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Short communication

On the impact of DNA extraction procedure on the recovery of condom evidence

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ABSTRACT

On the international level, different protocols exist for sampling condom traces, similarly to DNA traces. Usually collected with cotton swabs, some protocols use nylon swabs, which were found more efficient for the desorption of DNA, because they offer a better desorption of the compounds during the extraction. In addition, not all the protocols do contain swabs for other evidence than DNA. Depending on the protocol, the forensic scientist will either benefit from a swab purely dedicated to condom evidence or will have to prioritize which evidence to analyse first. It is more likely that priority will be set to DNA, but in the eventuality that no DNA would be recovered, it is necessary to know whether the swab can still be used for condom evidence recovery or not. This work aimed to use Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFTS-FTIR) to investigate whether DNA extraction affects the recovery of condom traces. Traces were simulated by either rubbing condoms on the swabs or soaking the swabs in solutions containing different concentrations of polydimethylsiloxane (PDMS), the most common condom lubricant found on the market. DNA extraction was found to generate a loss of silicones, when processed prior to condom silicone extraction. Therefore, results show that protocols should contain cotton swabs dedicated to condom traces collection.

1. Introduction

When it comes to rape or sexual assaults cases, forensic samples are collected on the victim, and usually focus on DNA evidence, which has become a sort of golden standard in the forensic area [1–3]. However, in some cases, it might appear that no DNA would be detected, which requires the forensic scientist to look for other types of evidence. Amongst different types of evidence, condom evidence was found to be of specific interest [4], as it is used to avoid sexual transmitted diseases or to avoid a DNA transfer [4–7].

Target compounds coming from condoms are lubricants, constituting the most important source of recovered evidence [8,9]. Lubricants are added to avoid the polymeric body of the condom (mostly latex) to stick to himself [8]. Polydimethylsiloxane (PDMS), glycerine and polyethylene glycol (PEG) are the most common lubricants according to a recent study [10,11]. If PEG and glycerine are water-based compounds presenting a short persistence in the vaginal matrix due to their chemical properties, PDMS is a silicone polymer which is not sensitive to aqueous matrix, such as the vaginal matrix, nor to micro-organism that can be found in the vagina [12–14]. These properties make it an interesting target compound for casework analysis.

Various instrumentations have been developed for the analysis of condom lubricants, and more specifically for silicone lubricants, from spectroscopic techniques [9,15–17] to more complex mass spectrometric methods [18–24], mostly focusing on the detection and discrimination of the lubricant. FTIR, and more specifically DRIFTS FTIR, has been reported as being a very good screening method [16] and was applied in multiple casework as reported in [9,25]. The availability in most forensic laboratories also makes it a more valuable tool in the forensic community.

On the international level, condom traces, similarly to DNA traces, are usually collected with cotton swabs [26–28], although some protocols are rather using nylon swabs, because they offer a better desorption of the compounds during the extraction [29,30]. Protocols can vary between different countries, but not all the protocols do contain swabs for other evidence than DNA. As a matter of example, the Swiss protocols have been harmonised on the police level. The harmonised procedure recommends the use of nylon

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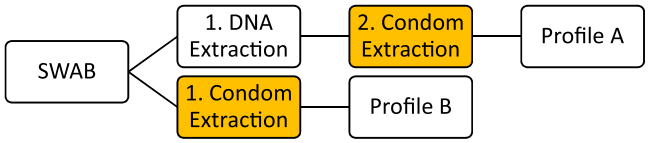

Fig. 1. Illustration of the extraction sequence.

swabs for sampling DNA, and do not offer additional swabs for condom evidence (Personal communication Dr. Vincent Castella, Centre Universitaire Romand de Médecine Légale (CURML)). On the other hand, the Sexual Assault Ressource Center (SARC) in Perth provides kits with over 20 cotton swabs, including some dedicated to condom evidence (Personal communication Dr. Maire Kelly, SARC). Their sampling form also contains specific questions to ask the victims regarding a recent use of condoms or lubricants. Therefore, on one side the forensic scientist will benefit from a swab purely dedicated to condom evidence, while on the other side, one will have to prioritize which evidence to analyse first. It is more likely that priority will be set to DNA, but in the eventuality that no DNA would be recovered, it is necessary to know whether the swab can still be used for condom evidence recovery or not.

The present paper aims to investigate how DNA extractions affect the recovery of silicone lubricants evidence compared to direct extraction of silicone traces, to guide the forensic practice and inform on the complementarity of DNA and condom evidence. The effect of DNA extraction on condom residues will be reported on both qualitative and semi-quantitative points of view.

2. Material and Methods

2.1. Material

Hexane and dimethylpolysiloxane 200 cSt were purchased from Sigma-Aldrich Potassium bromide (analytical grade) was purchased from Fluka Chemika and was manually grinded before use. The cotton swabs (COPAN 150 C) were purchased from COPAN Inc. (USA). The condom used were silicone-lubricated from the brand *COOP Qualité Prix*. QIAamp DNA Mini Kit and Investigator Lyse & Spin basket kits were purchased from Qiagen (Qiagen AG, Hombrechtikon, CH).

2.2. Solutions

A standard solution at 10 mg/mL of PDMS was prepared as a mother solution. Dilutions at different concentrations of respectively 0.1, 0.25, 0.5, 1.0 and 2.5 mg/mL in hexane were realised.

2.3. Sample preparation

Condoms were unrolled and a direct transfer was realised by swabbing the condoms directly with the cotton swabs, for a total of 10 swabs. For each solution prepared between 0.1 and 2.5 mg/mL, 10 swabs of each type were infused in the solutions for 1 min, and then left to dry prior to proceeding to extractions. A total of 100 swabs were obtained for the solution samples.

2.4. Extraction procedure

Prior to any extraction, the swabs were cut at the basis of the joint between the wooden stick and the swab itself. The extraction procedure was processed as follows: half of the swabs were processed firstly through DNA extraction, then followed by the condom extraction, to obtain a first profile. The other half of the swabs were directly extracted with the condom extraction procedure, to obtain a second profile. Blank swabs were processed at the same time to ensure there were no contaminations. The sequence is illustrated in Fig. 1.

2.4.1. DNA extraction

Spin baskets were placed in each sterile tube used for the extraction. The cut swab was first deposited in the spin basket. Then 180µl of ATL buffer were added, as well as 20µl of proteinase K. The tubes were vortexed and incubated for 1 h at 56 °C at 500 rpm on a thermoshaker, and then centrifugated 5 min at 12000 rpm. The spin basket was then removed, and the swab contained in it was deposited in a clean vial for condom extraction.

2.4.2. Condom extraction

Swabs were cut from the wooden sticks and individually put in a glass vial and extracted with 1 mL of hexane. The vials were vortexed for 1 min and sonicated for 15 min. The resulting samples were analysed in triplicates.

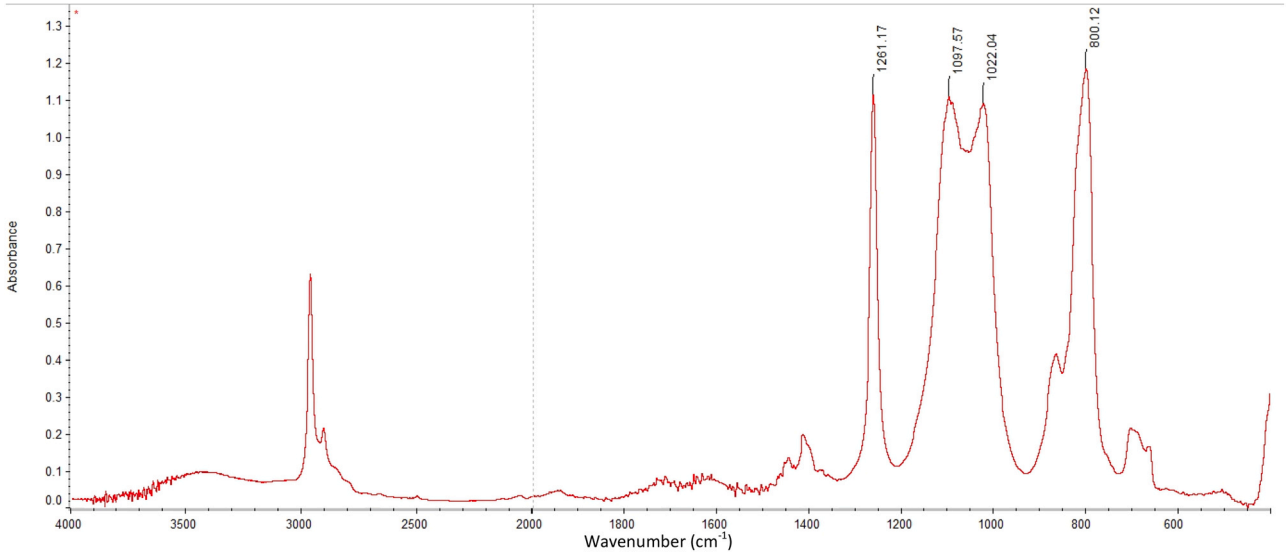

Fig. 2. : Illustration of a reference PDMS spectrum analysed using DRIFTS.

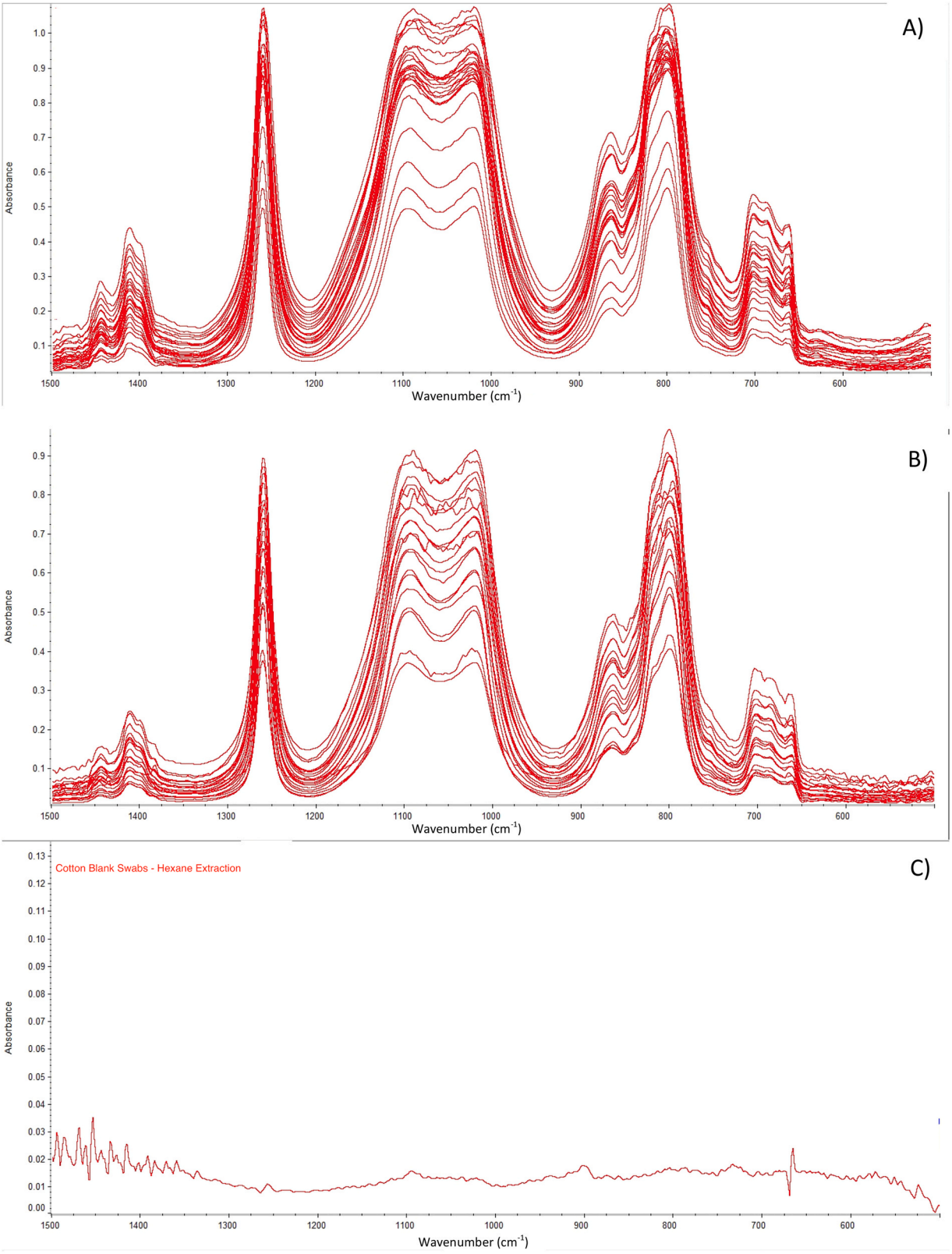

Fig. 3. Illustration of the spectra obtained A) after hexane extraction, B) after DNA followed by Hexane extraction, C) blank cotton swab.

2.5. Instrumentation and analytical conditions

DRIFTS spectra were acquired with a Digilab FTS 3000 Excalibur FTIR spectrometer, equipped with a Spectra-Tech 0030–05 Collector II diffuse reflectance accessory and DTGS detector. 64 scans with a resolution of 4 cm^{-1} were processed on the entire 4000 and 400 cm^{-1} wavenumber zone. Potassium bromide (KBr) was manually grinded to obtain a homogenous powder and deposited into metal sample cups for DRIFTS analysis. Manual pressure was applied with a spatula to the pellets to remove residual air, and the pellet batch stored

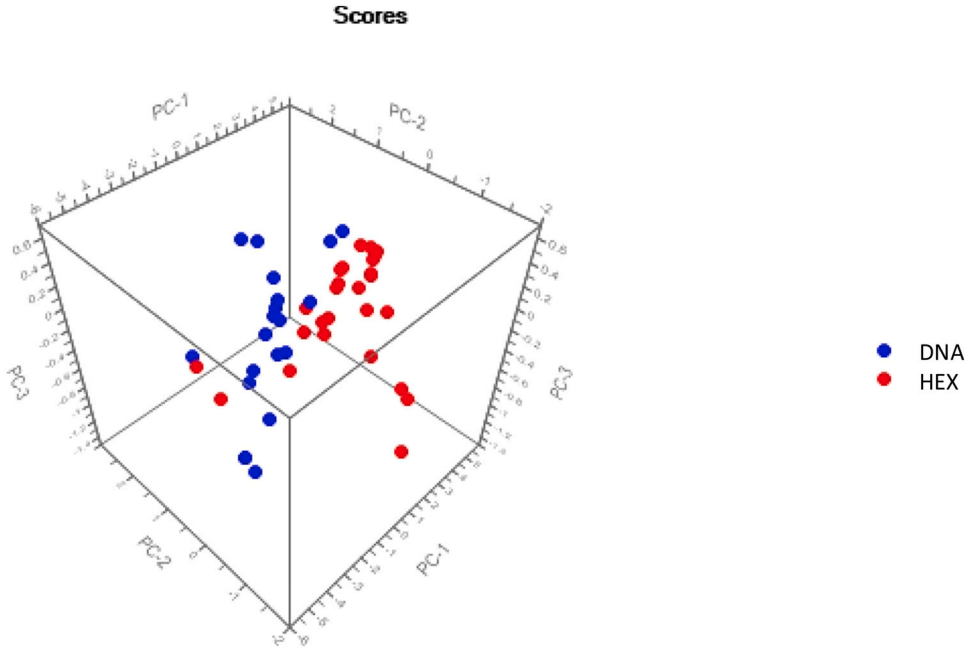

Fig. 4. 3-dimensional PCA score plot showing the distribution of the sample according to the extraction procedure which has been processed first. In blue the samples have been through DNA extraction prior to hexane extraction; in red, samples have been directly extracted with hexane for condom evidence.

in a 100 °C oven. For analysis, 10µl of sample in solution were spiked onto a pellet which was then placed in a 100 °C oven for 15 min to evaporate the solvent.

2.6. Data processing

All infrared spectra were acquired using ResolutionPro v. 4.0 from Agilent. Spectra were exported in.spc format. Spectra were the imported in ThermoFisher Omnic32™ software (v. 8.2.0.387), to process qualitative analysis and visualization of the spectra. All the spectra were subjected to a baseline correction using Savitsky-Golay algorithm.

Impact of the extraction was investigated by observing qualitatively and comparing chemical profiles obtained straight after proceeding to hexane extraction for condom lubricants, and after DNA followed by hexane extractions. The presence of PDMS peak was assessed and the comparison of the extraction was led by comparing the difference in the presence or absence of the PDMS bonds as well as the variation in their absorbance.

3. Results and discussion

3.1. Preliminary considerations

Reference raw PDMS was analysed to observe the expected chemical profile to obtain from the samples after the different extractions. The presence of PDMS is evident when the 4 peaks linked to PDMS vibrations are present, i.e. Si-O-Si symmetric and asymmetric stretching at 1020 and 1090 cm^{-1}, Si-C stretching at 1263 cm^{-1} and the dimethyl and trimethyl symmetric deformation near 807 cm^{-1} [9,15,16,25] (Fig. 2). Si-O-Si and Si-C vibrations are the most important ones to focus on when analysing for PDMS using vibrational spectroscopy, as they are directly linked to the backbone of the molecule, thus being diagnostic peaks attesting the presence of silicone-based products. The rest of the study will focus exclusively on the 500–1500 cm^{-1} region, given that it is where the diagnostic peaks of PDMS appear.

The four bands are correlated and the presence of the four of them is needed to assess the presence of PDMS. Therefore, when it comes to low concentrations, challenges in the detection of the peaks may be encountered.

In addition, both DNA and condom extraction procedures were taken from practitioners' practice and published literature [9,33,34]. The choice to compare nylon and cotton swabs was led by different practice around the world, although most laboratories use cotton swabs. The comparison of the swabs quality of recovery is important as it might affect the recovery of low concentration trace evidence. Similarly, the use of one dedicated swab for condom evidence sampling versus the use of only one swab for both DNA and condom evidence was also led by different practices amongst legal medicine laboratories.

3.2. Impact of the extraction procedure

Investigation of the extraction procedure and sequence was led on swabs which have been.

1. Extracted with hexane for condom evidence recovery
2. Extracted for DNA and then with hexane for condom evidence recovery.

Simulations were first realized on reference material obtained by swabbing silicone lubricated condoms. The qualitative observations of the spectra are presented in Figs. 3A and 3B for the results of the extraction, and Fig. 3C illustrates the blank swabs. Blank swabs were all found to be blank. Therefore, the peaks detected in the spectra were considered as relevant and coming from the sample. PDMS peaks are systematically present in all the spectra. The variability within the spectra extracted with the same procedure is very important, independently from the selected sequence. The absorbance was found to vary between 0.45 and 1.25 when the swabs were first extracted with hexane, to varying between 0.3 and 0.9 if DNA extraction had been processed first. The range is overlapping between the two extraction procedures, however a loss is highlighted, with smaller absorbance detected if DNA extraction comes first. It is also to keep in mind that part of the variation is due to the instrumental conditions, as KBr pellets were manually prepared, and samples

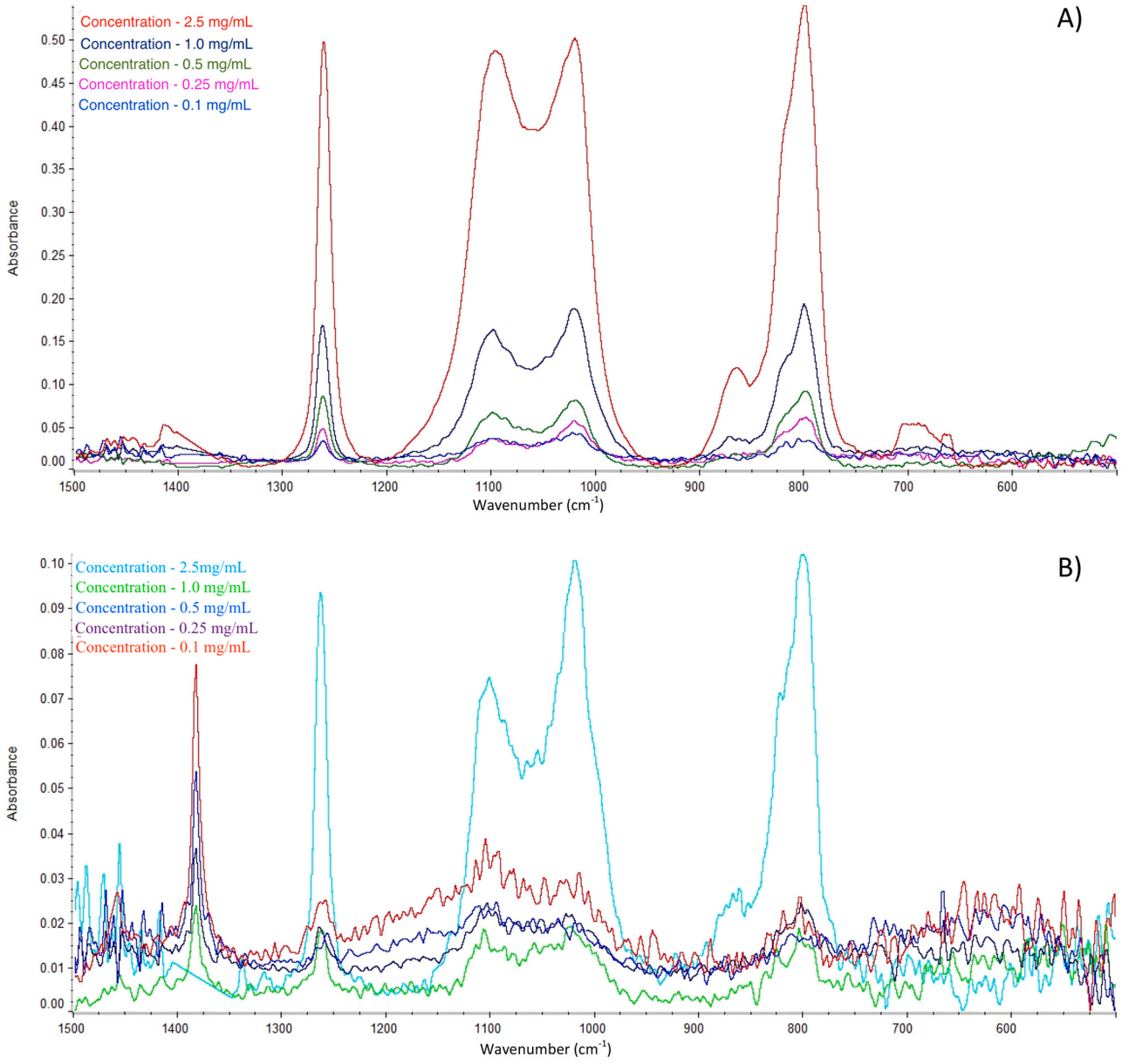

Fig. 5. : Illustration of the decrease of the concentration A) after direct hexane extraction, B) after DNA followed by hexane extraction.

were spiked manually on the pellet, thus causing a non-controlled deformation of the surface, affecting the quality of the results.

From a statistical point of view, all the data were baseline corrected and range normalized [11,31] and Principal Component Analysis (PCA) was performed on the whole dataset. This was used to investigate whether the extraction influenced statistically the discrimination pattern, given that it might affect the classification of samples within a given model to identify the source of the sample [35]. Observation of the PCA (Fig. 4) confirms previous qualitative observations of the significant overlap between the data and outlining the high variability of the data. Spreading of the data along PC2 and PC3 are similar for both populations, whereas PC1 allows to separate the data: spectra extracted first with hexane present higher positive scores than spectra extracted with DNA first. Observation of the loading plots does not allow to identify a region that would generate a higher discrimination of these samples. Therefore, a difference of concentration is more likely to be the source of the differences between the two groups. This hypothesis is even confirmed by the investigation of the overlapped points: blue dots (DNA) clustered within the red population (HEX) are the ones which present the highest absorbance (and therefore the highest concentration) of silicone residues, whereas the red dots (HEX) clustered within the blue population (DNA) are the ones which present the lowest absorbance in the population.

Based on the observation of the data acquired on a high amount of silicone trace, a strong variation due to the extraction procedure was noticed. Such variability can significantly affect trace evidence recovery, and therefore, prior to moving on trace evidence, diluted reference material transfer and extraction will be investigated. To this effect, samples prepared with known concentrations ranging between 2.5 and 0.1 mg/mL silicone were extracted through the whole extraction procedures. Fig. 5 illustrates the observations of the absorbance decrease as the concentration decreases in the samples. Data acquired after hexane extraction (Fig. 5A) at first present a strong decrease with peaks being visible up to 0.25 mg/mL, which is the limit of detection as described in [16]. Regarding the swabs run after DNA extraction, only the swabs containing 2.5 and 1.0 mg/mL silicone did provide a chemical pattern, no peaks were observed in

Table 1
Absorbance variation between the two extraction sequences. ND = non detected.

Concentration [mg/mL]	Absorbance after hexane extraction	Absorbance after DNA extraction followed by hexane extraction
2.5	0.426 ± 0.08	0.071 ± 0.04
1.0	0.199 ± 0.073	<0.02
0.5	0.104 ± 0.035	ND
0.25	0.069 ± 0.018	ND
0.1	0.034 ± 0.008	ND

any of the lower concentration samples. It is important to note in Fig. 5B that although the 1.0 mg/mL pattern is visible, the absorbance is very close to the background and is inferior to the limit of detection. Therefore, it can be concluded that DNA analysis affects the recovery of condom evidence when performed prior to hexane extraction.

Maximal absorbance was noted in all the replicates of the different specimen analysed. Mean and standard deviation were calculated for each concentration specimen and the results are gathered in Table 1.

As illustrated in Table 1, DNA extraction significantly affects the recovery of condom evidence. The results obtained for the hexane extraction procedure are in accordance with previous published papers which reported similar values of the absorbance after extraction of diluted silicone residues with hexane [35].

Sexual assaults samples are not usually collected straight after the aggression, and victims may wait for a long time before reporting the case either to the police or to medical examiners at the hospital. Traces, especially condom traces, might suffer from a long time interval between alleged assault and sample collection, as they not only decrease over time naturally as illustrated by [8,16] but may also be affected by the victim's activity in this interval [8,10]. The selected FTIR method for this study allows the detection of condom traces up to 18 h post-coitus, with a strongly decreasing absorbance. Based on data published in [16], the estimated maximal absorbance for an 18-hours post coital sample extracted only with hexane is around 0.03. Such absorbance fits an estimated concentration of 0.1 mg/mL according to the data gathered in Table 1, but that concentration is not detected if DNA extraction has been processed prior to hexane extraction for condom traces. This might be an issue when it comes to the interpretation of the evidence.

Therefore, based on the results observed in this study, it would be more adequate that the sampling kits for sexual assault contain some cotton swabs dedicated to condom evidence sampling, rather than having to use a same swab for both DNA and condom evidence analysis.

4. Conclusion

In sexual assault cases, DNA is the most investigated evidence. Recently, forensic examiners have observed an increase of the number of cases in which no DNA was detected, and where condom use were questioned. Condom evidence has therefore become a very interesting evidence, used in complementarity to DNA evidence.

In the forensic practice, sexual assault collection kits were initially not designed for condom evidence collection. Many different protocols exist in different countries and may even differ within the same country. Some use cotton swabs for DNA sampling, whereas other use nylon swabs. Similarly, some have added in their protocols swabs dedicated to condom evidence whereas others haven't yet, and would use the same swab for both DNA and condom evidence analysis. The potential of an extraction sequence with DNA and condom residues in the same sequence was investigated. It was found out that although no big differences were observed when extraction highly concentrated samples, DNA extraction was significantly affecting the recovery of lower concentrated silicone content coming from condoms. Therefore, it would be advised that sexual assault collection kits would contain some cotton swabs dedicated to condom evidence.

CRediT authorship contribution statement

Chloé Mbo: Writing – original draft, Methodology, Validation, Data curation, Visualization. **Florian Honoré:** Writing – review & editing, Resources. **Marie-Pierre Milon:** Writing – review & editing. **Geneviève Massonnet:** Writing – review & editing, Resources. **Céline Burnier:** Writing – review & editing, Conceptualization, Methodology, Validation, Investigation, Resources, Data curation, Visualization, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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