Anal Bioanal Chem (2009) 394:1995–2008
DOI 10.1007/s00216-009-2659-2

REVIEW

Forensic applications of ambient ionization mass spectrometry

Demian R. Ifa · Ayanna U. Jackson · Giuseppe Paglia · R. Graham Cooks

Received: 30 November 2008 / Revised: 23 January 2009 / Accepted: 27 January 2009 / Published online: 26 February 2009

Abstract This review highlights and critically assesses forensic applications in the developing field of ambient ionization mass spectrometry. Ambient ionization methods permit the ionization of samples outside the mass spectrometer in the ordinary atmosphere, with minimal sample preparation. Several ambient ionization methods have been created since 2004 and they utilize different mechanisms to create ions for mass-spectrometric analysis. Forensic applications of these techniques—to the analysis of toxic industrial compounds, chemical warfare agents, illicit drugs and formulations, explosives, foodstuff, inks, fingerprints, and skin—are reviewed. The minimal sample pretreatment needed is illustrated with examples of analysis from complex matrices (e.g., food) on various substrates (e.g., paper). The low limits of detection achieved by most of the ambient ionization methods for compounds of forensic interest readily offer qualitative confirmation of chemical identity; in some cases quantitative data are also available. The forensic applications of ambient ionization methods are a growing research field and there are still many types of applications which remain to be explored, particularly those involving on-site analysis. Aspects of ambient ionization currently undergoing rapid development include molecular imaging and increased detection specificity through simultaneous chemical reaction and ionization by addition of appropriate chemical reagents.

D. R. Ifa · A. U. Jackson · G. Paglia · R. G. Cooks (✉)
Department of Chemistry, Purdue University,
560 Oval Drive,
West Lafayette, IN 47907, USA
e-mail: cooks@purdue.edu

G. Paglia
Department of Biomedical Sciences, Faculty of Medicine,
University of Foggia,
Viale L. Pinto,
71100 Foggia, Italy

Keywords Forensics/toxicology · Mass spectrometry plasma mass spectrometry · Ambient analysis · Illicit drugs · Explosives · Counterfeit pharmaceuticals

Introduction

Many analytical techniques applied in the forensic sciences are well established and are routinely accepted as evidence in the courts. Examples include polymerase chain reaction and restriction fragment length polymorphism for paternity tests; infrared (IR) spectroscopy for the estimation of blood alcohol content by breath analysis; gas chromatography (GC) or liquid chromatography (LC) coupled with mass spectrometry (MS) and immunoassays for the detection and quantitation of drugs of abuse and their metabolites in biological fluids. Even though all of these current methods are frequently used, alternative techniques are continuously under development [1, 2]. One area of application which seems likely to grow, in spite of the cost of equipment and the skill required of the operator, is MS. MS has seen application in the form of LC-MS and GC-MS in drug overdose cases [3], in accelerant identification in fire investigations [4] and in industrial hygiene investigations [5], as well as in product safety [6, 7] and labeling cases. More recent MS studies have tended to use the powerful and rapid method of tandem MS (MS/MS), but mobile mass spectrometers have had virtually no impact except in the military [8, 9] and miniature instruments [10] are yet to be deployed. This review covers a new development in MS—that of ambient ionization methods which permit the ionization of samples in the ordinary atmosphere, with minimal sample preparation. While this capability will ultimately be applied in conjunction with miniature portable

instruments, it is already giving highly encouraging results in laboratory instruments, since it simplifies and speeds up chemical analysis.

Desorption electrospray ionization (DESI) [11] and direct analysis in real time (DART) [12] were reported in 2004 and 2005, respectively, and their development led to a family of ambient ionization techniques [13]. In these methods, condensed-phase samples, often complex mixtures, are treated in such a way that analytes are desorbed and ionized. The two steps can occur together under the influence of a particular agent, or they can occur separately. The desorption step involves a change in phase (e.g., solid to vapor phase) and the ionization step involves acquisition of charge by neutral analyte molecules. In DESI, a spray of charged droplets impacts a condensed-phase sample on a substrate and creates a thin film of liquid. Analytes dissolve in this liquid film and as additional droplets arrive they splash into the film, creating a shower of microdroplets containing dissolved analyte. The secondary droplets are drawn into the atmospheric inlet of a mass spectrometer, where the solvent is removed by heat and vacuum and the resulting ionized form of the analyte is mass-analyzed. In DESI, therefore, desorption is by the action of charged microdroplets and ionization is by the standard electrospray ionization (ESI) process. In DART, a plasma is used to create excited-state atoms and ions from a gas (nitrogen or helium) at atmospheric pressure by applying an electrical potential. The resulting plasma contains excited-state atoms and usually ions which are products of reactions with atmospheric gases such as water; this material is heated and blown onto the sample. Ions of analyte molecules (typically in the range 20–1,000 Da) are desorbed into the gas phase and analyzed by MS. Desorption is thermally assisted; ionization is principally by ion/molecule reactions with solvated hydronium ions and solvated hydroxyl anions. Sample analysis in these desorption ionization experiments is performed in the native environment at atmospheric pressure without the requirement for sample confinement in the vacuum of the mass spectrometer. Sample preparation or preseparation procedures are usually not essential, but if time allows, any simplification of the sample mixture is advantageous.

Many ambient ionization techniques other than DESI and DART have emerged recently and many are included in Table 1. They can be classified according to the desorption method (e.g., laser desorption, thermal desorption) and also according to the ionization method (e.g., atmospheric pressure chemical ionization, ESI). In some methods the desorption and ionization steps are integrated and performed using a single agent (e.g., energetic aqueous droplets in DESI) although in other cases the steps occur in succession (e.g., in laser-assisted ESI, laser desorption is followed by ESI). Detailed mechanistic aspects of each technique will not be discussed here. Readers interested in the mechanisms or in the classification of the ambient ionization techniques should refer to the original references in Table 1, or to recent reviews [14–16]. Although all ambient ionization techniques have the potential for forensic applications, only a few have been applied in this specific context. For instance, many papers can be found in the literature for tablet analysis, but only a few report investigations of illicit drugs or counterfeit tablets. This review will highlight specific forensic applications of ambient ionization MS. The material is organized in terms of the various forensic applications. The paper ends with a conclusion which is a critical summary of these methods.

Forensic applications

Explosives, toxic industrial compounds, and chemical warfare agents

Rapid, trace detection of explosives, toxic industrial compounds, and chemical warfare agents is of particular interest to the forensics community. There is a need to be able to analyze these compounds on-site from a variety of surfaces, in various states of matter, and especially directly within complex matrices. Several MS ionization methods are currently used to detect these compounds, but only a few of these methods can be used in the ambient environment with minimal sample preparation. Early in situ MS methods employed the trace atmospheric gas analyzers, including van-transported triple-quadrupole instruments, for the analysis of gas-phase trinitrotoluene (TNT) and dinitrotoluene [17]. With the continued development of ambient ionization methods, the number of publications illustrating the direct analysis of explosives, toxic industrial compounds, and chemical warfare agents, as well as metabolites of these compounds, in the ambient environment is increasing.

DESI analysis of explosives and chemical warfare agents

The ability to analyze explosives and chemical warfare agent stimulants in the ambient environment with minimal sample preparation has been shown using the DESI method [18–25]. The detection of traces of explosives from various surfaces, including metal, brick, paper, cloth, and skin, has been reported [23]. Some analytes, such as trinitrohexahydro-1,3,5-triazine (RDX), can be detected in either the positive ion or the negative ion mode; many others are better detected in the negative ion mode because of limited competition from matrix-derived ions. Nonconventional explosives which do not contain nitro functional groups can be detected too, an important example being the organic peroxides which

Table 1 Ambient mass spectrometry ionization techniques and their acronyms. (Adapted from [14–16])

Technique	Agent/ionization mechanism	Acronym
Ambient solid analysis probe	HGF/CI	ASAP [72]
Atmospheric pressure thermal desorption ionization	HGF/CI	APTDI [28]
Desorption atmospheric pressure chemical ionization	HGF/CI	DAPCI [37]
Desorption atmospheric pressure photoionization	HGF/PI	DAPPI [73]
Desorption electrospray ionization	DP/ESI	DESI [11]
Easy ambient sonic-spray ionization	DP/ESI	EASI [74]
Dielectric discharge barrier ionization	PLI/CI	DBDI [33]
Direct analysis in real time	HGF/CI	DART [12]
Electrospray laser desorption/ionization	L/ESI	ELDI [75]
Extractive electrospray ionization	GF/ESI	EESI [76]
Fused-droplet electrospray ionization	DP/ESI	FD-ESI [77]
Helium atmospheric pressure glow discharge ionization	GF/CI	HAPGDI [78]
Laser-ablation electrospray ionization	L/ESI	LAESI [79]
Low-temperature plasma ionization	PLI/CI	LTP [35]
MALDI-assisted electrospray ionization*	L/ESI	MALDESI [80]
Neutral desorption extractive electrospray ionization	GF/ESI	ND-EESI [67]
Plasma-assisted desorption/ionization	HGF/CI	PADI [81]

DP droplet projectiles, *GF* gas flow, *L* laser, *HGF* heated gas flow, *ESI* electrospray ionization, *CI* chemical ionization, *MALDI* matrix-assisted laser desorption/ionization, *PI* photoionization, *PLI* plasma ionization.

*Not strictly ambient because it requires addition of matrix.

are readily observed in DESI and confirmed by their product ion MS/MS spectra.

Analysis of explosives in mixtures and chemical warfare agents in complex matrices is readily achieved by DESI, often with femtogram absolute detection limits for the individual pure compounds. Even when explosives are obscured by other chemicals, they may be recognized. For example, the analysis of an explosive mixture (four explosives each in nanogram amounts) in the presence of a complex (thick) cream was performed in different layering formats: (1) with the explosives mixture spotted on top of the cream on a cotton substrate and (2) with the explosives mixture spotted on top of the cotton substrate and then masked by the cream [19]. In both experiments, the explosives mixture was successfully detected. However, in the case of the second experiment in which the explosives were masked by cream, they were not observed until the cream layer had been depleted by the solvent spray. These results are illustrated in Fig. 1a and b. Characteristic spectra can be obtained for individual constituents of mixtures by MS/MS. Alternatively, accurate mass measurements can be used to increase specificity of detection [26]. Explosives are recognizable by their characteristic heteroatom content which influences their accurate masses.

An emerging development in the ambient ionization methods is the use of added reagents that promote selective ionization. This allows the experiment to be specifically tailored to the analytes of interest. The DESI version of this experiment is termed "reactive DESI" [27–29]; specific reagents are added to the DESI spray to target a specific functional group or particular analyte by in situ formation of a characteristic reaction product. Frequently, the reaction is chosen so that a charged product results. This increases the selectivity and extent of ionization and is ideal when the analyte of interest does not ionize well, either because it is present in a complex mixture and other analytes compete to take the charge or because it is present at low levels. These experiments have been used to form new covalently bound reaction products in both the positive ion and the negative ion modes [20, 22, 25]; an illustration is provided in Fig. 1c.

D'Agostino et al. [30, 31] have used DESI analysis for the detection of chemical warfare agents from office media and solid phase microextraction (SPME) fibers. In these studies, the DESI solvent was sprayed directly onto the SPME fibers, desorbing and ionizing a complex tabun (also known as GA) standard or chemical warfare agent standards which were spiked onto representative indoor office media, including office carpet, office fabrics, photocopy paper, and Dracon sampling swabs (in the range from 5 to 25 μg/g). A sample of munition-grade tabun containing ethyl dimethylphosphoroamydocyanidate (tabun), ethyl tetramethylphosphorodiamidate, diethyl dimethylphosphoramidate, ethyl isopropyl dimethylphospoaramidate, diisopropyl dimethylphosporamidate, and triisopropyl phosphate was investigated. The product-ion mass spectra obtained

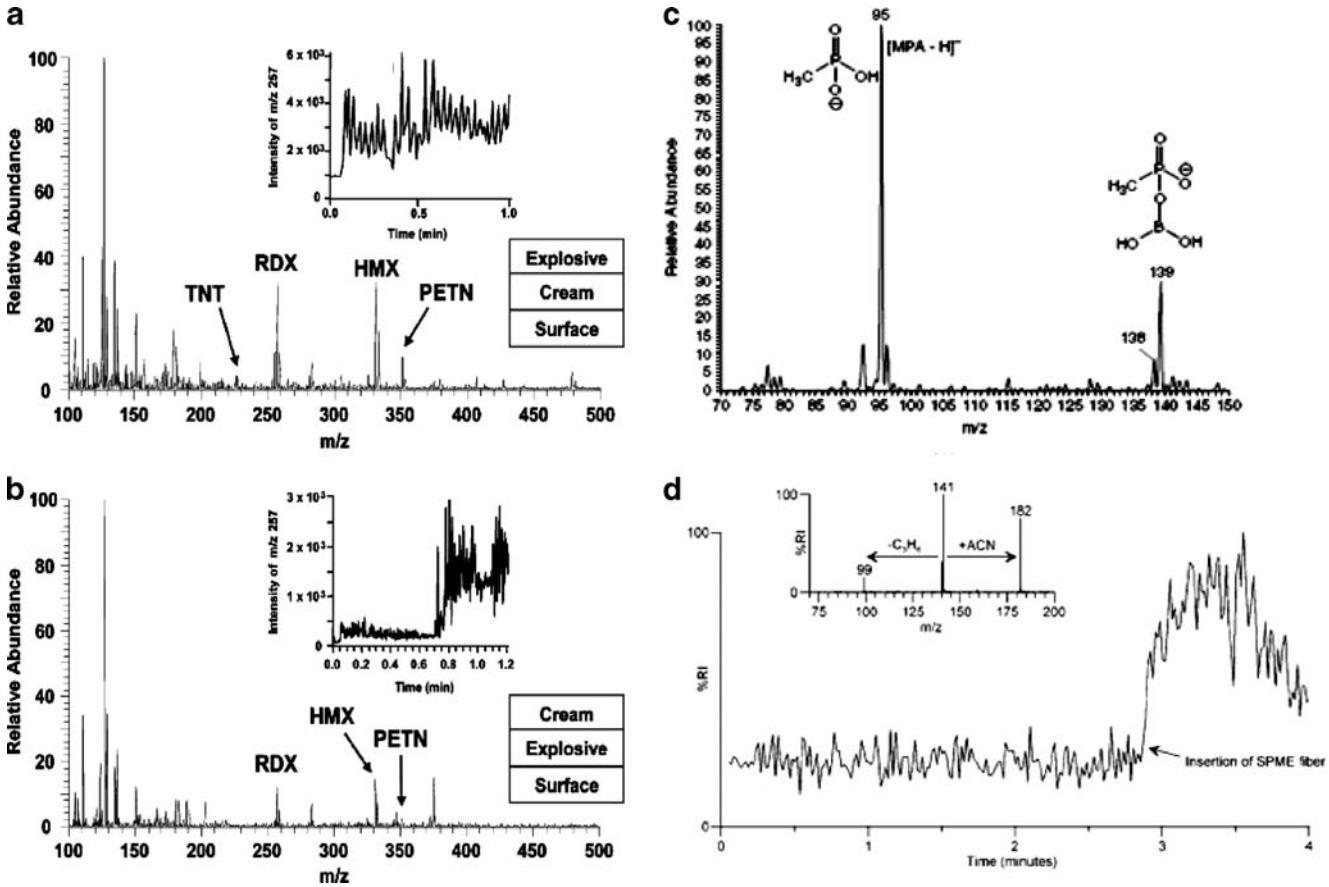

Fig. 1 Desorption electrospray ionization (DESI) analysis of explosives (**a**, **b**) and chemical warfare agents (**c**, **d**) A mixture of four explosives (trinitrotoluene, *TNT*; trinitrohexahydro-1,3,5-triazine (RDX); octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX); pentaerythritol tetranitrate, *PETN*) was analyzed from a cotton substrate in the presence of a complex cream matrix. **a** Explosives were spotted directly on top of the complex matrix and **b** the explosives were spotted then masked by the complex matrix. The *insets* in **a** and **b** show ion chronograms for each analysis type illustrating that the explosives were detected instantaneously when spotted on top of the matrix and only after depleting the masking matrix by solvent in **b**. **c** Reactive DESI experiment in which ethyl methylphosphonic acid, a hydrolysis product of a chemical warfare agent, was reacted with boronic acid in the DESI spray, demonstrating in situ chemical reaction product as a supplementary method of identification of the targeted analyte. **d** Ion chronogram associated with DESI analysis of a solid phase microextraction fiber from a mock training environment with chemical warfare agents illustrating the detection of sarin (*inset* tandem mass spectrometry, MS/MS, product ion spectrum). Note that protonated sarin, *m/z* 141, undergoes both an ion/molecule reaction with addition of acetonitrile (*ACN*) and collision-induced dissociation with loss of propene. (Reproduced with permission from **a**, **b** [19], **c** [25], and **d** [30])

were consistent with those obtained from the same compounds using LC-ESI-MS/MS. Other analytes, including 3-(fluoromethylphosphoryl) oxy-2,2-dimethylbutane (soman, or GD), and 2-(fluoromethylphosphoryl) oxypropane (sarin, or GB), were also analyzed by DESI-MS/MS (Fig. 1d) and again the results obtained were consistent with those obtained using LC-ESI-MS/MS and GC-MS. In the case of triethyl phosphate, which was analyzed by both DESI-MS/MS and LC-ESI-MS/MS, the signal-to-noise ratio (S/N) obtained in DESI (S/N 100:1) was better than that obtained in LC-ESI (S/N 30:1). Further, bis(2-chloroethyl) sulfide (sulfur mustard) was successfully detected by DESI from SPME, whereas it was not possible to detect the same compound using an LC-ESI-MS/MS method. These results suggest that the desorption using DESI was more efficient than the extraction procedure used by the authors for the LC procedure as well as having the advantage of a much faster and simplified analytical protocol.

DART analysis of explosives, toxic industrial compounds, and chemical warfare agents

The initial DART publication by Cody et al. [12] demonstrated the direct detection of solid-phase explosives and toxic industrial compounds. In addition, arson accelerants, inorganic explosives, as well as trace dynamite residues were successfully detected. Ions corresponding to the molecular weights (MWs) of these compounds are typically observed. To improve the ionization of some explosives, dopants can be added, including 1% trifluoroacetic acid octahydro-1,3,5,7-

tetranitro-1,3,5,7-tetrazocine (HMX) RDX, and dichloromethane for the detection of nitroglycerin. Toxic industrial compounds such as toluene vapor are readily analyzed by DART using either helium or nitrogen as a metastable atom source gas yielding the protonated molecule and precursor radical cation, depending on other analytical conditions. Chemical warfare agents detected by DART include G- and V-series nerve agents and HN-series blister agents. Additional work using DART has been applied to the characterization of amine peroxide explosives, hexamethylene triperoxide diamine, tetramethylene diperoxide dicarbamide, and tetramethylene diperoxide acetamide [32]. The results of this study were validated using Raman and Fourier transfer IR (FTIR) spectroscopy.

Analysis of explosives, toxic industrial compounds, and chemical warfare agents by plasma-based methods

Low-temperature plasmas (LTPs) are used to ionize organic compounds in several of the newer ambient ionization methods. Na et al. [33, 34] used the recently developed technique of dielectric barrier discharged ionization (DBDI) for the direct detection of traces of explosives from solid surfaces. DBDI is a desorption ionization method in which a nonequilibrium LTP is generated by applying a low-power, high-frequency alternating current to electrodes that sandwich a dielectric layer. Low nanogram to picogram detection limits were achieved for TNT, RDX, and pentaerythritol tetranitrate in the negative ion mode. Typical ions observed included simple deprotonated analyte as well as nitrate adducts. Successful trace analysis of TNT from cloth, paper, soil, and paints was achieved. Even from the different substrates this method demonstrated little deviation in sample analyses with respect to analyte signal, suggesting this ionization technique is fairy reproducible. The simple DBDI source, as well as being a solvent-free method, requires less source gas than either DESI or DART.

A related method and one of the newest ambient ionization methods, LTP ionization, has also been applied to the analysis of explosives. In the LTP probe, the electrodes are formatted so that the plasma is allowed to impinge directly on the sample of interest [35]. The method is related to plasma-assisted desorption/ionization and DBDI but uses notably lower power consumption and lower carrier gas flow. The LTP method is reported to achieve the detection of RDX and HMX from polytetrafluoroethylene surfaces with low (5 pg) detection limits. Another advantage of this method is the capability to analyze samples directly from an aqueous medium. For example, atrazine, a common pesticide, can be readily detected from a beaker of its aqueous solution. This offers an advantage over the DESI method, which normally requires time for the sample to dry, although recent reports show that DESI can be used to examine aqueous solutions [36] spread as a liquid film across a suitable surface. The LTP method is still being developed and optimized, but even at its current state of development it gives encouraging performance and its size and power requirements are such that it is well suited to be fitted onto a miniature mass spectrometer.

Another plasma-based method, desorption atmospheric pressure chemical ionization (DAPCI), uses a corona discharge ionization source to ionize organic vapors such as toluene and uses this combination to ionize organic solids on surfaces [37]. In an extension to this method, Chen et al. [38] have used water molecules in humid ambient air as the reagent as an alternative to solvents such as toluene for the ambient detection of explosives, chemical warfare agents, and herbicides from skin and cloth. Trace amounts of RDX and TNT were detected from cloth material and their presence was confirmed via MS/MS analysis. For instance, protonated RDX was observed at *m/z* 223 in the full mass spectrum and upon fragmentation the main fragment observed, *m/z* 177, corresponded to the loss of a nitro group, NO_2. The deprotonated species of both TNT and RDX were observed in the negative ion mode. With use of MS/MS, subpicogram detection limits have been achieved for these and other classes of compounds. Limits as low as 50 pg were achieved for RDX in the positive ion mode, with a measured linear dynamic range of 5 orders of magnitude. This method has also been applied to powder-based samples and the ability to work with fine particles is an advantage over most ambient ionization methods.

Illicit drugs

Plant material

The analysis of cannabinoids directly on cannabis leaves, blooms, and resins was performed by DESI [39] and by desorption atmospheric pressure photoionization (DAPPI) [40]. In DAPPI, a heated gas thermally desorbs analyte from the surface into the gas-phase and this is followed by the photoionization step. Dopants are normally added to the gas to facilitate ionization. Both protonated molecules and molecular cation radicals can be generated using DAPPI, depending on the molecular structure and the solvent used. For instance, when toluene is used, a charge exchange mechanism is promoted, while acetone promotes ionization thru a more conventional proton transfer mechanism [40].

DESI analysis of cannabis leaves showed one major peak at *m/z* 315. This peak is composed of protonated Δ^9-tetrahydrocannabinol (Δ9-THC), cannabidiol, and canabichroneme; each isomer has a MW of 314. Other lower-intensity peaks were observed at *m/z* 311, 327, 341, and 359, in agreement with minor cannabinoid components of cannabis such as cannabinol (MW 310) and the carboxylic

acid form of Δ^9-tetrahydrocannabinol (MW 358). DAPPI analysis of blooms (marijuana) showed peaks at *m/z* 314 and 315 when toluene was employed as the dopant solvent, whereas with acetone only the peak at *m/z* 315 was observed. These peaks corresponded to the molecular radical cations (*m/z* 314) and protonated molecules (*m/z* 315) of the cannabinoids as mentioned above. For resin (hashish) analysis, additional peaks at *m/z* 310 (toluene as solvent) and 311 (acetone or toluene as solvents) were observed. Confirmatory analysis by MS/MS identified these peaks as principally due to the molecular cation radical (*m/z* 310) and the protonated molecule (*m/z* 311) of cannabinol.

Currency and fabrics

Analysis of cocaine on currency was first shown by DART [12] using a time-of-flight (TOF) laboratory-scale instrument and later by DESI with a handheld mass spectrometer [41]. In addition to cocaine, heroin and methamphetamine were detected as protonated molecules in fabrics such as cotton and polyester by DESI [19]. Quantitative measurements were performed for cocaine on cotton fabric. Based only on the cocaine signal, without addition of an internal standard, the linearity of the response (judged by the correlation coefficient) was R^2=0.989 over the dynamic range 5–2,500 ng of cocaine spotted. When an intrinsic compound within the cotton which gives a signal at *m/z* 245 was used as an internal standard, the correlation coefficient improved to R^2=0.994 over the range 5–5,000 ng [19].

Pharmaceutical formulations

The ability to interrogate compounds directly from tablets and other formulations was demonstrated in early DART [12] and DESI [11] publications. Forensic analysis of drug formulations usually involves evaluation formulations for undeclared compounds, for formulations that lack active compounds, or for their presence in concentrations lower than those declared, as well as identifying confiscated tablets from the streets. In a typical study, ten ecstasy tablets seized from the local drug market in Switzerland were successfully interrogated by DESI-MS/MS [42]. The results showed methylendioxymethamphetamine (MDMA) as the major component in four of the tablets as demonstrated by MS/MS. Other analytes, such as *N*-methyl-1-(1,3-benzodioxol-5-yl)-2- butanamine, amphetamine, 4-methylthioamphetamine (4-MTA), and caffeine, were also identified in the tablets by direct ambient analysis. The results were confirmed by GC-MS and LC-MS and only in one case was DESI not successful in desorbing ions from a tablet containing 4-MTA.

In parallel work, Rodriguez-Cruz [39] employed DESI to interrogate five possible suspected ecstasy tablets. The full-scan mass spectra showed that the major component was protonated MDMA at *m/z* 194. Another analyte, associated with a signal detected at *m/z* 150, was consistent with methamphetamine. IR spectroscopy and high-performance LC were used to confirm the data obtained by DESI-MS.

DAPPI-MS/MS was used by Kauppila et al. [40] to recognize confiscated illicit tablets and blotter papers. Toluene and acetone were used as the spray solvent and the analysis of ecstasy tablets showed protonated amphetamine and methamphetamines with the use of both solvents. The DAPPI spectra obtained using acetone and toluene were similar to those obtained using DESI [39, 42]. Blotter paper containing lysergic acid diethylamide (MW 323) showed both the molecular ion (*m/z* 323) and the protonated molecule (*m/z* 324) using toluene with similar intensity, whereas only the protonated ion was detected when acetone was sprayed (Fig. 2).

Tablets containing phenazepam (MW 349) were also analyzed and a typical isotopic pattern characteristic of a compound which has one bromine and one chlorine atom at *m/z* 349, 351, and 353 was observed. A typical bromine isotopic ratio (*m/z* 294:*m/z* 296, 1:1) was observed when blotter paper containing bromobenzodiuranylisopropylamine (bromo-Dragonfly, or ABDF) (MW 294) was interrogated.

Biological fluids

The use of ambient MS has also been reported for the analysis of illicit and abused drugs in biological fluids. Most but not all the methods employ sample extraction of the drugs before their analysis. Kauppila et. al [43] reported a qualitative DESI method for screening benzodiazepines, opiates, cannabinoids, amphetamines, and their metabolites

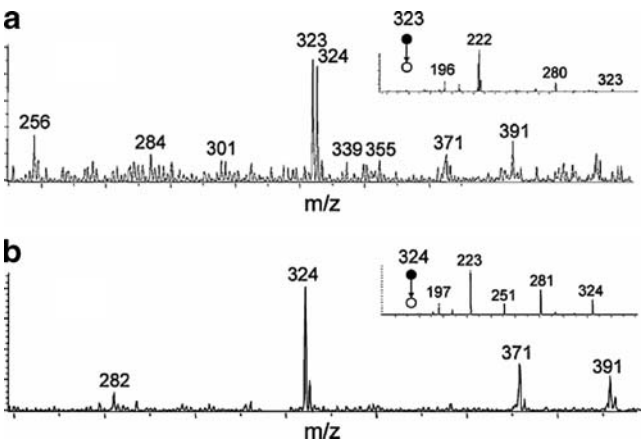

Fig. 2 Analysis of blotter paper which contained lysergic acid diethylamide by desorption atmospheric pressure photoionization in positive mode. Spray solvents **a** toluene and **b** acetone both at 2 μL/min. *Inserts* show the product ion MS/MS of the radical cation, *m/z* 323 (**a**) and the protonated molecule, *m/z* 324 (**b**). (Reproduced with permission from [40])

from urine of drug abusers after liquid–liquid extraction. The compounds studied were temazepam, oxazepam, *N*-desmethyldiazepam, *para*-hydroxytemazepam, codeine, morphine, oxymorphone, codeine, amphetamine, Δ^9-tetrahydrocannabinol, and 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol. The authors showed acquisition times of approximately 30 s per sample for DESI in contrast to GC, which typically takes several minutes per sample. The results obtained from DESI were compared with those obtained by GC-MS selected ion monitoring, and showed good correlations between the methods, with much faster analysis by the ambient ionization method.

Huang et al. [27] proposed a screening method for anabolic steroids in whole urine by combining reactive DESI and MS/MS. Reactive DESI employed hydroxylamine as the reagent and it was included in the water/methanol spray solution; analytes with carbonyl groups reacted with the amine to yield covalent adducts which dehydrated to give the oximes. Urine spiked with androsterone hemisuccinate, epitestosterone, and 5α-androstan-3β,17β-diol-16-one was analyzed without sample preparation and also after extraction using SPME. Use of SPME improved the concentration detection limits of these steroids, yielding a limit of detection (LOD) of 20 ng/mL.

Screening of the anabolic drug clenbuterol in urine by DESI was reported by Lin et al. [44]. They found that solid-phase extraction was necessary to obtain an adequate LOD, close to 2 ng/mL, which is the LOD required by the World Anti-Doping Agency. These authors also reported that the direct assay of urine by DESI-LTQ-MS allowed a LOD of 1 μg/mL. Good linearity was obtained for the calibration curve in the range 10–400 ng/mL using spiked urine samples without an internal standard. The recovery calculated from the use of aqueous standards ranged from 57.8 to 79%, whereas the recovery calculated using standard spiked urine ranged from 82 to 111%, suggesting that even with the solid phase extraction procedure, matrix effects were still present [44].

It is important to note that the presence of drugs in low concentration in complex matrices such as blood or urine may still require sample preparation. Ion suppression in MS affects all ionization techniques, including DESI and other ambient ionization methods. However, for bioanalysis even with the requirement of sample extraction, the ambient ionization techniques still offer advantages since they bypass much of the time-consuming separation process.

Counterfeit drug formulations

Counterfeit drugs are an increasing public health problem which affects both developed and developing countries. Many cases of counterfeit products that mimic the antimalarial artesunate have been reported and the consumption of these fake tablets can cause the death of the patients [45]. Classical methods of counterfeit tablet analysis are cumbersome as LC-MS requires about 1 h of sample preparation and chromatography run time and the analysis step involves expensive equipment. The new ambient desorption ionization techniques require minimal sample preparation and so offer the possibility of directly interrogating the tablets and represent a powerful tool for recognizing fake tablets. The cost of equipment is still high, but with its miniaturization (see "Conclusion") significant cost savings should occur.

Fernandez et al. [46] showed that the distinction between fake and genuine artesunate tablets is possible employing DART coupled to a TOF mass spectrometer. Artesunate was analyzed in the negative ion mode, through detection of the deprotonated molecule (*m/z* 382), and in the positive ion mode by adding NH_3 as a dopant agent to yield the ammonium adduct, $[M+NH_4]^+$. In both the positive and the negative ionization modes, fragments due to dissociation of the highly labile artesunate carboxylic acid side chain were detected. From the 52 tablets analyzed by DART, 43 were found to be fake, containing no detectable artesunate. This result was validated by colorimetric and LC-UV analysis. The authors showed mass spectra from fake tablets containing only excipients (palmitic and stearic acid), unexpected pharmaceuticals (chloramphenicol and metronidazole), and artemisinin (the naturally occurring chemical found in *Artemisia annua* and the precursor for artesunate synthesis). More alarmingly, the authors reported results on tablets containing just a fraction of the active ingredient artesunate. When a full-dosing regimen is not followed it can genetically select artesunate-resistant parasites, making this drug useless for the treatment of malaria.

In their continued work, these authors investigated the combined potential of DESI and microattenuated total reflection FTIR (ATR-FTIR) as orthogonal and complementary tools for rapid screening and characterization of counterfeit antimalarial tablets [47]. Imaging by ATR-FTIR reveals inhomogeneities in some tablets, but DESI improved specificity and sensitivity, being able to detect compounds such as sulfadoxine and pyrimethamine in fake tablets which are not detected by ATR-FTIR. It is interesting to note that the same counterfeit tablets containing low amounts of the active ingredient when analyzed by DESI or ATR-FTIR did not reveal any signal for artesunate. However, by adding dodecylamine (DDA) to the sprayed solvent (i.e., using reactive DESI), the authors were able to detect the low-intensity artesunate signals as a proton-bound noncovalent artesunate complex with DDA ($[A+DDA+H]^+$, *m/z* 570; Fig. 3); the complex with acetaminophen was also observed. The screening of antimalarial tablets by reactive DESI, which makes use of the reaction between primary

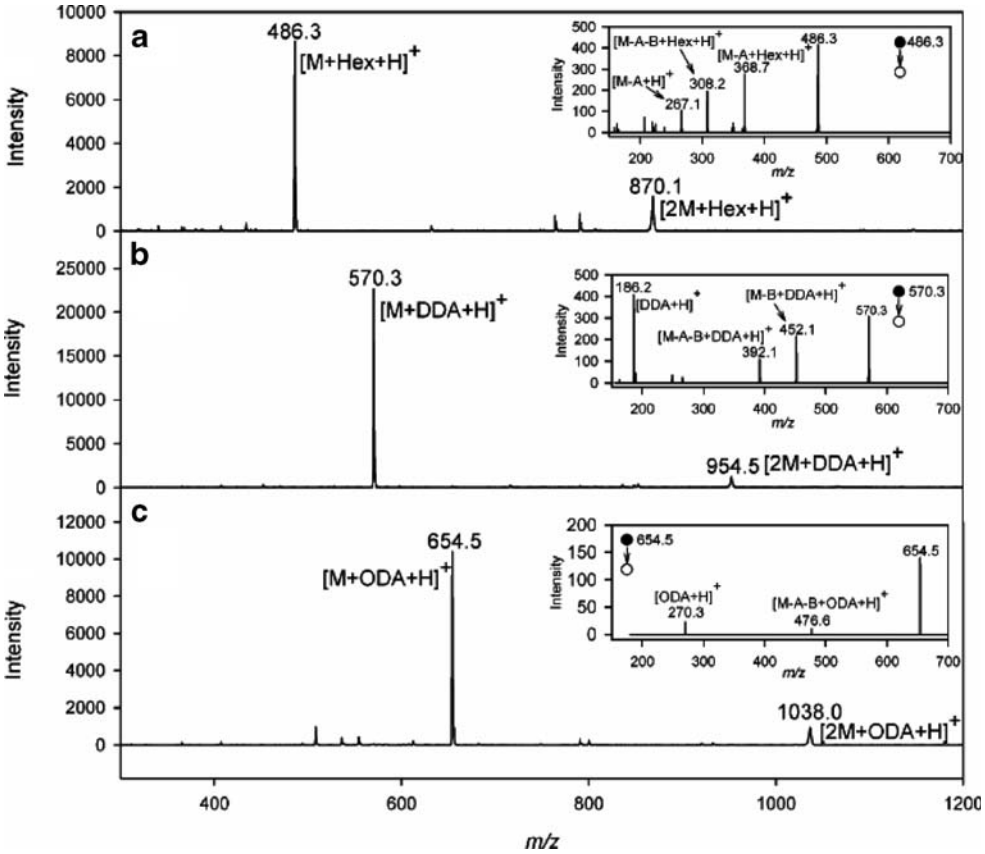

Fig. 3 Reactive-DESI mass spectra in positive mode for genuine artesunate tablets sprayed with solutions containing 100 μM **a** hexylamine (*Hex*), **b** dodecylamine (*DDA*), and **c** octadecylamine (*ODA*) in 75:25 CH_3CN–H_2O. *A* is $C_4H_6O_4$; *B* is $C_2H_4O_2$. The *inserts* depict MS/MS product ion spectra of the [M+amine +H]$^+$ ions with *m/z* 486.3, *m/z* 570.3, and *m/z* 654.5. (Reproduced with permission from [29])

alkylamines and artesunate, guaranteed better specificity and sensitivity compared with DART and conventional DESI [29]. Some of the newer counterfeit tablets contain small amounts of the therapeutic compound to defeat the current authentication colorimetric tests used for the screening of antimalarial tablets. Hence, quantitative screening methods are necessary, and the possibility to perform a quantitative analysis by DESI was investigated by Nyadong et al. [48]. DESI geometrical parameters strongly influence the DESI signal and moreover in the case of pharmaceutical solid dosage forms there is an additional dependence of the DESI signal on sample properties such as hardness and shape. The use of labeled internal standards enable DESI quantitation [49]. Nyadong et al. [48] investigated different sample pretreatment methods with labeled internal standard with pneumatically assisted electrospray deposition, addition of an internal standard into the DESI spray solution, and pipette deposition of a neat acetonitrile internal standard solution. They obtained better results by pipette deposition of a thin solution film onto the surface of the sample. A calibration curve in the range from 0.02 to 0.32 mg of artesunate showed good linearity, with a correlation coefficient of 0.9985.

Nyadong et al. [50] also developed a reactive-DESI method for the potential screening of Tamilu® tablets. Tamiflu® contains oseltamivir, a neuramidase inhibitor antiviral, as the active ingredient. The demand for those tablets has increased owing to fears of a pandemic caused by recent outbreaks of avian influenza. Its high cost and demand quickly made it a potential target for drug counterfeiters. Reactive DESI was employed for its ability to enhance the selectivity and the specificity of the method. With use of reactive DESI, it was possible to avoid the spread of the analyte signal intensity due to in-source fragmentation and formation of dimeric species in conventional DESI, resulting in a tenfold improvement in the LOD. The formation of complexes between oseltamivir and crown ethers was exploited to perform reactive DESI. Various crown ether hosts were assessed and the stability of the complexes generated was evaluated by solution and gas-phase studies. Using a mixture of crown ethers added in the solvent spray (15-C-5 and 18-C-8), they showed that quantitative prediction by reactive-DESI analysis and by high-performance LC of Tamiflu® tablets purchased on-line were both 91% accurate.

Chingin et al. [51] applied another amibient ionization method, extractive ESI (EESI), for the rapid classification of complex samples such as perfume formulations. In EESI, two spray sources are used—unlike DESI, which uses only one. The solvent is electrosprayed and it extracts analyte from the sample droplets, which are also nebulized but not deliberately ionized. Ten authentic perfumes were analyzed together with some of their analogs (inspired formulations)

and counterfeit formulations. Differentiation was based on characteristic compounds present in the mass spectra (fingerprinting) from the authentic and counterfeit samples. A similar approach was employed using the easy ambient sonic-spray ionization method to discriminate between authentic and fake perfumes [52].

Food adulterants

Melamine (2,4,6-triamino-1,3,5-triazine) is a chemical compound commonly used in the fabrication of plastics, glues, inks, and fertilizers. It has no approved use as an ingredient in human or animal food. When mixed with wheat gluten, milk, and other foods, it can make a product appear in commonly used low-specificity tests to have a higher protein level than is actually the case. Melamine-contaminated foods have caused illnesses and deaths [53]. DART was successfully applied to the direct determination of melamine in confiscated pet food (Fig. 4) [54]. The presence of melamine was confirmed by accurate mass measurements, isotope peak intensities, in-source fragmentation, and H/D exchange experiments for determination of the number of active hydrogens. The LOD of melamine in the pet food was 1 ppm on the basis of in-source fragmentation experiments. More recently, the LTP method has been used to detect trace melamine contamination in whole milk and related products [55]. In these experiments the milk is heated and the water evaporated; the solid is exposed to the LTP probe and the presence of melamine down to less than 10 ppb is determined and confirmed by MS/MS, all in less than 30 s. Ultrasound-assisted EESI has also been employed for the determination of melamine in milk and wheat gluten [56]. The LODs were 500 ppb (S/N 3) and 1 ppm (S/N 7) for melamine-spiked milk and 200 ppb (S/N 3) and 1 ppm (S/N 15) for methanol-extracted wheat gluten.

Sudan dyes are diazo-conjugated compounds illegally used as colorants in foodstuff. Owing to their carcinogenic potential, their addition to foods is forbidden in many countries. Chen et al. [38] suggested that this class of compounds might be detected in foods by DAPCI. In their experiment, a few nanograms (5–10 ng) of Sudan dyes

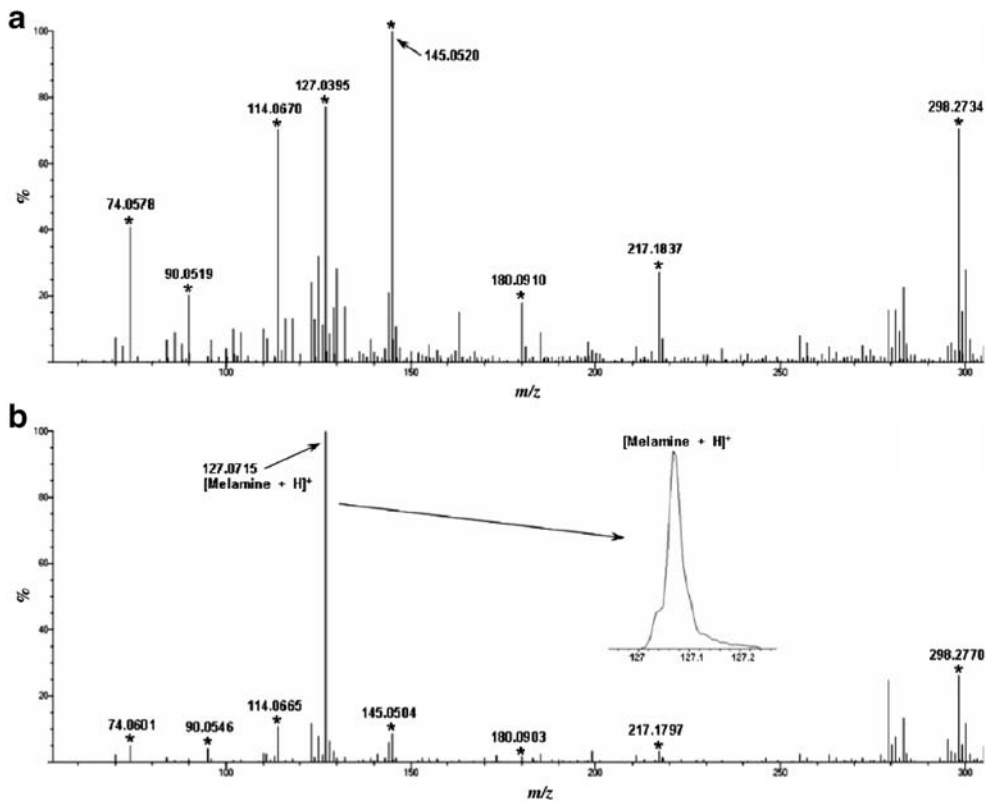

Fig. 4 Direct analysis in real time mass spectra of **a** uncontaminated and **b** contaminated dog food. Mass-spectral peaks endogenous to the dog food are marked with *asterisks*. The base peak in **a** is at *m/z* 145.0520. The base peak in **b** is at *m/z* 127.0715, consistent with the exact mass of a protonated molecule of melamine. Notice that all the endogenous mass-spectral peaks in the contaminated dog food have a much lower intensity relative to those of the protonated molecule of melamine in the mass spectrum of the contaminated dog food. A mass-spectral peak at *m/z* 127.0395 is present in the mass spectrum of the uncontaminated dog food; it is also visible in the profile display (*insert*) of the melamine-protonated molecule peak, but does not affect the peak assignment. (Reproduced with permission from [54])

(I–IV) were successfully detected and identified by MS/MS in a tomato sauce matrix.

Cajka et al. [57] demonstrated profiling of soft drinks by DART-TOF-MS. They showed that several components of soft drinks such as sweeteners, saccharides, and acidulates can be detected and therefore used for the determination of product authenticity and quality control.

Many countries have limitations on the nature and amounts of trace residues that are permitted in foods. Recently, DESI, DART, and atmospheric pressure glow discharge [58] have been applied in the analysis of wheat grain, fruits, and vegetables for pesticides, herbicides, and fungicides. As such, these techniques have not only the capability of detecting the various types of compounds, but they also allow quantitation at the low levels within extracts of the samples. In the case of DESI, pesticide residues [59] were detected directly from the skin of market-purchased fruit and vegetables. These methods give LODs within an order of magnitude below conventional LC-MS (ESI) methods applied after sample workup and fall within European regulated ranges of LODs in almost all cases studied.

Inks and document authenticity

Ink analysis of documents has been demonstrated by DART [60] and DESI [61]. Both techniques are useful for discrimination between different inks on the basis of their formulation. For example, DART was shown to be capable of discriminating between fluid and gel ink pens and a library containing DART mass spectra of various pen types has been created to identify the specific pen type used on a document [60]. When DESI is coupled to a two-dimensional moving stage, the chemical information (mass spectrum) can be recorded together with the *X* and *Y* position of the spray spot [62]. After the entire surface has been rastered, maps of specific ions can be created (Fig. 5). The resolution for DESI experiments was found to be around 150–300 μm (pixel size); however, under more highly controlled conditions, a resolution of 40 μm could be reached [63]. Ink analysis can also discriminate between counterfeit and authentic banknotes, as demonstrated by combining easy ambient sonic-spray ionization MS with principal component analysis (R. Haddad, R.C.S. Neto, R. G. Cosso, D.R.J. Maia, A.O. Maldener, G.B. Sanvito, L.S. Eberlin, and M.N. Eberlin, unpublished results). In addition, Huang et al. [64] demonstrated the ability to analyze pigments directly on a painting by electrospray laser desorption/ionization MS. Because the laser covered only a very small area, no visible degradation of the painting was observed after electrospray laser desorption/ionization MS analysis. This observation and related work on the examination of archeological and art objects by DESI opens another area of application of ambient desorption ionization.

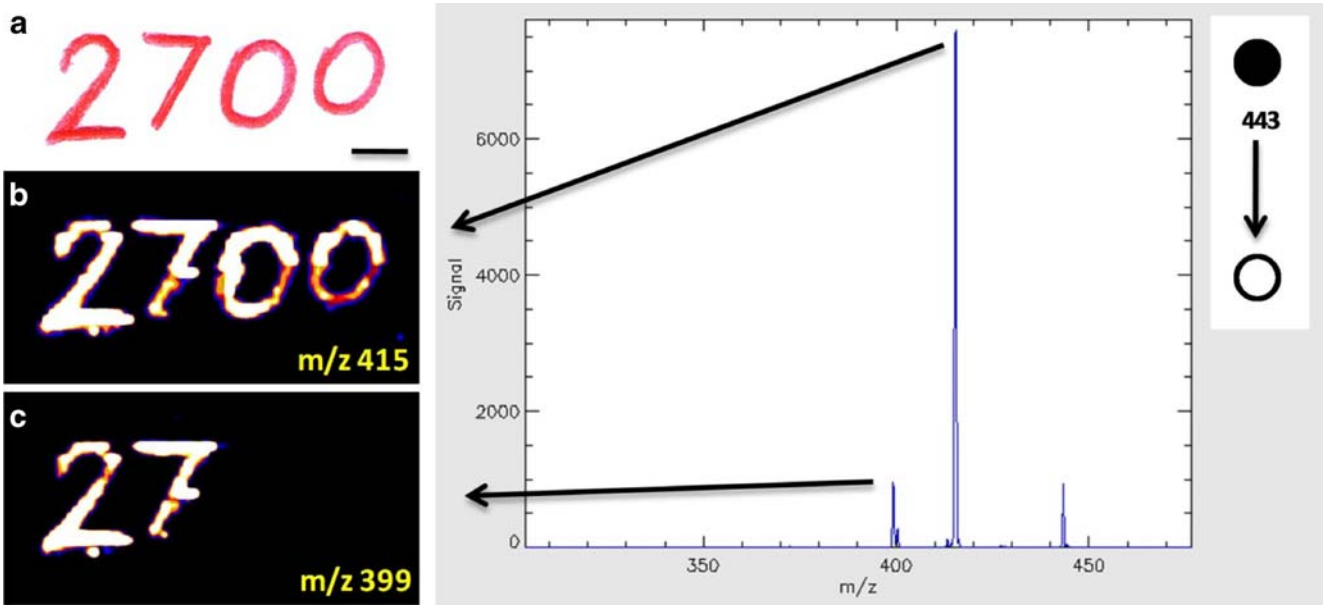

Fig. 5 **a** Optical image of handwritten text on paper. The red pen used to create the characters "27" contained a mixture of the pigments rhodamine B and rhodamine 6G. The second pen, used to create the characters "00", contained only rhodamine B. Although both pigments have the same mass-to-charge ratio (*m/z* 443), DESI-MS/MS distinguishes them by their fragmentation pattern. **b** Chemical imaging of rhodamine 6G followed by its main fragment *m/z* 415 (neutral loss of ethylene). **c** Chemical imaging of rhodamine B followed by its main fragment *m/z* 339 (neutral loss of CO_2)

Fingerprints and skin analysis

Ifa et al. [65] recently reported the application of DESI to the imaging of latent fingerprints (Fig. 6). DESI is capable of producing an image based on any particular ion in the mass spectrum recorded at different positions within a latent fingerprint. Endogenous compounds such as the fatty acids and lipids known to be present on the skin can be detected and identified. In tests of this capability, when the finger is doped with 5 μg of an analyte such as a drug of abuse or an explosive, these compounds are detected in the mass spectra taken on the latent print. The latent prints have been successfully imaged from a variety of surfaces, including glass, paper, and plastic. Note that individual compounds can be identified on the basis of the molecular ion in the mass spectrum, and/or any characteristic fragment ions. MS/MS can be used to further increase the specificity with which particular compounds in latent fingerprints are observed. The latent prints not only provide information on who the individual is, but also information on his/her recent activities. With advances in research methods, ambient MS can be used for on-site analysis of virtual fingerprints as well as for direct analysis of skin.

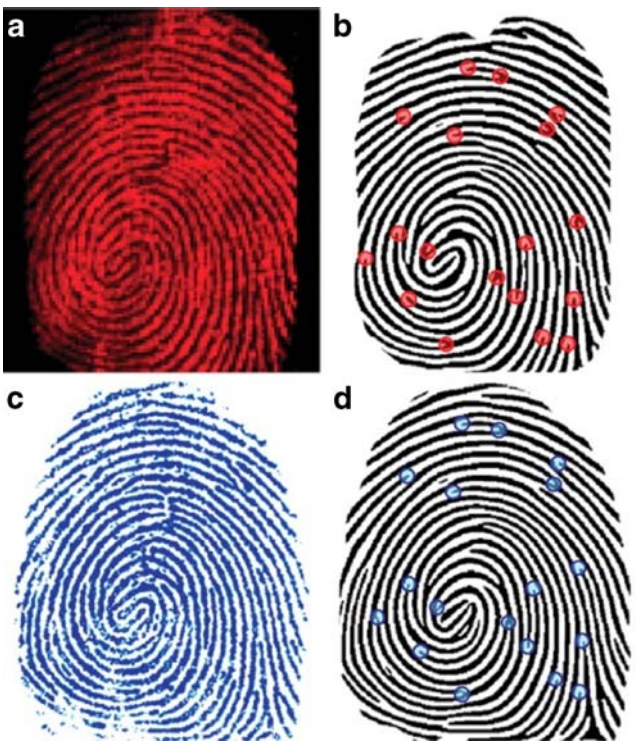

Fig. 6 **a** DESI image of the distribution of cocaine on a latent fingerprint blotted on glass. **b** Computer-generated fingerprint from the DESI image. **c** Ink fingerprint blotted on paper and optically scanned. **d** Computer-generated fingerprint from the optical image. Some of the automatically detected points of interest (minutiae) are represented by *dots* in **b** and **d**. (Reproduced with permission from [65])

The fact that DESI imaging of a virtual print gives the normal physical information connects the individual to the compounds found in the print. Overlapping prints can be distinguished. Insights may also be provided regarding an individual's diet and medication. Analysis of TNT and oxycodone (drug of abuse) on fingerprints blotted onto plastic and glass, respectively, was also demonstrated by DART [66], although chemical images of fingerprints have so far only been reported by DESI, but they are almost certainly accessible by other ambient ionization methods.

Several of the ambient ionization methods have been reported to be safe enough for direct analysis of skin, which suggests applications both within and outside forensics. Compounds of forensic interest have been detected from skin using various ambient ionization methods. With use of the DART method, skin analysis has been achieved [12], but specific compounds have not been reported. Chen et al. [67, 68] have used EESI for the analysis of biological samples, including those on skin. Caffeine from coffee has been detected using this method. After 30 and 60 min of consumption, there was a noticeable increase in the signal of caffeine, *m/z* 195, which was confirmed by tandem analysis through observation of the main fragment *m/z* 138. DAPCI has also been used in the detection of nicotine and dimethyl methylphosphonate (a chemical warfare agent stimulant) doped on the forearm of a smoker [38]. Confirmation of the stimulant was through ions *m/z* 111, 125, and 147, corresponding to a water adduct of the product ion, the protonated species, and the sodium adduct, respectively. Nicotine, evidence of the individual's smoking habit, was also detected in the full mass spectrum taken from the forearm. With use of the LTP ionization method, 1 μg of cocaine has been directly detected from the skin in the positive ion mode [35]. These methods are ideal for the analysis of skin as they are all solvent-free methods. DESI has also been used successfully for the detection of pharmaceutically active ingredients, explosives, drugs of abuse, and gunshot residues from skin. Loratidine, the active ingredient of Claritin®, was successfully detected from the finger 30 min after consumption [11]. Nanogram amounts of TNT, RDX, HMX, pentaerythritol tetranitrate, and their mixtures were successfully detected from skin using a spray solvent of methanol/water (1:1) doped with sodium chloride to create chloride adducts of several of the explosives in the negative ion mode; the radical anion of TNT was observed [23]. The presence of these explosives from skin was confirmed not only from the chlorine isotopes profiles, but also MS/MS using collision-induced dissociation, but also from the chlorine isotope profile. Methyl centralite and ethyl centralite, gunshot residues, were both detected from skin and confirmed via DESI-MS/MS analysis [69]. Monitoring of the main fragmentation pathway of the analytes, methyl centralite *m/z* 241 → 134

and ethyl centralite *m/z* 269 → 148, resulted in the finding that the residues could be detected from a person's skin approximately 12 h after shooting, after six hand washings. These analyses further demonstrate the sensitivity of the DESI technique and its direct application to the field of forensic science.

The detection of endogenous metabolites from skin also has forensic applications with regards to identifying individuals on the basis of their intrinsic chemical profiles. With use of a modified EESI source which included a heated region (80 °C) prior to the mass spectrometer inlet, to optimize the analysis of neutral molecules, the analysis of different skin surfaces such as the forehead, abdomen, foot, forearm, and finger was achieved [68]. Different mass-spectral profiles were observed from the skin surface. While this method has been used to evaluate different skin types, it has not been applied in the identification of or distinction between individuals.

A variation of the conventional DESI analysis, geometry-independent DESI permits the analysis of samples within an enclosed atmosphere [70]. The sampling inlet and DESI spray are mutually parallel and oriented normal to the surface, eliminating the need to reoptimize the geometry for different samples, surface textures, or curvatures as in the traditional analysis. Geometry-independent DESI is currently being used to determine if individuals have characteristic chemical fingerprints and if these can be diagnostic of metabolic processes.

Conclusions

As expected for a rapidly developing technology, the majority of the applications highlighted in this review are still at the proof-of-concept stage. Forensic applications of explosives, toxic industrial compounds, and chemical warfare agents by ambient ionization methods have been well explored by a variety of individual methods. However, these analyses are limited by the fact that they are usually done from standard solutions or from complex matrices doped with standards. Similarly, except for a few cases, pure chemicals were spiked onto surfaces of interest such as food or skin for drug analysis. Counterfeit tablets and drugs of abuse have been thoroughly explored by ambient methods as these samples are somewhat easier to obtain.

The majority of applications of ambient ionization found in the literature were performed by the established techniques DESI and DART, the two earliest and at present the only commercially available methods. However, more than a score of ambient ionization techniques have been reported and this number is still growing. Obviously, experiments now under way will determine the areas of most appropriate application of the individual methods. With the maturation of ambient MS techniques, ambient ionization seems set to become a valuable tool for the forensic sciences.

It is clear that the ambient ionization methods in general, DESI and DART in particular, are capable of detecting compounds of a wide range of chemical types in complex samples in short times, based on the minimal need to perform sample workup. The literature on DESI is larger than that on the other methods, so more problems have been solved this way. In the experience of this laboratory, there are few if any low MW compounds (below 1,000) that cannot be detected by DESI from a variety of matrices. The plasma methods appear to be slightly more limited in scope, based on our experience with DAPCI and LTP and the literature of DART. A reviewer asked about the relative merits of the various ionization methods and when they might be used. The advantage of DESI is that it is a solution-based process. By choice of an appropriate solvent, the underlying microextraction step can be performed on almost any analyte. The disadvantage of DESI is that the solvent has to be propelled and this requires high-speed gas; the result is that more power and weight is involved than (for example) with LTP or DART. Conceivably this could limit the size of miniature mass spectrometers equipped with ambient ionization sources (see below).

Matrix effects are a factor in low-efficiency ionization in each of the ambient methods but it is also clear from recent published and unpublished work that compounds that are difficult to ionize in one solvent system (e.g., the common methanol/water system, which is particularly good for polar or ionic compounds, either acidic or basic) can often be ionized in other solvent systems.

It is one of the great strengths of DESI that it is a method of ionization based on chemical processes: electrical charge applied to the solution produces charged droplets, which in aqueous solutions lead to an excess of hydronium ions or hydroxide ions and hence to protonated or deprotonated analytes, which are observable in the positive ion and negative ion modes, respectively. This strength as a chemical process extends also to the addition of reagents to the spray solvent as a means of changing the analyte to a compound which is preferentially ionized or of increasing ion yields. Consider, for example, the lipid cholesterol, which is not readily ionized using the methanol/water solvent system. However, when betaine aldehyde is added to the spray solvent, this quaternary species forms a complex with cholesterol which dominates the mass spectra of tissue sections previously dominated by phospholipids signals (C. Wu, D.R. Ifa, N.E. Manicke, and R.G. Cooks, unpublished results).

Turning to the subject of quantitative analysis, the performance of the ambient ionization methods is dictated by the constraints of addition of standards. If this can be done, then normal MS precision and accuracy are available,

e.g., 10% relative standard deviation and 10% accuracy in typical circumstances. If a natural material must be examined and it is not possible to mix in a standard, then an intrinsic compound of known concentration can be used as a standard, or the material can be compared with a standard material. Both procedures are likely to be less satisfactory in terms of quantitation.

Reactive or in situ derivatization experiments seem to be a unique advantage of ambient ionization MS techniques over conventional methods. By addition of a specific compound to the spray solvent or the gas flow, chosen so that it reacts selectively with the analyte of interest. The reaction product is normally easier to detect. For instance, spraying solutions of protonated hydroxylamine on steroids promotes the reaction between the hydroxylamine and the carbonyl groups on steroids. The reaction product, an oxime, retains a positive charge, making the new product less susceptible to ion suppression [27]. We foresee many forensic applications in the future that will depend on the enhanced specificity of reactive or in situ derivatization experiments.

Another significant development is that of standoff, or nonproximate, detection [20]. In these experiments the mass spectrometer is some distance (up to a few meters) from the sample and an extended ion transfer tube is used to transport ions and any associated solvent to the mass spectrometer. With the recent coupling of DESI to miniature mass spectrometers, using the highly effective discontinuous atmospheric interface [71], on-site detection of chemical warfare agent stimulants such as dimethyl methylphosphonate and other compounds is achieved [24]. These are important experiments since they presage the future use of portable handheld mass spectrometers for immediate on-site chemical analysis.

The ionization source must be minimized in weight, size, and expendables to make it suitable for portable applications. The interfacing of ambient ionization sources to portable mass spectrometers to use on-site for immediate detection of specific analytes is a development that is under way. It should be particularly important in detecting compounds of interest to first responders at incident scenes. If this capability is successfully developed, it will represent a major step forwards in dispersing the powerful capabilities of MS, especially those which are simple to apply to real-world, complex samples in a wide variety of milieu.

References

1. Brettell TA, Butler JM, Almirall JR (2007) Anal Chem 79:4365–4384
2. Brettell TA, Butler JM, Saferstein R (2005) Anal Chem 77:3839–3860
3. Costello CE, Hertz HS, Sakai T, Biemann K (1974) Clin Chem 20:255–265
4. Kelly RL, Martz RM (1984) J Forensic Sci 29:714–722
5. Choudhary G, Cooper CV (1979) Am Ind Hyg Assoc J 40:39–46
6. Angeletti R, Gioacchini AM, Seraglia R, Piro R, Traldi P (1998) J Mass Spectrom 33:525–531
7. Di Donna L, Maiuolo L, Mazzotti F, De Luca D, Sindona G (2004) Anal Chem 76:5104–5108
8. Hart KJ, Wise MB, Griest WH, Lammert SA (2000) Field Anal Chem Technol 4:93–110
9. Griest WH, Wise MB, Hart KJ, Lammert SA, Thompson CV, Vass AA (2001) Field Anal Chem Technol 5:177–184
10. Ouyang Z, Cooks RG (2009) Annu Rev Anal Chem 2 (in press)
11. Takats Z, Wiseman JM, Gologan B, Cooks RG (2004) Science 306:471–473
12. Cody RB, Laramee JA, Durst HD (2005) Anal Chem 77:2297–2302
13. Cooks RG, Ouyang Z, Takats Z, Wiseman JM (2006) Science 311:1566–1570
14. Van-Berkel GJ, Pasilis SP, Ovchinnikova O (2008) J Mass Spectrom 43:1161–1180
15. Harris GA, Nyadong L, Fernandez FM (2008) Analyst 133:1297–1301
16. Venter A, Nefliu M, Graham Cooks R (2008) Trends Anal Chem 27:284–290
17. Reid NM, Buckley JA, French JB (1977) UTIAS Tech Note 213:7
18. Cotte-Rodriguez I, Chen H, Cooks RG (2006) Chem Commun 8:953–955
19. Talaty N, Mulligan CC, Justes DR, Jackson AU, Noll RJ, Cooks RG (2008) Analyst 133:1532–1540
20. Cotte-Rodriguez I, Cooks RG (2006) Chem Commum 28:2968–2970
21. Cotte-Rodriguez I, Hernandez-Soto H, Chen H, Cooks RG (2008) Anal Chem 80:1512–1519
22. Cotte-Rodriguez I, Takats Z, Talaty N, Chen HW, Cooks RG (2005) Anal Chem 77:6755–6764
23. Justes DR, Talaty N, Cotte-Rodriguez I, Cooks RG (2007) Chem Commun 21:2142–2144
24. Mulligan CC, Talaty N, Cooks RG (2006) Chem Commun 16:1709–1711
25. Song Y, Cooks RG (2007) J Mass Spectrom 42:1086–1092
26. Williams JP, Lock R, Patel VJ, Scrivens JH (2006) Anal Chem 78:7440–7445
27. Huang G, Chen H, Zhang X, Cooks RG, Ouyang Z (2007) Anal Chem 79:8327–8332
28. Chen H, Zheng OY, Cooks RG (2006) Angew Chem Int Ed 45:3656–3660
29. Nyadong L, Green MD, De Jesus VR, Newton PN, Fernandez FM (2007) Anal Chem 79:2150–2157
30. D'Agostino PA, Chenier CL, Hancock JR, Lepage CRJ (2007) Rapid Commun Mass Spectrom 21:543–549
31. D'Agostino PA, Hancock JR, Chenier CL (2008) J Chromatogr A 110:86–94
32. Pena-Quevedo AJ, Cody R, Mina N, Ramos M, Hernandez-Rivera SP (2007) Paper presented at the conference on sensors and command, control, communications and intelligence (C3I) technologies for homeland security and homeland defense VI, Orlando, 2007
33. Na N, Zhao MX, Zhang SC, Yang CD, Zhang XR (2007) J Am Soc Mass Spectrom 18:1859–1862
34. Na N, Zhang C, Zhao MX, Zhang SC, Yang CD, Fang X, Zhang XR (2007) J Mass Spectrom 42:1079–1085
35. Harper JD, Charipar NA, Mulligan CC, Cooks RG, Ouyang Z (2008) Anal Chem 80:9097–9104
36. Miao Z, Chen H (2009) J Am Soc Mass Spectrom (in press)
37. Takats Z, Cotte-Rodriguez I, Talaty N, Chen HW, Cooks RG (2005) Chem Commun 1950–1952
38. Chen HW, Zheng J, Zhang X, Luo MB, Wang ZC, Qiao XL (2007) J Mass Spectrom 42:1045–1056

39. Rodriguez-Cruz SE (2006) Rapid Commun Mass Spectrom 20:53–60
40. Kauppila TJ, Arvola V, Haapala M, Pol J, Aalberg L, Saarela V, Franssila S, Kotiaho T, Kostiainen R (2008) Rapid Commun Mass Spectrom 22:979–985
41. Keil A, Talaty N, Janfelt C, Noll RJ, Gao L, Ouyang Z, Cooks RG (2007) Anal Chem 79:7734–7739
42. Leuthold LA, Mandscheff JF, Fathi M, Giroud C, Augsburger M, Varesio E, Hopfgartner G (2006) Rapid Commun Mass Spectrom 20:103–110
43. Kauppila TJ, Talaty N, Kuuranne T, Kotiaho T, Kostiainen R, Cooks RG (2007) Analyst 132:868–875
44. Lin ZQ, Zhang SC, Zhao MX, Yang CD, Chen DP, Zhang XR (2008) Rapid Commun Mass Spectrom 22:1882–1888
45. Fernandez FM, Green MD, Newton PN (2008) Ind Eng Chem Res 47:585–590
46. Fernandez FM, Cody RB, Green MD, Hampton CY, McGready R, Sengaloundeth S, White NJ, Newton PN (2006) ChemMedChem 1:702
47. Ricci C, Nyadong L, Fernandez FM, Newton PN, Kazarian SG (2007) Anal Bioanal Chem 387:551–559
48. Nyadong L, Late S, Green MD, Banga A, Fernandez FM (2008) J Am Soc Mass Spectrom 19:380–388
49. Ifa DR, Manicke NE, Rusine AL, Cooks RG (2008) Rapid Commun Mass Spectrom 22:503–510
50. Nyadong L, Hohenstein EG, Johnson K, Sherrill CD, Green MD, Fernández FM (2008) Analyst 133:1513–1522
51. Chingin K, Gamez G, Chen HW, Zhu L, Zenobi R (2008) Rapid Commun Mass Spectrom 22:2009–2014
52. Haddad R, Catharino RR, Marques LA, Eberlin MN (2008) Rapid Commun Mass Spectrom 22:3662–3666
53. FDA (200) Pet food recall/tainted animal feed. http://www.fda.gov/oc/opacom/hottopics/petfood.html
54. Vail TM, Jones PR, Sparkman OD (2007) J Anal Toxicol 31:304–312
55. Huang G, Ouyang Z, Cooks RG (2009) Chem Commun 556–558.
56. Zhu L, Gamez G, Chen H, Chingin K, Zenobi R (2009) Chem Commun 559–561.
57. Cajka T, Vaclavik L, Riddellova K, Hajslova J (2008) LC GC Eur 21:250–256
58. Jecklin MC, Gamez G, Touboul D, Zenobi R (2008) Rapid Commun Mass Spectrom 22:2791–2798
59. García-Reyes JF, Jackson AU, Molina-Díaz A, Cooks RG (2009) Anal Chem 81:820–829
60. Jones RW, Cody RB, McClelland JF (2006) J Forensic Sci 51:915–918
61. Ifa DR, Gumaelius LM, Eberlin LS, Manicke NE, Cooks RG (2007) Analyst 132:461–467
62. Ifa DR, Wiseman JM, Song QY, Cooks RG (2007) Int J Mass Spectrom 259:8–15
63. Kertesz V, Van Berkel GJ (2008) Rapid Commun Mass Spectrom 22:2639–2644
64. Huang MZ, Hsu HJ, Wu CI, Lin SY, Ma YL, Cheng TL, Shiea J (2007) Rapid Commun Mass Spectrom 21:1767–1775
65. Ifa DR, Manicke NE, Dill AL, Cooks RG (2008) Science 321:805–805
66. Laranee JA, Cody RB, Nilles JM, Durst HD (2007) In: Blackledge RD (ed) Forensic analysis on the cutting edge: new methods for trace evidence analysis. Wiley, Hoboken, pp 180–181
67. Chen H, Yang S, Wortmann A, Zenobi R (2007) Angew Chem Int Ed 46:7591–7594
68. Chen HW, Wortmann A, Zenobi R (2007) J Mass Spectrom 42:1123–1135
69. Zhao MX, Zhang SC, Yang CD, Xu YC, Wen YX, Sun LS, Zhang XR (2008) J Forensic Sci 53:807–811
70. Venter A, Cooks RG (2008) Anal Chem 79:6398–6403
71. Gao L, Cooks RG, Ouyang Z (2008) Anal Chem 80:4026–4032
72. McEwen CN, McKay RG, Larsen BS (2005) Anal Chem 77:7826–7831
73. Haapala M, Pol J, Saarela V, Arvola V, Kotiaho T, Ketola RA, Franssila S, Kauppila TJ, Kostiainen R (2007) Anal Chem 79:7867–7872
74. Haddad R, Sparrapan R, Kotiaho T, Eberlin MN (2008) Anal Chem 80:898–903
75. Shiea J, Huang MZ, Hsu HJ, Lee CY, Yuan CH, Beech I, Sunner J (2005) Rapid Commun Mass Spectrom 19:3701–3704
76. Chen HW, Venter A, Cooks RG (2006) Chem Commun 2042–2044
77. Shieh IF, Lee CY, Shiea J (2005) J Proteome Res 4:606–612
78. Andrade FJ, Shelley JT, Wetzel WC, Webb MR, Gamez G, Ray SJ, Hieftje GM (2008) Anal Chem 80:2654–2663
79. Nemes P, Vertes A (2007) Anal Chem 79:8098–8106
80. Sampson JS, Hawkridge AM, Muddiman DC (2006) J Am Soc Mass Spectrom 17:1712–1716
81. Ratcliffe LV, Rutten FJM, Barrett DA, Whitmore T, Seymour D, Greenwood C, Aranda-Gonzalvo Y, Robinson S, McCoustrat M (2007) Anal Chem 79:6094–6101