Electronic Supplementary Material (ESI) for Analytical Methods.

Electronic Supplementary Information For:

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

* 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: mulligan@ilstu.edu

Table S1. Summary of Established and Emerging MS-Based Techniques for Forensic Chemical Analysis
(*NOTE:* included reference numbers correspond to those found in the main publication)

Separation Techniques and Related Ionization Sources		
Technique	**Short Description**	**Typical Applications**
Gas Chromatography – Mass Spectrometry (GC-MS)†‡*	Considered the "gold standard" for forensic chemical analysis, thanks to high repeatability and standard spectral databases. Molecules are separated in the gas phase via a variety of column stationary phases and then analyzed by mass spectrometry after elution, typically by electron ionization (EI)[9, 35] Has been employed with multiple stages of MS analysis (MS/MS, or MS^n)	A majority of casework involving controlled substances,[11] toxicology,[29,36] and fire debris analysis[37] is processed via GC-MS.
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 by allowing for increased peak capacity.[43]	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]
Headspace Solid-Phase Microextraction (HS-SPME)†‡*	An extraction technique used to help trap and concentration volatile vapors from headspace emanating from evidence before chemical analysis, often coupled with GC.[47] Employs coated fused silica fibers of various phases.	When coupled with high resolution MS (HRMS), this separation can improves the confidence of volatile organic compound (VOC) detection and speciation by allowing preconcentration of trace analytes. Employed for decomposition odors from soil at death scenes.[47]
Liquid Chromatography – Mass Spectrometry (LC-MS)†‡*	A compliment to GC-MS, as LC-MS is capable of analyzing a wider range of forensic compounds, including polar and less volatile analytes,[9, 29, 55] via a variety of column stationary phases and then analyzed by mass spectrometry after elution, typically by electrospray ionization (ESI). Has been employed with multiple stages of MS analysis (MS/MS, or MS^n).	LC-MS has been employed in forensic workflows in the public laboratory system, but is not as prevalent as GC-MS. Typically used for toxicological assessments, such as screening drugs and their metabolites in bodily fluids[56]

Capillary Electrophoresis – Mass Spectrometry (CE-MS) ‡*	A separation technique that utilizes a strong electric field to separate compounds.[67, 68] CE is amenable to portability and on-site analysis due to minimal sample and solvent volume requirements, minimal waste, and separation can be obtained in ~1 min., if coupled with portable MS.	CE-MS has been used for analyzing complex drug mixtures and dye compounds found in textile fibers[73]
Membrane Inlet Mass Spectrometry (MIMS) ‡,*	MIMS allows for direct sample probing via a semi-permeable membrane capable of selectively introducing target analytes found in ambient air [195] and water for MS analysis.	MIMS has been used for volatile explosives and chemical warfare agent screening, as well as continuous sampling of ambient air around clandestine synthetic labs to screen for target volatile effluents[195]
Electron Ionization – Mass Spectrometry (EI-MS)†‡*	Utilized in GC-MS to produce highly reproducible mass spectra with a high degree of confidence for compound identification which coupled to standard spectral databases. EI-MS is a "hard" ionization source, producing representative fragmentation patterns of analytes, with little to no molecular ions typically seen.	Due to mechanistic aspects, EI-MS is broadly applicable, but is typically used in the forensic setting for controlled substances,[11] toxicology,[29, 36] and fire debris evidence[37]
Electrospray Ionization (ESI)†‡*	ESI is a "soft" ionization source that typically creates molecular ions (e.g., protonated, deprotonated, alkali metal adducts, etc.), and little to no 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.	Commonly employed on LC-MS systems for drugs of abuse and metabolites in bodily fluids[56]

Laser-Based Techniques		
Technique	**Short Description**	**Typical Applications**
Matrix-Assisted Laser Desorption/Ionization – Mass Spectrometry (MALDI-MS)†*	MALDI-MS utilizes a laser energy that coincides with adsorption characteristics of an applied matrix to induce energetic desorption and secondary ionization in the selvedge region above the sample surface. Like ESI-MS, molecular ions (e.g., protonated, deprotonated, alkali metal adducts, etc.) and little to no fragmentation are observed. MS imaging can be easily accomplished via MALDI-MS. As the sample is rastered, mass spectra are collected at each "pixel" where the laser is fired,[75] providing informative images of chemical information.	In forensic-related work, MALDI has most notably been used for imaging fingerprints,[74] but has been investigated for DNA typing, as well.
Laser Ablation Inductively-Coupled Plasma Mass Spectrometry (LA-ICP-MS) †*	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] Known for its high sensitivity.	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.

Ambient MS Ionization Sources		
Technique	**Short Description**	**Typical Applications**
Ambient Ionization – Mass Spectrometry (AI-MS)†‡*	An emerging class of MS ionization sources designed to allow collection of target analytes from target samples with minimal to no sample preparation.[10, 24, 95] Inherent simplicity makes them appealing for use by non-technical operators, but few techniques have been validated.	See the specific ambient MS sources subsequently described for target applications.
Desorption Electrospray Ionization (DESI)†‡*	A spray of charged solvent droplets is directed towards a sample of interest (e.g., solid material, surface residue, etc.), where analyte present is solvent extracted. Primary, incoming droplets then produce secondary droplets containing analyte after surface impact. Secondary droplets undergo ESI-like processes for MS detection.[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, 95, 99]

Direct Analysis in Real Time (DART)[†‡*]	Excited-state gas species via glow discharge ionizes target analytes via ion-molecule reactions.[24, 111] DART provides rapid sample screening and little to no sample pretreatment, and desorption is aided by the high temperature of this ion source. DART-MS has been validation for forensic workflows in the public laboratory system.	Forensic applications of DART are wide-ranging, including illicit drugs,[111-113] toxicology,[111] explosives,[114] CWAs,[111] ignitable liquids,[115] GSR,[116, 117] paint analysis,[118] and inks.[119, 120] DART-MS has the ability to analyze stains on fabric, rodenticide adulterant in drug mixtures, and identify insect life stages to help determine time since death.
Paper Spray Ionization (PSI)[†‡*]	PSI utilizes a triangular paper substrate as a disposable ionization source, as well as for sample collection when employed for surface swabbing. When compatible spray solvent is applied to the substrate, it wicks through the paper, eluting analytes to the paper tip. Application of high voltage then produces an ESI-like processes from the paper for MS analysis. PSI is marked by its highly simplistic design, low cost, and ease of use for non-scientists, [28 24, 139-142] making it of interest for portability.	Current literature demonstrates PSI-MS for the analysis of inks and documents,[143-145] drugs of abuse,[145] chemical warfare agent (CWA) simulants in soil,[146] air,[147] and in blood and urine,[148] authentic CWAs in the ambient atmosphere,[149] protein toxin simulants from surfaces,[150] and explosives.[151, 152] It has been extensively demonstrated on portable MS systems.[28, 194, 196, 205, 220, 228]
Atmospheric Solids Analysis Probe (ASAP)[†‡*]	Analytes are thermally desorbed from a sampling probe by the heated nitrogen gas and ionized via corona discharge in standard APCI sources.[161] Little preparation, besides probe sampling, is required.	ASAP has been applied to rodenticide-adulterated drug samples, black tar heroin and associated impurities, and crack cocaine,[162] "spice" packets for synthetic cannabinoids and cathniones,[163] and amphetamines in urine.[164]
Direct Sample Analysis (DSA) [†*]	DSA combines features of both DESI and APCI.[164] 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]	DSA has been used to analyze drugs of abuse,[166] potentially adulterated and contaminated herbal medicines,[167] synthetic phenylethylamines in blotter paper paraphernalia,[168] writing inks,[169] and fentanyl and its analogs[170]
Dielectric Barrier Discharge Ionization (DBDI)[‡]	DBDI utilizes a low-power, non-thermal plasma to desorb and ionize surface-bound or liquid-phases analytes.[170] Similar/variant DBDI ion sources include active capillary plasma[177] and low temperature plasma probe.[178-180]	DBDI and like sources have been extensively applied for explosive analytes, including the novel coupling of LC and DBDI for multi-class explosives found in water and soil matrices.[175]

Surface Enhanced Raman Spectroscopy Coupled with Paper Spray Ionization – Mass Spectrometry (SERS-PSI-MS)‡	The novel coupling of SERS analysis with PSI-MS by utilizing nanoparticle-infused paper substrates compatible with each technique. Both commercial[218] and novel/experimental[220] substrates have been demonstrated on portable MS systems. This two-tiered analysis strategy meets the recommendations set by the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG)[7] for drug identification methods. Also referred to as pSERS-PSI-MS.	SERS-PSI-MS has been demonstrated for explosives, CWA simulants,[218] and various drugs, such as fentanyls[219] and difficult isomeric combinations.[220] Efforts to validate a portable variant of this method for forensic drug confirmation were recently reported.[220]
Swab Touch Spray Ionization (STSI)‡	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]
Filter Cone Spray Ionization (FCSI) †‡	A 3D variant on PSI where filter paper is crafted into a pyramidal cone shape to sample, extract, and analyze bulk drug evidence types. [196, 214, 215] Spray solvent is added to the conical reservoir holding the sample of interest, and when high voltage is applied, extracted and filtered analytes flow to the tip where they undergo ESI-like ionization.[216]	FCSI-MS has been used to analyze drug evidence from authentic drug casework (e.g, powders, synthetic cannabinoid seizures, etc.), prescription and counterfeit pharmaceutical tablets, and veterinary toxicology samples.[216] Filtration media employed for forensic vacuum of trace evidence can also be analyzed for chemical attributes.[216]

† **Utilized for authentic forensic casework**

‡ **Portable and/or demonstrated on portable devices**

* **Commercially-available instrument and/or ionization source**