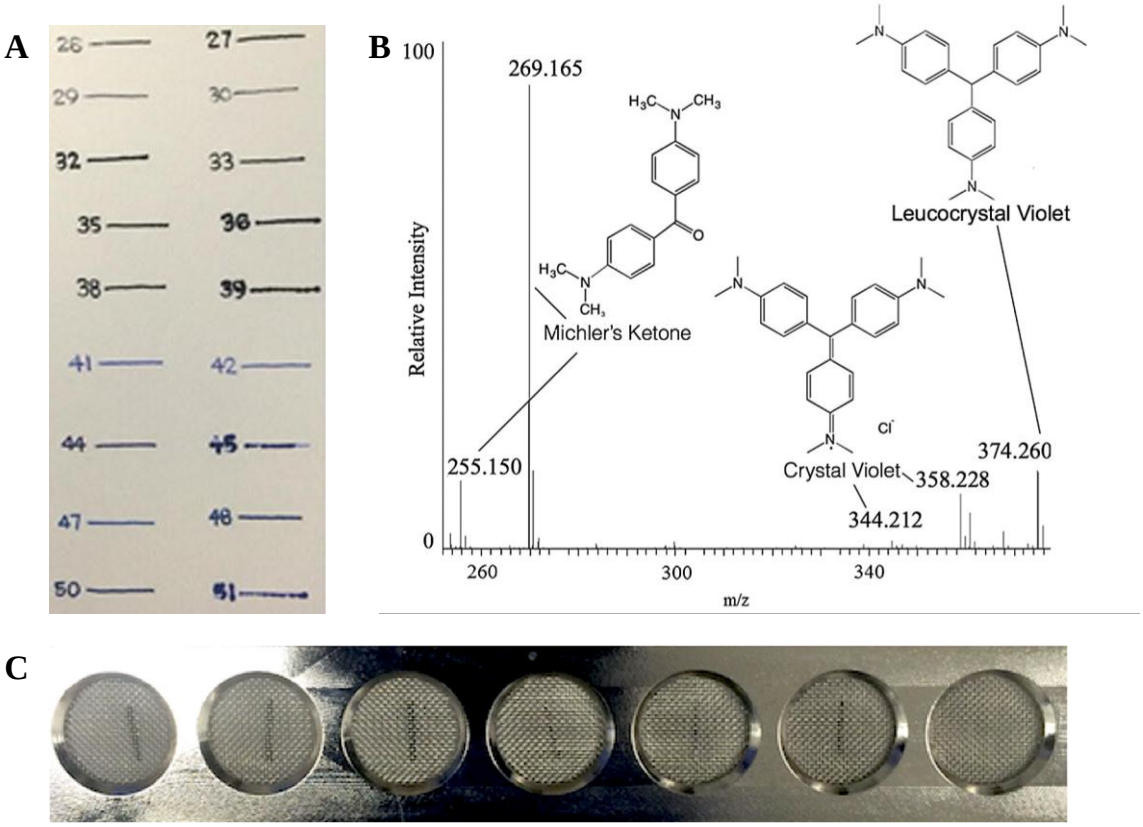
A
26
27
29
30
32
33
35
36
38
39
41
42
44
45
47
48
50
51
B
100
0
Relative Intensity
269.165
255.150
344.212
358.228
374.260
260
300
340
m/z
Michler's Ketone
Leucocrystal Violet
Crystal Violet
Cl
C

Figure 9

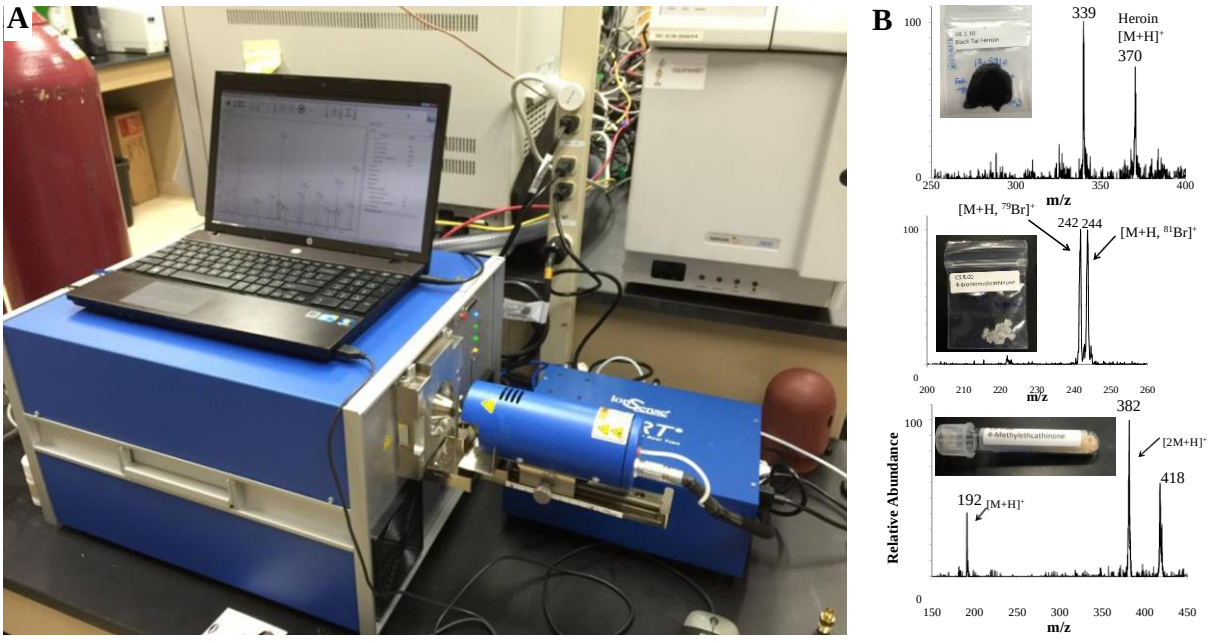
A
B
339
Heroin
[M+H]+
370
m/z
[M+H, 79Br]+
242 244
[M+H, 81Br]+
382
[2M+H]+
418
192 [M+H]+
Relative Abundance

Figure 10

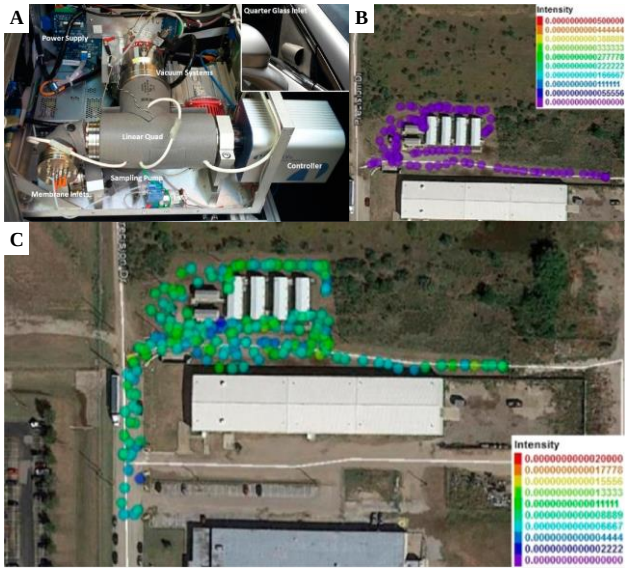
A
Power Supply
Quarter Glass Inlet
Vacuum System
Linear Quad
Controller
Sampling Ports
Membrane Inlets
B
Intensity
C
Intensity

Figure 11

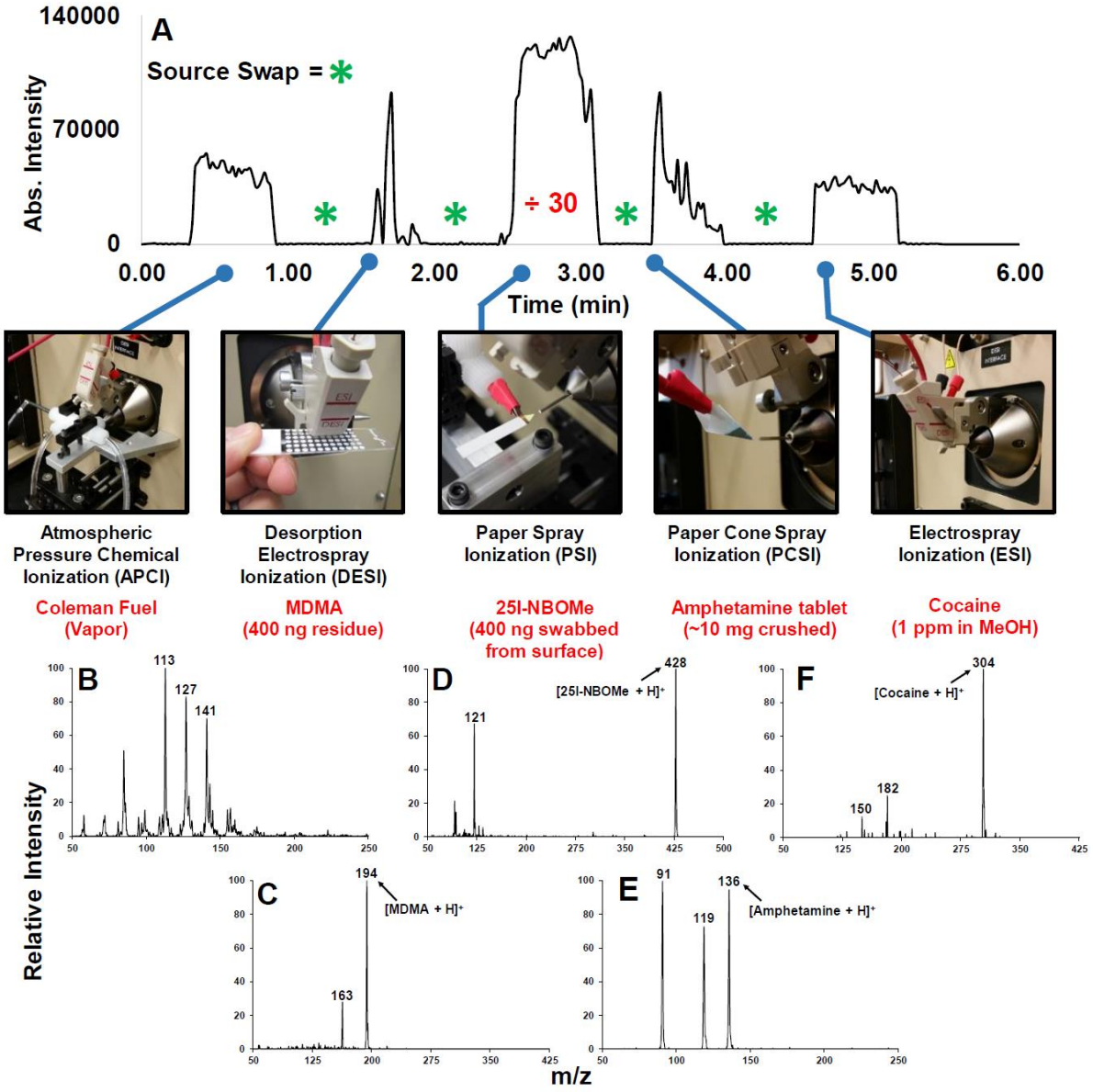
A
Source Swap = *
140000
70000
0
Abs. Intensity
÷ 30
0.00
1.00
2.00
3.00
4.00
5.00
6.00
Time (min)
Atmospheric Pressure Chemical Ionization (APCI)
Desorption Electrospray Ionization (DESI)
Paper Spray Ionization (PSI)
Paper Cone Spray Ionization (PCSI)
Electrospray Ionization (ESI)
Coleman Fuel (Vapor)
MDMA (400 ng residue)
25I-NBOMe (400 ng swabbed from surface)
Amphetamine tablet (~10 mg crushed)
Cocaine (1 ppm in MeOH)
B
113
127
141
D
121
428
[25I-NBOMe + H]+
F
304
[Cocaine + H]+
182
150
C
194
[MDMA + H]+
163
E
91
136
119
[Amphetamine + H]+
Relative Intensity
m/z

Figure 12

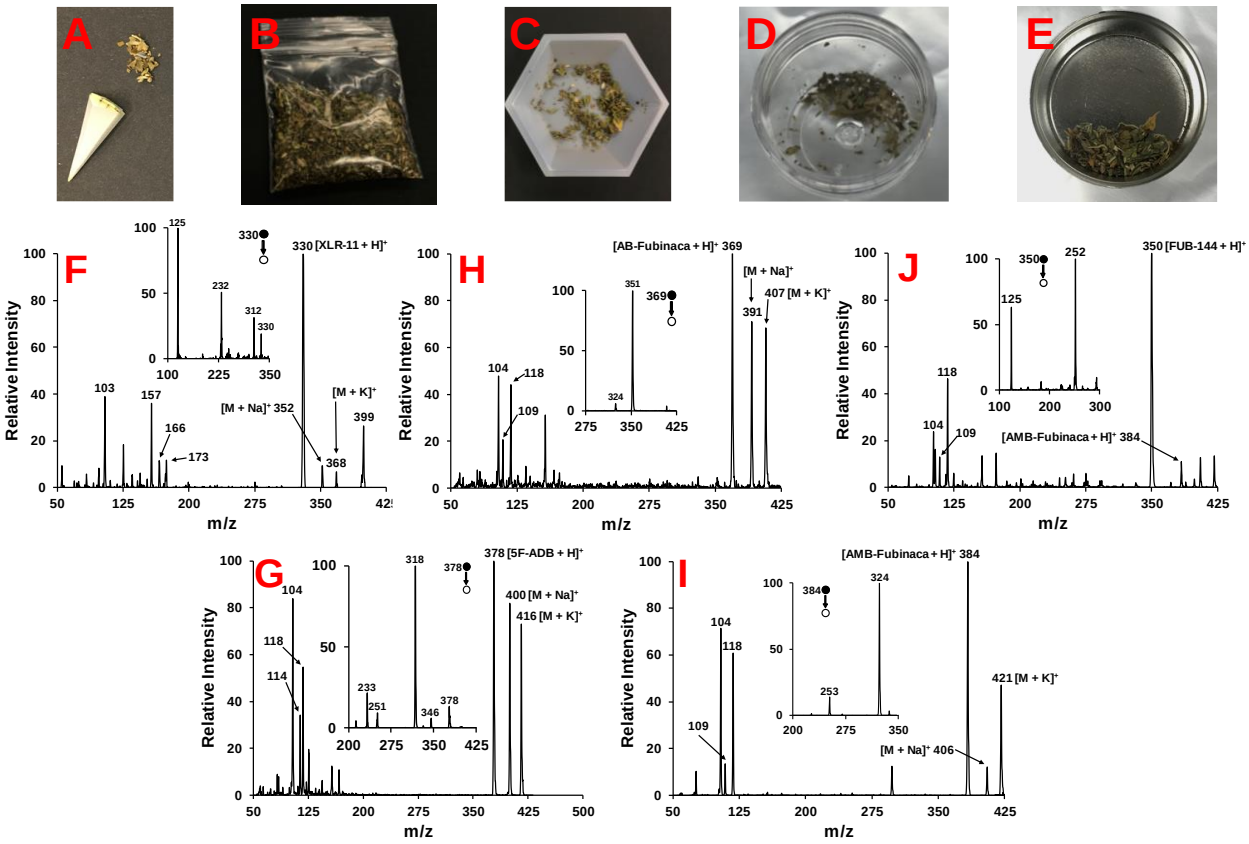
A
B
C
D
E
F
330 [XLR-11 + H]+
[M + Na]+ 352
[M + K]+
G
378 [5F-ADB + H]+
400 [M + Na]+
416 [M + K]+
H
[AB-Fubinaca + H]+ 369
[M + Na]+
407 [M + K]+
I
[AMB-Fubinaca + H]+ 384
[M + Na]+ 406
421 [M + K]+
J
350 [FUB-144 + H]+
[AMB-Fubinaca + H]+ 384
Relative Intensity
m/z

Figure 13

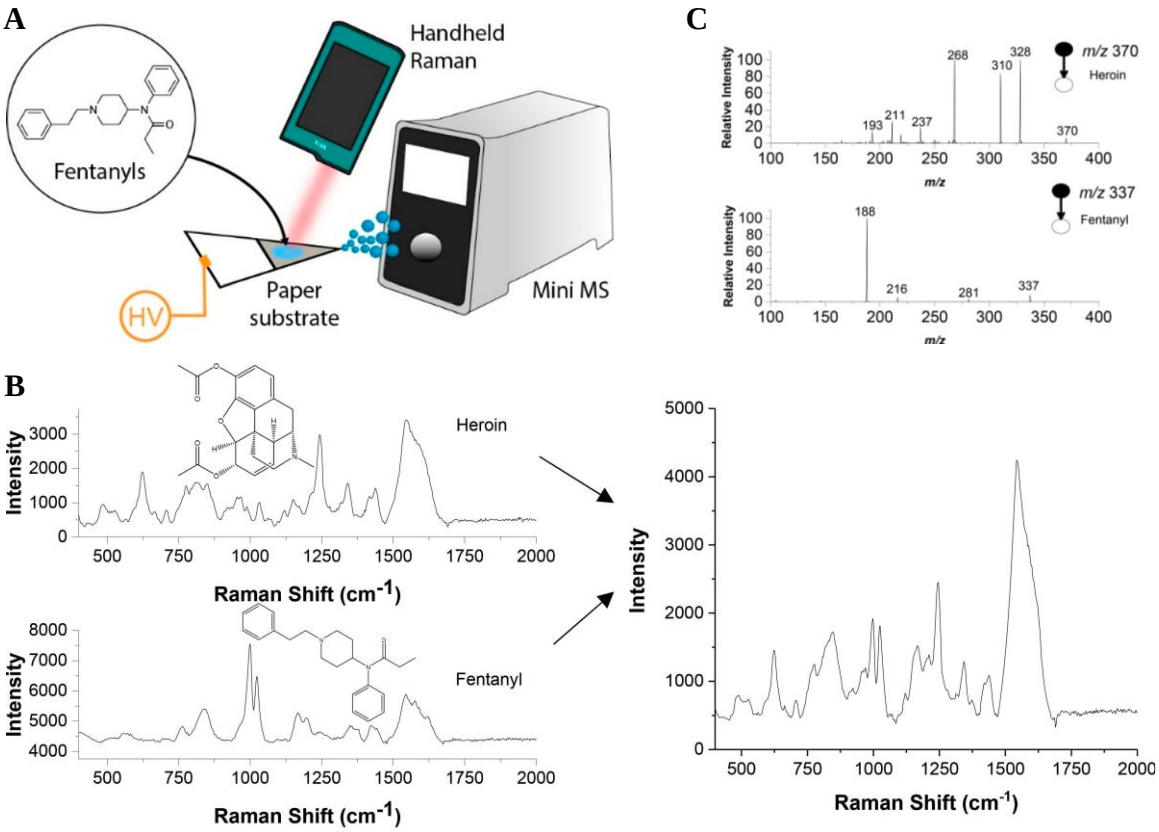
A
Handheld
Raman
Fentanyls
Paper
substrate
HV
Mini MS
B
Heroin
Intensity
Raman Shift (cm-1)
Fentanyl
Intensity
Raman Shift (cm-1)
C
m/z 370
Heroin
Relative Intensity
m/z
m/z 337
Fentanyl
Relative Intensity
m/z
Intensity
Raman Shift (cm-1)

Figure 14

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A critical review of the role that mass spectrometry currently plays in forensic science is provided, as well as emerging techniques aimed at assisting the future forensic practitioner.