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Optimization of a Py-GC/MS method for silicone-based lubricants analysis

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ABSTRACT

Condom evidence can be analysed using several analytical techniques, such as FTIR, MALDI-MS or DART-TOF-MS, but the only one that was used on real samples for transfer and persistence studies in the context of sexual assault or rape cases was Py-GC/MS. However, there has been no study to identify which specific pyrolysis parameters were the most suitable for the analysis of silicone-based lubricants, especially in terms of repeatability of the analyses.

This study looked at the different reported pyrolysis parameter with the aim of optimizing these parameters for polydimethylsiloxane (PDMS) analysis and detection. Experimental parameters were refined while performing a full factorial experimental design (FFD) for the screening, extended to a face centered central composite design (FCCD) for the optimisation. Analyses were led on standard PDMS reference material for the optimisation. Two-way ANOVA statistics and surface responses were used to define the most adequate parameters for the analysis.

The adequate parameters were then applied to five condom extracts that were analysed in replicates. Chemometrics was used to evaluate within and between sample variations. Separation of the samples was investigated and was not found to be applicable to the limited set of samples. Issues in reproducibility were highlighted and further investigation on different instruments are necessary to improve the reported study.

1. Introduction

In the last decades, condom evidence has become an increasing topic of forensic concern, with several cases reported in the literature [1–4], from police statistics [5,6] or from Court Appeal [7]. Although medical studies have shown that condoms were the second most used contraception device after oral contraception, condoms are designed to protect from sexually transmitted diseases (STDs) during a sexual intercourse. There has been an increasing need to be able to detect condom evidence in sexual assault cases, especially when no DNA was recovered. The recovery and characterisation of such evidence may provide associative evidence and help establishing corpus delicti [3,4,8]. Condom evidence will therefore be used to check if there was a penetration [3,4,8], as well as to support the allegations of the victim or of the aggressor. In this case, it is not the sexual act but the way it happened that is questioned [9].

Modern condoms primarily consist of latex covered with solid particles, lubricants and in some cases spermicide and aromas or flavourings. Latex is the bulk of the product, offering protection for pregnancy and STD transmission. Lubricants, allow a proper lubrication during condom use, are present at around 500 (± 50) mg on the condoms and are generally PDMS- or PEG-based lubricant [8–12]. These are the only two types of lubricants that can be found on condoms as they are not altering latex properties, as stated by international regulations [13–15]. Some condoms also contain spermicide, usually around 5–10% of the total lubricant weight [10,16]. The remainder of the products consists of additives, such as solid particles (e.g. corn starch or polyethylene powder), antioxidants, flavourings, aromas, anaesthetics and preservatives. These may be present to extend product's lifetime, give a specific smell, delay ejaculation, or enhance the polymer protection [2,9,10,17].

Condoms are typically the type of mass-produced consumer products, and although different brands and models are present on the market, there are limited possibilities to individualise any of them [9,18–20]. However, forensic scientist analysing condom evidence faces currently two different challenges, the first being the detection and discrimination between different types of condoms, the other being the use of a method applicable to real samples, allowing an accurate detection in real cases, when found in swabs collected by medical examiners.

Literature offers a very diverse panel of analytical techniques used for condom analysis, from non-destructive techniques (e.g. FTIR,

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Raman) [4,11,21–23] to more complex ones (e.g. MALDI-MS, DART-TOF-MS) [18–21,24,25]. Among all these techniques, pyrolysis-GC/MS (Py-GC/MS) has been referenced several times as a powerful confirmation technique or as a way of detecting traces up to 48 h after a sexual intercourse [12,26,27]. This method is subject to many critics [19,20] because pyrolysis would degrade minor compounds that could be interesting in forensic investigations and therefore the information obtained from these analyses would be limited. Indeed, Py-GC/MS has a number of advantages, but also disadvantages which are discussed here after.

In terms of disadvantages, Py-GC/MS can be a very challenging and complex method, and no study to date has presented complete and optimized pyrolysis parameters based on a strong experimental design and thus statistical evaluation [11,12,26,27], although these are key points in this type of analysis [28,29]. In addition, the repeatability of the results has not been published or presented and there is no complete indication of the data processing methods or database of chemical pyrolysis profiles of the compounds of condoms. In terms of advantages, Py-GC/MS offers the possibility to analyse non-volatile compounds (such as silicone-based lubricants), with a very good sensitivity. It is also the only method that has, up to now, successfully been applied to real samples and was found to be adequate for the evaluation of transfer and persistence of silicone-based products in a human matrix [12,26,27]. However, there's never been any investigation on the discrimination potential of condoms using this method on a massive sample set.

The present paper aims to determine which factors, between the temperature and the time of pyrolysis, most significantly affect Py-GC/MS analysis in order to obtain a more adequate understanding of how to analyse silicone-based condom residues. This type of research is absolutely mandatory in forensic sciences, especially when dealing with instruments such as Pyrolysis-GC, as it was previously outlined that pyrolysis parameters as well as the amount of sample deposited for analysis were significantly affecting the quality of the analytical response [28–31]. Experiments were carried out using a full factorial experimental design (FFD) followed by an extension to face central composite design (FCCD) to explore the possible combinations of parameters using multivariate statistics. Interaction between the different factors were also investigated thus leading to the construction of response surface plots to understand how parameters affect the analytical results and how to set up proper instrumental parameters to allow repeatable and sensible analyses. The optimised parameters were then applied to condom extracts to ensure the potential applicability to real sample analysis.

2. Material and methods

2.1. Material

Hexane of analytical grade was from Sigma Aldrich (USA) and was used as received. PDMS 200 centiStokes (cSt) obtained from Sigma Aldrich (USA) was diluted in hexane at concentrations of 0.1 mg/mL and 1 mg/mL. Quartz tubes for pyrolysis and glass wool both come from CDS Analytical (USA). A 5 µl syringe eVol XR ® from SGE Analytical Science was used to deposit the samples into the quartz tubes.

2.2. Instrumentation and chromatographic conditions

The instrumentation used in this study is a resistively heated filament Pyroprobe 5150 from CDS Analytical Inc. The pyrolysis device was coupled to an Agilent GC 6890 N GC system interfaced with an Agilent 5975C mass spectrum detector, the software used were respectively Pyroprobe 3.21 from CDS and ChemStation v. D00.01.27 from Agilent.

Separation was achieved on a HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm) using helium as a carrier gas at a flow rate of 1 mL/min. Injections were carried out in splitless mode, the injector temperature being set at 280 °C. The chromatographic program was as follows: 50 °C for 2 min, 10 °C/min to 230 °C, 20 °C/min to 300 °C, and hold at 300 °C during 5 min. Concerning mass spectral detection, the transfer line was set at 250 °C, the ion source at 230 °C and the quadrupole at 150 °C. Data were acquired in full scan mode (30 − 550 m/z), with a sampling rate of 3.

Table 1
Factors and levels used for the identification of the surface response, using an FFD design.

Factor	Level -1	Level 0	Level 1
Temperature (°C)	420	620	920
Time (s)	10	20	30

2.3. Experimental design

Several experimental designs were conducted in this study, as an iterative process in order to obtain the most repeatable results. All the designs were realised using standard solutions of bulk PDMS diluted in hexane. The first experimental cycle used was a two-level FFD (Full Factorial Design) experimental plan, generated using Unscrambler X (Camo Software, Norway) to observe the response surface. The parameters used are described in Table 1. The chosen FFD plan used two replicas of each point (420 °C/10 s; 420 °C/30 s; 920 °C/10 s; 920 °C/30 s) and three replicas for the central point (620 °C/20 s). This resulted in a total of 11 randomized program experiments. The central point was defined at 620 °C and 20 s because it is the closest to the pyrolysis conditions presented in the literature [13,27]. Eight additional analyses were added to the plan, to study the variability of the extreme points, namely the couples 420 °C /10 s (3 replicates), 920 °C /30 s (3 replicates) and 620 °C /20 s (2 replicates).

The second experimental cycle was led to estimate the effects of each factor. A new FFD was designed, with new temperature levels chosen within ± 100 °C from the central point. The time variables have not been modified but correspond to a variation of ± 10 s around the value of the central point. Finally, based on the response obtained on the FFD, the latest was extended into a central composite design (CCD), more specifically here, a face central composite design (FCCD). To capture the true relation between the factors and the response, the FCCD was designed using 9 points (520 °C/10 s; 520 °C/20 s; 520 °C/30 s; 720 °C/10 s; 720 °C/20 s; 720 °C/30 s; 920 °C/10 s; 920 °C/20 s; 920 °C/30 s). For each experimental cycle, effect significance, lack-of-fit, regression significance and curvature were evaluated.

2.4. Data processing

Visual and qualitative analyses of GC/MS data were performed on Agilent Technologies' Enhanced Data Analysis MSD ChemStation software (v. D.02.00.275). The National Institute of Standards and Technology (NIST08) database was used to characterize the various components of the samples.

Six pyrolysis compounds, i.e. cyclic DMS oligomers, (Table 2) were chosen for the semi-quantitative analysis, because they were the most abundant compounds found and also well separated and known to be characteristic of siloxane degradation [32,33]. Additionally, literature also illustrated that the ratios between these major cyclosiloxane oligomers were varying within different polymers, and thus might be used for discrimination purposes [34]. Other products present were not selected because their abundances were very small (< 10,000 A.U). The choice of the target compounds was also based on the literature [12,26,27,35,36].

The R software (v. 1.2.1335) was used for statistical processing and for the choice of appropriate pre-treatments. Integrated area of the

Table 2
Chemical compound, retention time (on HP-5MS column), extracted ion for the selected compounds.

Compound	Abr	RT [min]	Target Ion (m/z)	Qualifiers (m/z)
Hexamethylcyclotrisiloxane	D3	4.51	207	96, 133, 191
Octamethylcyclotetrasiloxane	D4	7.14	281	249, 265, 191
Decamethylcyclopentasiloxane	D5	9.56	355	73, 267, 268
Dodecamethylcyclohexasiloxane	D6	12.03	429	73, 147, 341
Tetradecamethylcycloheptasiloxane	D7	14.27	503	281, 327, 415
Hexadecamethylcyclooctasiloxane	D8	16.26	593	355, 73, 221

target compounds was normalised to the total sum of areas and processed by double square root. The coefficients of variation (CV) were computed for each compound and the variability was figured out.

Data analyses of experimental designs were performed in Unscrambler X and two-ways ANOVA calculations was used to determine the effects of the factors. For all the models sketched on the data, the significance of the effects, the adjustment of the model (lack-of-fit), the significance of the regression and the curvature of the plans were evaluated. The lack-of-fit was assessed according to a Snedecor's test [37], and the curvature of the plan according to a Student's test [38]. Several regression models of different complexity (from linear to quadratic) were fitted on the data. The model describing the best relation between the factors was then selected based on the highest lack-of-fit p-value and the lowest regression significance *p*-value.

2.5. Application to condom samples

In order to make sure that the proposed method was suitable for real samples after being developed on PDMs standards, there is a need to observe the application on condom samples. Five condoms of different brands and models were purchased from the Swiss market for analyses (Table 3). Condom were individually opened and unrolled before being put in a 100 mL glass bottle and covered with 50 mL of hexane. The bottles were then closed and put in the ultrasonic bath for 15 min. Bottles were then stored at −18 °C until analytical runs. Before analysis, samples were aliquoted and diluted 10 times. 3 µl of the solution were spiked in the quartz tube on the glass wool and the analysis was processed.

Five replicate samples were prepared from each condom to probe the composition homogeneity of the sample as well as the variation due to the instrumentation and the sample preparation.

These condoms were then analysed with the optimised Py-GC/MS method established during this work. Qualitative and semi-quantitative analyses were performed, based on a selected pyrolysis compound and its relative abundance respectively.

3. Results and discussion

3.1. Preliminary considerations

To date, the majority of studies concerning the optimization of samples analysis in forensic sciences use standard solutions or real samples. The use of standard solution is not often reported when optimizing Py-GC/MS parameters, because real samples are more adequate, as they allow to consider all the potential interferences and recombination with other components present in the forensic sample when overcoming the pyrolysis [28,29]. Real samples extract of condom residues may mainly contain PDMS; therefore, the optimisation of the parameters in this study was realised using PDMS with a viscosity of 200 cSt, as it was previously highlighted that this was the most common PDMS used for condoms [39].

Table 3
Condoms used in this study.

No	Producer	Brand	Model
01	Reckitt Benckiser	Durex	Natural
02	Lamprecht	Ceylor	Blue band
03	Ansell	Manix	Orgazmax Plus
04	Ansell	Manix	Skyn Original
05	Ansell	Manix	Strawberry

Analytical parameters presented in the literature were first set on the instrument: pyrolysis temperature of 600 °C, 40 °C (hold for 2 min) to 300 °C (hold for 10 min), at a rate of 10 °C/min, with a split ratio of 1:100. However, the pyrolysis time was not indicated in any of the previous researches. Based on background knowledge on pyrolysis [28,29,40] and on preliminary experiments, a pyrolysis time between 15 and 20 s should allow the proper degradation of the polymer. Therefore, 20 s was chosen as an adequate pyrolysis time for a central point in the experimental design. Both pyrolysis temperature and time were set values on the instrument. Slight variations (± 2 °C) can be expected. As silicones and siloxanes are ubiquitous compounds, blanks were performed between each sample or solution analysis. Precautions were taken to avoid any contaminations, by cleaning the material with pure hexane between each analysis.

Instrumentation was set up with all the aforementioned parameters, and low concentration diluted PDMS (around 0.1 mg/mL) was then analysed. Only the D3 oligomer was clearly observed and other oligomers from the PDMS degradation provided weak signals. The method was then modified up to a splitless injection mode, so that a consistent and adequate profile could be obtained with low concentration samples. Moreover, the use of splitless mode ensures that the whole sample is injected in the instrument, helping to reach adequate semi-quantification or quantification process if needed.

Some other short modifications were introduced in the instrumental setup to allow a proper analysis. A drying step for 10 s at 70 °C was found to help the evaporation of hexane used as solvent, and therefore avoid its pyrolysis and recombination with other pyrolysis products. A 3-minutes solvent delay was also added after remarking that nothing was getting out of the column during this moment. Finally, the oven temperature program was increased to 20 °C steps from 230 °C to 300 °C after noticing that no other compounds were getting out of the column after 230 °C.

Finally, concerning data preprocessing, several different data treatments were tested: area sum normalisation, logarithm, square root and double square root. Among the proposed processing treatments, area sum normalisation is the most dedicated one as it allows to compare all the results without the need to use an internal standard. Previous researches showed out that there was no real need of an internal standard when doing pyrolysis [41,42] and that it did not reduce the variability [43]. Considering working in splitless mode also reduces the need of an internal standard given that the whole of the sample will be injected in the instrument [44]. In additional, the internal standard would be pyrolysed at the same time than the sample and could generate random recombination with pyrolysis residues coming from the sample, especially considering the instrumentation built up with a long transfer line between the pyrolysis device and the GC. Area sum normalisation followed by a double square root pre-treatment was found to be the most adequate preprocessing to interpret properly Py-GC data.

3.2. Experimental design

3.2.1. Response surface screening

Analyses carried out on the Full Factorial Design led to screen the surface response were first visually analysed to evaluate the variability, based on the presence of given peaks and their number among the all replicates.

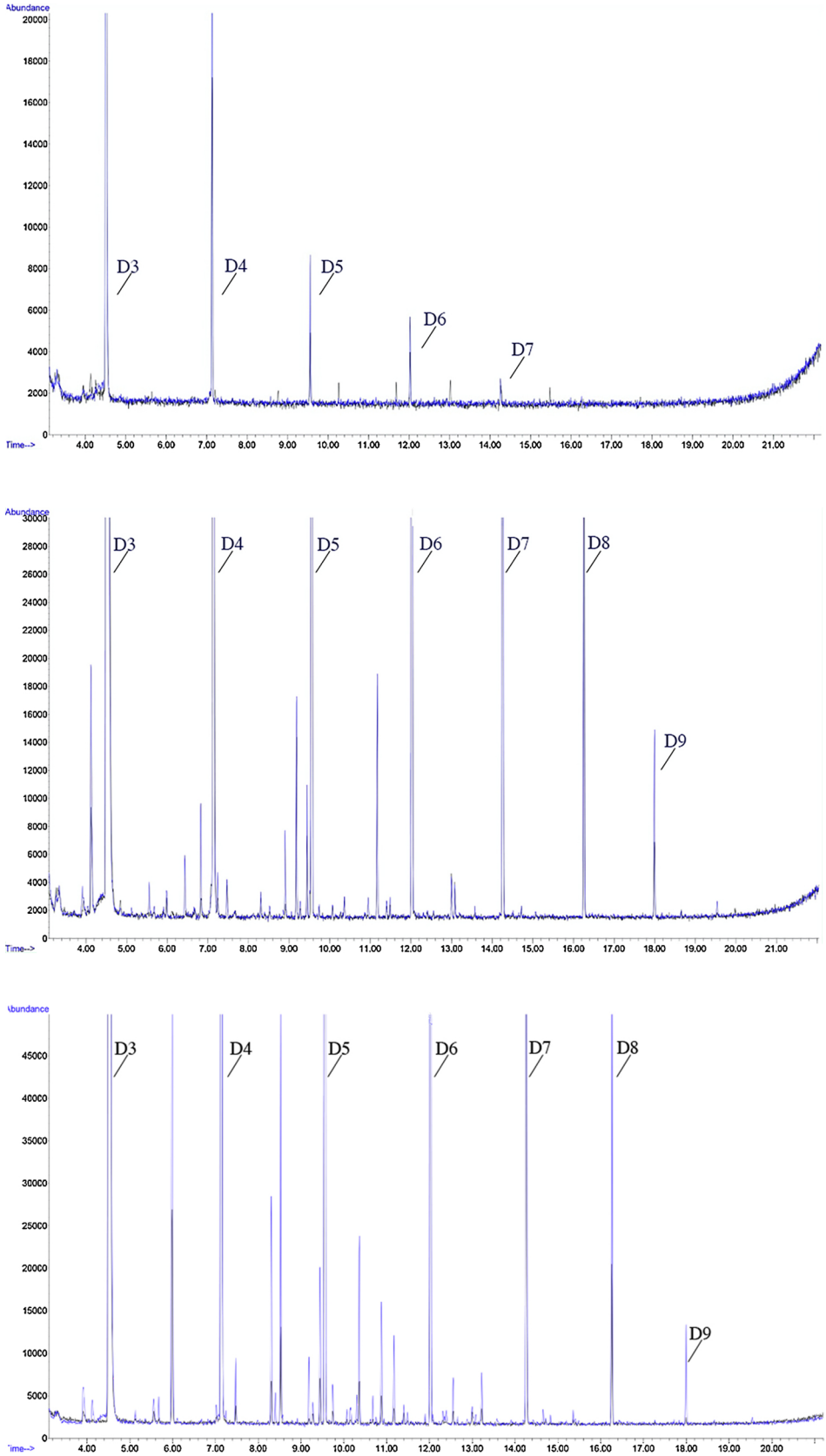

Fig. 1. Illustration of pyrograms acquired under different pyrolysis conditions (temperature/pyrolysis time), two replicates per design point are presented a) 420 °C/10 s, b) 620 °C/20 s, c) 920 °C/30 s. Variation in terms of number of peaks and their position as well as their abundance can be observed. The pyrograms obtained at 420 °C present a low intensity compared to the ones obtained at 620 °C and 920 °C, and less compounds. Cyclic oligomers D3-D9 are indicated after identification using NIST database.

The visual comparison of replicates carried out at 420 °C showed the highlighted five major peaks (Fig. 1a) with abundances greater than 3000 A.U., this abundance being considered as a quality threshold over which peaks are distinguished from the background. These six peaks correspond to the D3-D7 oligomers and are the only repeatable observed peaks. Other smaller peaks can sometimes be seen but are neither reproducible nor present in sufficient relative abundance to be considered as significant peaks. Same results were obtained with a 30 s pyrolysis time. These observations confirm that the pyrolysis temperature is a crucial parameter influencing the reproducibility of the data as well as their quality.

When carried out at 620 °C and 20 s, the pyrograms still presented seven major peaks, D3-D9, with abundances greater than 3000 A.U. (Fig. 1b). Moreover, a zoom on the zone from 0 to 20,000 A.U. highlighted the presence of about ten smaller peaks that are clearly above the signal to noise ratio. The overlay of the replicates showed that the number and retention times of those significant peaks were repeatable. However, the relative abundances sometimes seem to vary between the different replicas, which is usually observed in Py-GC/MS [28,29].

Analyses led at 920 °C (Fig. 1c) presented a good repeatability whatever the pyrolysis time. Several smaller additional peaks other than the principal cyclic DMS were found, but their relative abundance seemed to be very variable.

Variability of the results and confirmation of the variation observed during qualitative analyses were carried out after peak area extraction and preprocessing as described in section 2.4. The Table 4 highlighted the low variability of the central point (620 °C/20 s), about 4 times smaller than the 920 °C points, and so without any significative increase of the coefficient of variation (CV) above the 5% threshold. These results indicate that the temperature as well as the pyrolysis time influence the variability of the relative abundance of the target compounds. The number of values over 5% for the centre points is 2 out of 6 compounds, which is significantly high. However, none of the presented combination did present all the compounds to be lower than 5%. Analyses carried out at 920 °C/ 30 s and 420 °C/10 s also showed out that CVs of 2 out of the 6 peaks were over 5%, but their total variance was also found to be higher than the one of the central points. All the others have over 50% of the compounds over 5%, which means their variability is too high to be considered as interesting parameters for further analysis.

To understand why the number of CVs over 5% was the same between several analyses, CV were plotted as a function of the analysis parameters for each target compound (Fig. 2). As illustrated in Fig. 2, there is an evident pattern of exponential increase of the CV as a function of the retention time of the cyclic DMS. Indeed, compounds D7 and D8 have much larger CVs than the 5% limit. The CVs of the compounds at the end of the pyrograms present a greater averaged value than those at the beginning of the pyrograms. As observed for all pyrolysis conditions, it was assumed that this variation was not linked to the pyrolysis process itself. Although this may be due to the automated integration procedure, peak area were manually corrected on each peak to limit the variation. D7 and D8 are exhibiting a weak intensity, the determination of the integration limits remains unprecise, so higher variability on peaks of weak abundance has to be considered.

All these observations allowed to conclude that a low pyrolysis temperature gives results qualitatively exploitable in term of the presence of oligomers D3 to D8, but not repeatable and therefore not appropriate to our studies. Therefore, these conditions were judged to be non-optimal and analysis at 420 °C were discontinued for further investigations. Higher temperatures induce higher variance of the six oligomers if the temperature is too high but although reproducibility is improved compared to low pyrolysis temperature.

Table 4
Results of the variability study after data processing. Total variance was calculated on the six cyclic DMS D3-D8.

Point of the plan (Temperature (°C)/Time (s))	CV > 5%	Total variance
920/30	2	~0.0020
920/10	3	~0.0020
620/20	2	~0.00058
420/30	4	~0.0021
420/10	2	~0.0026

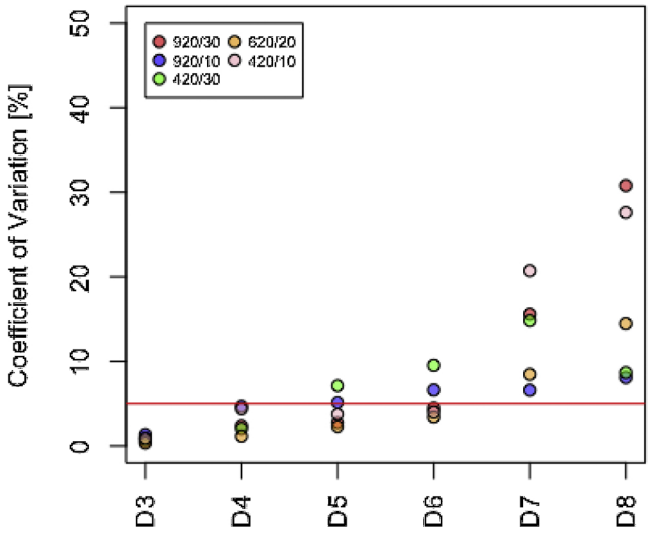

Fig. 2. Illustration of the distribution of the CVs according to the analysed compounds, after area sum normalization and double square root pre-treatment.

The surface screening showed better results for a temperature near the central point. At this point, in order to grasp the effects of each variables of interest, a new design of experiments was carried out, focusing the setting values close to this central point.

3.2.2. Calculation of the main effects

The knowledge acquired in the first cycle of experiment allowed to reduce the factors closer to the central points. A new two-level factorial design of experiment was run with the aim of estimating the effects of the factors. Each point was analysed twice to get replicates except for the central point which was measured 3 times. The first cycle of experiments shows that the D3 oligomer is the one with the best abundance and a sufficient repeatability to be used as a reference compound. Furthermore, this compound is encountered in every analysis whatever the pyrolysis temperature and time, the sample type and, moreover, its relative abundance is only slightly impacted by the variation of conditions. Thus, the following cycles of experiments will be focused only on this oligomer. Same normalisation procedure than for the FFD plan was used.

A first design was set up around the 620 °C and 20 s central point. After strict consideration of the response surface obtained for these parameters, it was found that the response surface never reached its extremums. When evaluating design of experiments models, validation of the models is done by minimising p-value regression significance and maximising the p-value of the lack-of-fit. However, in our experiments, the p-value obtained for the lack-of-fit was found to be very low (10^{-4}) indicating that the model was not fitting the surface response. Thus, the plan was modified for potential optimization by increasing the temperatures including both the central point and the extreme temperature points. Therefore, a new central point was set at 720 °C and 20 s of pyrolysis, which was found to offer a total variance lower than the one obtained for the 620 °C and 20 s point of the planification.

Calculation of the main effect of each parameter were realized as described in [38] and respective effects of ~ -0.0126 for the temperature and ~ -0.0157 or the time were obtained. The effects are thus

equivalent and both parameters impact the abundance of D3 in the same way, i.e. an augmentation of the temperature or time will decrease the abundance of D3. The effect of the interaction has also been calculated and is ~ -0.0126, almost as much as the effects of the main factors. These results allow to conclude that there is a threshold above which an increase of the parameters would generate an increase of the results variability.

This design was still not sufficient to have a complete coverage and understanding of all the interactions underlying this complex pyrolysis phenomenon. Thus, an extension to a FCCD design which allow to compute more complex interactions and create a final response surface modelling with the best understanding of the impact of each parameter was achieved.

3.2.3. Response surface modelling

FCCD was used to estimate and evaluate first and second order models of regression. The analytical results were used to build a full regression model of the first order, firstly using only the temperature and the time (Equation: Amount of D3 = X0 + X1*Temperature + X2*Time, with X0 a constant, X1 and X2 the effect attributed to each parameter) and in a second approach considering their interaction as well (Equation: Amount of D3 = X0 + X1*Temperature + X2*Time + X3*Temperature*Time, with X0 a constant, X1, X2 and X3 the effect attributed to each parameter). A full regression model of the second order was also tested. The different models were all compared using the adjusted R^2 with a partial Fisher-test. The following model was finally retained:

$$\text{Amount of D3} = 0.941 - 0.007 \times \text{Temperature} - 0.005 \times \text{Time} - 0.006 \times \text{Temperature} \times \text{Time}$$

The multiple determination coefficient for this model was 83.64%, which was considered as satisfactory. The model quality was checked using classic methods of regression and error normality conditions. Q-Q plots were used as well as the plot of the studentised residues against the predictive variables and the standardised residuals against the fitted values. No points stood out from the rest of the data, thus leading to conclude that the model was adequate for fitting values and could be used for subsequent application.

The modelled surface response is illustrated in Fig. 3. Depending on the relative amount of D3 oligomer, the area around 520 °C and 10 s of pyrolysis appeared to be a statistic optimum. The area between 520 and 720 °C was close to the value of 94% and therefore be considered as a local maximum. A diminution of the D3 relative abundance was observed as the couple time/temperature gradually increased. The knee-point seems to be around 620 °C–720 °C which is in agreement with the literature [12,26,27,35,36]. As illustrated in Fig. 3b, if the pyrolysis time is too high or too low, the abundance of oligomer D3 decreases. A maximum zone around 20 s of pyrolysis time was found to allow the maximization of the relative abundance of the target oligomer.

The observation of the current model and its surface response allowed to highlight an optimal area for the pyrolysis, with a temperature varying between 620 °C and 720 °C and a pyrolysis time of 20 s. The first one is widely reported in the literature and the second one, which is not fully documented, presents the smallest variability. Only an application to real samples will be able to highlight if a temperature of 720 °C or 620 °C is more adequate, based on the analysis of several replicas.

3.3. Application to real samples

3.3.1. Identification of the best pyrolysis temperature

Two samples were both analysed, with 5 replicates, within the two different pyrolysis temperatures, i.e. 620 and 720 °C, and 20 s of pyrolysis. Qualitative and semi-quantitative analysis of the data were led on the acquired replicates.

At a temperature of 620 °C, the oligomers coming from PDMS degradation, from D3 to D9, presented an excellent reproducibility in terms of peak shape and retention time being independent from the sample. However, different replicates presented obvious differences and lack of reproducibility when smaller peaks were considered. For example, 2,5-Hexanedione was found to be present in the pyrograms, but its retention time shifted randomly between 7.00 and 8.00 min in the replicates. Chemical profiles obtained at a temperature of 720 °C offered a better reproducibility and more consistency when considering the whole profile. Cyclic oligomers from PDMS degradation are highly reproducible and there were no variable peaks as previously highlighted in the pyrograms acquired at 620 °C.

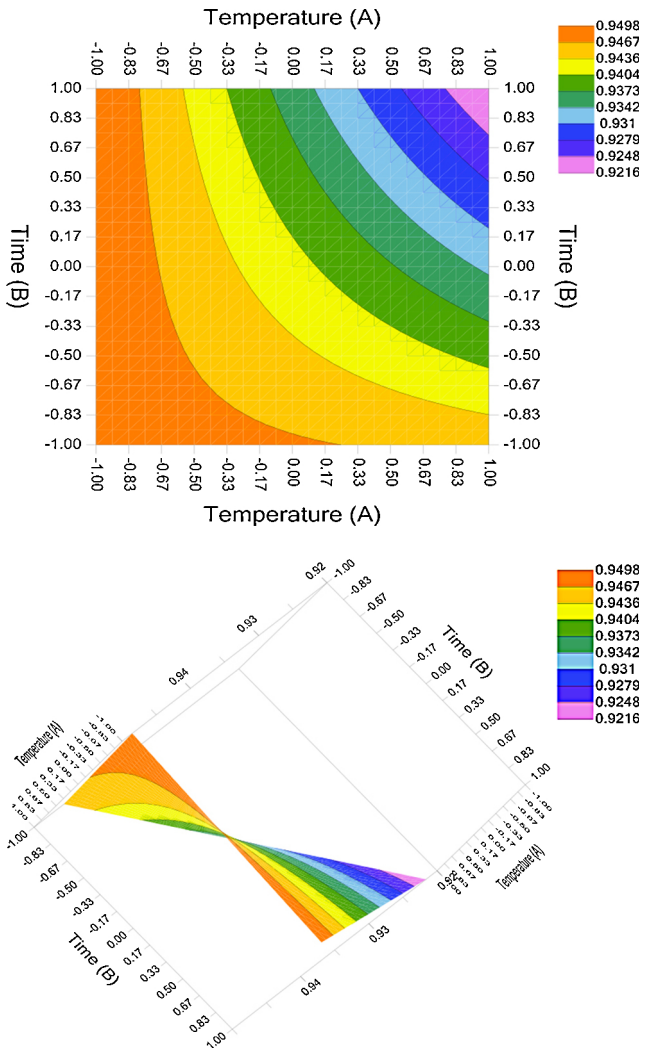

Fig. 3. Illustration of the response surface obtained from the FCCD, a) 2D surface, b) 3D surface. Axes contain the coded values used to draw the surface response. Interaction on the 3D space refers to the interaction between Time and Temperature. The numbers refer to the amount of styrene obtained along the different points of the design.

The semi-quantitative analysis showed out that the coefficient of variation for the all different cyclic oligomers were lower than 5%, and the total variance was found to be 3.17×10^{-4} and 2.38×10^{-4} at 620 °C, and 9.15×10^{-5} and 6.33×10^{-4} at 720 °C respectively. As a better visual quality was assessed on chemical profiles acquired at a temperature of 720 °C, based on the number of peaks and their reproducibility and repeatability, these conditions were selected as the adequate pyrolysis temperature. The final pyrolysis parameters were 720 °C and 20 s of pyrolysis.

3.3.2. Homogeneity and classification potential

The five first different condom samples presented in Table 3 were

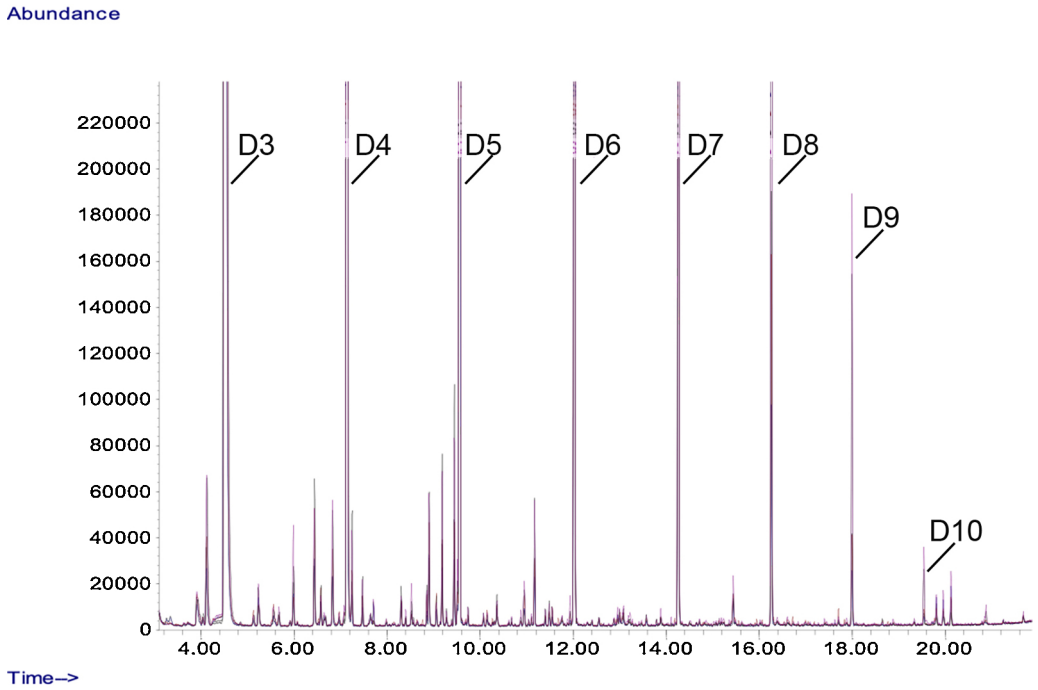

Fig. 4. Illustration of the repeatability of the pyrograms on the five replicates sample Manix Skyn. Displayed between 4 and 23 min for better readability. Cyclic oligomers D3 -D10 are indicated on the pyrogram after identification in the NIST database.

analysed five times with the following pyrolysis conditions: 720 °C during 20 s. Pyrograms acquired for each sample were found to be highly repeatable in terms of compound number, retention time and relative intensities, between 3.00 and 22.00 min (Fig. 4).

Interestingly, the obtained chemical profile gathered from extracted condom lubricants were found to be exactly similar to the ones obtained on the standard material. No compounds were identified after 22.00 min. The overlay of the different samples highlighted that the profiles were visually not significatively different and most of the residues were common between all the samples. However, variation in terms of relative abundance was visually observable and thereby, these compounds can be used for discrimination purposes. It is important here to highlight that, despite the well described retention of the PDMS in the vagina's matrix [12,26,27]., these studies did not investigate the presence of other condom residues *in vivo*. Thus, in term of application on real cases, further investigations must be done in order to understand the properties of these compounds.

Up to 31 residues over 3000 AU were characterized using NIST database, but not all of them presented a hit in the database with sufficient quality to be attributed to the proposed component. This is not surprising as previous researches outlined the difficulties of identifying the many compounds generated during the pyrolysis process [28,29,40,45]. 8 could be identified as coming from the cyclic oligomers generated during the PDMS pyrolysis, i.e. D3-D10, based on the comparison with the database and literature [12,27,46]. The remaining 23 compounds could not be identified in the databases. However, observing the mass spectra regarding the literature [46] allowed to identify these compounds as coming exclusively from siloxane degradation, and not from other compounds. Table 5 presents the 31 compounds resulting from the characterization and that were integrated for the overall analyses for further statistical analysis.

For all samples, within samples variation (intravariability) and between samples variation (intervariability) were calculated and the boxplots of the calculated coefficient of variation for the 31 selected compounds are shown in Fig. 5.

Although the condoms presented similar chemical patterns, the boxplots obtained for the five condoms show very dispersed and highly variable results. The boxplot of the CV calculated for the intervariability is clearly distinguishable from the others, showing a higher median.

All five condoms present more than 75% of their compounds under

Table 5
Characterization of the compounds identified from GC/MS analyses including retention times, target ions and qualifiers.

Peak no.	RT [min]	Compound name	Target ion m/z	Qualifiers m/z
1	3.91	Toluene	91	92, 78
2	4.12	Unknown 4.1	149	133, 75, 115
3	4.51	D3	207	96, 133, 191
4	5.99	Unknown 5.9	207	193, 221, 177
5	6.44	Unknown 6.4	193	209, 97, 135
6	6.83	Unknown 6.8	207	191, 223, 133
7	7.14	D4	281	249, 265, 191
8	7.24	Unknown 7.2	267	126, 251, 193
9	8.30	Unknown 8.3	265	249, 191, 125
10	8.52	Unknown 8.5	281	295, 267, 163
11	8.90	Unknown 8.9	267	283, 126, 193
12	9.18	Unknown 9.1	341	325, 155, 73
13	9.45	Unknown 9.4	341	325, 73, 163
14	9.56	D5	355	73, 267, 268
15	10.88	Unknown 10.8	369	267, 355, 73
16	10.99	Unknown 10.9	341	325, 163, 123
17	11.18	Unknown 11.1	327	415, 399, 73
18	11.40	Unknown 11.4	327	415, 73, 207
19	11.49	Unknown 11.5	327	415, 73, 399
20	12.03	D6	429	73, 147, 341
21	13.00	Unknown 13	401	489, 73, 475
22	13.57	Unknown 13.5	401	489, 385
23	13.88	Naphthalene,2,1-methyl	155	170, 128, 76
24	14.27	D7	503	281, 327, 415
25	15.44	Naphthalene, 1,7-methyl	169	184, 154, 115
26	16.26	D8	401	355, 73, 221
27	17.99	D9	429	355, 147, 221
28	19.54	D10	503	281, 221, 147
29	19.82	Unknown 19.8	239	165, 141, 195
30	19.96	Unknown 19.9	197	239, 254, 281
31	20.12	Unknown 20.1	239	254, 199, 141

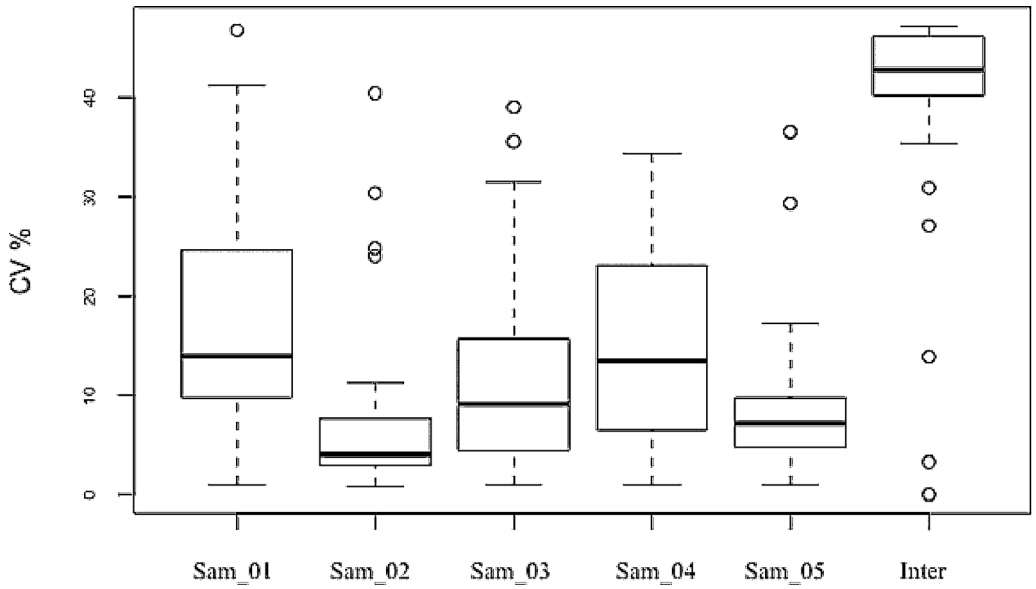

Fig. 5. Boxplots of the CV calculated within each sample and between the five condoms on 31 variables. Inter refers to between sample variability (intervariability).

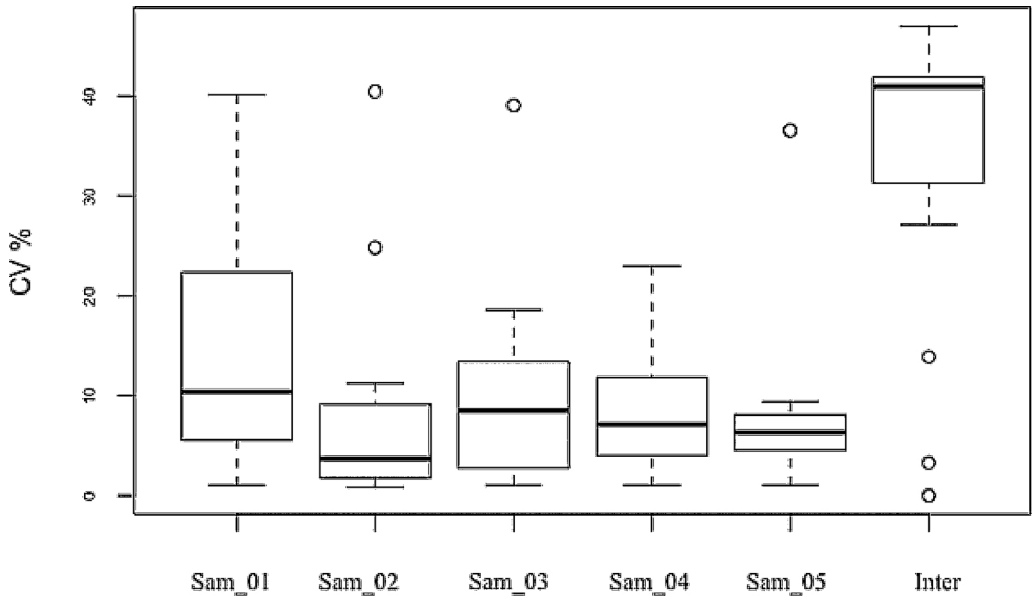

Fig. 6. Boxplots of the CV calculated within each sample and between the five condoms on 15 variables. Inter refers to between sample variability (intervariability).

40%. This suggests that some of the considered variables were not important for a discrimination. A reduction of the variables was conducted by deleting all the variables whose variability was close to 0 or too small to offer any discrimination potential. This led to a new dataset containing only fifteen variables. Boxplot were replotted to observe the new separation (Fig. 6).

The separation between the intravariability and the intervariability was slightly enhanced. Most of the condoms present a variability lower than 20% except for sample 1. The intervariability boxplot shows that most of the compounds have a CV over 30%. The intravariability is slightly lower than the intervariability. Clustering of the samples was tested using non supervised classification such as hierarchical cluster analysis (HCA) and principal component analysis (PCA) on the reduced dataset using only fifteen variables. As shown on Fig. 7, PCA applied on the pre-processed data did not allow a proper separation of the different samples nor clustering of the replicates of the same sample, except for sample 4, when modelling in a 3-dimensional space. Indeed, most of the clusters observed along the different PC highlighted clusters grouping replicates from different samples. Separation can be observed along PC2, as sample 1 and 3 and sample 2 and 5 were found to be separated. Observations of the loadings along PC2 showed that the cyclic oligomers were not responsible for the separation of the samples. Indeed, compound 6 (Table 5, Fig. 8) has a positive influence on the separation, which means that sample 2 and 5 contain more of this compound than sample 1 and 3. In addition, compounds 6, 11and 16 (Table 5, Fig. 8) were found to have a strong negative influence on the separation. Other PCs did not help enhancing the separation of the samples. These observations suggest that separation of different condoms would be enhanced using minor compounds instead of the cyclic oligomers that allow confirming the presence of PDMS in the sample. The dispersion of the data was found to be rather high along PC1 and PC2 to figure out a proper discrimination, but more samples are necessary to confirm these observations.

The dispersion of the data can be explained by several sources of variations. Inhomogeneity of the sample could be one of them, but vortexing the sample before any analysis was done and it was thus

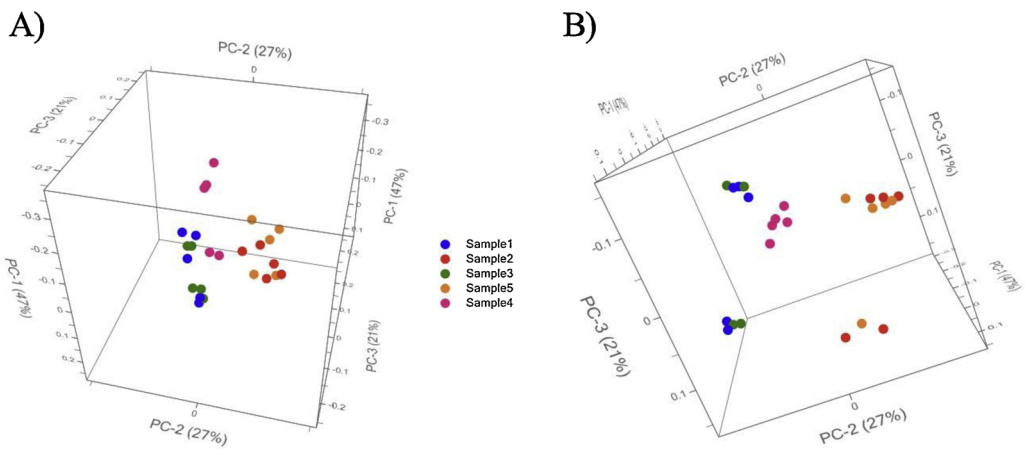

Fig. 7. PCA realised on the five studied samples, using the 15 compounds, illustrating the problematic of the clustering. Replicates coming from sample 1 and sample 3 (blue and green dots) are clustered together, although they are not from the same source. Same observations are outlined with sample 4 and sample 5.

assumed that it would not affect the repeatability of the sample. The amount of quartz wool present in the quartz tube was manually inserted and may be varying between the tubes. Therefore, a different absorption of the sample on the wool can be expected and might affect the repeatability of the sample. An incomplete adsorption of the sample on the quartz wool or an incomplete desorption of the latest during the pyrolysis process (variable amount of compound entering in the GC column) are also sources of variations. However, these do not seem to make sense as all the profiles were consistent in terms of relative abundance on a qualitative point of view. Finally, a last possible source of variation remains into the integration of the chromatographic peaks and especially for those of weak intensities, which may lead to an increase of the whole variation. Nevertheless, it is worth investigating the source of these variations using another instrument to evaluate the potential reduction of the error on the acquired results. Then only a proper classification and homogeneity study can be led as well as a discrimination model built using LDA.

4. Conclusion

The present research fits into an investigative approach intended to allow detection of condom traces after sexual assault. We focused on the PDMS, which has a high persistence period in the vaginal matrix, in order that it may be used also in cases involving long time delays. Py-GC/MS was used to skirt the problem inherent in the analysis of PDMS while giving a representation of the pyrolytic degradation of this compound.

Thus, in order to optimize the pyrolysis parameters, we used a Face-Centered Composite Design of the experiment to analyse the PDMS present in condom lubricants. The optimal combination of parameters was determined using standard PDMS materials. This allowed us to obtain an objective and robust method offering the most repeatable results.

This method was then applied on five real samples of condom lubricants found in various brands and extracted with hexane. This was done in order to stay as close as possible to a real trace extract where

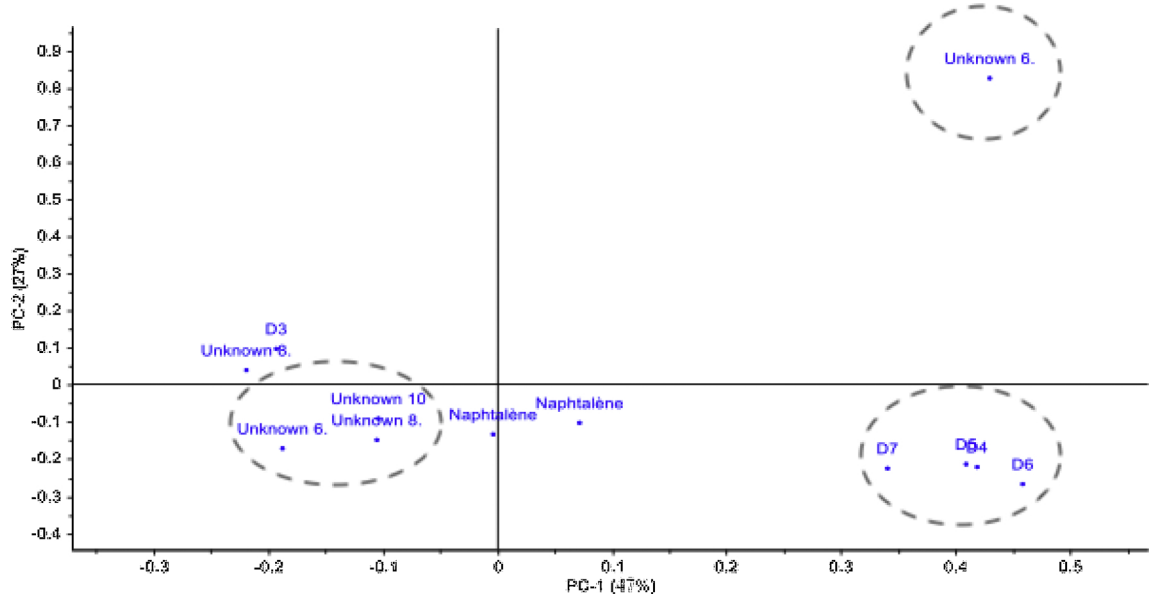

Fig. 8. Loading plots obtained from the PCA plots, indicating which variables describe PCs and are responsible for the separation of the samples. PC1 and PC2 are presented.

swabs are used. To estimate the discrimination power of the analytical method, variations within and between samples were studied. It appeared that the chemometrics tools applied on the dataset did not discriminate samples that originated from different sources. Indeed, not only were samples not clearly distinguishable between themselves, but also within sample variation did not allow proper clustering of replicates. These results highlight the need to pursue the investigation to identify if the source of variations observed originates from the sample or from the instrumentation used.

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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CRediT authorship contribution statement

Jonathan Maurer: Formal analysis, Data curation, Visualization. **Kévin Buffaz:** Data curation, Formal analysis, Writing - original draft, Visualization. **Geneviève Massonnet:** Writing - review & editing, Supervision, Conceptualization. **Christophe Roussel:** Writing - review & editing, Conceptualization. **Céline Burnier:** Conceptualization, Formal analysis, Methodology, Data curation, Software, Writing - original draft, Writing - review & editing, Supervision.

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