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DOI
10.1016/j.forsciint.2019.110005

Publication date
2019

Document Version
Final published version

Published in
Forensic Science International

Citation (APA)
van Helmond, W., Begieneman, M. P. V., Kniest, R., & de Puit, M. (2019). Classification of condom lubricants in cyanoacrylate treated fingerprints by desorption electrospray ionization mass spectrometry. *Forensic Science International*, *305*, Article 110005. https://doi.org/10.1016/j.forsciint.2019.110005

Forensic Science International 305 (2019) 110005

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

Classification of condom lubricants in cyanoacrylate treated fingerprints by desorption electrospray ionization mass spectrometry

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ARTICLE INFO

Article history:
Received 6 August 2019
Received in revised form 16 October 2019
Accepted 21 October 2019
Available online 23 October 2019

Keywords:
Mass Spectrometry Imaging
DESI-MSI
Polydimethylsiloxane
Polyethylene glycol
Principal component analysis
Linear discriminant analysis

ABSTRACT

Traces of condom lubricants in fingerprints can be valuable information in cases of sexual assault. Ideally, not only confirmation of the presence of the condom but also determination of the type of condom brand used can be retrieved. Previous studies have shown to be able to retrieve information about the condom brand and type from fingerprints containing lubricants using various analytical techniques. However, in practice fingerprints often appear latent and need to be detected first, which is often achieved by cyanoacrylate fuming. In this study, we developed a desorption electrospray ionization mass spectrometry (DESI-MS) method which, combined with principal component analysis and linear discriminant analysis (PCA-LDA), allows for high accuracy classification of condom brands and types from fingerprints containing condom lubricant traces. The developed method is compatible with cyanoacrylate (CA) fuming. We collected and analyzed a representative dataset for the Netherlands comprising 32 different condoms. Distinctive lubricant components such as polyethylene glycol (PEG), polydimethylsiloxane (PDMS), octoxynol-9 and nonoxynol-9 were readily detected using the DESI-MS method. Based on the analysis of lubricant spots, a 99.0% classification accuracy was achieved. When analyzing lubricant containing fingerprints, an overall accuracy of 90.9% was obtained. Full chemical images could be generated from fingerprints, showing the distribution of lubricant components such as PEG and PDMS throughout the fingerprint, while still allowing for classification. The developed method shows potential for the development of DESI-MS based analyses of CA treated exogenous compounds from fingerprints for use in forensic science.

1. Introduction

Sexual assault is a major health problem and a violation of human rights [1]. When the identity of the perpetrator is unknown, the most important traces in sexual assault cases are often of biological origin, such as blood, semen, saliva and hair. This is due to the fact that DNA can be retrieved and analyzed from these traces, allowing for the identification of possible criminals. Nowadays, biological material becomes increasingly harder to find in cases of sexual assault, potentially because awareness of the importance of DNA in forensic techniques and evidence in the justice system is growing among criminals [2]. Because of this awareness, as well as alertness to sexual transmitted diseases, criminals are becoming more vigilant in leaving biological traces and the use of condoms in sexual assault cases has increased over the past decades [3–6]. Other types of trace evidence may thus be required to establish a link between victim and criminal. In the past, studies have shown that a condom can be a critical piece of evidence in sexual assault cases [4,7,8]. The analysis of condom derived traces can thus be of significant associative evidential importance. Ideally, not only confirmation of the presence of the condom but also determination of the type of condom brand used can be retrieved.

In the last decades several studies have been performed to develop methods to detect traces of condoms. Most of these studies focused on the lubricants that are added to the condoms by manufacturers. Indeed, primary components of lubricants such as polydimethylsiloxane (PDMS) and polyethylene glycol (PEG) were found to be detectable by desorption chemical ionization mass spectrometry [9], pyrolysis gas chromatography mass spectrometry (pyGC-MS), GC–MS [10], Raman spectroscopy [11] and Fourier

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http://dx.doi.org/10.1016/j.forsciint.2019.110005

transform infrared spectroscopy (FT-IR) [9,12]. Another specific component in case of spermicide containing condoms, nonoxynol-9 (N9), could also be identified by FT-IR [9], GC–MS [13] and liquid chromatography mass spectrometry (LC–MS) [12]. Multiple studies examined the possibility to discriminate different types of condoms. Maynard et al. described a two-step method using FT-IR as a first screening tool, followed by either GC–MS, pyGC-MS or LC–MS as a confirmation method, enabling them to uniquely identify 11 types of condoms [12]. Burger et al. showed that capillary electrophoreses may also be a promising technique for classifying both condom and personal lubricants, although it remained unclear which discriminating constituents were used in the analysis [14].

However, most of these analytical techniques require sample preparation and/or extraction which may be time consuming and result in loss of the initial trace evidence. Additionally, the method itself may also limit the amount of information retrieved from the sample. For instance, analysis of silicone lubricants by GC–MS requires pyrolysis of the lubricant [10], that can cause degradation of minor components. These minor components were found to be an important differentiating factor in distinguishing sexual lubricants and personal hygiene products, which contain similar major components such as PDMS and PEG [15]. Also, preservation of the original evidential trace can be of great interest in forensic science. In this respect, the use of ambient ionization mass spectrometry techniques is more favorable. A popular technique that has been used in recent years is direct analysis in real time (DART) MS, that has been shown to be a very effective tool for the detection of both the major and minor components of condom lubricants, without the need to extensively prepare the sample or potential loss of evidence [16–20]. Furthermore, DART analysis is highly effective in discriminating lubricants. Baumgarten et al. and Maric et al. successfully discriminated condom and personal lubricants using DART-Time-of-Flight (TOF) MS analysis [19,18]. Using a DART-High Resolution MS (HR-MS) analysis technique, Coon et al. could rapidly generate diagnostic chemical fingerprint signatures of 110 condoms, enabling them to discriminate condoms of 16 different brands [20]. However, a disadvantage of DART analysis is that this technique is unable to retrieve spatial chemical information from the samples, such as fingerprints containing lubricants.

Lubricated fingerprints are likely to be found at a crime scene of sexual assault, as handling of a condom will transfer the outer coating of the condom onto the perpetrator's fingerprints, potentially leaving condom lubricant contaminated fingerprints behind [21,22]. Detection of a lubricant from a fingerprint found at a sexual assault scene, would greatly increase the strength of the evidence, as it not only establishes contact with a condom but also indicates the presence of the criminal at the crime scene [21]. Bradshaw et al. developed a method for the visualization of condom lubricant within a fingerprint by mapping the fingerprint ridge pattern using Matrix Assisted Laser Desorption/Ionization Mass Spectrometry Imaging (MALDI-MSI) [21]. In a follow-up study they showed that differentiation of 6 different condoms in lubricated fingerprints was possible using MALDI-MSI, Raman spectroscopy and ATR-FTIR imaging in a synergistic manner [22]. Besides MALDI-MSI, desorption electrospray ionization (DESI) MSI has also been shown to be a powerful analytical tool. Whereas MALDI offers excellent spatial resolution ~20 μm, one of the main disadvantages is the necessity to apply matrix solution to your sample [23]. Applying the matrix solution in too large droplets will be detrimental for the spatial resolution as diffusion will occur within these droplets. One of the main advantages of DESI-MSI is that samples require no sample preparation and can be readily analyzed. Using DESI, typically spatial resolutions of ~100 μm can be achieved [23]. However, the choice of the electrospray solvent composition is essential, as the interaction between the electrospray and the surface greatly influences the sensitivity and spatial resolution [24]. Mirabelli et al. were able to generate chemical images of latent lubricated fingerprints deposited on different surfaces and of different ages using DESI-MS [25]. The data acquired from DESI-MS analysis of lubricated fingerprints, combined with supervised pattern recognition statistical analysis (linear discriminant analysis (LDA) and soft independent modeling of class analogy (SIMCA)), enabled Mirabelli et al. to distinguish 10 different condom types with a 94% prediction ability for both LDA and SIMCA [26].

However, fingerprints in practice often need to be detected first, as they appear latent. One of the most used visualization techniques for latent fingerprints on non-porous substrates is cyanoacrylate (CA) fuming [27,28]. Fingerprints from a sexual assault scene that have been analyzed at a forensic lab, are potentially treated with CA. In this regard, the aim of our study was to develop a method to analyze lubricated fingerprints, which was compatible with CA fuming and able to differentiate between different types of condoms. The developed method, based on DESI-MS analysis, is capable of generating chemical images, mapping common lubricant components in a lubricated fingerprint. Additionally, using this method, combined with a statistical approach, PCA followed by LDA, we were able to differentiate between 32 types of condoms from 21 different brands.

2. Materials & methods

2.1. Materials

UPLC-grade acetonitrile and formic acid were purchased from Biosolve (Valkenswaard, Netherlands). UPLC-grade methanol was purchased at Merck (Darmstadt, Germany). Cyanoacrylate was purchased from BVDA (Haarlem, Netherlands). Microscope glass slides were purchased from Thermo Fischer Scientific (Breda, Netherlands). 24-wells slides were purchased from Prosolia (Indianapolis, USA). Reference masses purine (5 mM) and hexakis(1H, 1H, 3H-tetrafluoropropoxy)phosphazine) (HP-0921, 2.5 mM) were purchased from Agilent Technologies (Santa Clara, USA). Mitra microsampler tips (10 μL) were purchased from Neoteryx (Torrance, USA). A range of 32 different condoms were purchased at online pharmacy and condom websites (Table 1).

2.2. Lubricant samples

Lubricant of each condom was collected by swiping the interior of the condom package and both sides of the condom with a 10 μL microsampler until saturation. The microsampler is a volumetric absorption microsampling device (VAMS), that only absorbs 10 μl of sample. Because of this, it provides more control over the amount of lubricant to be sampled, in comparison to cotton swabs, that absorbed too much lubricant. The condom lubricant was carefully transferred onto the 24-wells slide by slightly touching each well once with the lubricated microsampler tip. Each lubricant was spotted 12 times (n = 12) on separate wells. As a control, each sample well was followed by a blank well. Slides were left to dry for at least 1 h at room temperature (RT). Next, cyanoacrylate (CA, 0.5 g heated to 120 °C) fuming was performed on all slides, in a MVC1000 fuming system (Foster and Freeman LTD, Worcestershire, UK) for 10 min at 80% humidity. Slides were then left to dry overnight at RT before analysis.

2.3. Fingerprint samples

Fingerprints were donated voluntarily by a female and male donor, after giving informed consent. No ethical approval was

Table 1
The 32 condoms used in this study, the abbreviation used, the manufacturer and their country of origin.

Condoms	Abbreviation	Manufacturer	Country
Billy Boy Extra Lubricated	BBEL	Mapa Health Care	Germany
Balance Condoom	BC	Condoom-anoniem	Netherlands
Beppy Soft Comfort	BSC	Beppy	Netherlands
Durex Classical Natural	DCN	Durex	UK
Durex Extra Safe	DES	Durex	UK
Durex Feeling Sensitive	DFS	Durex	UK
Durex Orgasmic	DO	Durex	UK
Durex Performa	DP	Durex	UK
Durex Real Feeling	DRF	Durex	UK
Durex XL Power	DXLP	Durex	UK
Euroglider	EU	Asha International/Euroglider	Netherlands
EXS Regular	EXS	LTC Health Care	UK
Fair Squared Original	FSO	Fair Squared GMBH	Germany
Glyde Ultra Naturelle	GUN	Glyde Health	Australia
Just Safe Standaard	JSS	Safe	Netherlands
Kruidvat Classic	KC	Kruidvat	Netherlands
Kruidvat Extra	KE	Kruidvat	Netherlands
Kruidvat Sensation Banana	KSb	Kruidvat	Netherlands
Kruidvat Sensation Chocolate	KSc	Kruidvat	Netherlands
Kruidvat Sensation Strawberry	KSs	Kruidvat	Netherlands
Kruidvat Ultra	KU	Kruidvat	Netherlands
LELO HEX Condooms	LH	Lelo	Sweden
Level Popular	LP	Your Levels BV	Netherlands
MoreAmore Soft Skin	MASS	Bizzy Diamond BV	Netherlands
MySize	MS	R&S Germany	Germany
Mates SKYN Original	MSO	Lifestyle Healthcare	Australia
ON Natural Feeling	ON	R&S Germany	Germany
Playboy Lubricated	PL	Playboy	USA
Pasante Naturelle	PN	Pasante Healthcare Ltd/Karex	UK
Startex	ST	ForeSee line	Belgium
Uniq Pull	UP	Uniq International	Colombia
Wingman	WI	Wingman	Netherlands

obtained as the material was gathered in a noninvasive manner and did not infringe on any privacy of the donors. All experiments were carried out following institutional guidelines and according to relevant laws. All condoms (n = 32) were handled by each donor (n = 2). After touching the condom and the inside of the packaging, the lubricant was distributed over the finger. After 5 min of drying, fingerprints of each condom lubricant were deposited on microscope slides. From each donor, a blank fingerprint was used as a control (n = 2). Slides were left to dry for at least 1 h at RT followed by CA fuming, as described above. Fingerprints were then left to dry overnight at RT.

2.4. DESI-Q-TOF MS

Desorption electrospray ionization mass spectrometry (DESI-MS) data were acquired using an Agilent technologies (Santa Clara, USA) 6530 quadrupole time-of-flight (TOF) MS equipped with a Prosolia (Indianapolis, USA) 2D-DESI. 24-wells sample slides were analyzed in dwell mode using positive polarity with the following parameters: spray voltage, 5 kV; nitrogen sheath gas pressure, 6.0 bar; drying gas flow, 8 L/minute, source gas temperature, 300 °C; acquisition time, 200 ms; mass range, *m/z* 100–1200; inlet-to-surface distance, ~1 mm and tip-to-surface distance, ~3 mm. Combinations of several spray incident angles (52°, 45° and 35°) and tip-to-inlet distances (4, 5, 6, and 8 mm) were tested. Best results were achieved with an angle of 45° and a tip-to-inlet distance of 6 mm. A larger tip-inlet distance led to decreased carry-over, also described by Mirabelli et al. [26]. A dwell time of 20 s was used, with a post-acquire-delay time of 30 s in between the wells. To further avoid carry-over after analysis of each sample well, the next blank well was dwelled for 5 min before measuring the next sample. Additionally, the MS-inlet was cleaned after analysis of 3–4 slides to avoid carry-over, as also indicated by Mirabelli et al. [26]. Different spraying solvents were tested, namely a mixture of acetonitrile and water (90:10 v/v), acetonitrile, methanol and a mixture of methanol with water (90:10 v/v). All solvents contained 0.4% formic acid, 0.02 mM Purine and 0.025 mM HP-0921. Best result were achieved with a mixture of acetonitrile and water (90:10 v/v) when sprayed at a constant volumetric flow rate of 3 μL/minute, delivered by a syringe pump (Fusion 100, Chemyx, Stafford, USA). The 64 lubricated and 2 blank fingerprints were analyzed using the same settings, but instead of dwelling, 5 scans of 3 mm were measured within each fingerprint, using a 150 μm/second scan rate (totaling to 20 s) and a step size of 1 mm. MS full scan data were acquired with Agilent Mass Hunter Data Acquisition software (version B.08.00). Before data analysis, the first line of each analyzed fingerprint was removed from the results, as these often contained spectra with low intensities. This was likely a result of sample wetting, as described by Bodzon-Kulakowska et al. [29]. Lubricant components were putatively annotated using the online METLIN mass spectral metabolite database [30] and comparison with previously obtained results from literature.

2.5. Fingerprint imaging

Chemical images (12 × 20 mm) of a cyanoacrylated blank and EXS lubricated fingerprint were acquired using the same parameters as described above. The MS-inlet was cleaned after acquisition of each chemical image. Images were acquired using a 150 μm/second scan rate, resulting in a 30 μm pixel width and step size (totaling to 400 rows). Data were converted to imzML, using FireFly (v. 3.0.1.1, Prosolia, Indianapolis, USA), and subsequently analyzed using MSIReader (v1.01) [31].

2.6. Statistics

Data were converted to mzXML and the 5000 most abundant peaks were filtered using msConvert [32]. Data were subsequently

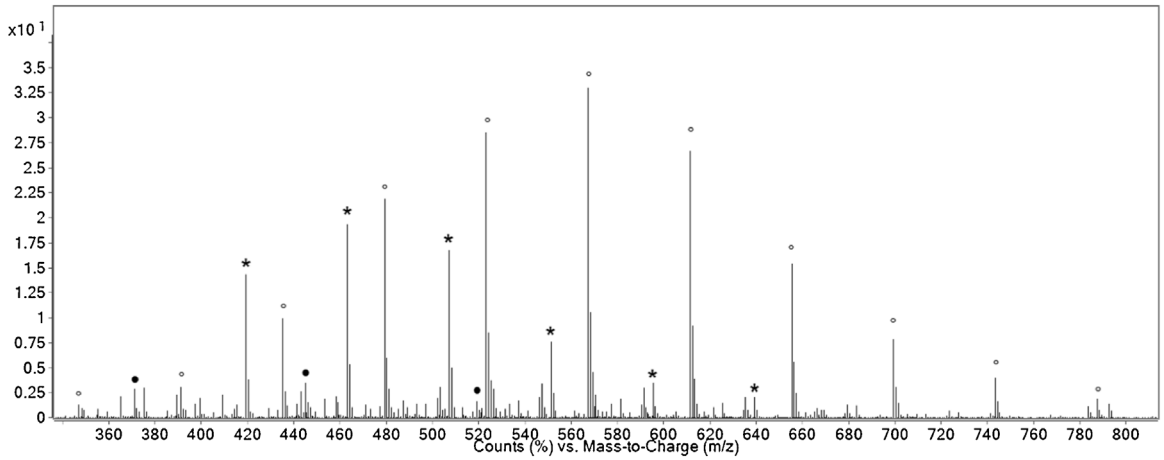

Fig. 1. Spectrum obtained from DESI-MS analysis of spots of lubricant from *BSC* condom, showing ion series corresponding to PDMS (•) and nonoxynol-9 $[M+Na]^+$ (*) and $[M+K]^+$ (°).

processed with R (version 3.4.2) using R studio (Version 1.1.456), using the MALDIquant package [33]. Mass spectra were square-root transformed and normalized using the total ion current (TIC). After aligning and averaging the spectra, peaks were detected using the corresponding MALDIquant functions. Principal component analysis (PCA) was then executed to reduce the data dimensionality. After splitting the data in a 75% training and 25% test set, the first 12 PCs (explaining 90% of the cumulative proportion of variance) were used to generate a linear discriminant analysis (LDA) model, using the MASS package [34]. Classification accuracy was evaluated by generating confusion matrices using the caret package [35].

3. Results

3.1. Detection of condom lubricants

As a first screening of the chemical components of condom lubricants, a detection method was developed based on the analyses of the 24-wells cyanoacrylated sample slides. Typical scans of lubricants from 4 different condoms are shown in Figs. 1–4. As can be deduced from these spectra, distinctive patterns, originating from polymers that make up a large part of the lubricants, were found. Closer analysis of the detected *m/z* values that form these ion series lead to the putative annotation of the major components of the condom lubricants (Table 2). A large component of many lubricants appeared to be poly(ethylene glycol) (PEG), whereas *m/z* values corresponding to polydimethylsiloxane (PDMS) were detected as well. In some lubricants, the polyethoxylated phenol nonionic surfactants octoxynol-9 or nonoxynol-9, serving as spermicides, were observed (Table 2). Next to these chemical components, multiple ion series corresponding to the fatty alcohol ethoxylates PEG decyl ether and PEG dodecyl ether were found, often used as non-ionic surfactants (Table 2) [36]. Poly(propylene glycol) (PPG) was putatively annotated in some of the lubricants as well. In addition to the ion series resulting from the polymers largely present in condom lubricants, a few molecular ion species were also detected and putatively annotated. An example is the detection of benzocaine, a local anesthetic used in two of the *Durex* condoms (*Performa* and *Orgasmic*) (Table 2). Furthermore, masses corresponding to undecylamine and dodecylamine were observed. As all samples were subjected to cyanoacrylate fuming, a commonly used detection technique for latent fingerprints within the forensic setting, a mass corresponding to a cyanoacrylate (CA) fragment was also found (Table 2). Finally, in controls, except CA, none of the above mentioned chemical components were found (data not shown).

3.2. Differentiation of condom lubricants

To differentiate between the 32 condoms, principal component analysis (PCA) and linear discriminant analysis (LDA) were performed, as both are shown to be effective in discriminating

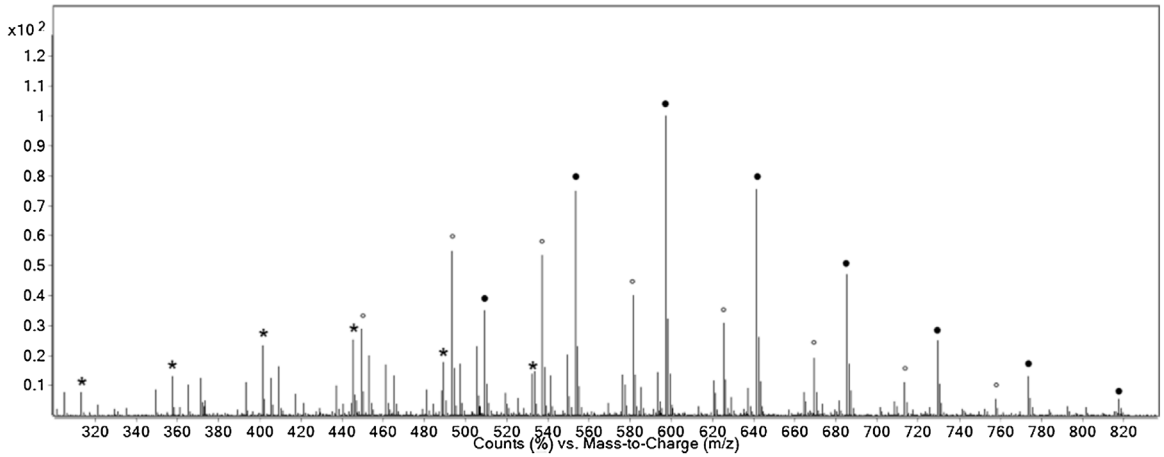

Fig. 2. Spectrum obtained from DESI-MS analysis of spots of lubricant from *MSO* condom, showing ion series corresponding to poly(ethylene glycol) decyl ether (*), octoxynol-9 $[M+K]^+$ (•) and $[M+Na]^+$ (°).

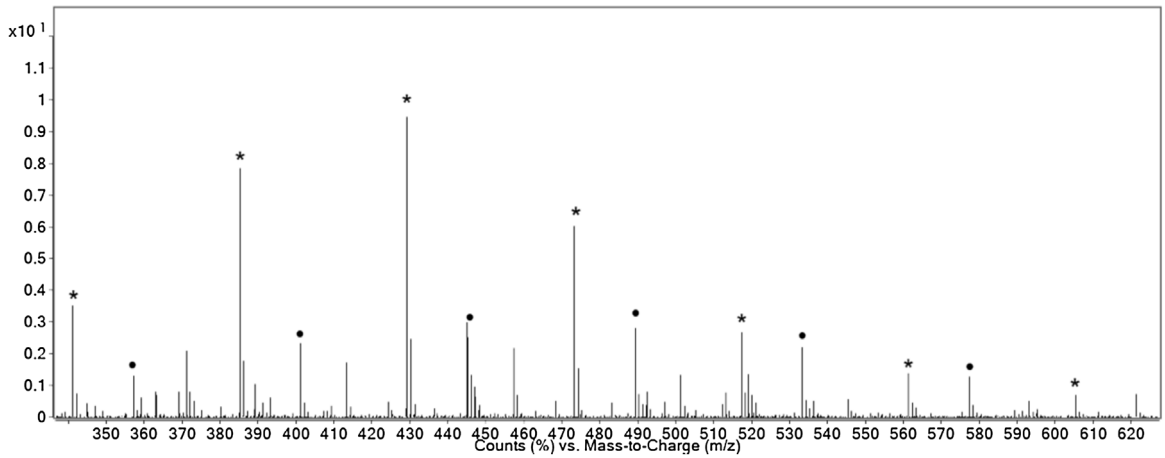

Fig. 3. Spectrum obtained from DESI-MS analysis of spots of lubricant from *KC* condom, showing ion series corresponding to poly(ethylene glycol) dodecyl ether $[M + Na]^+$ (*) and poly(ethylene glycol) decyl ether $[M + Na]^+$ (•).

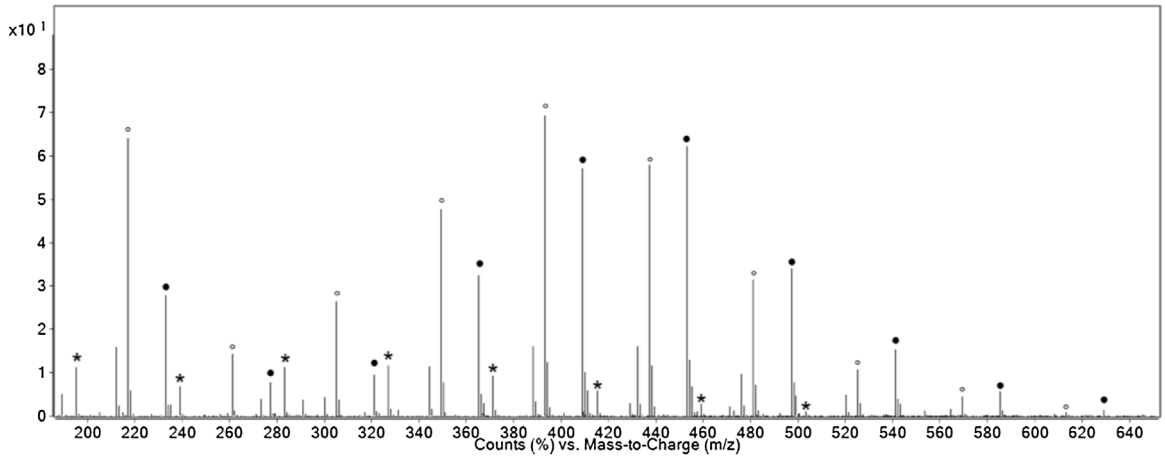

Fig. 4. Spectrum obtained from DESI-MS analysis of spots of lubricant from *DO* condom, showing ion series corresponding to poly(ethylene glycol) $[M+H]^+$ (*), $[M + K]^+$ (•) and $[M + Na]^+$ (°).

condom lubricants based on mass spectra [26,18,19]. PCA was performed to reduce data dimensionality, using the mass spectra acquired from the 32 different condom lubricants (Fig. 5). Using only the first two principal components (PCs), a distinction between major lubricant classes could already be made. To gain more insight in which components can be used to differentiate condom lubricants, the loadings of the first 5 PCs were analyzed (Fig. 6). The first PC contains *m*/*z* values corresponding to the $[M + Na]^+$ and $[M + K]^+$ ion series of PEG, whereas in the second PC *m*/*z* values of nonoxynol-9 (both $[M + Na]^+$ and $[M + K]^+$) are incorporated. In the third PC, putatively, octoxynol-9 (both $[M + Na]^+$ and $[M + K]^+$) was found to be the major component. Undecylamine and an unidentified *m*/*z* of 848.6672 are the major contributors to the fourth PC. In the fifth PC, dodecylamine, PEG, PPG and an unidentified *m*/*z* of 125.9863 were the strongest differentiating factors.

Next, linear discriminant analysis (LDA) was used to generate a classification model using the first 12 PCs (explaining 90% of the cumulative proportion of variance (Fig. S1)), based on the training data (75% of lubricant data obtained from analysis of the 24-wells slides). The generated model was subsequently evaluated by classification of the test data (25% of lubricant data obtained from analysis of the 24-wells slides). Analysis of the resulting confusion matrix shows that the model is able to classify condom lubricants with high accuracy (99.0%) (Table S1). Only 1 sample was predicted incorrectly; a sample containing KSb was predicted by the PCA-LDA model as KSs (Table S1), which both originate from the same brand of flavored condoms (*Kruidvat Sensations*). These lubricants likely contain the same basis, while different colorants and flavorings are added. The fact that many components of the lubricant are likely to be identical, could explain the missclassification of the generated model. Subsequently, we generated the PCA-LDA classification model based on the data from the lubricated cyanoacrylated fingerprints in the same manner. Using this data, an overall accuracy of 90.9% was achieved (Table S2). A few missclassification were present but seem to be comprehensible, such as the prediction of DES as DCN (both *Durex* condoms) and the prediction of DP as DO (both *Durex* condoms that contain benzocaine). However, the model performed poor for one specific condom lubricant, namely PL (sensitivity of 40%). Further analysis of the PL data revealed the low intensity of many of the chemical components, possibly explaining the poor performance of the model in this case.

3.3. Imaging of condom lubricants in fingerprints

Visualisation of the presence of condom traces within fingerprints would greatly enhance the strength of the evidence, as it both establishes the presence of the suspect at the crime scene and contact with a condom. Therefore, full chemical images were

Table 2
Detected *m/z* values, their putative annotation and corresponding formula.

Putative annotation	Experimental *m/z* values	Formula	n	Ref
Benzocaine	166.0862	$C_9H_{11}NO_2$ $[M+H]^+$	–	[30,37,18]
Undecylamine	172.2058	$C_{11}H_{25}N$ $[M+H]^+$	–	[30]
Dodecylamine	186.2217	$C_{12}H_{27}N$ $[M+H]^+$	–	[30]
Ethyl cyanoacrylate	556.1794	$(C_6H_7NO_2)_n$ $[M+H-C_4H_8N]^+$	n = 5	–
Poly(ethylene glycol)	195.1226, 239.1489, 283.1753, 327.1017, 371.2279, 415.2540, 459.2808, 503.3059	$H(C_2H_4O)_nOH$ $[M+H]^+$	n = 4 . . . 11	[38,12]
	217.1046, 261.1308, 305.1572, 349.1836, 393.2098, 437.2359, 481.2622, 525.2883, 569.3160, 613.3404, 701.4077	$H(C_2H_4O)_nOH$ $[M+Na]^+$	n = 4 . . . 14	
	233.0785, 277.1041, 321.1298, 365.1567, 409.1832, 453.2097, 497.2360, 541.2620, 585.2889, 629.3150	$H(C_2H_4O)_nOH$ $[M+K]^+$	n = 4 . . . 13	
Poly(ethylene glycol) decyl ether	313.2348, 357.2611, 401.2873, 445.3135, 489.3393, 533.3649, 577.3712	$C_{10}H_{21}(C_2H_4O)_nOH$ $[M+Na]^+$	n = 3 . . . 9	–
Poly(ethylene glycol) dodecyl ether	341.2662, 385.2923, 429.3183, 473.3441, 517.3681, 561.3913, 605.4167	$C_{12}H_{25}(C_2H_4O)_nOH$ $[M+Na]^+$	n = 3 . . . 9	[39]
Poly(propylene glycol)	273.1674, 331.2093, 389.2514	$H(C_3H_6O)_nOH$ $[M+Na]^+$	n = 4 . . . 6	[38]
	347.1857	$H(C_3H_6O)_nOH$ $[M+K]^+$	n = 5	
Poly(dimethylsiloxane)	371.1013, 445.1200, 519.1382	$(C_2H_6SiO)_n$ $[M+H]^+$	n = 5 . . . 7	[38]
	429.0882	$(C_2H_6SiO)_n$ $[M+H-CH_4]^+$	n = 6	
Octoxynol-9	449.2877, 493.3136, 537.3400, 581.3661, 625.3919, 669.4184, 713.4439, 757.4698	$C_{14}H_{21}(C_2H_4O)_nOH$ $[M+Na]^+$	n = 5 12	[38,40]
	509.2876, 553.3138, 597.3399, 641.3662, 685.3925, 729.4182, 773.4447, 817.4707	$C_{14}H_{21}(C_2H_4O)_nOH$ $[M+K]^+$	n = 6 . . . 13	
Nonoxynol-9	419.2772, 463.3031, 507.3291, 551.3552, 595.3811, 639.4076	$C_{15}H_{23}(C_2H_4O)_nOH$ $[M+Na]^+$	n = 4 . . . 9	[38,40,12,21]
	347.1982, 391.2244, 435.2509, 479.2771, 523.3033, 567.3295, 611.3555, 655.3815, 699.4078, 743.4352, 787.4599	$C_{15}H_{23}(C_2H_4O)_nOH$ $[M+K]^+$	n = 2 . . . 12	

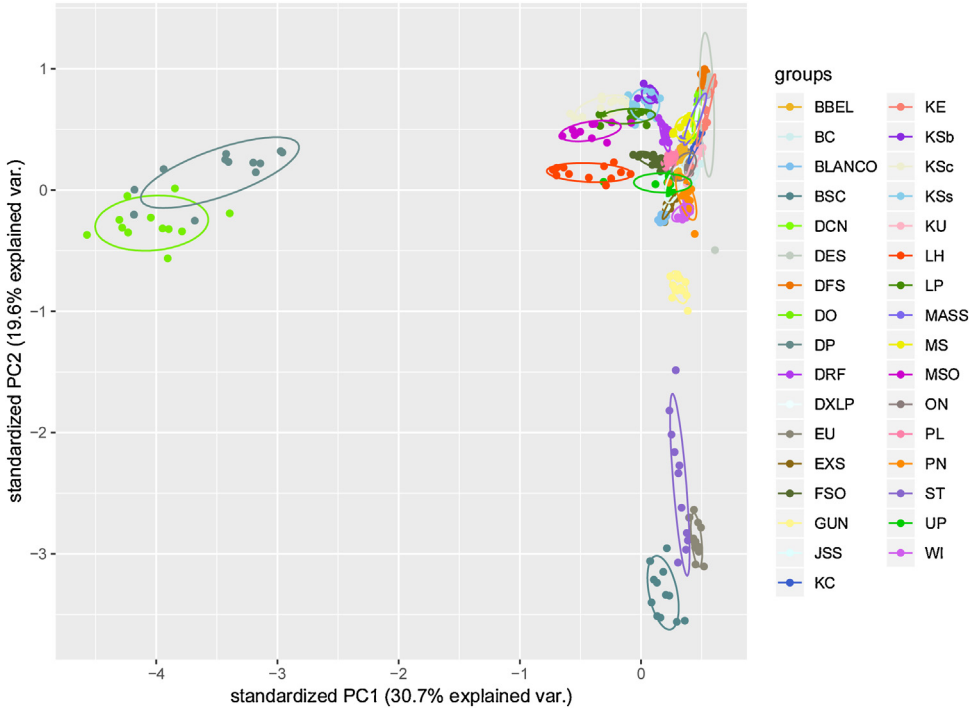

Fig. 5. Principal component analysis score plot based on the analysis of 32 condoms and blank (n = 12) using the first two principal components. Separate clustering of several lubricant groups is observed.

obtained from fingerprints that handled an EXS condom and a blank (natural) fingerprint, both treated with CA fuming (Fig. 7). As expected, CA (*m/z* 556.1794) was present in both fingerprints, and reveals the friction ridge pattern of the fingerprint in both cases (Fig. 7C and D). When rendering the chemical distribution of PDMS (*m/z* 445.1200) for both fingerprints, only in the lubricated fingerprint a distinctive image was acquired, that was absent in the blank (Fig. 7A and B). A similar result was obtained for the

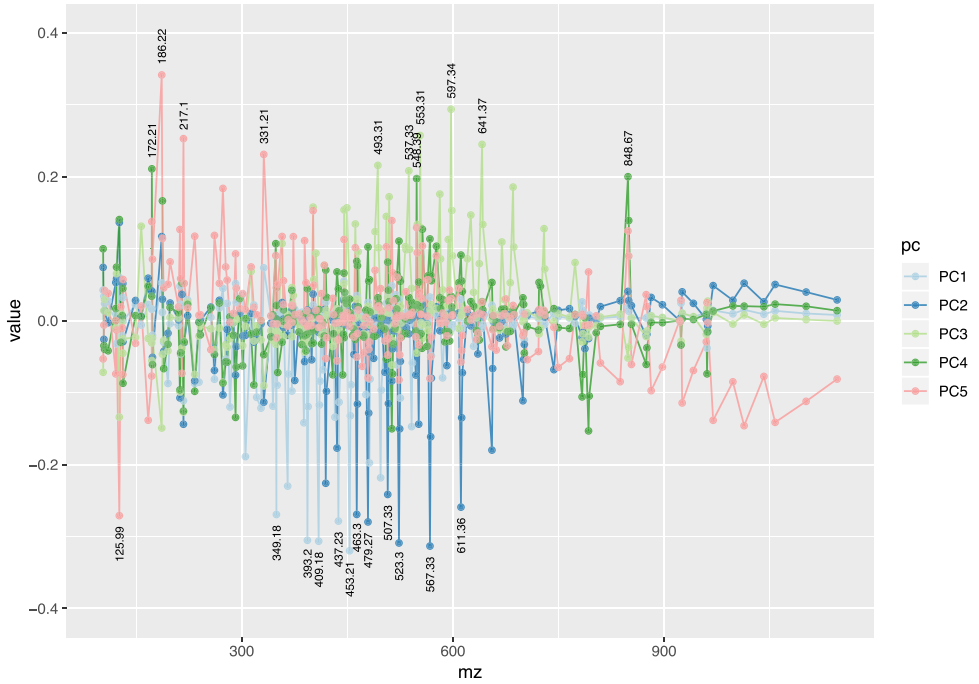

Fig. 6. Plot of the absolute values of component loadings of the DESI-MS spectra from condom lubricant for the first 5 principal components.

major lubricant component PEG (*m*/*z* 585.2889, Fig. 7E and F). PEG was found to be highly abundant in the lubricated fingerprint, while only minor abundance was found in the blank. Importantly, in case of the lubricated fingerprint, classification using a subset of the data (to get the same number of averaged scans), correctly predicts the source of the lubricant as EXS (Table S3).

4. Discussion

To the best of our knowledge, this is the first study describing a method for the differentiation of condom lubricants from CA treated fingerprints using DESI-MS combined with a PCA-LDA classification model. The generated model showed high accuracy for both direct analysis of condom lubricant spots (99.0%), as well as lubricated fingerprints (90.9%). Moreover, since a large range of different brands and types of condoms, commonly sold in the Netherlands, were analyzed, a representative database was collected.

The detection, discrimination and visualization of condom derived traces from fingerprints is of significant evidential importance in sexual assault cases, as it provides crucial information on the presence of a criminal at a crime scene as well as contact with a condom and type of condom used, thereby greatly increasing the strength of the evidence. In previous studies, it has already been shown that condom lubricants can be detected and discriminated, solely or within fingerprints, using several MS techniques, including DART-MS [18–20], MALDI-MS [21,22] and DESI-MS [25,26]. However, in these studies the effect of cyanoacrylate (CA) was not examined, while in forensics CA fuming is frequently performed to visualize fingerprints as they often appear latent. We now show that the current described method is compatible with cyanoacrylate fuming, rendering it more suitable for application to forensic casework. Additionally, full chemical images could be acquired from CA treated lubricated fingerprints, showing the spatial distribution of lubricant components such as PEG and PDMS throughout the fingerprint, which can be combined with classification of condom lubricants. The spatial information provided by chemical imaging, confirms that the lubricant was transferred by fingerprint contact as it links the presence of condom lubricant to the fingerprint ridge detail, making it of more evidential value than the sole analysis and comparison of condom components.

MALDI-MS was previously shown to have the potential to discriminate between different condom brands or types, combined with chemical imaging in a multidisciplinary analytical approach, by Bradshaw et al. [22]. However, the advantage of using DESI-MS as compared to MALDI-MS techniques, is that no matrix or sample preparation is needed and analysis can be performed at ambient pressure. DART-MS analysis offers straightforward analysis without the need for sample preparation, and was shown to be able to achieve high classification accuracies based on condom lubricant spectra [18–20], but lacks the capability to generate chemical images. We now found that DESI-MS combines the easy and direct analysis of condom lubricant samples with the ability to perform chemical imaging resulting in high accuracy detection and discrimination of condom traces. Although MALDI-MS is capable of achieving higher spatial resolutions, the chemical images generated using DESI-MS show clear ridge detail, which we found to be sufficient for the purpose of this method.

Using the developed DESI-MS method, we found multiple condom lubricant components. Among the most commonly encountered compounds were ion series corresponding to PEG, PDMS, nonoxynol-9, octoxynol-9 and PEG dodecyl ether. Based on the loadings of the first PCs, PEG, nonoxynol-9 and octoxynol-9 seem to be the most discriminatory lubricant components. Being an essential part of many lubricant bases, PDMS, PEG and nonoxynol-9 have been analyzed from condom lubricant traces using various analytical techniques, and have, not surprisingly, been included in many recent condom lubricant classification studies [18–20,25,26]. The detection of octoxynol-9 in condom lubricants is less commonly encountered, but has been described by Thomas et al. [40] and Bradshaw et al. [21]. The putative annotation of two fatty alcohol ethoxylates (PEG decyl ether and PEG dodecyl ether), that possible serve as ethoxylate lubricants, are in agreement with findings by Mirabelli et al., who already mentioned the possible presence of ethoxylate lubricant in certain

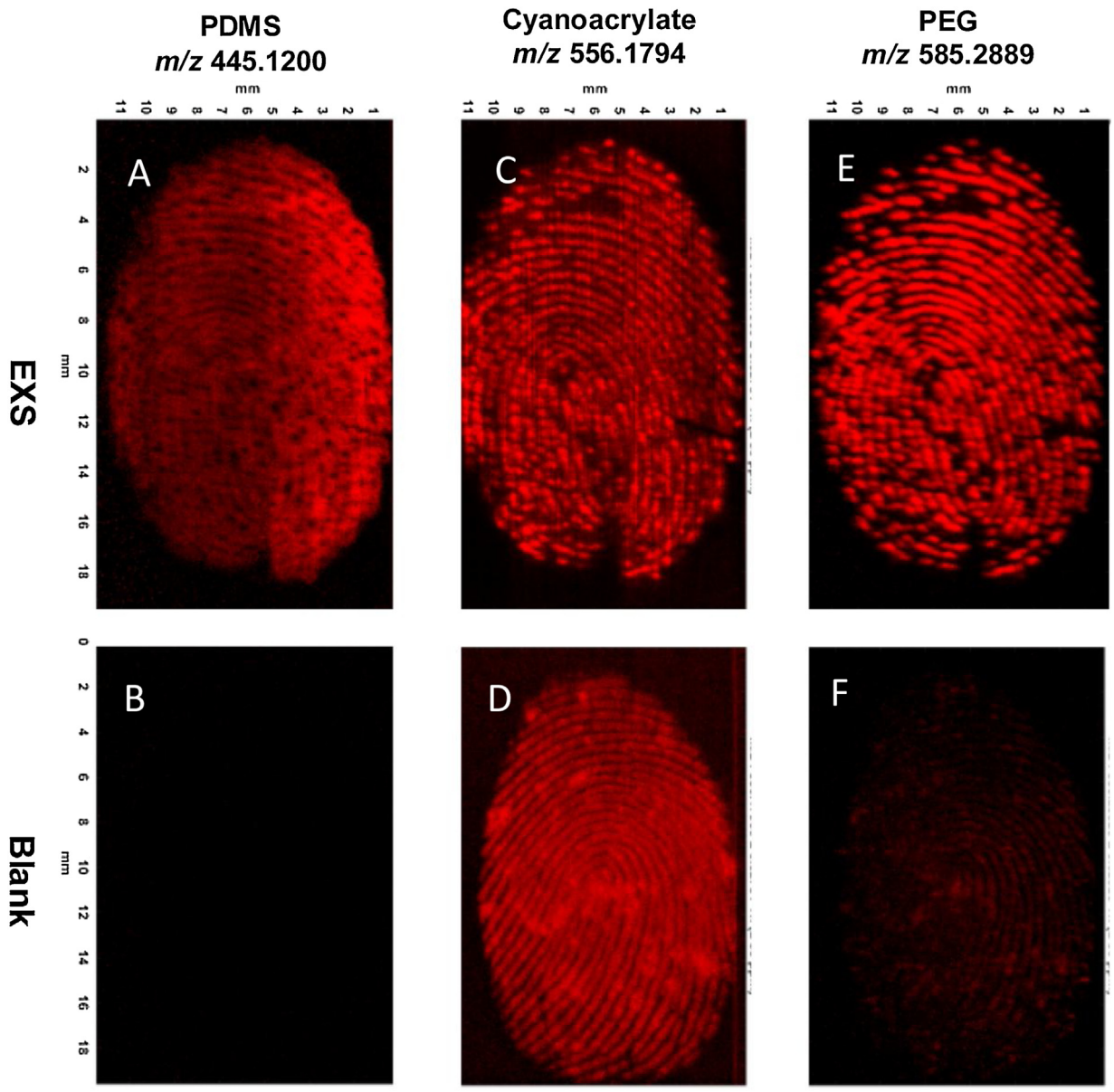

Fig. 7. Chemical images (12 × 20 mm) showing the distrubution of PDMS (*m*/*z* 445.1200, **A** and **B**), cyanoacrylate (*m*/*z* 556.1794, **C** and **D**) and PEG (*m*/*z* 585.2889, **E** and **F**) throughout a fingerprint containing EXS lubricant (**A**, **C** and **E**) and a natural blank (**B**, **D** and **F**) fingerprint.

types of condoms [26]. The *m*/*z* values used for the putative annotation of poly(ethylene glycol) dodecyl ether in our study, correspond to previously described polymer fragments from an unknown ethoxylated polymer species by Mirabelli et al. [26]. Additionally, Musah et al. reported the detection of octyl alcohol ethoxylate from *Skyn* condoms, after DART-MS analysis [16]. We also detected *m*/*z* values corresponding to undecylamine and dodecylamine, which, to our knowledge, are not commonly detected in condom lubricants, although octylamine was identified in many of the previous studies, mainly used as emulsifier, dispersant or lubricant [16,25,18–20].

There are numerous alternative approaches available to generate classification models based on analytical data. In our approach, we used PCA as a first step, to reduce data dimensionality, making the data easier to perceive. LDA was subsequently chosen as classification method as it showed to be an easy and fast classification method, which had already proven to be effective in discriminating condom lubricants based on mass spectra in previous studies by Maric et al., Baumgarten et al., and Mirabelli et al. [18,19,26]. In terms of classification accuracy based on lubricant spectra using DART-MS, Maric et al. achieved a 98.7% accuracy based on classification of 90 lubricants to one of 12 distinctive groups [18], Baumgarten et al. acquired a 88.9% accuracy when classifying 18 different lubricants [19], while Coon et al. discriminated 110 condom types from 16 different brands with a 97.4% accuracy [20]. Classification of lubricants from 10 different condoms using DESI-MS by Mirabelli et al. resulted in a 94% accuracy [26]. Our results are largely in line with these previous studies, as we gained a 99.0% accuracy when analyzing condom lubricant spots, and a 90.9% accuracy based on analysis of lubricant containing fingerprints. Additionally, these results show that the presence of CA does not interfere with the detection and discrimination of condom lubricants, and high accuracy classification of CA fumed lubricant traces using DESI-MS and PCA-LDA analysis is attainable.

Some of the misclassifications in our study seem to be caused due to lubricants originating from the same condom brand. When analyzing lubricant spots, a sample containing KSb was predicted

as KSs (both *Kruidvat* condoms), while in fingerprints containing lubricants, DES was predicted as DCN (both *Durex* condoms) and DP was predicted as DO (both *Durex* condoms that contain benzocaine). The misclassification of condom lubricant originating from two different *Durex* sources was also experienced in one occasion by Mirabelli et al. [26], likely being the result of similarities between condom lubricants originating from the same brand. This was shown by Maric et al. and Coon et al., who classified condom lubricants to a major lubricant group/brand with high accuracy [18,20]. Predicting the condom lubricant traces by brand only, instead of brand and type, would presumably lead to an increased classification accuracy in our study as well. For one particular condom (PL), we found a low sensitivity (40%) in lubricated fingerprints, which seemed to be the result of low ion intensities, possibly explaining the poor performance of the statistical model in this case.

As the major components of condom lubricants are known contaminants in mass spectrometry [38], we encountered carry-over problems during method development and optimization, that were similar to the effects described by Mirabelli et al. [26]. In their study, it was found that the most relevant parameters determining the 'memory effect' were the distance between the spray tip and ion transfer line and between the ion transfer line and sample. Too short distances resulted in contamination of the ion transfer line, as sample material could be sucked into the MS inlet [26]. Indeed, we also found that increasing the ion transfer line-to-surface distance and spray tip-to-ion transfer line distance, together with cleaning the MS inlet after 3–4 samples, resulted in avoidance of sample carry-over, indicating that these are crucial settings and actions for reliable results when analyzing condom lubricant traces with DESI-MS. Also, when imaging lubricated fingerprints using DESI-MS, we found that high amounts of condom lubricant in the fingerprints did not generate high quality chemical images, due to a decrease in clear ridge detail as a consequence of high abundances of PDMS and PEG ion signals. However, the classification model still predicted the source of the lubricant correctly, indicating that a discrimination could still be made.

In this paper, we solely focused on the analysis of condom lubricant traces in CA treated fingerprints. However, the main components of these condom lubricants, such as PEG and PDMS, can also be found in many personal care products [15]. As a result, analysis of fingerprints that possibly contain traces of any of these personal care products, may lead to misclassifications. Although the ability to discriminate between personal care products and condom lubricants in fingerprints was not analyzed in the present study, a recent study performed by Moustafa and Bridge showed that discrimination between these classes of products is possible using DART-MS and LDA [15]. The addition of discriminating factors from other classes of personal care products to the current developed model would further increase the forensic applicability of the generated method. Furthermore, we only measured fingerprints with condom lubricant traces from glass substrates, while in practice, fingerprints can be found on all available substrates. Further optimization of the analysis of fingerprints containing condom lubricant traces on several different substrates would also benefit the developed method. Indeed, Mirabelli et al. showed that chemical analysis and imaging of fingerprints containing condom lubricant is possible on metal and paper surfaces [25,26]. However, spectra obtained from paper surfaces had lower signal intensities due to sorption effects, and a wash-out effect was encountered when analyzing on metal surfaces [25,26].

5. Conclusion

We developed a DESI-MS method for the detection and discrimination of condom lubricant traces from fingerprints that, combined with a PCA-LDA classification model, has an overall accuracy of 90.9% and is compatible with CA fuming, making it more applicable for forensic casework. Additionally, full chemical images of fingerprints containing condom lubricant traces could be acquired, visualizing the spatial distribution of condom lubricant compounds, such as PDMS and PEG. This confirms that the condom lubricant is originating from the fingerprint and not the substrate, thereby increasing evidential strength. These results are promising leads for further development of DESI-MS methods to qualitatively analyze exogenous compounds from fingerprints for use in forensic science.

CRediT authorship contribution statement

Ward van Helmond: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Visualization. **Mark P.V. Begieneman:** Conceptualization, Methodology, Investigation, Writing - original draft. **Roos Kniest:** Investigation, Writing - review & editing. **Marcel de Puit:** Conceptualization, Methodology, Writing - review & editing, Project administration, Funding acquisition.

Declaration of Competing Interest

There are no conflicts to declare.

Acknowledgement

WvH acknowledges a RAAK-PRO research grant (no. 2014-01-124PRO), the Netherlands.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.forsciint.2019.110005.

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