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Direct analysis in real time mass spectrometry for analysis of sexual assault evidence

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RATIONALE: Sexual assault crimes are vastly underreported and suffer from alarmingly low prosecution and conviction rates. The key scientific method to aid in prosecution of such cases is forensic DNA analysis, where biological evidence such as semen collected using a rape test kit is used to determine a suspect's DNA profile. However, the growing awareness by criminals of the importance of DNA in the prosecution of sexual assaults has resulted in increased condom use by assailants as a means to avoid leaving behind their DNA. Thus, other types of trace evidence are important to help corroborate victims' accounts, exonerate the innocent, link suspects to the crime, or confirm penetration.

METHODS: Direct Analysis in Real Time Mass Spectrometry (DART-MS) was employed for the comprehensive characterization of non-DNA trace evidence associated with sexual assault. The ambient ionization method associated with DART-MS is extremely rapid and samples are processed instantaneously, without the need for extraction, sample preparation, or other means that might compromise forensic evidence for future analyses.

RESULTS: In a single assay, we demonstrated the ability to identify lubricant formulations associated with sexual assault, such as the spermicide nonoxynol-9, compounds used in condom manufacture, and numerous other trace components as probative evidence. In addition, the method can also serve to identify compounds within trace biological residues, such as fatty acids commonly identified in latent fingerprints.

CONCLUSIONS: Characterization of lubricant residues as probative evidence serves to establish a connection between the victim and the perpetrator, and the availability of these details may lead to higher rates of prosecution and conviction, as well as more severe penalties. The methodology described here opens the way for the adoption of a comprehensive, rapid, and sensitive analysis for use in crime labs, while providing knowledge that can inform and guide criminal justice policy and practice. Copyright © 2012 John Wiley & Sons, Ltd.

Locard's Principle, a mantra of forensic science, states that "every contact leaves a trace". In this regard, trace evidence can take numerous forms, be it macroscopic, microscopic, visible, or undetectable with the naked eye. Examples of evidence that is macroscopic and visible are numerous and logical in context, such as paint chips from a hit-and-run vehicular incident, a ransom note, or a simple footprint. On the other hand, latent and/or microscopic trace evidence is also commonplace, such as undeveloped fingerprints or residues associated with arson or the use of explosives. Arguably the most significant demonstration of a link between a criminal and a crime scene is DNA evidence.^[1–4] DNA analysis is associated with statistics so definite that a perfect match between a suspect's DNA profile and biological evidence retrieved from a crime scene is considered as an irrefutable connection between the two. Despite this fact, sexual assault crimes are vastly underreported, and suffer from alarmingly low prosecution and conviction rates.^[5–8] While forensic DNA testing

of semen, saliva, or other biological evidence can identify a perpetrator of sexual assault or exonerate a suspect, the presence of DNA alone does not prove that a crime was committed. Even when biological evidence is recovered, trace evidence can help define the case or put other evidence into context. Forensic labs can analyze trace fibers found at the crime scene, test for drugs used to incapacitate the victim, and identify lubricants used to facilitate the crime, among other determinations. However, when biological evidence is not recovered, trace evidence can play an even more important role in the investigation, by establishing an associative link between victim and perpetrator or as evidence of penetration which may lead to a higher degree of charges brought against a suspect.^[11,12]

The general awareness and universal acceptance of the importance of DNA in forensic analyses and the dramatic increase in prosecution and conviction rates associated with rape cases where DNA matches are established have resulted in criminals employing various measures to avoid leaving trace biological evidence at a crime scene. In a similar fashion to the awareness shown by a burglar who wears gloves for fear of leaving fingerprints at the scene of a robbery, a disturbing twist on this tendency also exists; as DNA testing of seminal fluid can identify a rapist, criminals committing sexual assault are knowledgeable to the extent

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that sizeable rates of condom usage have been documented.^[9–11] Thus, other types of trace evidence are required to establish a connection between a victim and the criminal. The availability of such particulars may lead to higher rates of prosecution and conviction, as well as to more severe penalties.^[12] In this regard, analysis of lubricant residues associated with condom use in sexual assaults can provide probative evidence to support a victim's claims that a crime has occurred, establish the extent of the crime, link a suspect to a victim, or confirm penetration.^[11] Several case studies have been documented where a condom has served to establish a critical link between the victim and the perpetrator.^[11,13,15]

The experimental methods that have been developed to characterize lubricants associated with sexual assault include those that utilize techniques that are commonplace in crime labs, such as microscopy, infrared analysis, and various mass spectrometry (MS) methods.^[16,17] In addition, more sophisticated MS techniques not routinely found in crime laboratories have been applied to lubricant analysis, including liquid chromatography/mass spectrometry (LC/MS) and matrix-assisted laser desorption/ionization (MALDI)-MS analysis.^[16–21] In particular, MALDI imaging-mass spectrometry^[22,23] has been applied to the forensic analysis of trace evidence, such as lubricant identification and fingerprint analysis, where visualization of the spatial distribution of sample components is possible along with mass spectrometric analysis.^[19,24] With specific regard to the analysis of lubricant formulations as

probative evidence, the aforementioned methods have deficiencies that can limit their utility. Gas chromatography/mass spectrometry (GC/MS) and LC/MS testing are time-consuming, sample preparation can be cumbersome, and the analyses involve the consumption and/or modification of the evidence. Desorption methods, such as MALDI, may be problematic as the sample needs to be pre-treated with a matrix to enable analysis, while it is desirable to maintain the evidence in its native form so as to not compromise any necessary further analyses, such as DNA testing. In addition, ion suppression has been shown to occur in the MALDI-MS analysis of biological residues, including semen and saliva, both of which are likely to be found in the context of sexual assault.^[20] Accordingly, the development of complementary procedures that can offer improvements on these methods is critical for the analysis, identification, and characterization of forensic evidence.

Direct Analysis in Real Time (DART)-MS is a powerful technique that can be used for the identification of non-DNA trace evidence associated with sexual assault.^[25–27] We demonstrate here the power and utility of the technique in revealing information on multiple aspects of non-DNA sexual assault evidence. The ambient ionization method associated with DART-MS is extremely rapid; samples are processed instantaneously, without the need for extraction, sample preparation, or other procedures that might compromise the evidence for future analyses.^[25] Because the technique does not require sample

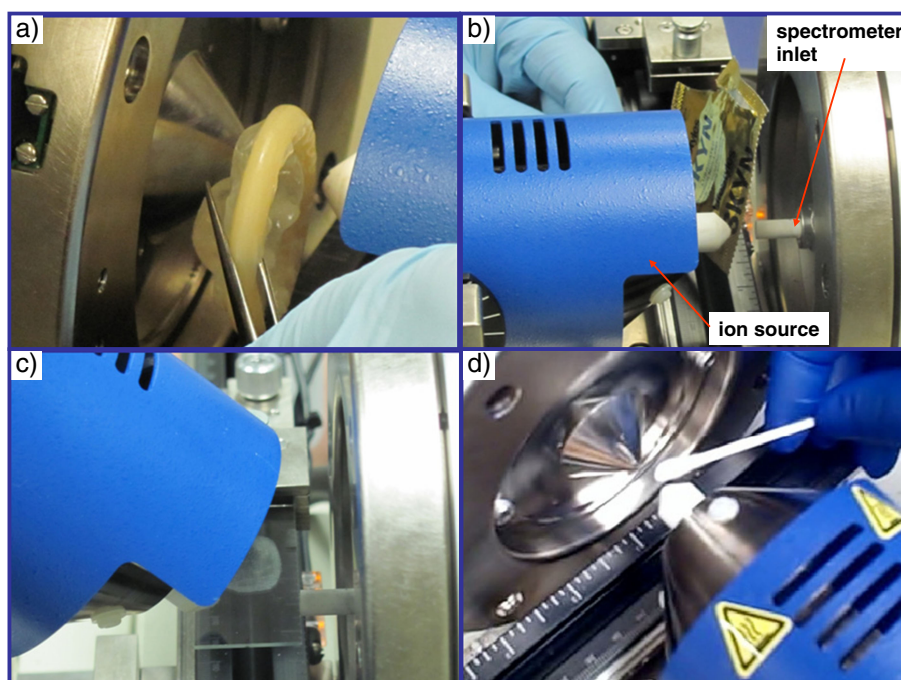


Figure 1. The utility of DART-MS. Samples of any type (solid, liquid, or gas) can be tested simply by placing the sample between the ion source and the spectrometer inlet, without requiring sample extraction or other means of preparation. In terms of evidence related to sexual assault, we show DART-MS sampling of (a) a condom, (b) a condom wrapper, (c) a fingerprint on a glass microscope slide, and (d) a swab, similar to that found in a rape test kit.

preparation, evidentiary materials can be analyzed in their native state, with ionization taking place directly on the sample surface. The ionization source produces excited-state species that interact with the sample and atmospheric water or oxygen, and the heated energetic gas desorbs and ionizes the sample. This ionized sample travels with the gas beam and is pulled into the spectrometer inlet of a time-of-flight (TOF) mass analyzer. The resulting mass spectrum appears as a plot of an ion from each molecular species versus its relative abundance. We employed the DART ion source in positive-ion mode, resulting in simple mass spectra which showed protonated or ammoniated molecular ion peaks (i.e. $[M+H]^+$ or $[M+NH_4]^+$, respectively). Importantly, DART-MS is considered to be minimally destructive and it does not require treatment or sample preparation prior to analysis, allowing samples to be tested directly. The methodology described here opens the way for the adoption of a comprehensive, rapid, sensitive, and definitive analysis of such evidence, while providing knowledge that can inform criminal justice policy and practice.

EXPERIMENTAL

Materials

Lifestyles[®], Trojan[®], and Lifestyles SKYN[®] condoms were purchased at a local drug store. Fluorescent fingerprint powder was purchased from Evident Crime Scene Products (Union Hall, VA, USA). Puritan[®] cotton-tipped applicators, used as swabs, were obtained from Fisher Scientific (Pittsburgh, PA, USA).

DART-MS parameters

An AccuTOF-DART (JEOL USA, Inc., Peabody, MA, USA) time-of-flight (TOF) mass spectrometer was used for mass measurements. The mass spectrometer resolving power was 6000 FWHM (full width at half maximum) measured for protonated reserpine. A mass spectrum of poly(ethylene glycol) with an average molecular weight of 600 was included in each dataset as a reference standard for exact mass measurements. The atmospheric pressure interface was typically operated at the

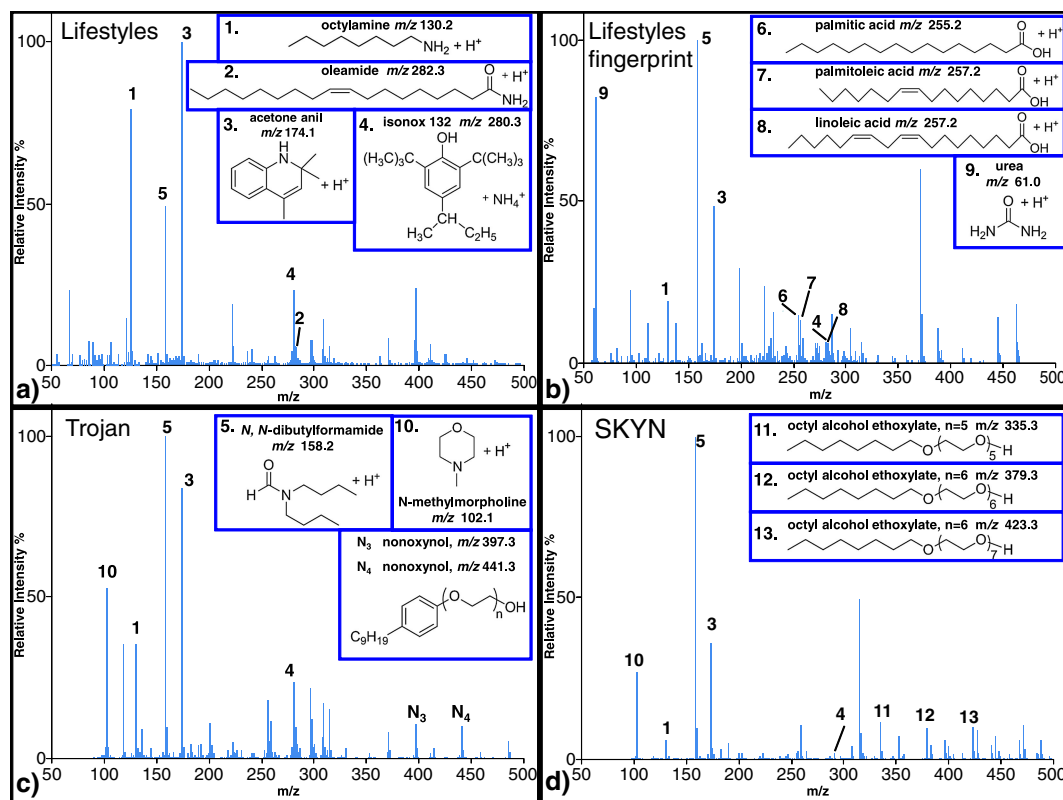


Figure 2. Four DART-MS time-of-flight mass spectra of three different lubricated condoms and a lubricant-contaminated fingerprint. Data related to this figure, including relative abundances and calculated and observed masses, are listed in Table 1. Inserts show species identified in the mass spectra, demonstrating that the lubricant formulations are readily differentiated. The desorption temperature was set at 200 °C which preserved the integrity of the condom material while enabling desorption of small molecules. (a) DART-MS spectrum of a Lifestyles[®] condom; (b) DART-MS spectrum of a latent print created by exposing a digit to the lubricant of the Lifestyles[®] condom, and applying the lubricant-laden print to a microscope slide. The slide was then placed between the ion source and spectrometer inlet as shown in Fig. 1(c); (c) DART-MS spectrum of a Trojan[®] condom. The term 'nonoxynol' and its symbol 'N' in the inset refer to the molecule's core structure as shown, with the numerical subscript indicating the number of associated ethoxymer units; (d) DART-MS spectrum of a SKYN[®] condom.

following potentials: orifice 1 = 20 V, orifice 2 = 3 V, ring lens = 3 V. The RF ion guide voltage was set to 550 V to allow detection of ions greater than m/z 55. The DART simplified voltage and pressure (SVP) ion source (IonSense Inc., Saugus, MA, USA) was operated with the helium gas heater temperature set at 200 to 350 °C, depending on the analyte. The helium flow rate was set at 2 L min⁻¹. The grid electrode was operated at 250 V. TSSPro3 software (Shrader Analytical, Detroit, MI, USA) together with Mass Spec Tools 2 programs (ChemSW Inc., Fairfield, CA, USA) was used for data processing and data interpretation. In these experiments, the condoms, condom packaging and fingerprints were introduced directly to the DART source.

Fingerprint image

A lubricated condom was handled and a latent fingerprint saturated with lubricant was placed at the center of a condom wrapper and on a microscope slide. DART-MS was

performed on both fingerprints. Subsequently, the fingerprint that had been applied to the condom wrapper was developed with fluorescent powder and visualized using a Foster + Freeman visual spectral comparator 2000 (Sterling, VA, USA). The developed fingerprint image was adjusted using the Paint.net v3.5.10 software, available free of charge on the internet.^[28]

RESULTS AND DISCUSSION

An open-air gap exists between the ion source and the spectrometer inlet that allowed our samples, including latent fingerprints, condoms, condom wrappers, and swabs similar to those found in a rape test kit, to be held directly in the path of the ion beam (Fig. 1). Studies by Francese and coworkers have demonstrated that MALDI imaging MS analysis of a variety of surfaces relevant to forensic analyses can be performed.^[19,24] Analogously, it is demonstrate here that

Table 1. High-resolution data for the mass spectra shown in Fig. 2 (measured masses are given as m/z values). Three datasets are indicated by the brand name of the condom sampled (Lifestyles[®], Trojan[®], Lifestyles SKYN[®]), while the fourth dataset aligns with analysis of a lubricant-contaminated fingerprint (from a Lifestyles[®] condom)

Sample	Component	Composition	Meas.	Calc.	Rel. Abund. %
Lifestyles [®]	<i>n</i> -octylamine ¹	[C ₈ H ₁₉ N + H] ⁺	130.1605	130.1596	78.8
	oleamide ²	[C ₁₈ H ₃₅ NO + H] ⁺	282.2795	282.2797	7.2
	acetone anil ³	[C ₁₂ H ₁₅ N + H] ⁺	174.1292	174.1283	100.0
	isonox 132 ⁴	[C ₁₈ H ₃₀ N + NH ₄] ⁺	280.2633	280.2640	20.2
	<i>N,N</i> -dibutyl formamide ⁵	[C ₉ H ₁₉ NO + H] ⁺	158.1550	158.1545	56.9
Fingerprint Lifestyles [®]	<i>n</i> -octylamine ¹	[C ₈ H ₁₉ N + H] ⁺	130.1577	130.1596	17.9
	acetone anil ³	[C ₁₂ H ₁₅ N + H] ⁺	174.1293	174.1283	48.6
	isonox 132 ⁴	[C ₁₈ H ₃₀ O + NH ₄] ⁺	280.2631	280.2640	3.4
	<i>N,N</i> -dibutyl formamide ⁵	[C ₉ H ₁₉ NO + H] ⁺	158.1524	158.1545	100.0
	palmitic acid	[C ₁₆ H ₃₀ O ₂ + H] ⁺	255.2285	255.2324	14.2
	palmitoleic acid	[C ₁₆ H ₃₂ O ₂ + H] ⁺	257.2467	257.2481	13.6
	linoleic acid	[C ₁₈ H ₃₂ O ₂ + H] ⁺	281.2517	281.2481	6.2
	urea	[CH ₄ N ₂ O + H] ⁺	61.0404	61.0402	79.6
Trojan [®]	<i>n</i> -octylamine ¹	[C ₈ H ₁₉ N + H] ⁺	130.1577	130.1596	35.3
	acetone anil ³	[C ₁₂ H ₁₅ N + H] ⁺	174.1292	174.1283	83.6
	isonox 132 ⁴	[C ₁₈ H ₃₀ O + NH ₄] ⁺	280.2631	280.2640	10.5
	<i>N,N</i> -dibutyl formamide ⁵	[C ₉ H ₁₉ NO + H] ⁺	158.1526	158.1545	100.0
	<i>N</i> -methylmorpholine ⁶	[C ₅ H ₁₁ NO + H] ⁺	102.0910	102.0919	52.9
	[N ₄] ⁷	[C ₂₃ H ₄₀ O ₅ + H] ⁺	397.2968	397.2954	10.6
	[N ₅] ⁸	[C ₂₅ H ₄₄ O ₆ + H] ⁺	441.3234	441.3216	10.0
	SKYN [®]	<i>n</i> -octylamine ¹	[C ₈ H ₁₉ N + H] ⁺	130.1577	130.1596
acetone anil ³		[C ₁₂ H ₁₅ N + H] ⁺	174.1305	174.1283	35.9
isonox 132 ⁴		[C ₁₈ H ₃₀ O + NH ₄] ⁺	280.2631	280.2640	1.0
<i>N</i> -methylmorpholine ⁶		[C ₅ H ₁₁ NO + H] ⁺	102.0934	102.0919	26.8
<i>N,N</i> -dibutyl formamide ⁵		[C ₉ H ₁₉ NO + H] ⁺	158.1514	158.1545	100.0
octyl alcohol ethoxylate n = 5 ⁹		[C ₁₈ H ₃₈ O ₅ + H] ⁺	335.2778	335.2798	11.5
octyl alcohol ethoxylate n = 6 ⁹		[C ₂₀ H ₄₃ O ₆ + H] ⁺	379.3065	379.3060	9.4
octyl alcohol ethoxylate n = 7 ⁹		[C ₂₂ H ₄₇ O ₇ + H] ⁺	423.3348	423.3322	10.2

¹Used as a germicide, emulsifier, dispersant or lubricant. ²Used as a slip agent. ³Used as a rubber/latex antioxidant.

⁴Refers to 2,6-di-*tert*-butyl-4-*sec*-butyl phenol; used as an antioxidant and UV stabilizer. ⁵Used as a cosurfactant and catalyst. ⁶Used as an accelerator in rubber manufacture. ^{7,8}Components of nonoxynol-9 comprised of a nonylphenol core with a *para*-substituted side chain containing four [-CH₂CH₂O-] units and five [-CH₂CH₂O-] units, respectively.

⁹Non-ionic surfactants.

DART-MS can be utilized to perform MS analyses on various other surfaces, including glass, a cotton swab, or even a condom or its wrapper directly. Three different lubricant formulations associated with condoms were characterized: Trojan[®] natural latex with spermicide, Lifestyles[®] latex without spermicide, and Lifestyles SKYN[®] polyisoprene condom without spermicide. Our method is striking in its simplicity. Each sample in its native form was held between the DART ion source and the inlet of the mass spectrometer to register the spectra shown in Fig. 2.

Trace lubricant evidence associated with sexual assault can originate from a condom, or from supplementary use of a lubricant used to facilitate the criminal act. Condoms consist of a sheath of solid polymeric material, such as latex or polyisoprene, coated with specific lubricant formulations that vary slightly from brand to brand. Lubricant formulations contain a wide variety of specific trace chemicals that include flavorings, odorants, desensitizers, and spermicides. Of particular interest is the spermicide nonoxynol-9, which is the sole spermicide approved for use in the United States and a common additive in both male and female condoms and various other lubricants or contraceptive products. Ultimately, materials used to facilitate sexual assaults, including condoms, lubricants, or additives used in the manufacture of condoms such as plasticizers and antioxidants, are components that could conceivably be detected as trace evidence in sexual assault.^[14,16–19,29,30]

Four mass spectra that reveal the presence of several substances that enabled distinctions to be made between different condom brands are shown in Fig. 2, and the pertinent details of the components identified are shown in Table 1. The species detected in each sample can be traced back to either the condom manufacturing process or various trace components within the specific lubricant formulations used in each brand. Similar spectra were observed from analysis of cotton-tipped swabs applied to the condoms (data not shown). The compounds shown in Fig. 2 were identified as a result of the high mass resolution possible with DART-MS using a TOF mass analyzer, and, in total, allow for the differentiation of each formulation. While many of the specific formulations used by manufacturers are proprietary and not outlined on ingredient lists, we show that DART-MS enables observation of compounds that can be used to distinguish one formulation, and by extension, one brand, from another. For example, the two latex condoms (Lifestyles[®] and Trojan[®]) were not only differentiated clearly from a polyisoprene condom (SKYN[®]), but also resulted in spectra that were distinguishable from each other (Figs. 2(a), 2(c), and 2(d)). The series of peaks in each spectrum in Fig. 2 represent signature profiles that can be the basis for identification, differentiation, and/or match comparison. For example, the spermicide nonoxynol-9 was identified in the lubricant from only one of the three condoms (Trojan[®], Fig. 2(c)), but was also detectable in the lubricant from a swab taken of the condom (Fig. 3), and in a fingerprint contaminated with the same spermicidal lubricant (data not shown).

The presence of nonoxynol-9 has been used as a probative link in sexual assault cases.^[14–16,18,29,31] The DART-MS spectrum of the spermicide is intricate but fully characteristic of the substance, as nonoxynol-9 is actually a mixture of similarly structured nonylphenol ethoxylates that manifests as a series of peaks in the mass spectrum.^[32,33] The individual

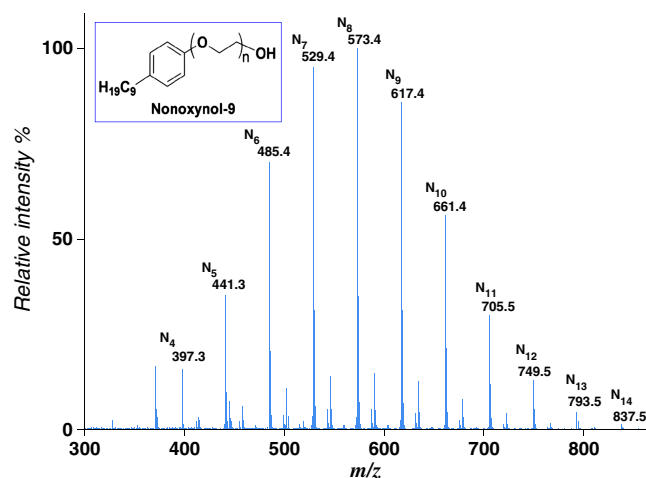


Figure 3. DART-MS spectrum of a cotton swab that was exposed to a Trojan[®] condom having a lubricant containing nonoxynol-9. Nonoxynol-9 is actually a complex mixture of closely related substances that differ by the number of ethylene oxide units and manifests in the mass spectrum as a Poisson distribution. The generic structure of nonoxynol-9 is included as an insert, where 'n' refers to the number of ethoxymer units associated with each component. Each component in the mixture is indicated by the observation of peaks at m/z values that correspond to the nonoxynol-9 core structure with the indicated number of ethoxymer subunits, seen as $[M+H]^+$ peaks. In order to view the entire range of nonoxynol-9 congeners, the desorption temperature was set at 350 °C. Data related to this figure, including relative abundances and calculated and observed masses, are listed in Table 2.

components of nonoxynol-9 all have a core nonylphenol structure, with each having a poly(ethylene oxide) side chain. The components of the mixture differ from one another by the length of the side chain and the branching of the nonyl substituent (Fig. 3, insert).^[19,32–34] The various components that are resolvable based on this repeating $[-OCH_2CH_2-]$ side chain result in a series of peaks. Each peak identified in the spectrum represents a single constituent of the mixture, having between four and fourteen repeats (N_4 – N_{14}). In positive-ion mode, the distribution of peaks begins at $[M+H]^+ = 397.3$ (corresponding to N_4), and continues with increases of 44 m/z units ($[M+H]^+ = 441.3, 485.3, 529.3$, etc.), due to the addition of subsequent ethylene oxide units, up to a maximum of m/z 837.6 which corresponds to N_{14} .

Another form of probative evidence is a fingerprint which, although informative in its own right, is now much more so due to the increasing use in forensic analysis of 'touch DNA'.^[2–4] Forensic DNA analysis can be used to develop a profile from objects touched by a suspect, such as a telephone or doorknob. The technique is currently applied to investigations of criminal acts such as burglaries and property crimes, where DNA evidence can be gathered from fingerprints on stolen goods, or other general contacts. It has been proposed by Francese and coworkers that a lubricant-contaminated fingerprint is a likely occurrence at a sexual assault crime scene and therefore would have obvious probative value.^[19] Our results support this assertion, as we were able to identify trace residues of biological components within fingerprint residues, even within the background of a larger, more complicated matrix. Specifically, palmitic,

Table 2. High-resolution data for the mass spectrum shown in Fig. 3 (measured masses are given as m/z values). DART-MS of a swab of a nonoxynol-9-containing Trojan[®] condom. Entries in the first column refer to components of bulk nonoxynol-9, each comprised of a nonylphenol core with a *para*-substituted side chain containing the indicated number of [-CH₂CH₂O-] units

Component	Composition	Meas.	Calc.	Abund.
[N ₄]	[C ₂₃ H ₄₀ O ₅ + H] ⁺	397.2971	397.2954	16.0
[N ₅]	[C ₂₅ H ₄₄ O ₆ + H] ⁺	441.3204	441.3216	35.3
[N ₆]	[C ₂₇ H ₄₈ O ₇ + H] ⁺	485.3471	485.3478	70.2
[N ₇]	[C ₂₉ H ₅₂ O ₈ + H] ⁺	529.3784	529.3740	95.0
[N ₈]	[C ₃₁ H ₅₆ O ₉ + H] ⁺	573.4029	573.4003	100.0
[N ₉]	[C ₃₃ H ₆₀ O ₁₀ + H] ⁺	617.4279	617.4265	85.9
[N ₁₀]	[C ₂₃ H ₆₄ O ₁₁ + H] ⁺	661.4565	661.4527	56.5
[N ₁₁]	[C ₃₇ H ₆₈ O ₁₂ + H] ⁺	705.4773	705.4789	30.1
[N ₁₂]	[C ₃₉ H ₇₂ O ₁₃ + H] ⁺	749.5115	749.5051	12.9
[N ₁₃]	[C ₄₁ H ₇₆ O ₁₄ + H] ⁺	793.5361	793.5313	4.6
[N ₁₄]	[C ₄₃ H ₈₀ O ₁₅ + H] ⁺	837.5659	837.5575	1.4

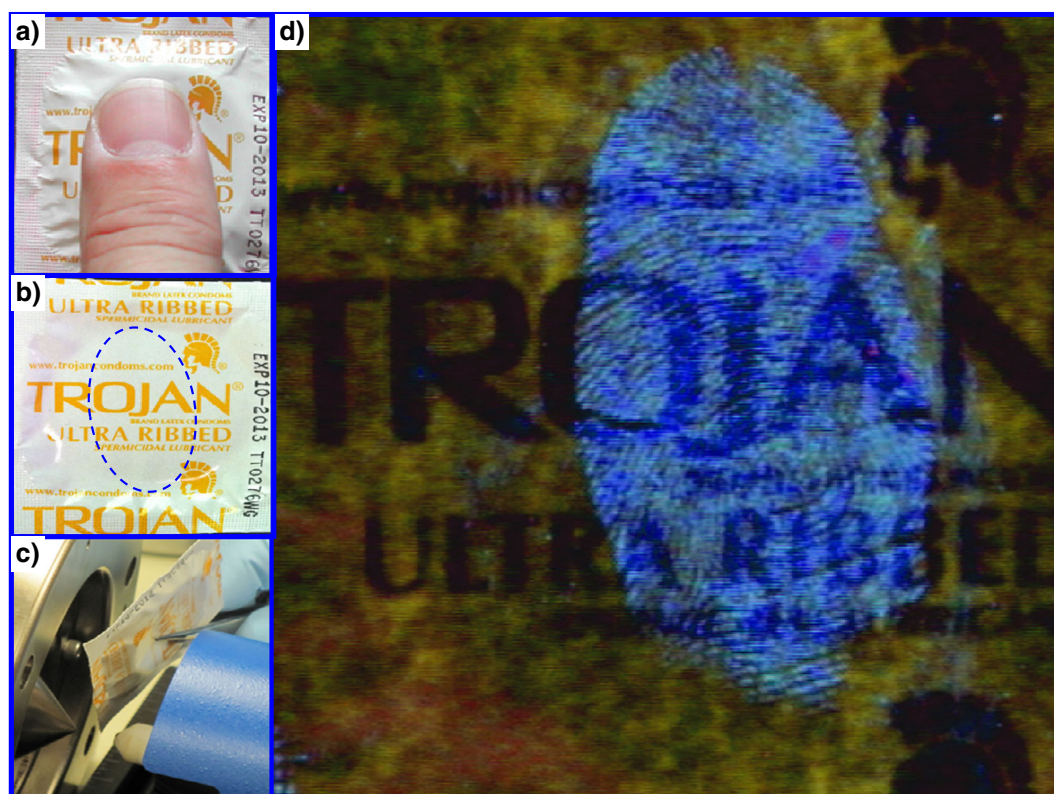


Figure 4. Identification by DART-MS of a latent fingerprint. (a) A latent print laden with condom lubricant was deposited on the center of the condom wrapper; (b) the condom wrapper on which the print was deposited, showing that the print was not visible against the complex background of the condom wrapper. (c) The wrapper was held between the DART-MS ion source and the mass spectrometer inlet to identify peaks known to be from the lubricant, as well as biological residues associated with the fingerprint. A DART mass spectrum of a latent fingerprint contaminated with lubricant is shown in Fig. 2(b). (d) The latent print developed with fluorescent powder *after* DART-MS analysis.

palmitoleic, and linoleic acids, as well as urea, were identified as trace biological residues within a latent fingerprint that contained overwhelming quantities of lubricant.^[19,20] These biological residues are routinely identified in the chemical analysis of fingerprints.^[19,35,36] A second study by Francese

and coworkers used MALDI imaging MS to develop a latent print in a conventional manner, prior to employing the matrix to aid in desorption of the chemical components for analysis.^[24] The DART-MS analysis method used herein permitted the exact opposite sequence of steps; mass spectral chemical

analysis was performed directly on the latent print (with no matrix necessary), and the latent print was then developed by conventional methods. Specifically, lubricated condoms were handled and a fingerprint saturated with lubricant was placed at the center of the condom wrapper (Fig. 4) and on a microscope slide (Fig. 1(c)). Figure 4 shows images of the condom wrapper before and during DART-MS analysis. Our results show that this technique can be used to establish the presence of the latent print on the condom wrapper as the wrapper was held between the ionization source and the mass spectrometer inlet. Figure 4 also shows the fingerprint on the wrapper, developed with a fluorescent dye after DART-MS analysis. Figures 2(a) and 2(b) show mass spectra of a condom compared with the spectrum from a lubricant-containing fingerprint. The spectra matched one another to the extent that peaks common to both spectra were identified, but, in the fingerprint, multiple trace biological residues common to fingerprints were also apparent.

CONCLUSIONS

DART-MS exhibits several favorable characteristics that make it an excellent method for use in forensic analyses, such as high-resolution mass detection and the ability to sample items without extraction or sample preparation.^[25–27] It allowed rapid sample testing and the TOF mass analyzer provided high-resolution, accurate mass determinations, unambiguous peak assignments, and determination of isotopic ratios and elemental compositions. Destructive methods or those that consume evidence, which are characteristic of techniques such as standard GC/MS, are less desirable in forensic analyses where preservation of evidence in its native state can be of paramount importance. In addition, desorption MS techniques, which require that the sample be treated with a matrix material, may also be problematic because of the need to preserve evidence in its native form for further testing, such as forensic DNA analysis. DART-MS requires no such matrix, and no sample preparation whatsoever, since samples can be tested directly (Fig. 1). In addition, DART mass spectrometers have the potential to be made portable, making it possible for them to be introduced directly into a crime scene for processing evidence. The extension of DART-MS analysis to a broader range of substances and surfaces related to crime scene analyses is the subject of on-going studies.

Forensic analyses often require comparisons to be made between a known sample and questioned evidence. Our method identified numerous major and trace components of each sample, resulting in the ability to differentiate lubricants associated with sexual assault and preliminary identification of biological residues. DART-MS enabled us to perform rapid analyses of many forms of evidence directly, identifying aspects of the specific formulations pertinent to each sample. To keep pace with an ever-changing landscape of 'educated' criminals, we expect to continue developing methods further employing DART-MS to definitively characterize other forms of trace evidence.

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