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Analysis of Condom Evidence in Rape and Sexual Assault Cases - Development of an analytical and interpretative framework and application

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Development of an analytical and interpretative framework and application

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IMPRIMATUR

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« Analysis of Condom Evidence in Rape and Sexual Assault Cases - Development of an analytical and interpretative framework and application »

La Présidente du Jury

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- *The man who passes the sentence should swing the sword. If you would take a man's life, you owe it to him to look into his eyes and hear his final words. And if you cannot bear to do that, then perhaps the man does not deserve to die –*

Lord Eddard Stark, A Song of Ice and Fire

Résumé

Lorsqu'une enquête pour viol ou agression sexuelle est ouverte, la première trace forensique recherchée est la trace ADN, en plus de l'examen gynécologique. En effet, s'il n'y a pas eu utilisation de préservatif, l'ADN de l'agresseur devrait être retrouvé sur la victime, en quantité variable. Mais que se passe-t-il si aucune trace ADN n'est retrouvée ? Un préservatif a-t-il été utilisé ? L'étude de quelques cas présentés dans la littérature montre que les experts se prononcent sur la source d'une trace même si cela est basé sur une comparaison qui ne prend pas en compte les erreurs possibles, en termes de faux positifs ou faux négatifs. Toutefois, la problématique de l'analyse et de l'interprétation des traces de préservatifs est rencontrée au niveau international, et le nombre limité de protocoles fiables et valides ainsi que l'absence de modèles interprétatifs soulignent la nécessité d'effectuer des études sur le sujet.

Le but de ce travail est donc de développer une approche forensique qui permette d'identifier une trace de préservatifs sur un support de traces (i.e. les écouvillons de prélèvements de la médecine légale utilisés pour le prélèvement ADN). Les questions entourant la mise au point d'une telle procédure ne sont pas nouvelles, différentes recherches ont déjà été publiées depuis plus de 40 ans. Le présent travail les a étudiées de manière critique, et souligne les limitations de ces méthodologies notamment l'absence de validation ou d'application dans les cas pratiques. Néanmoins, certaines techniques analytiques se sont montrées prometteuses lors d'applications à des échantillons humains. Ainsi, un recensement détaillé de la littérature a été conduit afin d'identifier les composés présents dans les préservatifs et dans divers produits d'hygiène intime ainsi que les techniques analytiques capables de les détecter. Les techniques analytiques choisies sont la spectroscopie infrarouge à transformée de Fourier (FTIR), la chromatographie gazeuse couplée à la spectrométrie de masse (GC/MS) ainsi que le couplage avec la technique de pyrolyse (py-GC/MS).

Dans un premier temps, ce travail traite de l'optimisation des conditions analytiques à l'aide de plans d'expériences, principalement pour ce qui concerne les mesures infrarouges et les conditions de pyrolyse. Les conditions nécessaires afin d'obtenir des résultats reproductibles et minimisant la variation au sein d'un même échantillon (intravariabilité) ont été étudiées et sont présentées. Diverses procédures de corrections des données ont été proposées au moyen de prétraitements et de sélections de variables, afin de maximiser l'information chimique pertinente et de renforcer le potentiel discriminatoire des techniques.

Ensuite, une étude de marché internationale couvrant les marchés suisses, australiens et néo-zélandais a été effectuée sur 166 échantillons de préservatifs, lubrifiants ainsi que des produits d'hygiène intime susceptibles d'entrer en contact de manière légitime avec la matrice vaginale, et par conséquent de générer des faux positifs. La discrimination entre les échantillons a été testée sur des échantillons de nature différente (préservatif, lubrifiant, produits d'hygiène intime), puis sur des préservatifs de marques et de modèles différents, mais également sur des préservatifs de même marque et différents modèles, ou

encore de même marque, même modèle mais provenant de différents lots de production. Les résultats ont montré que la composition en lubrifiant était le principal facteur de discrimination des échantillons.

La spécificité des composés a été étudiée sur les deux populations principales rencontrées, à savoir les préservatifs et les autres produits. Bien que majoritairement composée d'échantillons contenant de l'huile de silicone, la population des préservatifs a également mis en évidence des échantillons lubrifiés avec de la glycérine. La complémentarité de la GC/MS avec la py-GC/MS a permis de séparer des populations de préservatifs dont le contenu était différent. Parmi les lubrifiants et produits d'hygiène intime, certains ont présentés un profil infrarouge indifférenciable de celui des préservatifs, mais l'analyse par pyrolyse-GC/MS a permis de différencier ces échantillons de ceux provenant des préservatifs.

Le cadre analytique développé a été appliqué à des tests de compétences de laboratoire ainsi que dans des cas réels, afin d'évaluer la qualité de la méthodologie. La complémentarité des différentes techniques a été confirmée et il a été possible de déterminer la nature de la source d'une trace avec succès. Si ces résultats sont prometteurs, l'application dans les cas réels a soulevé d'importantes questions relatives à l'interprétation de l'indice, notamment en ce qui concerne la non-détection de la trace. Il est impératif de conduire des recherches supplémentaires afin d'évaluer les voies d'interprétation de la preuve au niveau de l'activité, et la compréhension des phénomènes qui affectent la trace.

Dans une perspective d'intégration dans le cadre forensique de l'interprétation, une étude pilote a également été effectuée par spectroscopie infrarouge afin d'obtenir des informations quant au bruit de fond, au transfert et à la persistance des traces de préservatifs. Plus précisément, la capacité de cette technique à mettre en évidence l'effet de certains facteurs d'influence a été investiguée. L'étude du bruit de fond et de la matrice vaginale a permis de mettre en évidence que la prévalence des composés siliconés dans la matrice vaginale pour des raisons légitimes est pratiquement nulle. Aucune différence significative entre les volontaires n'a été observée. En termes de transfert, l'information analytique obtenue a été comparée avec celles obtenues par les spectres FTIR de l'étude de marché et une confrontation entre les observations visuelles et statistiques a été réalisée. Il en a ainsi résulté que la FTIR était une méthode de dépistage puissante, mais que son utilisation pour la modélisation de paramètres de transfert et persistance à but forensique avait des limites. En effet, dans cette recherche, cette technique a montré des désavantages majeurs, à savoir une variabilité analytique extrêmement importante, sur le plan quantitatif, qui masque les variations intrinsèques à l'échantillon.

Finalement, les résultats obtenus dans ce travail ont permis la proposition et la discussion d'une approche pragmatique pour aborder les questions liées aux traces de préservatifs. Cette approche permet d'identifier l'information que le scientifique peut apporter aux enquêteurs à l'heure actuelle. Le canevas proposé décrit également les différentes étapes du développement qui devraient être réalisées afin de parvenir à la validation de la méthodologie, tant sur le plan analytique que sur le plan interprétatif, dont les capacités et limites ne sont, à l'heure actuelle, ni totalement connues ni documentées.

Abstract

When a rape investigation is opened, the first forensic trace sought is the DNA trace, in addition to gynecological examination. In fact, if there was no use of a condom, the perpetrator's DNA should be found on the victim, in variable quantities. But what happens if no DNA trace is found? Was a condom used? The study of a few cases reported in the literature shows that experts decide on the source of a trace even if it is based on a comparison which does not take into account possible errors, such as false positives and false negatives. However, the problem of analysis and interpretation of traces of condoms is encountered at international level, and the limited number of reliable and valid protocol as well as the absence of interpretative models underline the need to carry out studies on the subject.

The aim of this work is therefore to develop a forensic approach which makes it possible to identify a trace of condoms on a support of traces (i.e., the swabs of forensic samples used for DNA sampling). Since the questions surrounding the development of such a procedure are not new, various studies have already been published for over 40 years. The present research has studied them critically, and highlights the limitations of these methodologies, in particular the lack of validation or application in practical cases. However, certain analytical techniques have shown promise in applications to human samples. Thus, a detailed literature review was conducted in order to identify the compounds present in condoms and in various intimate hygiene products as well as the analytical techniques capable of detecting them. The analytical techniques chosen are Fourier transform infrared spectroscopy (FTIR), gas chromatography coupled to mass spectrometry (GC/MS) as well as coupling with the pyrolysis technique (py-GC/MS).

First, this work deals with the optimization of analytical conditions using experimental designs, mainly with regard to infrared measurements and pyrolysis conditions. The conditions necessary to obtain reproducible results and minimizing variation within the same sample (intravariability) have been studied and are presented. Various data correction procedures have been proposed using preprocessing and variable selection to maximize the relevant chemical information and enhance the discriminatory potential of the techniques.

Then, an international market study covering the Swiss, Australian and New Zealand markets was carried out on 166 samples of condoms, lubricants as well as personal hygiene products likely to come into legitimate contact with the vaginal matrix, and therefore generate false positives. The discrimination between the samples was tested on samples of different nature (condom, lubricants, personal hygiene products), and then on condom from same brand and models, but also on condoms of the same brand and different models, or even of the same brand, same model but different production batch. The results showed that the lubricant composition was the main factor of discrimination of the samples.

The specificity of the compounds was studied on the two main populations encountered, namely condoms and other products. Although mostly made up of samples containing silicone oil, the

population of condoms also highlighted samples lubricated with glycerin. The complementarity of GC/MS with py-GC/MS made it possible to separate populations of condoms whose content was different. Among lubricants and personal hygiene products, some presented an indistinguishable infrared profile from that of condoms, but analysis by pyrolysis-GC/MS made it possible to differentiate these samples from those from condoms.

The analytical framework developed was applied to laboratory skills tests as well as in real cases, in order to assess the quality of the methodology. The complementarity of the different techniques was confirmed, and it was possible to determine the nature of the source of a trace successfully. While these results are promising, the application in real cases has raised important questions relating to the interpretation of the evidence, particularly with regard to the non-detection of the trace. It is imperative to conduct additional research in order to assess the ways of interpreting the evidence at the activity level, and the understanding of the phenomena that affect the trace.

With a view to integration into the forensic framework of interpretation, a pilot study was also carried out by infrared spectroscopy in order to obtain information on the background noise, the transfer and the persistence of traces of condoms. More specifically, the ability of this technique to highlight the effect of certain influencing factors was investigated. The study of background noise and the vaginal matrix has shown that the prevalence of silicone compounds in the vaginal matrix for legitimate reasons is practically zero. No significant difference between the volunteers was observed. In terms of transfer, the analytical information obtained was compared with that obtained by the FTIR spectra of the market study and a comparison between visual and statistical observations was carried out. As a result, FTIR was a powerful screening method, but its use for modeling transfer and persistence parameters for forensic purposes had limits. Indeed, in this research, this technique has shown major disadvantages, namely an extremely high analytical variability, from the quantitative point of view, which masks the variations intrinsic to the sample.

Finally, the results obtained in this work allowed the proposal and the discussion of a pragmatic approach to tackle the questions linked to traces of condoms. This approach identifies the information that the scientist can currently provide to investigators. The proposed outline also describes the different stages of development that should be carried out in order to achieve validation of the methodology, both analytically and interpretatively, whose capacities and limits are, at present, neither fully known or documented.

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Publications

This dissertation contains work which is published or has been submitted for publication in the following peer reviewed journals or newsletter.

Article 1: Burnier C., Massonnet G., (2019) *Pre-analytical considerations of condom traces: A review of composition, background, transfer and persistence*, Forensic Science International, Vol. 302, pp. 135-141

Article 2: Burnier C., Massonnet G., (2020) *Forensic analysis of condom traces: Chemical considerations and literature review,* Forensic Science International, Vol. 310

Article 3: Maurer J., Buffaz K., Massonnet G., Roussel C., Burnier C., (2020) *Optimization of a py-GC/MS method for silicone-based lubricants analysis*, Journal of Analytical and Applied Pyrolysis, Vol. 149

Article 4: Burnier C., Van Bronswijk W., Massonnet G., (2020) *Comparison of spectroscopic methods in detection of silicone-based condom lubricants evidence,* Analytical Methods, pp. 657-665

Article 5: Burnier C., Massonnet G., Coulson S., DeTata D., Pitts K., (2020) *Characterization and classification of water-based compounds in condoms and personal hygiene products using GC/MS*, Forensic Science International, Vol. 317

Article 6: Burnier C., Coulson S., Massonnet G., Pitts K., Sauzier G., Lewis S.W., (2021) *Forensic investigation of condom and personal hygiene products market using ATR-FTIR coupled to chemometrics*, Science & Justice

Article 7: Burnier C., Massonnet G., Coulson S., DeTata D., Pitts K., (2021) *Characterization and classification of silicone-based compounds in condoms and personal hygiene products using pyrolysis-GC/MS*, Manuscript submitted to Forensic Science International

Article 8: Burnier C., Kelly M., DeTata D., Pitts K., (2020) *Investigation of condom evidence in cases of sexual assault: case studies,* Manuscript submitted to Forensic Science International: Reports

Article 9: Hermelin A., Fabien L., Fischer J., Saric N., Massonnet G., Burnier C., (2021) *Analysis of Condom Evidence in Forensic Science: Background survey of the human vaginal matrix using DRIFTS and pyrolysis-GC/MS,* Forensic Science International

Article 12: Saric N., Fabien L., Fischer J., Hermelin A., Massonnet G., Burnier C., (2021) *A preliminary investigation of transfer of condom lubricants in vaginal matrix*, Forensic Science International

Article 14: Burnier C., Favre V., Massonnet G., (2021) *The use of an optimized DRIFTS-FTIR method for the forensic analysis and classification of silicone condom lubricants*, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

Burnier C., DeTata D.A., Pitts K. M., Coumbaros J., ChemCentre Physical Evidence Newsletter Issue 5, February 2020

Book Chapter: Challinor J. M., DeTata D. A., Pitts K. M., Burnier C., (2021) *Chapter 8: Examination of Forensic Evidence*, Handbook of Applied Pyrolysis, Third Edition

In addition, the following articles are waiting for co-authors approval for submission or under redaction:

Article 10: Burnier C., Monzo M., Massonnet G, Lewis S.W, Sauzier G. (2020) *Reporting negative results on quantification of PDMS using optimized DRIFTS analysis*, Manuscript in redaction

Article 11: Burnier C., Maurer J., DeTata D, Pitts K, (2020) *Outlining the importance of adequate analytical instrumentation: an example of pyrolysis-GC/MS*, Manuscript in redaction

Article 13: Fischer J., Saric N., Fabien L., Hermelin A., Massonnet G., Burnier C., (2021) *Persistence of condom silicone-lubricants in the vaginal matrix in sexual assaults,* manuscript submitted to Forensic Science International

Article 15: Burnier C., Grisoni C., Kelly M., DeTata D, Pitts K, Hicks T., (2020) *Considerations on the interpretation of condom evidence in forensic casework,* Manuscript in redaction

Conference Presentations

Selected aspects of the work contained within this thesis have been presented, or have been accepted for presentation at the following conferences:

Burnier C., *Condom Evidence Analysis*, presented for the visit of Chris Palenik at the Ecole des Sciences Criminelles, Lausanne, 13th October 2016

Burnier C., Monzo M., Massonnet G., *FTIR analysis of condom lubricant traces: method development, transfer, persistence and background study*, presented at the 6th Summer School of the Ecole des Sciences Criminelles, Les Diablerets, 29th August 2017

Burnier C., Monzo M., Massonnet G., *Analyse de traces de lubrifiants de préservatifs par FTIR : développement de méthode, transfert, persistance et bruit de fond*, presented at the Swiss Microtraces Group, Aarau, 6th September 2017.

Burnier C., Monzo M., Massonnet G., *FTIR analysis of condom lubricants in sexual assaults: background, transfer, and persistence study,* presented at the ANZFSS 24th International Symposium, Perth, Western Australia, 10th September 2018

Burnier C., Massonnet G., *How to detect PDMS using Raman Spectroscopy?,* presented at the 7th Swiss Raman Meeting, Affoltern am Albis, 8th November 2018

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Burnier C., Massonnet G., DeTata D., Pitts K., *Challenges in forensic sciences – Condom evidence,* presented at Sexual Assault Resource Centre Doctor's meeting, Perth, 9th January 2020

Abbreviations and Acronyms

ADN	Acide désoxyribonucléique
a.m.u	Atomic mass unit
ATR	Attenuated Total Reflectance
APCI	Atmospheric Pressure Chemical Ionization
AU	Abundance Unit
BHT	Butylhydroxytoluene
CCD	Central Composite Design
CE	Capillary Electrophoresis
CHUV	Centre Hospitalier Universitaire Vaudois
CI	Chemical Ionization
CPS	Code Pénal Suisse
CPR	Cardiopulmonary resuscitation
cSt	centi-Stokes
CURML	Centre Universitaire de Médecine Légale
CV	Coefficient of Variation
DAD	Diode Array Detector
DART	Direct Analysis in Real Time
DCI	Desorption Chemical Ionisation
DCM	Dichloromethane
DESI	Desorption Electrospray Ionisation
DHB	Dihydroxybenzoic acid
DIOS	Desorption ionisation on silicon
DMSO	Dimethylsulfoxide
DOE	Design of Experiment
DRIFTS	Diffused Reflectance Infrared Fourier Transform Spectroscopy
DTG	Derivative Thermogravimetric Curves
DTGS	Deuterated triglycine sulfate detector
EFSA	European Food Safety Authority
EI	Electronic impact
EPFL	Ecole Polytechnique Fédérale de Lausanne
ESC	Ecole des Sciences Criminelles
ESI	Electrospray ionisation
ESR	Environmental Science and Research Institute (NZ)
FCCD	Faced Centered Composite Design
FFD	Full Factorial Design
FTIR	Fourier Transform Infrared
FTMS	Fourier Transform Mass Spectrometry
FSD	Fourier Self Deconvolution
GC	Gas Chromatography
GLC	Gas Liquid Chromatography
GLY	Glycerin
GPC	Gel Permeation Chromatography
ISO	International Standardisation Organisation
IST	Infection sexuellement transmissible
IUMSP	Institut universitaire de médecine sociale et préventive (now labelled as Unisanté)
IUPAC	International Union of Pure and Applied Chemistry
HCA	Hierarchical Cluster Analysis
HEX	Hexane

HIV	Human Immunodeficiency Virus
HPLC	High Pressure Liquid chromatography
K-M	Kubelkla-Munk
LAVI	Loi sur l'aide aux victimes
LC	Liquid chromatography
LDA	Linear Discriminant Analysis
LR	Likelihood Ratio
MALDI	Matrix Assisted Laser Desorption Ionisation
MEKC	Micellar ElectroKinetic Chromatography
MS	Mass Spectrometry
MS/MS	Tandem Mass Spectrometry
MSC	Multiplicative Scatter Correction
MSI	Mass Spectrometry Imaging
MST	Maladie Sexuellement Transmissible
NCIS	Naval Criminal Investigation Service
NDBA	N-nitrosodibutylamine
NDEA	N-nitrosodiethylamine
NDMA	N-nitrosodiméthylamine
NIPALS	Nonlinear Interative Partial Least Square
NMR	Nuclear Magnetic Resonance
N9	Nonoxynol-9
OCT	1,2-octanediol
PCA	Principal Component Analysis
PDMS	Polydimethylsiloxane
PE	2-Phenoxyethanol
PEG	Polyethylene Glycol
PEG-Hex	Hexaethylene Glycol
PEG-Hept	Heptaethylene Glycol
PEG-Oct	Octaethylene Glycol
PPG or PG	Propylene Glycol
Py-GC	Pyrolysis-Gas Chromatography
RN	Range Normalisation
SARC	Sexual Assault Resource Centre
SDS	Sodium Dodecyl Sulfate
SEC	Size exclusion chromatography
SNV	Standard Normal Variate
TEA	Thermal Energy Analyzer
TG	ThermoGravimetric
TLC	Thin Layer Chromatography
TOF	Time of Flight
UFC	Unité de formation de colonie
UGF	Unité de génétique forensique
UML	Unité de médecine légale
UVN	Unit vector normalisation
WHO	World Health Organisation

Summary

Chapter 1: Introduction

1.1 Research Context

Condoms are today the most widely used and widely known contraceptive devices, regardless of an individual's socio-economic background, age or education [1–3]. However, the condom was originally designed and marketed to prevent the spread of sexually transmitted diseases [4]. Furthermore, internationally, condoms are defined as a "medical device, not sterile, used by consumers, which covers and stands on the penis during sexual activity, for the purpose of contraception and prevention of sexually transmitted infections transmissible" [5]. Sexual behaviour as well as the perception of the risks of pregnancy or transmission of sexual transmitted diseases (STDs) will significantly affect the use of such a device [1–3]. Condom traces are increasingly detected from victims of sexual assault. American statistics indicate that traces of condoms were found in 2% of cases [6]. In Switzerland, the Centre Universitaire Romand de Médecine Légale (CURML) reported around 15 samples that could potentially contain traces of condoms, out of around 300 samples collected during over a year (5%)[*]. In Western Australia, the director of Sexual Assault Resource Center (SARC) reported carrying out at least one analysis a week for condom traces[†].

Concretely, the issue of condom traces in forensic science has been illustrated since the 1980s, especially in cases of sexual assaults on women [7–9]. According to the information obtained during an investigation, it may become necessary to determine whether a trace of a condom is present, in order to confirm or deny the allegations of the parties: it is not the occurrence of a sexual act but the way it happened that is questioned [10]. The condom trace is also used as associative evidence to establish the *corpus delicti* and verify penetration [8,11,12]. Several different but complementary approaches have thus been defined and are utilised throughout this study:

- of the *composition*: in order to determine the components of a given sample, and thus to be able to define which traces are to be sought

- of the *discrimination*: in order to determine whether the samples present on the various markets have chemical characteristics which render them differentiable

[*] Personal Communication Dr. Frédéric Grosjean, CURML, CHUV, Lausanne

[†] Personal communication, Dr. Maire Kelly, SARC, Perth, WA

- of the *chemical markers in the vaginal matrix*: in order to determine, for example, which compounds are the most easily detected and what can be found in traces.

- of *interpretive parameters*: in order to analyse the alteration of the traces over time. If parameters such as background noise, transfer and persistence can be modelled, then it is possible to interpret physical traces.

All of these approaches are essential to forensic investigation, the aim of which is to determine if a trace is present (detection), if it is possible to link this trace to a sample class (classification) and if it is possible to interpret this result, in particular if questions of persistence come into play (interpretation) [13]. In general, detection of a physical trace depends on the compounds targeted and the possibility of detecting these markers in a living matrix. Analysis of the trace makes it possible to decide on the interpretation of the results, based on the chemical profile obtained. However, despite the importance of these four main stages in the question of the analysis of a trace that can be considered as "new"(given that it is only forty years old) few published works are devoted to them - in particular in the case of interpretative parameters - or else the results obtained are either contradictory or inapplicable in real cases. This is likely due to barriers related to the type of study. Indeed, the variations observed in a profile are influenced by various factors which are difficult or even impossible to control [14,15], and whose effects are for the most part myriad and unknown (e.g. the effects of contact itself, or even of the vaginal matrix) [14]. Added to this are ethical considerations, which make these studies long and tedious but demonstrate the need to increase research on the subject. It is a question of exploring the concrete possibilities and limitations in order to be able to claim the development of methods that can be operationalized by practitioners and recognized by the forensic community.

This thesis deals with the notion of the condom trace in forensic science in a global manner, by tackling the problem of the structure of the market, as well as that of the establishment of an analytical sequence for detection and interpretation of traces in a biological matrix. These traces are found more and more frequently in victims of sexual assault, but very little research is concerned with the detection and interpretation of such. However, reflections on this subject date back to the 1980s, as for example by Blackledge in his study on two real cases [11]. However, analytical diversity as well as many assumptions complicate the situation, such as if an analytical instrument is very sensitive then there is no need to consider parameters such as transfer and / or persistence during interpretation [9,11]. In addition, the complexity of the analyses to be carried out, and the difficulties encountered when interpreting the observations [7,9,11], lead researchers to focus on technical developments, without dealing with the practical

application of the methods developed [10,15–21]. Therefore, the present study proposes to keep a fundamental and pragmatic manner of approach, keeping in mind an ultimate applicability to the forensic system.

1.2 Aims and overview

The aim of this work is therefore to develop a forensic approach which makes it possible to identify condom traces on a collection substrate (i.e., the swabs used to obtain forensic samples). The developed approach must be simple and quick to implement in order to be applicable in routine, while guaranteeing the lowest error rate achievable. The general aim of this thesis is expressed as follows:

To study the possibility of developing an analytical sequence for the analysis of traces of condoms, based on the analysis of certain characteristic chemical compounds and on the specificity of these compounds within the market, as well as on modelling of interpretative parameters, by clearly identifying its potential and limitations while taking into account aspects of applicability in the forensic context.

In order to achieve this goal, precise knowledge regarding the current state of the art was carried out on studies of the composition of condoms as well as on the instrumental techniques used, by identifying the compounds of interest and their forensic potential (**Chapter 2**). This literature review allowed the definition of detailed objectives (**Chapter 3**) leading to the collection of results using three different analytical techniques (**Chapter 4, 5 and 6),** thus helping to understand where sources of variations can be observed and what type of variation is identified. The structure of the manuscript will be dictated by the analytical instrumentation: **Chapter 4** will consider FTIR analysis, **Chapter 5** py-GC/MS and **Chapter 6** GC/MS instrumentation. It is important to evaluate these variations as well as being able to explain them, before applying the developed methodology to forensic cases (**Chapter 7**). **Chapter 8** will consider which FTIR technique is more adequate for trace evidence and quantitative analysis as a needed for the investigation of interpretive parameters which will be explored in **Chapter 9, 10 and 11**. The discussion of the various results obtained led to a reflection on the operationalization and the questions of interpretation of the traces of condoms (**Chapter 12**). Finally, a conclusion will summarise the main results (**Chapter 13**) and will be followed by the bibliography of this thesis (**Chapter 14**).

The realization of this research saw the submission and publication of several articles and book chapters concerning the state of the art, the results and their discussion, as well as technical

notes on the instrumental conditions. Thus, the chapters of this manuscript were written by summarizing the articles and inserting them in Appendix I, while retaining their original format (language, layout). Various additional information concerning the main methodological aspects as well as the details of the experimental plans and the justifications for strategic choices are also presented in Appendix.

The present research is a collaboration between Ecole des Sciences Criminelles (University of Lausanne, CH), Curtin University (Perth, WA), ChemCentre (Perth, WA), ESR (Auckland, NZ) and was initiated by operational needs from Swiss police forces and CURML (CHUV, Lausanne, CH).

Chapter 2: Literature overview

This chapter is based on the following article (Appendix I):

Article 1: Burnier C, Massonnet G (2019) *Pre-analytical considerations of condom traces: A review of composition, background, transfer and persistence*, Forensic Science International, Vol. 302.

Article 2: Burnier C., Massonnet G (2020) *Forensic analysis of condom traces: Chemical considerations and literature review,* Forensic Science International, Vol. 310.

2.1 Forensic investigation

2.1.1 Case studies

When condom evidence is investigated, either because the victim or aggressor has indicated that a condom was used, or in cases where the victim does not remember this happened, detection of evidence (sensitivity of the analytical technique) is the main question. In a second step only, the evidence will be evaluated in order to determine if its source is indeed a condom.

In the United States, an expert from the Navy already attempted to answer this question based mainly on analytical observations [11]. Without claiming to offer here an exhaustive list of cases investigated, three major cases are discussed from the literature. In these cases, the experts obtained positive [11,22,23] or negative [11] profiles, and in only one case was a comparison between a trace and a reference condom performed [23]. When the profile was positive, the experts concluded that a condom was present, while when the profile was negative, the only possible option was the absence of the use of a condom [11]. The possibility of another condom or another type of product being the source of the evidence was not assessed, and not even mentioned. However forensic scientists should be aware that sharing an undistinguishable chemical profile does not mean that the reference sample is the source of the trace evidence. The statement that polydimethylsiloxane (PDMS) should be present if the condom had been used assumes that (only) the initial condom contains PDMS.

In the United Kingdom, the *Regina v. Andrew Nicholas Malkinson* case (2006) [22,24] is one of the most well-known cases reporting condom evidence analysis. Experts detected PDMS traces on the victim's underwear. The victim's statement was that she never used cosmetics as she had very sensitive skin. When the expert reported the presence of PDMS traces, the court stated that if the victim's statement was correct and considering the expert's testimony, it was more likely that the traces originated from a condom [22,24].

However, at the time of the publication of these cases, i.e. between 1994 and 2006, there was no published model that identified the error rates related to these analyses, nor that allowed concrete interpretation of a negative result. False positives generated by hygiene products or other lubricants have never, up to now, been investigated.

2.1.2 Sampling

The protocol for collecting condom evidence has never been investigated for condom themselves, and the current protocols mostly rel on the principles of DNA sampling. The analogy works quite well, but a few clarifications must be made, as well as some guides on the collection and identification of traces of condoms in cases of sexual assault.

The first recommendations concern the gynaecological examination, as the examination gloves used by doctors may contain lubricants or solid particles that are indistinguishable from those found on condoms. Thus, recommendation is to wear non-lubricated latex or plastic examination gloves, which is also relevant if solid particles are the evidence of interest [8]. The current recommendation is to start by taking the swabs before proceeding to the in-depth gynaecological examination [8], in order to avoid contamination (in particular those coming from examination gloves). Samples should be taken from inside and then outside of the vagina, anus and mouth. Cotton swabs should be preferred to nylon and/or foam swabs, as issues in forensic analysis of the evidence, such as interferences during analysis were observed [25]. In Switzerland, DNA kits generally consist of at least 4 nylon FLOQ swabs (one for vulva sampling, one for vaginal sampling, one for endocervical sampling and the last for anal sampling), which are intended for DNA analysis [26–29]. In Australia, DNA kits for evidence collection contain extra cotton swabs for condom sampling (Personal Communication, Dr. Maire Kelly, Sexual Assault Resource Centre), but only one is usually used. Condom sampling is usually done before the rest of the gynaecological examination and DNA sampling to avoid contaminations with other lubricants used. There is no existing optimal sequence of evidence collection.

Doctors collecting evidence should be aware that uncollected evidence is lost evidence. Sample collection can be done on the victim themselves, where doctors are principally involved, at the crime scene if there is any, as well as at the victim's residence (if any) or clothing. Sampling includes the swabbing of the genital area of the victim, collection of packaging and/or used condoms from the crime scene or at the victim's place, as well as collection of victim's underwear.

2.2 Condom Chemical Composition

The composition of condoms is regulated by international standards [5,30–33]. The intrinsic composition of the samples is therefore a very stable system, at the level of the initial source of the sample. The intrinsic composition of the trace thus depends on this system, depending on the compounds which are more likely to transfer, but is affected by various factors.

2.2.1 Sample composition

Modern condoms primarily consist of a latex or polyurethane body, mainly covered with lubricants and solid particles, but spermicide or flavourings can also be found. During the manufacturing process, nitrosamines and dithiocarbamates can be produced as undesirable products of vulcanisation [5,34–38]. Solid particles, such as corn starch or polyethylene powder, are first added to the latex, to avoid it sticking to itself [39,40]. Lubricants are then added to facilitate penetration. Most commonly used lubricants cannot be used as they may interact with the latex, making it be porous, and therefore losing all efficiency as a contraceptive device. This is heavily regulated by international norms [5,30–32]. This is the reason why, as shown in Table 1, more than 85% of the condoms found on the market are lubricated with a silicone-based lubricant, more commonly known as PDMS, with the rest being either PEG-lubricated or, in most cases, non-lubricated condoms [9–11,14,16,41].

Table 1: Number of condoms studied in different studies and proportion of PDMS and PEG lubricated condoms. NA = non available * These data come from a market study led in Switzerland. Reproduction from *Burnier C, Massonnet G (2019) Pre-analytical considerations of condom traces: A review of composition, background, transfer and persistence, Forensic Science International, Vol. 302, pp. 135-141*

Ref.	Number of condoms types	Containing PDMS	Containing PEG	Other
[41]	56	46.4 %	5.3%	-
[11]	40	72.5%	12.5%	15% non lubricated
[18]	25	92%	8%	-
[13]	53	88.6 %	NA	6 other lubricants than PDMS
[42]	35	91.5%	5.7%	2.8% glycerine based
[25]*	204	87.8%	6.4%	3% non-lubricated 2.8% non identified

Non-lubricated condoms usually contain a significant amount of solid particles (i.e. cornstarch or polyethylene powder). Spermicide is found in lower quantities compared to lubricants and accounts for approximately 5% of the composition [14,43]. Additives such as antioxidants or flavourings may be incorporated to produce more satisfaction to the consumer [14].

Personal hygiene products (soaps, shampoo, cosmetics) and various lubricants or creams are likely to contain the same types of compounds as found on condoms, thus leading to potential misinterpretation of a condom as the source of the recovered forensic evidence. Usually, intimate lubricants can contain any type of lubricants, such as oils or glycerine, the only condition for these products being that they should be bio absorbable [33]. The exact composition of creams and other intimate products is not known, but previous research indicated that they would be likely to contain glycerine, PEG or PDMS in various amounts and would be distinguishable from lubricants or condoms [10,16,17,21,44].

2.2.2 Trace composition

The initial composition of condom traces as forensic evidence depends on the likelihood the various components can transfer to the vaginal matrix. This firstly depends on the quantity of each component on the condom before contact. Dithiocarbamates and nitrosamines might transfer but have never been studied, and their quantities on a condom are not known [35–38,40].

Lubricants and solid particles are known to be present in given quantities (Table 2), as regulated by international norms [30–32] and WHO recommendations [33]. Concentrations immediately following transfer can be estimated knowing the quantity on a condom and parameters regarding the vaginal secretions. Considering that lubricants can vary from a minimum 400mg and maximum 800mg [14] and given a vaginal secretion production of 4 mL per day [45,46] the maximal concentration that can be obtained is of 200mg/ml right after intercourse, assuming that all the material present on the condom transfers [14]. Given the chemical properties of silicone lubricants, another hypothesis to consider is that residues are deposited on the surface of the vaginal matrix. Therefore, when sampling, trace is anyway collected. Disappearance of the compounds in the matrix would be due to the secretions which would drive the compounds out of the vagina.

Regarding the spermicide content, its quantity is usually around 5-10% (w/w) of the rest of the added compounds [43]. Considering a maximal lubricant quantity of 800mg, spermicide content would be approximately max. 80mg, and the concentration right after intercourse would be estimated to 20mg/ml [14]. Finally, other additives have never been reported as detected in traces and were not investigated for forensic purposes.

Table 2 resumes the possibility of transfer for each component, expected quantity, if they have already been studied in a living matrix and if they are of forensic interest. The aforementioned compounds or type of compounds were reported as being the most likely traces

to be recovered in case work. This composition is set up right after transfer, which means right after intercourse. However, trace composition does evolve with time, causing changes in the detected compounds. This highlights the importance of understanding persistence, which may vary between compounds, but also its influence factors. Major changes are due to absorption of water-based compounds in the vaginal matrix.

Table 2: Transfer, quantities on condom and forensic interest. Reproduced from *Burnier C, Massonnet G (2019) Pre-analytical considerations of condom traces: A review of composition, background, transfer and persistence, Forensic Science International, Vol. 302, pp. 135-141*

Compound	Transfer	Quantity expected on the condom	Studied as a trace in living matrix	Forensic interest
Latex	No	-	No	No
Dithiocarbamates	Yes	Not indicated	No	No
Nitrosamines	Yes	Not indicated	Few [5,35,36]	Limited
Solid Particles	Yes	500 ± 50 mg [31]	Yes [39,47]	High
Lubricants	Yes	550 ± 150 mg [31]	Yes [7,9,11–13,15,18,41,43,44,48–51]	Very High
Spermicides	Yes	5-10% (w/w) of the whole other added compounds [43]	Yes [19,43,48,49,52,53]	High
Other components	Expected	Not indicated	Not found	Likely

As illustrated in Table 3, water-based compounds persist no longer than 24h, whereas silicone-based lubricants might be detected up to 48h after transfer. This can be easily be explained as silicones are apolar compounds and are not sensitive to the activity of micro-organisms in the vaginal matrix [18].

Table 3: Persistence of different compounds from condoms. Reproduced from *Burnier C, Massonnet G (2019) Pre-analytical considerations of condom traces: A review of composition, background, transfer and persistence, Forensic Science International, Vol. 302, pp. 135-141*

Compound	Studied	Persistence	Surface	Reference
Solid particles	No	Estimated: 24h		-
PDMS	Yes	24h	Vaginal matrix	[11]
		Up to 12h	Vaginal matrix	[41]
		4.5-12 h	Vaginal matrix	[18]
		24 - 48h	Vaginal matrix	[15]
		Up to 48h	Vaginal matrix	Unpublished, mentioned in [41]
PEG		7-8h	Vaginal matrix	[41]
		24h	Skin	[51]
Oil-based lubricants		24h	Skin	[51]
Spermicides		12-24h	Simulated vaginal fluid	[54]
		4h	Vaginal matrix	[43]
		8h	Vaginal matrix	[55]

2.2.3 Influence factors

In addition to variation over time, the traces are affected, both at the level of the initial composition (transfer) and of the composition after a given time (persistence) by various influencing factors, classified in five categories: the donor (i.e. the condom), the recipient (i.e. the vaginal matrix), the contact, the activity of the victim and the time elapsed (Figure 1).

It has thus been reported that the chemical as well as physical characteristics of the donor influence the composition of the trace found, mainly on the qualitative level (type of lubricant used) but also on the semi-quantitative level, considering the condom size or different texture (i.e. ribbed, stubbed).

The pressure and the duration of contact between the donor and the recipient as well as the donor used (conditions of transfer) affect the initial composition of the trace, and consequently the composition after a given time. Various parameters linked to sexual intercourse are also part of the deposition conditions affecting the initial composition of the trace.

The vaginal matrix is the receiver (trace support) in this research. The vagina is an organ subject to significant variations due to its constant regeneration and its evolution as a function of the menstrual cycle since, as Wagner and Levin (1980) show, "(…) the vaginal fluid of women has a distinct ionic characteristic throughout the menstrual cycle (…)" [56]. The vagina is an expandable surface and the surface area exposed to a condom or penis can therefore vary between 80 and 400 cm^2 [54]. The composition of vaginal secretions is known but the concentration of the various constituents is variable. Indeed, the production of vaginal lubricant would be 6g/day and 0.5-0.75g would be present in the vagina at a given time [46,57]. The vaginal matrix is constantly lubricated, at a rate of 1-4 ml/day [45,46]. In addition, the physico-chemical structure of the matrix is also affected by sexual stimulation, hormone therapy, or infections. These parameters affect the thickness of the vaginal matrix, and therefore its ability to retain and absorb compounds [54,58]. It is therefore a complex matrix.

The time elapsed as well as the concurrent activity of the living victim very clearly influence the composition of the traces collected. By analogy with DNA traces, the longer the time between the assault and the time of collection of the trace, the more likely the target compounds are to be degraded by the vaginal microbiota, to be absorbed by the matrix or to be evacuated in secretions [14,26,59,60]. Water-based compounds such as propylene glycol or glycerin disappear more quickly from the vaginal matrix, while silicone residues persist for a longer time because they are not affected by the microbiota and are not absorbed by the matrix [18]. The impact of human intervention (i.e. internal washing) on the trace has not been studied

in detail, though according to the literature, condom traces (PDMS) are not significantly affected by such activity [15].

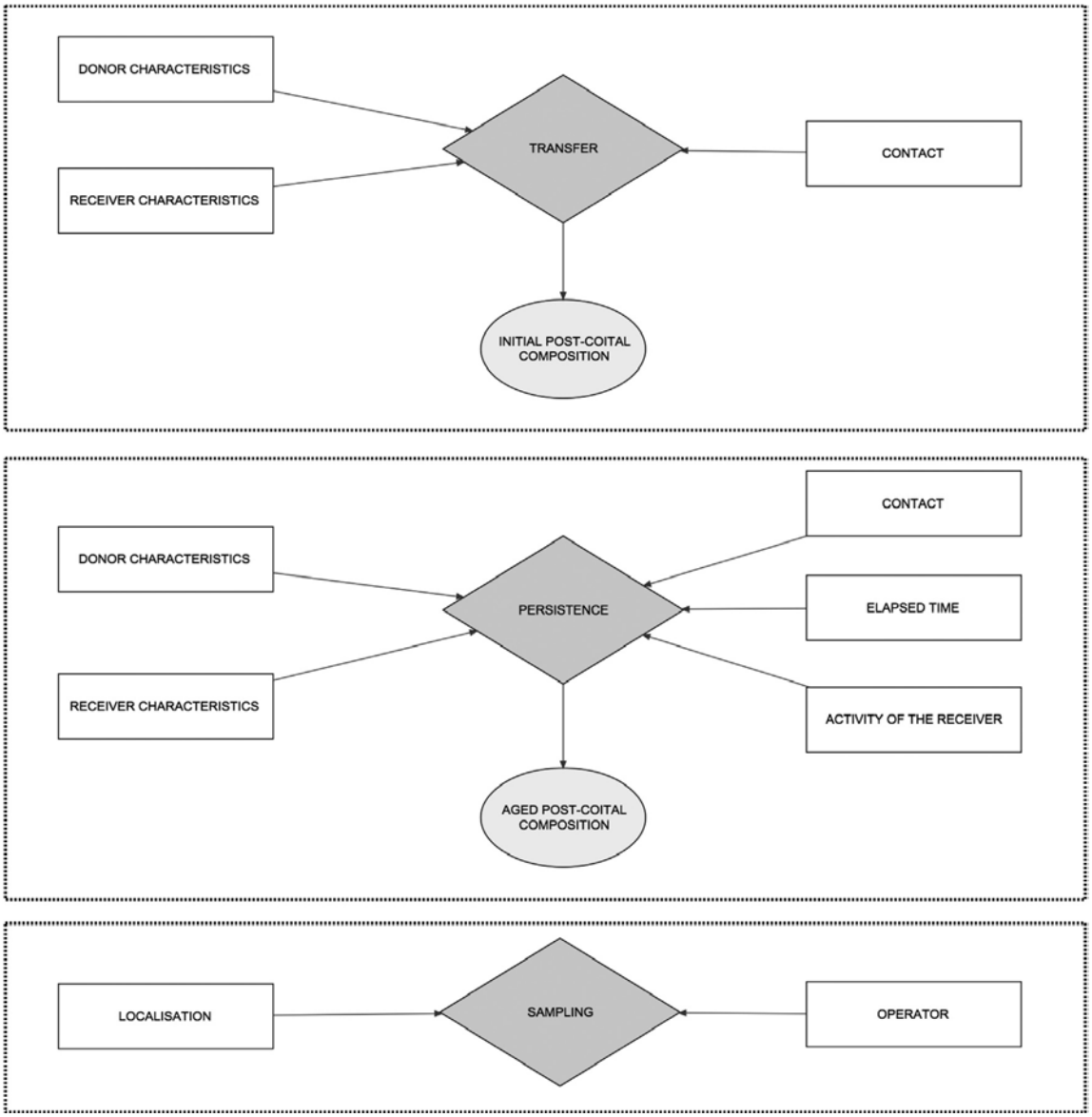


Figure 1:Schematic representation of the different influence factors and their impact at the different stage of the degradation procedure. Reproduced from *Burnier C, Massonnet G (2019) Pre-analytical considerations of condom traces: A review of composition, background, transfer and persistence, Forensic Science International, Vol. 302, pp. 135-141*

Finally, sampling of the trace using swabs similar to those used in DNA samples has not been studied in detail but has been the subject of several observations. It is necessary to take these samples first, before the gynecological examination so as to avoid contamination. The use of cotton swabs is recommended rather than the use of foam swabs or nylon swabs as these provide poor desorption of the compounds or an analytical response interfering with the target compounds [25].

2.3 Physical and chemical considerations

This section will focus only on the chemical and physical properties of materials that are most likely to be found in case work, i.e., solid particles, lubricants and spermicides.

2.3.1 Solid particles

Condoms can only contain two types of solid particles, which are cornstarch and polyethylene powder. Neither is soluble in polar solvents. Dimethyl sulfoxide (DMSO) is the only solvent known to solubilize cornstarch [61]. Starch is also known to exhibit birefringent characteristics when observed via microscopy [62]. In addition, starch was not found to be subject to digestion by bacterial enzymes present in the vaginal vault [63,64].

2.3.2 Lubricants

As aforementioned, two types of lubricants can be recovered from condoms: water-based and silicone-based ones. Polydimethylsiloxane (PDMS), also called dimethicone or simethicone, is the most common silicone-based lubricant found on condoms. Information regarding its synthesis process can be found in the literature [65,66]. A siloxane monomeric unit O-Si-C_2H_6 is repeated a various number of times (Figure 2), following the polymerization reaction, offering a great variety of polymers with different viscosities. The molecule is highly apolar and is selectively soluble in apolar solvents, but the most relevant ones are hexane, iso-octane or dichloromethane [67]. According to the literature [10,11,18,41], the polymer predominantly used on condoms is methyl-terminated-PDMS as shown on Figure 2. Hydroxyterminated PDMS can be found on spermicide-containing condoms.

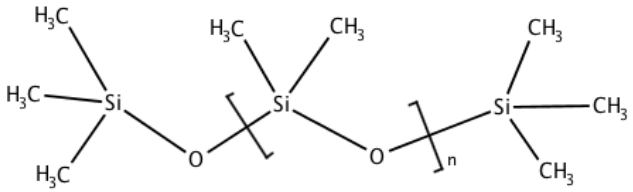


Figure 2 : Configuration of PDMS molecule, according to literature. Reproduced from *Burnier C, Massonnet G (2020) Forensic analysis of condom traces: chemical considerations and review of the literature, Forensic Science International, Vol. 310.*

Regarding water-based lubricants, the most frequently reported on condoms is polyethylene glycol (PEG), which is a polar compound, soluble in water, ethanol or acetone as well as in toluene or dichloromethane, given its chemical structure (Figure 3). Low molecular weight liquid PEG (mainly 300 and 400 g/mol) is used for condoms [18,68].

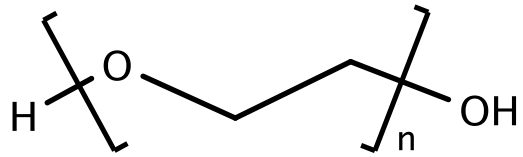


Figure 3: Chemical structure of polyethylene glycol. Reproduced from *Burnier C, Massonnet G (2020) Forensic analysis of condom traces: chemical considerations and review of the literature, Forensic Science International, Vol. 310.*

2.3.3 Spermicides

Nonoxynol-9 is the only spermicide found on condoms. Designed to kill spermatozoa by lysis of the cellular membrane, nonoxynol-9 is an amphiphilic molecule (Figure 4), containing ethoxy units, $-O(CH_2)_2-$ repeated nine times. Due to its highly apolar aliphatic chain C_9H_{19} as well as a polar chain due to - $[O-(CH_2)_2]$-OH, it is soluble in both polar and apolar solvents.

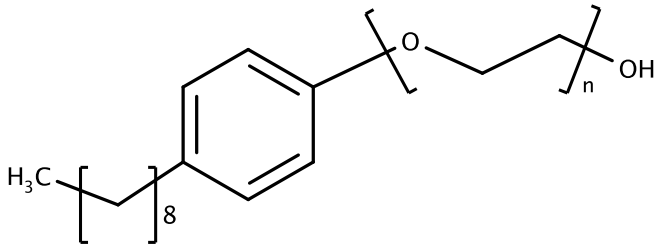


Figure 4 : Chemical structure of nonoxynol-9, n = 9. Reproduced from *Burnier C, Massonnet G (2020) Forensic analysis of condom traces: chemical considerations and review of the literature, Forensic Science International, Vol. 310.*

2.4 Analytical framework

2.4.1 Sample preparation

Two types of specimen preparation were reported from the literature review: *in situ* analysis and extraction procedure.

In situ analysis, i.e. directly on the cotton swab used for sample collection, is very relevant in forensic sciences as it limits contamination, and by avoiding extractions, reduces loss of any important traces and analysis time while eliminating the need for storage considerations. Such a methodology is usually restricted to certain types of analytical instruments, such as spectroscopy (i.e. Raman analysis) [13,69] or direct desorption of the analyte to run in using mass spectrometry instrumentation, such as Desorption electrospray ionization mass spectrometry (DESI-MS) [19,48,70], Direct Analysis in Real Time (DART)-MS [16,17,21,49,55], or desorption/ionization on silicone (DIOS)-MS [23].

However, the most common sample preparation is an extraction procedure, where the cotton swab is soaked in a solvent selected based on the chemical properties of the target

compounds. Although chlorinated solvents are usually widespread, recent research [25,41] highlighted incomplete extraction of silicone components and instead suggested using hexane for silicone-based components and methanol for water-based components.

2.4.2 Analytical instrumentation

The results of the literature presented above were obtained using different analytical techniques depending on the targeted compounds. For solid particles, the main techniques used are optical microscopy and scanning electron microscopy.

Regarding silicone-based lubricants, reported instruments are:

- Fourier transform infrared spectroscopy (FTIR), various mode used (Transmission, ATR, DRIFTS)
- Raman spectroscopy
- Nuclear Magnetic Resonance (NMR)
- Gas chromatography coupled to mass spectrometry (GC/MS)
- Capillary Electrophoresis coupled to ultraviolet-visible detector (CE-UV/Vis)
- Gel Permeation Chromatography (GPC)
- Pyrolysis-Gas chromatography coupled to mass spectrometry (Py-GC/MS)
- Electrospray Ionisation – Mass spectrometry (ESI-MS)
- Matrix assisted Laser Desorption Ionisation – Mass spectrometry (MALDI-MS)
- Desorption Chemical Ionisation - Mass spectrometry (DCI-MS)
- DESI-MS
- DART-(TOF)-MS

Regarding water-based lubricants, reported instruments include:

- Fourier transform infrared spectroscopy (FTIR), various mode used (Transmission, ATR, DRIFTS)
- Raman spectroscopy
- Nuclear Magnetic Resonance (NMR)
- Gas chromatography coupled to mass spectrometry (GC/MS)
- Capillary Electrophoresis coupled to ultraviolet-visible detector (CE-UV/Vis)
- Matrix assisted Laser Desorption Ionisation – Mass spectrometry (MALDI-MS)
- Desorption Electrospray Ionisation – Mass spectrometry (DESI-MS)
- Desorption ionization on Silicone – Mass spectrometry (DIOS-MS)

Regarding spermicides, reported instruments are:

- Fourier transform infrared spectroscopy (FTIR), DRIFTS mode.
- Raman spectroscopy
- Nuclear Magnetic Resonance (RMN)
- Gas chromatography coupled to mass spectrometry (GC/MS)
- Liquid chromatography coupled to mass spectrometry (LC-MS)
- Electrospray Ionisation – Mass spectrometry (ESI-MS)
- Desorption Electrospray Ionisation – Mass spectrometry (DESI-MS)
- Direct Analysis in Real Time Mass spectrometry (DART-MS) or coupled to time of flight mass spectrometer (DART-TOF-MS)
- Desorption ionization on Silicone – Mass spectrometry (DIOS-MS)

Regarding other additives, FTIR, GC/MS, DART-MS, MALDI-MS or even DESI-MS techniques enable these compounds to be detected in various samples [7,10,12,16,17,19–21,41,48,49,53,71].

The most frequent analytical methods used in the literature are GC (25%, 15/60 papers), infrared spectroscopy (16.6%, 10/60 papers), py-GC (15%, 9/60papers), LC (11.6%, 7/60 papers) and MALDI (10% 6/60 papers). The other analytical methods are used in less than 10% of cases. As the number of available analytical techniques is large and increasing with further technological developments, it is difficult to determine the most suitable techniques for the study of condom traces. The variety amongst the analytical methods is complex, and certain methods seem much more relevant than others depending on the aim of a particular investigation. In this case, it is a question of positioning the work with respect to the detection of the previously targeted compounds. The techniques were initially chosen to allow the analysis of the targeted compounds and the possibility of sequencing the various methods, based on the results presented in the literature [10,11,18,71–75]. Additional criteria pertain to the ease of implementation and availability of instrumentation. Four of the most well-known and commonly available techniques were thus selected, namely FTIR, Raman, py-GC/MS and GC/MS. Spectroscopy techniques (FTIR, Raman) are extremely powerful screening techniques that allow the detection of all chemical compounds [10,11,25,41,42,65,69,71,76–78]. Regarding confirmation techniques, py-GC/MS analysis has already been used in various cases [15,18,50,79] and several recent studies and allows the analysis of silicone residues and GC/MS allows the analysis of volatile compounds [53]. Although the other techniques identified in the literature have also made it possible to detect, discriminate or even classify samples, many of these analytical techniques are either not chemically adequate (for example type of solvent used) or not readily available in forensic laboratories in routine.

2.5 Forensic interpretation of evidence

2.5.1 Discrimination and statistical analysis

As with other consumer products, condoms are mass produced, and so individualisation using their lubricants properties is not realistic. Regardless, many formulations are characteristic, and thus ideal candidates for forensic discrimination. For example, the presence or absence of spermicides or flavourings, the lubricant type and its relative viscosity can be a source of variation. Recent years have also seen the emergence of condoms containing different types of additives, such as benzocaine or lidocaine added to increase pleasure delay ejaculation [21,42]. Based on these considerations, one could reasonably expect that condoms, intimate

lubricants and other type of samples would present different chemical profiles and could therefore be differentiated. By analogy with other types of evidence, different condom brands use different chemicals and therefore exhibit different chemical profiles. Samples bought in different geographic locations, as well as different model of condoms from a same brand, might also be expected to present different chemical profiles.

In the forensic literature, several discrimination studies were reported for silicone-based condom lubricants [10,21] or for water-based lubricants [16,17,20] and a few of them tried to differentiate lubricants from personal hygiene products, using chemometrics as a statistical method for sample discrimination. Discrimination of silicone-based lubricants (n=56) using DART-TOF-MS was possible with over 78% of explained variance on five principal components [21]. Discrimination of water-based lubricants (n=19) was possible with over 90% of the variance explained by five principal components and using only 7 compounds found in the samples [16]. When analysing both lubricants and condom populations, a first dataset (n=90) acquired using DART-TOF-MS was discriminated on the first 3 PC explaining 72% of the total variance [17] while a second dataset (n= 26) acquired using MALDI-MS was discriminated based on the first 3 PCs, which explained 87% of the total variance [10]. Finally, when investigating markers to profile condom lubricants on 110 samples with MALDI-MS, it was possible to distinguish samples from different brands and to identify markers specific to the respective brands [20]. Results acquired with DART-TOF-MS were also used to build classification models and various quality of classification were obtained, between 88.8% -100% [16,17,21]. However, it is worth noting that all these discrimination models were mainly built on minor compounds detected and not on the major lubricant itself. In addition, all those researches were led using very highly specialised instrumentation, i.e. MALDI-MS, DART-TOF-MS, which are rather expensive instruments not likely to be found in all forensic case work laboratories. In the future, these instruments might become a standard, but in the actual context of the present research, a more pragmatic selection of the instrumentation might be advisable.

2.5.2 Bayesian interpretation

Some authors have dwelt on the question of the conclusion of the results according to the results of the analyses. What if the result is positive? And if it is negative? [13,69]

The use of Bayesian approach can assist interpreting the evidence. Considering the hierarchy of the propositions developed by [80–82], source and activity levels can be investigated. Indeed, at the source level, the question to solve is to know, for example, whether the obtained chemical profile comes from a condom in general, or more specifically from a

specific condom, or from another source. This interpretation of evidence questions the frequency of a given chemical profile in the population (usually noted γ) and is frequently used for investigative purposes as well as for evaluative comparison of chemical profiles. The source level also contains multiple dimensions, and in an investigative process will question the background (i.e. analytical background or multiple traces) as well as the persistence, as indicators of unicity and constancy, At the activity level, two additional parameters are considered and are respectively background, transfer and persistence, those last two being studied together and considered as one. Background considers the prevalence of the given profile in the population for legitimate purposes, whereas transfer and persistence take into account whether or not the compounds can be transferred to the matrix and how long after intercourse they might be possibly detected. These parameters have been reported for various types of evidence [83–86] but have not yet been completely modelled and developed for condom evidence, although there is existing research in the area [15].

Investigation of parameters assisting Bayesian interpretation of evidence are crucial as it would allow to understand the significance of a positive (detected traces) and a negative (non-detected traces) result. If the result is positive, conclusion is likely to be that there has been contact with a product containing this type of molecule. However, such a conclusion is reductive, and it is necessary to take precautions when claiming that a condom has been used, since other products may also contain the targeted molecules [56]. Furthermore, drawing conclusions from a negative result in forensic science is difficult because the absence of evidence does not mean evidence of absence. Indeed, a negative result can come from a condom that does not present a classic chemical profile, the absence of a transfer, a lack of persistence or of sensitivity of the method, as well as the absence of evidence, which all affect interpretation in various ways [87,88]. However, in condom evidence, there is a pressing need to develop interpretative networks for forensic scientist more broadly.

2.6 Discussion

The previous sections demonstrate the complexity of the field of condom traces as well as the challenges related to detection and interpretation, which is frequently highlighted in recent publications. In addition, despite existing studies [15], in-depth knowledge is still missing in this area. This knowledge is however necessary for forensic science in order to pursue two main objectives requiring the identification and knowledge of target compounds: the development of an analytical framework, as well as the development of models helping in the interpretation of the measurements of a trace and their meaning.

With a view to the development of interpretative models of traces, it is particularly necessary to conduct more in-depth research on the specificity of the chemical profiles obtained as well as on the prevalence of these chemical profiles in the population. The next step is to understand how these traces interact with the vaginal matrix by focusing more specifically on the collection of data on living volunteers, in order to be able to study the parameters necessary for interpretation, which are background noise, transfer and persistence as well as the effects of the influencing factors. Water-based and silicone-based lubricants can be useful targets in this context. However, silicone compounds are more categorically detectable than water-based compounds [10,12,13,15,18,20,41,49], which is an advantage in the construction of models of persistence, considering that silicone compounds are the most widespread in condoms [10,12,18,41]. Regarding analytical techniques, in view of the results obtained by the various studies identified, a spectroscopy technique such as FTIR, followed by py-GC/MS and GC/MS techniques are particularly interesting because they are very often accessible and capable of analysing the silicone-based and aqueous-based compounds. FTIR serves as a screening method and is non-destructive. It makes it possible to determine which instrument will be most suitable subsequently, given that the silicone compounds can be analyzed in py-GC/MS while the water-based compounds are in GC/MS.

Highlights

This chapter aimed to review previous research from the literature. The main outcomes are the following:

- Casework analysis illustrates the need for further research on condom evidence.
- Sampling is similar to the one used for DNA.
- A condom is made of latex, lubricant, solid particles, spermicides and other additional minor compounds (aromas, flavouring)
- Trace analysis is mainly focused on lubricant detection.
- Analysis highly depending on the chemical properties of the target compounds.
- A large analytical panel is proposed in the literature for condom traces detection.
- Original composition influences the transfer of condom traces in a given matrix.
- Transfer and persistence of the evidence are affected by the donor, the matrix, the contact, the elapsed time and the activity of the victim.
- Interpretation of the results is so far incomplete.

Chapter 3: Practical Objectives

3.1 Preliminary considerations

Given the state of the art presented in the previous chapter, several choices were made concerning the nature of the targeted compounds, the analytical techniques, the matrix of interest, sampling and ethical considerations.

The present study firstly focuses on the general detection of all the compounds present in the samples in order to understand, using various analytical techniques, what the discriminatory capacities and the limitations of the instrumentations are. This study also makes it possible to collect data useful for understanding the market and therefore the compounds which can possibly be detected in a real case. Thus, the procedure proposed in this work will also help answering many additional questions which generally arise for all types of evidence such as:

- Production: how are condoms produced? Are there differences between production batches?
- Marketing: what is the diversity of condoms? Are there condoms of different brands with a similar chemical composition?
- Use: can chemical differences appear between the traces and the condoms used?
- Effects due to use: do the traces of condoms undergo modifications when they come into contact with the vaginal matrix? How does this influence the chemical composition? Can we estimate the time elapsed since using a condom?
- Sampling: what are the risks of contamination during the sampling of traces? How to prevent them? How does sampling affect the chemical compounds (constancy) ?
- Chemical analysis: what are the most suitable methods for the analysis of traces of condoms? What are the best parameters for analysis and sample preparation? How to minimize variations and laboratory errors? How to get a good chemical signal?
- Comparison of the analytical results: what is the best method of comparing analytical results? How can statistical methods help with analytical comparison?
- Evaluation of the results: how to assess the “condom evidence” index using a continuous Bayesian approach?

The main aim of the work is therefore not to reproduce exhaustively all the studies carried out in the field of condoms, but rather to provide a global procedure which allows the chemical analysis of traces of condoms and the statistical management of the results obtained.

In a second step, a preliminary study on the modelisation of the interpretive parameters will be investigated by prioritizing the silicone-based compounds. These are the compounds most frequently identified [14] in the literature and which have already been the subject of preliminary investigations in terms of transfer and persistence [15]. Their extraction from a given support (i.e. cotton swabs, textile) is possible. These are also common traces to investigate in case work [7,11,15,18,22,23].

As far as analytical techniques are concerned, their choice is related to the detection of previously targeted compounds. The techniques were chosen initially so as to allow the analysis of the targeted compounds and the possibility of sequencing the various methods. Then come the criteria of the ease of implementation and availability of devices for screening methods, which allows the selection of **FTIR** and **Raman.** The **py-GC/MS** should make it possible to obtain condom profiles, which are characteristic according to the brands and models of condoms, which makes it an interesting technique with a view to tracing the various brands existing on the market. Similarly, **GC/MS** allows the easy analysis of water-based compounds, which are volatile molecules. These are instruments that are readily available in forensic laboratories.

As infrared spectroscopy (FTIR) and Raman techniques are known and described in various specialized works, their operation will not be described here, but the interested reader can refer to various works, such as that by Stuart (2002), in regard to polymer analysis [77]. GC/MS is one of the most common and well-known analytical instruments in forensic laboratories. Its operation will not be described here, but the interested reader can refer to various specialized books [89,90]. Pyrolysis is on the contrary less common. The advantage of using py-GC/MS is that very heavy and non-volatile polysiloxanes, which cannot reasonably be separated using GC otherwise, are made more compatible with GC/MS analysis. More information can be gathered from [91–94].

3.2 Specimen selection

3.2.1 Market study

The specimen used has been established so as to be representative of the international market. According to data collected by the IUMSP in 2012 [95], at least 45 brands of condoms are present on the Swiss market and from each of them a large number of models are available, with 1,071 in total. A market study in Switzerland [25] found more than 200 brands/models of condoms; in Australia, no less than 80 different brands/models. In New Zealand, more than 100

different condoms have been identified. The most common brands on the overall market are Ansell (i.e. Ceylor, Manix, LifeStyles), Durex and Astroglide. Swiss market also presents a strong proportion of Coop and Migros brands, which are big supermarket brands. Widely available condoms were first targeted. It was decided that the most conventional condoms should be analyzed first and that condoms with other properties could be of interest in the classification of the samples. The sample set contains condoms from five possible categories: latex-free, classic, unlubricated, flavored or containing benzocaine. Other sources of lubricants, including intimate lubricants, should be explored. To this end, several intimate lubricants will be selected from those produced by the major manufacturers of condoms. Creams, personal hygiene products and massage oils that may come into contact with the vaginal matrix for legitimate (or other explainable) reasons were also selected as part of the sampling. A total of 166 samples were purchased for the purpose of this work (127 condoms, 6 creams, 6 personal hygiene products, 1 massage oil and 26 lubricants). The complete list of samples, as well as their brand, model, batch number and all chemical information linked to them following their analysis are presented in Appendix III.

3.2.2 Human samples

One of the main motivations for carrying out this work was potential application to cases of sexual assault on female victims. The vaginal matrix is therefore the most suitable for looking for this type of traces. However, the detection of traces of condoms can be made very complex by the influence of biological parameters (period of the menstrual cycle, thickness of the vaginal matrix, influence of the use of contraceptives) and the presence of certain organisms. (e.g. bacteria). The characteristics of the vaginal matrix are therefore neither constant nor unique [45,56–58,96,97]. The irregularity of the surface of the vaginal mucosa and the complex phenomena involved in the absorption and secretion of compounds also influence the conditions of the vaginal matrix [45,46,54,56–58,96,97]. The detection of target compounds in the vaginal matrix therefore depends on a large number of inherent factors. This matrix also involves working with varying concentrations over time, due to constantly changing vaginal secretions [45,46,54,56–58,96,97]. The aim to model interpretive parameters in a living matrix therefore involves working with living beings and is subject to the Federal Ordinance on Research on Human Beings (ORH). Thus, in order to carry out Chapter 9 and 10 of this manuscript, a research protocol was submitted to the ethics committee, who accepted the present study *Etude 2018-00690*. All ethical protocols are provided in the appendix (Appendix IV)

3.2.3 Sample preparation and analysis

Sample preparation highly depends on the type of instrumentation selected and the type of sample. The procedures were inspired from the literature, more specifically from Maynard et al (2001) work, as they have compared multiple extraction solvents for the extraction of both silicone-based and water-based residues [41]. Although briefly described in the next pages, the number of samples, their extraction and the number of replicates will be addressed individually in each chapter.

3.3 Research objectives

The research plan of this research can be visualised from an analytical point of view but also from a more generic point of view, as illustrated in Figure 5. Analytical development and market study will be processed according to the analytical instrumentation, thus following an iterative process. Each step as completed in the rest of the research will be briefly discussed and objectives set up.

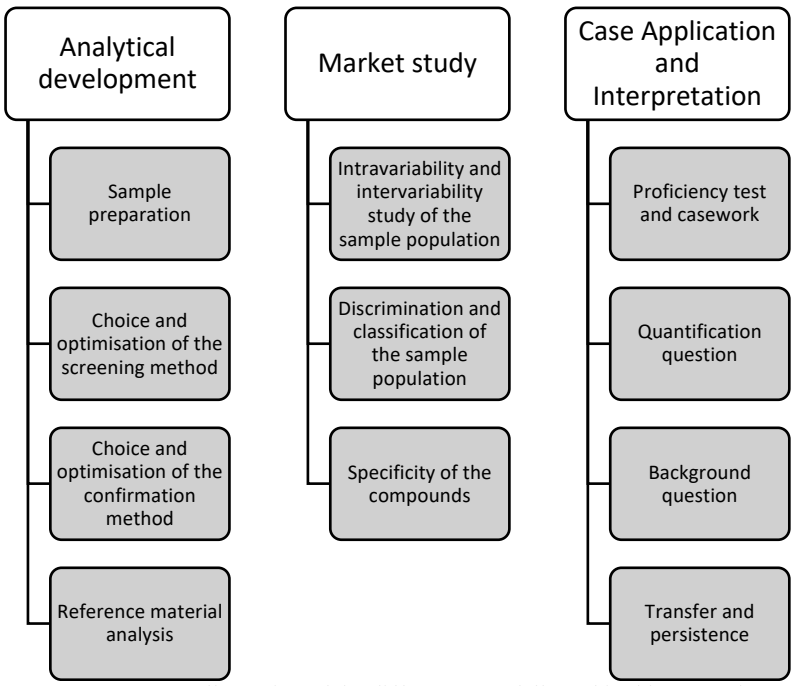


Figure 5: Illustration of the different steps followed in this research

3.3.1 Objective 1: Infrared Spectroscopy – Market Study

Casework studies have shown the value of distinguishing condom residues from other types of personal products used by women. However, up to now, there has been no investigation of their chemical variability within an international context. This work employed attenuated

total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) as a rapid screening method, coupled with chemometrics to provide objective characterization of condom lubricants and personal hygiene products from the international market. Reference material coming from silicone lubricants, glycerine and polyethylene glycol are also analysed to be able to visually separate the samples if possible. 166 samples were obtained covering five major classes of products likely to be used by women. This step was carried out in order to obtain qualitative and semi-quantitative data on the initial composition of the samples studied to understand the structure of the market population.

This type of objective makes it possible to study the variability between samples from different brands, models or batch numbers, or between samples belonging to different classes. It was also possible to assess the prevalence of the compounds in each of the sample classes analyzed. This is primarily a qualitative study which aims to establish the list of profiles present in the sample set, in order to identify the molecules of interest and to investigate their analytical behavior. Secondly, chemometrics (more specifically Principal Component Analysis) was used to assess the separation of the samples and the discrimination between the various profiles obtained. Finally, this step also aimed at studying the possibilities of classifying samples according to their composition, using classification algorithms. Validation of the classification procedure was also conducted. This classification could indeed be useful in any type of investigation in order to know the nature of the residues

3.3.2 Objective 2: Pyrolysis-gas chromatography and mass spectrometry (py-GC/MS) study

3.3.2.1 Step 1: Optimization of analytical parameters

The various studies on py-GC/MS have only reported the pyrolysis temperature [15,18]. The pyrolysis time is not listed; however, it is an important parameter that can significantly affect the observed result [98]. The reported pyrolysis temperatures seem not to have been optimized to obtain repeatable results. A trial-and-error approach centred around the peaks of interest from TGA curves [79,99,100] can be considered to find the most appropriate parameters, but this may take a long time. A more statistically rigorous approach involves the use of multivariate chemometrics to identify and model the interactions between several factors [101–103]. Experimental designs, more specifically Central Composite Design (CCD), can be used to identify which factors most affect the target response. In addition, it is possible to identify the relationships between several factors and the resulting analytical response.

Separation parameters were investigated based on literature parameters [15,18] and adapted to offer good resolution and reasonable time of analysis.

3.3.2.2 Step 2: Market Study

Silicone-based samples were analysed to evaluate the composition and the variation within a given population. Qualitative observations were performed using ChemStation software (v. F.01.03.2357, Agilent) and samples were visually classified into different groups according to their chemical profiles. Overlay was assessed to evaluate the repeatability of the chromatograms and to compare the samples due to the chemical pattern observed as well as, when indistinguishable patterns were observed, to compare retention time and mass spectra obtained between the different samples.

Principal component analysis (PCA) was performed on the total acquired dataset. This method was used to visually explore the structure of the data, by reducing their dimensionality and therefore evaluate the variation. PCA is also used to determine which variables most affect the principal components by studying the loadings, which are coefficient of linear combination, associated to each principal component. Finally, discriminant analysis was investigated on the entire dataset. Classification models were built on the basis of categories containing only the classes of the samples, i.e. condom, lubricant, cream, oils and intimate hygiene products.

3.3.3 Objective 3: Gas chromatography and mass spectrometry (GC/MS) study

3.3.3.1 Step 1: Optimization of the analytical parameters

The various studies on GC/MS analysis always reported the same type of column used and similar temperature program and analytical conditions as the ones that were used for py-GC/MS. Therefore, it was decided to rely on these studies and not to proceed to an optimization of the GC/MS analytical conditions.

3.3.3.2 Step 2: Market Study

Qualitative analysis of the samples acquired in GC/MS was performed with ChemStation software (v. F.01.03.2357, Agilent). NIST18 (*National Institute of Standards and Technology*), TOX3 (*Wiley Drug and Pesticides*) and NBS75K (*National Bureau of Standards*) databases were used to characterize the diverse components present in the samples.

Semi-quantitative analysis was conducted by extracting peak areas using the automated integration feature in ChemStation. Data were then exported to Excel®. Data were normalized to the internal standard. Further processing for discriminative purposes was investigated using

chemometrics. Within and between samples variations were studied, and chemometrics was used to evaluate sample discrimination. Chemometrics instruments are described in each chapter.

3.3.4 Objective 4: Application to case work

One way to assess the applicability and relevance of an analytical framework is to test it on simulated cases, such as proficiency trials, before applying it to case work investigation. ChemCentre provided the opportunity to process such application and validation.

ChemCentre (Perth, WA), in collaboration with the Sexual Assault Resource Centre (SARC) and the West Australian Police Force, has recently implemented advancements in the collection and analysis of condom evidence. This procedure involves forensic clinicians, as well as police officers and forensic scientists, as they are all part of the forensic investigation. Recently, an analytical and statistical framework was developed, using py-GC/MS and GC/MS, for the analysis and classification of condom residues as well as lubricants and personal hygiene products [104,105] The application of the method to known casework simulants in the form of proficiency trials is necessary to validate the framework, before proceeding to casework analysis. The implementation of this new framework in Western Australia has generated an increase in case numbers in which condom evidence is submitted and analysed. Case samples were processed, and gaps that affect the interpretation of this type of evidence highlighted within the current framework.

This objective aims to address the challenges for the Clinical Forensic Physician and the Forensic Scientist from the point of condom evidence collection, through the analysis and the interpretation. Data from proficiency trials will be presented to ensure validation of the analytical framework. Then casework examples will illustrate different issues in terms of sample collection and sample analysis. Finally, the interpretation of the evidence will be discussed.

3.3.5 Objective 5: Infrared spectroscopy – Trace Oriented questions

3.3.5.1 Step 1: Choice of the adequate analytical procedure

These development and optimization steps were necessary since the literature reports the use of various infrared and Raman techniques as well as various analysis parameters [11,13,34,42,69,71,76]. This choice was made by carrying out the analyses with five infrared techniques (Transmission, ATR, micro-transmission, micro-ATR, DRIFTS) and with Raman with four lasers, which were the operating conditions reported in the various publications

[11,13,34,42,69,71,76]. The priority target compounds were silicones, given their presence on the market. The analyses were carried out on pure reference standards of PDMS and then diluted in a solvent, so as to be representative of reality.

3.3.5.2 Step 2: Optimization of the analytical parameters

FTIR analytical parameters were optimized using an experiment plan (Full Factorial Design) was designed to identify the parameters affecting the signal-to-noise ratio (SNR) as the parameter to be maximized. Experimental designs, more specifically Central Composite Design (CCD), were used to identify the relationships between several factors and the resulting analytical response, as well as to optimize the results by enhancing the SNR. In addition, Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) were used to investigate the reproducibility of the spectra. Different analytical parameters were investigated based on literature [15,18] and adapted to offer good signal-to-noise ratio and reproductibility.

3.3.5.3 Step 3: Data pattern recognition

One of the current questions to investigate is to determine whether it is possible to combine data acquired within the market study with the ones acquired with the adequate method selected in the Step 1 of the present objective. The models previously created with ATR-FTIR in the market study objective should ideally be possible to be used for predicting samples acquired with other IR analysis technique and could be supplemented with additional samples from real cases. In the event that this approach is unsuccessful, it would be of great interest to examine data dispersion using chemometrics and if the same type of clustering than observed within the market study can be obtained, to find out if patterns acquired are transposable.

3.3.5.4 Step 4: Quantification & Semi-Quantification

The possibility of conducting quantitative analysis is to be investigated, with respect to questions of repeatability, linearity, trueness, accuracy, uncertainty and recovery. This is compulsory as it significantly eases the modelisation of interpretative parameters, by using quantified values of a concentration in a sample.

3.3.6 Objective 6: Modelling of parameters assisting interpreting evidence

The state of the art has emphasized that there is currently no method in the field which allows the interpretation of traces of condoms other than by the presence or absence of a

chemical profile. Indeed, studies on the subject have been carried out in a non-systematic or incomplete manner and the data reported in the literature generates enormous uncertainties. Practical applications are rarely discussed, which does not encourage constructive development. Thus, based on the results obtained concerning the composition of condoms, this research aimed to produce results which would help to interpret the data obtained in a real case.

3.3.6.1 Step 1: Background study

It is recognized that there is a need to identify not only the analytical parameters that would allow evidence to be detected, but also the factors that would influence their detection in casework [14]. Previous researchers have highlighted issues regarding the lack of knowledge on the interaction between the target compounds and the vaginal matrix [13,14,51]. Other investigators have conducted research to answer questions concerning the transfer and persistence of condom residues [15,18,76], but the question of the background is still pending.

Samples were collected from a small population of donors to explore the range of qualitative and semi-quantitative variation, and to identify the composition of the matrix, using literature comparison and various databases (e.g. NIST14). Several approaches were taken to identify the composition, such as comparison with previously reported data on human skin, latent fingermarks or vaginal matrix. The prevalence of silicone-based lubricants traces in the given population will be discussed.

3.3.6.2 Step 2: Transfer study

Several factors are likely to influence the trace and its recovery by influencing the transfer and persistence of the evidence. Despite the recurrence of such questions, there's is no existing model to assist the forensic scientist to understand what happens during the transfer as well as after a sexual intercourse, and thus to allow adequate interpretation of the evidence.

It is interesting to investigate the classification of real traces, to see whether they classify the same way pure lubricant samples do. It is also interesting in a forensic context to understand how the target compounds react when in contact with the matrix, and the effects of the donors (condoms) and receivers (human vaginal matrix).

The aim of this step is on one hand to study the classification of traces and their discrimination, and on the second hand to study the transfer of the silicone residues exposed to the different influence factors, using DRIFTS-FTIR. Whether the residue variability could be reduced in such a way that robust models could be developed and used for condom evidence interpretation was assessed. Principal component analysis (PCA) was used to evaluate the

transfer, in order to collect fundamental knowledge about the variability brought by the effect of known factors (i.e. donor and receiver).

3.3.6.3 Step 3: Persistence study

Persistence It is of great importance for research to be based on real world challenges to assist the judicial system. This step of the research aimed to generate data to demonstrate the persistence of condom evidence in the vaginal matrix in a way that is applicable to sexual assault caseworks. A contribution to an empirical evidence base is needed, so as to establish the nature of the persistence of transferred condom silicone lubricants and develop an evaluative framework for the interpretation of condom evidence.

Therefore, the effect of factors such as time and activity, in addition to the effect of the receiver were evaluated for the persistence, using principal component analysis (PCA), partial least square regressions (PLSR) and linear-exponential regressions to develop a model assisting the forensic scientist at the interpretation of the evidence.

Highlights

This chapter presented the structure of the present research. The main results are the following:

- 166 selected samples, i.e. condom, lubricants and personal hygiene products, covered 3 international market: Australia, New Zealand and Switzerland. This was a strategic choice given the previous research led by Australia and New Zealand in the field.
- Pragmatic selection of instruments led to analysis using FTIR, py-GC/MS and GC/MS
- The present manuscript is structured following the analytical instrumentation.
- For each analytical instrumentation, optimization and sample preparation will be discussed, followed by a market study and a classification model built.
- Application to proficiency tests and case work analysis will be presented and questions related to the interpretation will be raised.
- A prevalence study will be led in order to be able to inform on the background parameter, needed for interpretative purposes.
- A preliminary investigation of transfer and persistence of silicone-based residues will finally be presented as an opening on further research.

Chapter 4: FTIR Market Study

This chapter is based on the following articles (Appendix I) :

Article 6: Burnier C., Coulson S., Massonnet G., Pitts K., Sauzier G., Lewis S.W. (2021) *Forensic investigation of condom and personal hygiene products market using ATR-FTIR coupled to chemometrics*, Science & Justice.

Casework presented in **Chapter 2** in addition to earlier studies reported the use of vibrational spectroscopy as a non-destructive means to characterize a trace. Several such studies have used infrared spectroscopy as a screening method to examine condom lubricants [9,11,41,42,71] and classify them as a function of their viscosity [9], while Raman spectroscopy has also been used for trace evidence detection [13,69]. As these methods are recommended for identifying potential condom residues in casework [11,41], it is of prime importance to define the discriminating capabilities of the technique to face issues of sample differentiation or source identification. One common way to evaluate discriminatory performance is to conduct a statistical exploratory study on a large population set. Although a recent study found that DRIFTS-FTIR [76] showed greater potential for characterizing lubricants, ATR-FTIR was chosen for this study as it readily available in most forensic laboratories and requires minimal sample preparation. Assertions on the content of the samples present on the market have never been verified and the discrimination of similar chemical profiles have been verified only on a small set of samples. Previous papers [16,17,21,41,42] offered an overview of data acquired on a small set of samples, rarely going over 100 samples.

This study made use of bulk samples (i.e. personal hygiene products, condom lubricants and intimate lubricants) found on the Australian, New Zealand and Swiss markets, in order to first evaluate any structure in the market and assess the ability of ATR-FTIR to distinguish bulk lubricants based on their chemical profile. The spectra obtained were qualitatively analysed based on visual observation before being subjected to chemometrics. As highlighted in both the National Academy of Sciences (NAS) [106,107] and more recently in the PCAST [108] report, visual interpretation of forensic traces, especially fingerprints, can suffer from operator bias or subjectivity. Applied to more chemical traces, a visual comparison is not sufficient to quantify the results obtained by the comparison. The use of chemometrics can minimise this bias as it uses mathematical and statistical methods to explore differences in a given dataset. Chemometrics is also known to be useful for pattern recognition and may help to reveal trends not clearly visible otherwise. This is useful in observing the structure in the data.

4.1 Market study – Qualitative chemical observations

Based on previous researches [42,109–112], ATR-FTIR was selected for the present market study in order to gather more information on the market structure, in terms of chemical profiles.

4.1.1 Samples

100 condoms representing 8 brands present on the New Zealand market were purchased from major distributors and manufacturers. 20 condoms representing 6 brands present on the Swiss market were purchased from Swiss supermarkets and pharmacies; and 6 condoms and representing 4 brands present on the Australian market were purchased from Western Australian supermarkets and pharmacies, for a total of 127 condoms. 6 creams, 5 personal hygiene products, 1 massage oil and 26 lubricants that may be used by women on a daily basis, representing 9 brands from the Australian and international market, were purchased from pharmacies. The samples obtained were considered representative of the market share of the major condom and personal hygiene brands and sub-brands available to consumers. In total, 166 samples were analysed. The entire listing of the samples is available in Appendix III.

4.1.2 Instrumental conditions, data acquisition and sample preparation

Infrared spectra were collected using Nicolet iS50 FTIR spectrometer equipped with different ATR instruments according to the place where the analysis were run: single-bounce diamond crystal ATR accessory (Australian samples), Smart Orbit attachment (New Zealand samples) or Golden Gate Single Reflection Diamond ATR system (Swiss samples). Data collection was carried out using the OMNIC software. This adds both instrumental and operator variability. Spectra were collected over the 4000 to 400 cm^{-1} range with 4 cm^{-1} resolution and 32 co-added scans. ATR correction was performed on all spectra to account for variations in penetration depth based upon wavelength.

Condoms were rubbed directly on the ATR crystal and analysed with no further preparation. All other products were applied as thin films to cover the ATR crystal and analysed with no further preparation. The sampling window was cleaned using ethanol and lint-free tissue before each sample, and a background scan of the clean crystal obtained between each replicate acquisition. For each sample, 5 replicates were acquired, by depositing 5 times the samples on the crystal and cleaning in between all the replicates, to be able to statistically consider the sample variation. In a single sample, if there was variation amongst the 5 replicates and more

than one chemical profile could be observed, an additional 5 replicates were run to ensure adequate representation of this variability. A total of 830 analyses were used for the present study.

4.1.3 Data analysis

4.1.3.1 Qualitative analysis

Qualitative observations were carried out using OMNIC software and samples were visually classified into different groups according to their chemical profiles. Data pre-processing and chemometric analysis were performed using the Unscrambler® X 10.5 software (Camo Software AS, Oslo, Norway). Spectra were truncated to omit the 2340-1880 cm^{-1} region due to interference from the diamond crystal. Then range normalisation was applied to remove variation related to the amount of sample deposited and therefore the sample layer thickness on the crystal. Before performing the complete exploratory study, it was important to know whether consistent results were obtained when analysing the same types of condoms. Tests were performed to evaluate if different lots of the same brand presented different chemical profiles.

4.1.3.2 Chemometrics

Using the Unscrambler X (v. 10.5), principal component analysis (PCA) was carried out using mean-centered spectra and non-linear iterative partial least square (NIPALS) algorithm, with 1000 iterations. Samples were plotted using up to the first three principal components (PCs) to visualize the distribution within the samples and identify any clustering. 12 different pre-processing methods (available in Appendix IX) were applied and the resulting PCA plots compared to see which combination allowed the best visual discrimination of the samples.

4.1.3.3 Discrimination Model creation and comparison

Given that visual observation of principal component clustering can still suffer from operator's subjectivity, supervised classification was used to provide more objective discrimination. Models were constructed using the first three PCs, treating each replicate of each sample as a separate sample, as they were analysed as if they came from different samples. Discrete classes were attributed to each replicate based on the observations of the chemical profile and the knowledge of the samples (i.e. sample type, sample content, brand, model). Five classification algorithms; linear discriminant analysis (LDA), quadratic discriminant analysis

(QDA), and support vector machine (SVM) with linear, polynomial (3rd degree) and radial basis function algorithms, were used and compared. Confusion matrices showing the actual and predicted classes were obtained, and model performances were calculated for each discrimination model created. The predicted classes using the test set were compared to the actual class, to evaluate the accuracy of the model using performance analysis parameters, such as classification error rate, false positive and false negative rate.

4.1.3.4 Discrimination Model validation

Performance on an external sample set is crucial to rigorous validation, as using the same data to build and test a model can result in overly optimistic discrimination and classification accuracies. Therefore, the different models were evaluated using an external sample set and the performances were calculated. The models were then tested on three external sample batches involving different analysts and different instruments: a known-matching samples set (i.e. a batch of samples already represented in the model); a known non-matching samples set (i.e. a batch of samples not yet represented in the model); and a blind validation consisting of samples unknown to the analyst. Regarding the blind validation procedure, and a second operator acquired new data, from selected samples taken in the dataset, and the results were interpreted by another operator. Such an approach reinforces the quality of the model as experimental factors may significantly affect the observation, due to instrumental and operator variation. Therefore, the higher the correct classification, the stronger the model is for further applications. Samples were subjected to the same data pre-processing as the samples used to build the model and were then projected and classified using each model.

4.1.4 Preliminary considerations

Suitable replicates are essential in a forensic context. In the case of this study, replicates not only allow consideration of sample or instrumentation variability, but also operator variation such as in depositing samples on the ATR crystal. It was observed during the analysis that, when analysing non-condom samples, if the droplet on the crystal was too thick then minimal spectral variation was seen between samples. Water-based chemical profiles would be observed, and the profile would change with water evaporating. Therefore, the deposition strategy to ensure proper qualitative observation of a full and adequate chemical profile was to deposit the sample on a gloved finger and then rub it on the crystal to ensure that the sample was applied as a thin film.

As previously reported in the literature [14,41,42], at least 3 major classes, i.e. silicone-based, water-based, or oil-based (vegetable or mineral oils), are most likely to be encountered on the market. Within the water-based class, the most common lubricants are PEG and glycerine. Reference materials were run to observe the differences between the different lubricants. Chemical profiles from PDMS, PEG and glycerine are presented in Figure 6. PEG and glycerine were distinguished based on the peak number and position, as well as the OH peak shape. For the oil content, comparison with ATR spectra found in the literature and NIST webbook online database (https://webbook.nist.gov/chemistry) was used.

Any sub-groupings within these main categories could be due either to variation in the deposition and analysis approach or to variation in the sample composition. The use of replicates, in this case, is needed to ensure that any observed characteristics are consistent in all the profiles and hence differences are attributable to compositional changes rather than variation in the analysis.

After collecting each replicate profiles for each sample, an insight into qualitative spectral identification was carried out to determine whether different chemical profiles were observed within the overall dataset, and if it was possible to visually classify them. Four main categories were expected to be present as different chemical profiles were observed, some containing silicone compounds (Figure 6A), some containing water-based compounds (Figure 6B and C) and some being characteristic of oil-based content (not presented on Figure 6).

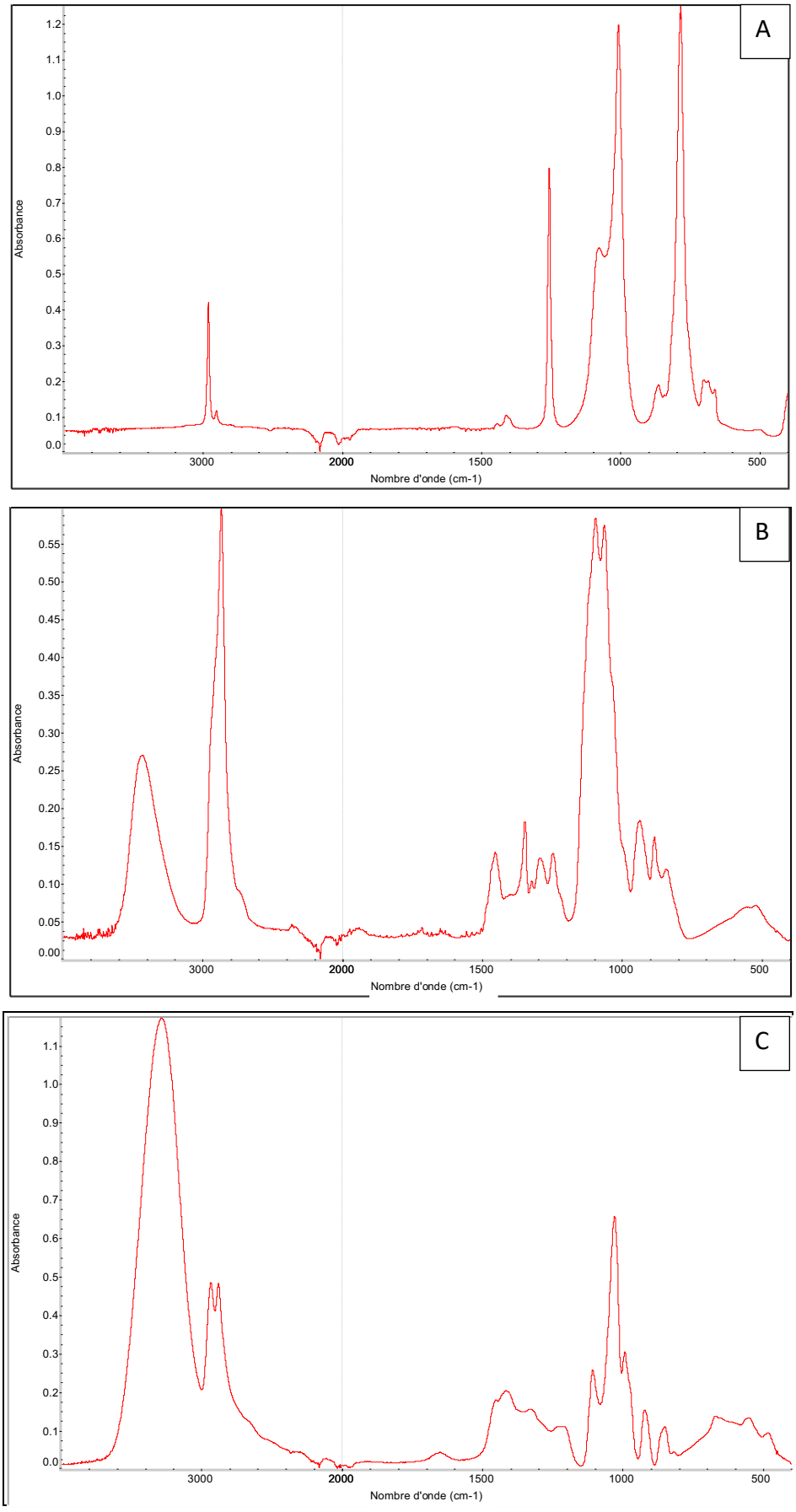


Figure 6 : Reference FTIR chemical profiles used to visually discriminate spectra from the overall dataset, a) silicone-based, b) PEG-based c) Glycerine-based

4.1.4 Chemical Qualitative Classification

Qualitative spectral identification was carried out to determine whether different chemical profiles were observed in the overall data and if it was possible to visually classify them. Spectra were overlaid in Omnic software, and evaluation was based on the presence of peaks as well as their number, position and shape. Systematic comparison with reference materials was performed to ensure correct identification of the composition.

Five main categories representing different chemical profiles were obtained based on the chemical composition. The first four were silicone-based, oil-based, glycerine-based, and PEG-based as expected. Category 5 is made of an unknown water-based component presenting a different chemical profile than glycerine and PEG. An additional sixth category gave a chemical profile of products dominated by water, which were hence unable to be reliably assigned to any of the specific water-based sub-groups. Spectra were then examined within each category, and sub-groups within the 2 major groups (silicone-based and glycerine-based), were identified (Table 4). Subgroups were defined based on peak presence or absence, peak position and peak shape. Table 4 lists the number of samples in each category, and the IR bands characteristic of each chemical profile.

Silicone-based samples (Group 1 in Table 4, Figure 6A) were the main category observed with the presence of characteristic peaks associated with PDMS corresponding to symmetric and asymmetric Si-O stretching at 1020 and 1090 cm^{-1}, a Si-C stretching at 1263 cm^{-1} and a C-H dimethyl and trimethyl deformation around 800 cm^{-1} . Group 1 samples only presented these four peaks, thus suggesting that either PDMS is not mixed with any other component when applied to a condom or in a lubricant, or PDMS is predominant in the spectrum and other components are present in too low a relative amount to be detected. Sub-clusters 1a to 1d were created based on the observation of variations within the chemical profiles (Figure 7). Category 1a was the most commonly observed profile and was matching the reference spectrum of PDMS, without any noticeable differences (Figure 7A). Other sub-clusters were distinguished by the presence of additional peaks to the classic silicone pattern (Figure 7B), a single peak instead of a doublet for the silicone double bond (Figure 7C), or an inverted relationship between symmetric and asymmetric Si-O vibrations (Figure 7D). These differences may be explained by the presence of other components (group 1b) or a different viscosity of the PDMS (1c and 1d). These differences are not linked to different viscosities, as analysis of reference material of different viscosities have not illustrated such differences. Category 1 samples only presented these four peaks in the IR spectra, thus suggesting that either PDMS is not mixed

with any other component when applied to a condom or PDMS is predominant in the spectrum and other components, if any, are present in insufficient amounts for detection. Therefore, the selectivity of the instrumentation is very high for PDMS, although this might be due to fairly high concentration of silicones on the sample.

Table 4: Categories of chemical profiles obtained from qualitative analysis of the dataset, along with the number of samples in each group or subgroup, their characteristic infrared absorption frequencies and corresponding components identified through comparison with reference materials.

Group	Number of samples	Sample no	Infrared Absorption Frequencies (cm^{-1})	Components
1	129		790, 1012, 1090, 1260, 2960	PDMS
1a	124	1-13; 15-18; 20-21; 23-56; 58-69; 71-100; 109-111; 113-123; 125-133, 169,171, 178-181	790, 1012, 1090, 1260, 2960	
1b	3	14, 19, 22	790, 865, 1012, 1090, 1260, 2960	
1c	1	174	700, 790, 1050, 1260, 2960	
1d	1	156	790, 1012, 1090, 1260, 2960	
2	15		850, 921, 992, 1029, 1108, 1410, 2879, 2936, 3280	Glycerine
2a	9	57, 70, 108, 175, 182, 165, 172,176, 177	799, 839, 922, 992, 1036, 1108, 1261, 2879, 2936, 3310	
2b	5	135, 137, 140, 141, 142	922, 992, 1040, 1112, 1414,1641, 2888, 2944, 3320	
2c	1	163	994, 1043, 1112, 1639, 2848, 2916, 3345	
3	2	138, 158, 159, 173	722, 1098, 1160, 1237, 1464, 1743, 2853, 2922, 3008, 3408	Oils
4	1	124	519, 840, 885, 935, 1063, 1095, 1248, 1294, 1348, 1459, 2865, 3430	PEG
5	3	112, 168, 170	802, 837, 921, 990, 1040, 1078, 1136, 1640, 2881, 2935, 2975, 3330	Unknown water-based component
6	13	160, 161, 162, 164, 157, 154, 134, 136, 166, 167, 155, 132, 139	1636, 2853,2924, 3350	Water-dominated spectra

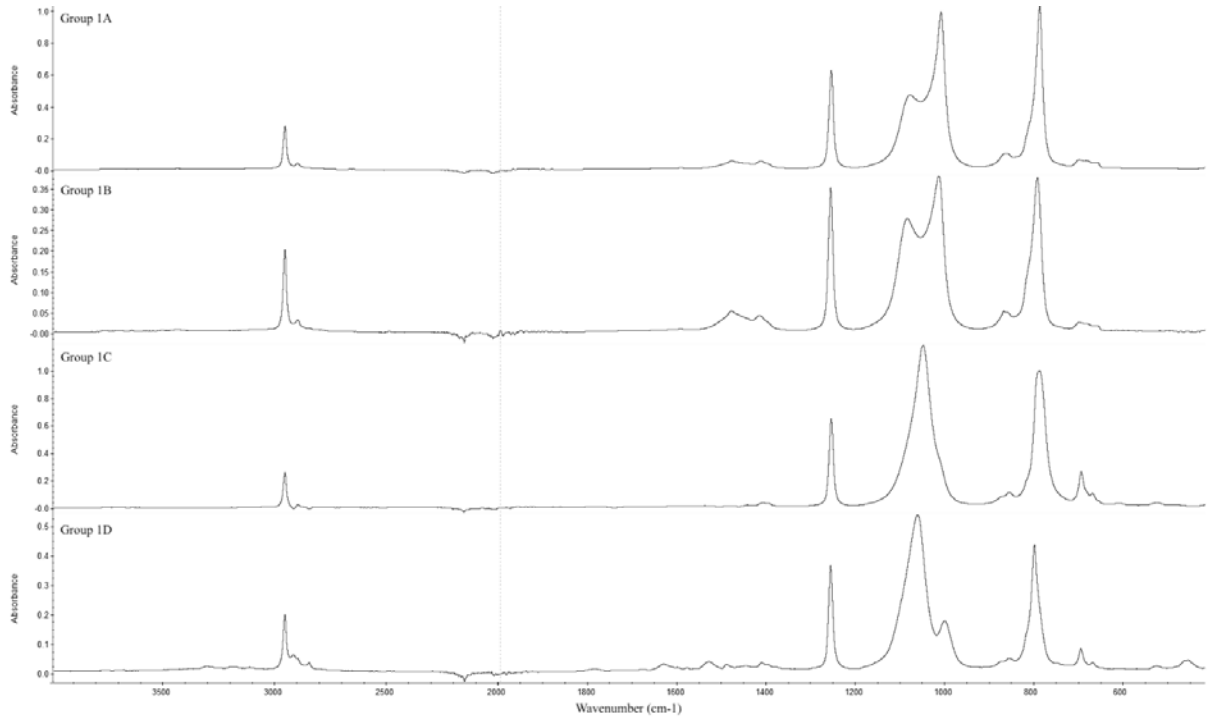


Figure 7: Sub-groups created within Category 1 samples (silicone-containing samples). All the presented bands are exclusively linked to PDMS. These observations were reproducible within the dataset.

Oil-based samples (Group 3) did not present any typical vibration of O-H or silicone bonds, whereas all other sample types presented a strong O-H peak between 3700-3000 cm^{-1}. This peak was found in all samples containing PEG (Group 4), glycerine (Group 2) or more generally water-based products (Groups 5-6). However, differences between the O-H peak shape as well as additional peaks allowed these samples to be separated into different categories (Figure 8). The presence of additional peaks as indicated in Table 4, for example peaks at 800, 1261, or 1640 cm^{-1} in the glycerine group, allowed the creation of sub-categories within this group. These peaks are potentially due to other water-based additives.

A more careful observation of the different groups highlighted that water-based type samples (Groups 2, 4, 5 and 6) did not present any strong vibrations linked to silicone-based products. Likewise, silicone-based samples did not present any peaks specific from glycerine- or PEG-based lubricants. These observations suggest that condom lubricants appear to be either silicone-based or water-based, but not a mixture of the two. This is surprising considering that some of the silicone-lubricated condoms are flavoured or expected to contain additives dedicated to long-lasting pleasure. In addition, Maynard *et al* in 2001 [41] reported that some silicone-lubricated condoms or lubricants were also containing nonoxynol or glycerine. Such differences might be explained by the difference of the spectroscopic instrumentation used for the study as well as a modification of the market within the last 20 years.

However, such classification should be handled with great care. The present classification may also suffer from operator's subjectivity. Unsupervised classification techniques, such as PCA, may allow more reliable and objective data assessment, reducing operator bias and ensuring a more transparent discrimination [107,108,111].

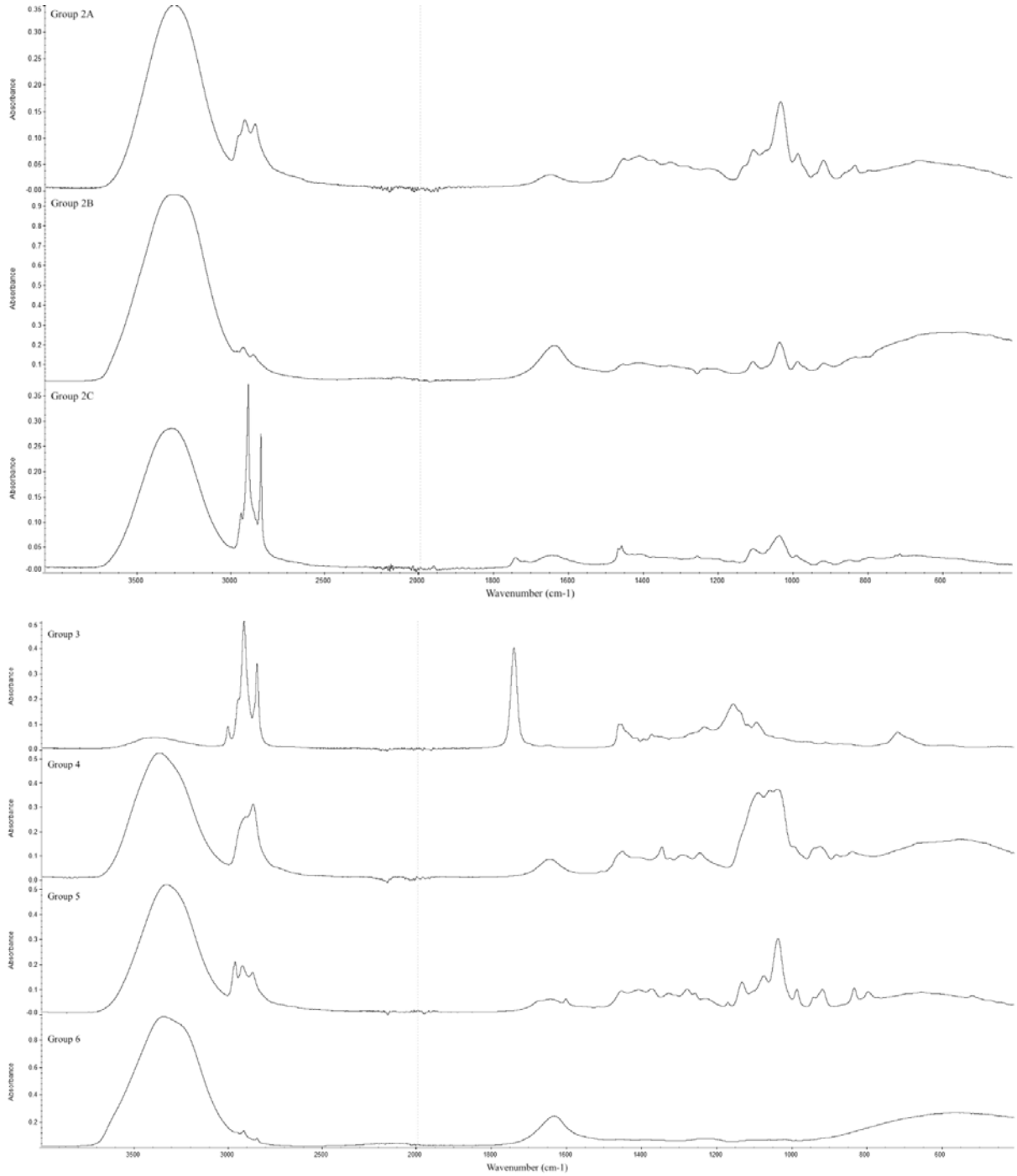


Figure 8 : Spectra obtained for water-based samples. Differences in peak shapes, position and presence/absence can be noted, Group 6 presents significantly less peaks than others and is clearly dominated by water.

4.2 Market study – Statistical discrimination

The aim of these experiments was to determine whether samples coming from condoms and from other sources could be distinguished, as well as to evaluate the potential discrimination of different types of condoms. As a reminder, this was led on results acquired with ATR-FTIR instrumentation and not DRIFTS-FTIR analysis.

4.2.1 Sample Homogeneity

Before commencing the complete exploratory study, it is important to know whether or not consistent results can be obtained when analysing the same types of condoms and if statistical analysis can distinguish them. Condoms of the same brand and same model but different production lots were analysed, and Figure 9 presents the results of the statistical clustering obtained. No major differences were observed, as all the samples are mixed all together, suggesting a certain homogeneity between the different lot numbers. This is not surprising considering the limited amounts and types of products that can be used on a condom due to the international regulations concerning their production.

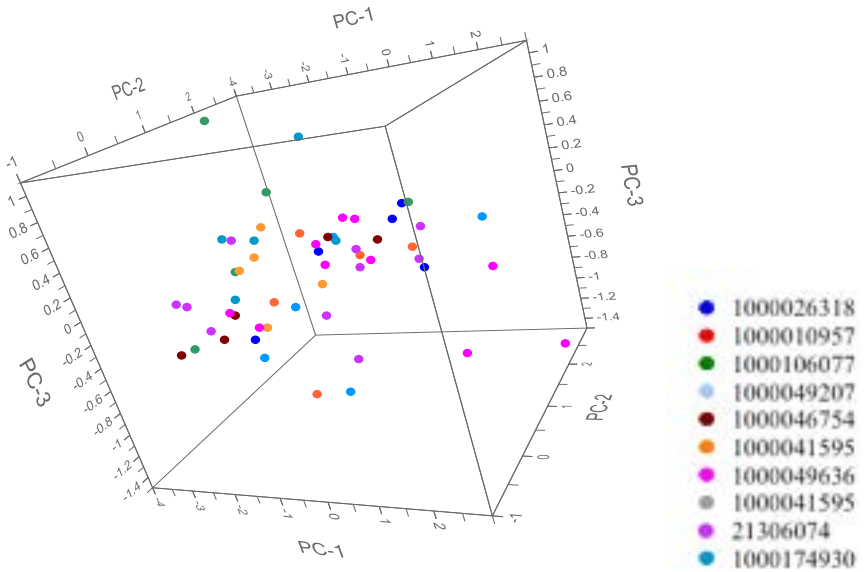


Figure 9: Separation of same brand, same model (Durex Classic) but different lot number condom samples. Different colors indicate different lot number condoms.

4.2.2 Population diversity

The purpose of this diversity study was to differentiate as many individual samples or sample clusters as possible. 12 pre-processing methods were considered in this study, based on previous studies reporting the importance of pre-treatment on the discrimination of samples [113–117]. PCA was carried out for each set of pre-processing, but none were found to improve sample

discrimination compared to the initial corrected dataset (baseline correction and range normalisation).

PCA was performed on the entire dataset without further pre-processing to visualize any clusters. Based on literature [10,14,41,42] and the composition of the different products composing the dataset, at least four different clusters were expected: silicone-based clusters, glycerine-based clusters, PEG-based clusters and oil-based clusters.

PCA performed on the dataset revealed that 93% of the total variance was described by the first three PCs. From the scree plot (Figure 10), it was determined that up to 5 principal components accounted for 98% of the variance. The first PC explaining 90% of the variance indicates that our data are highly correlated.

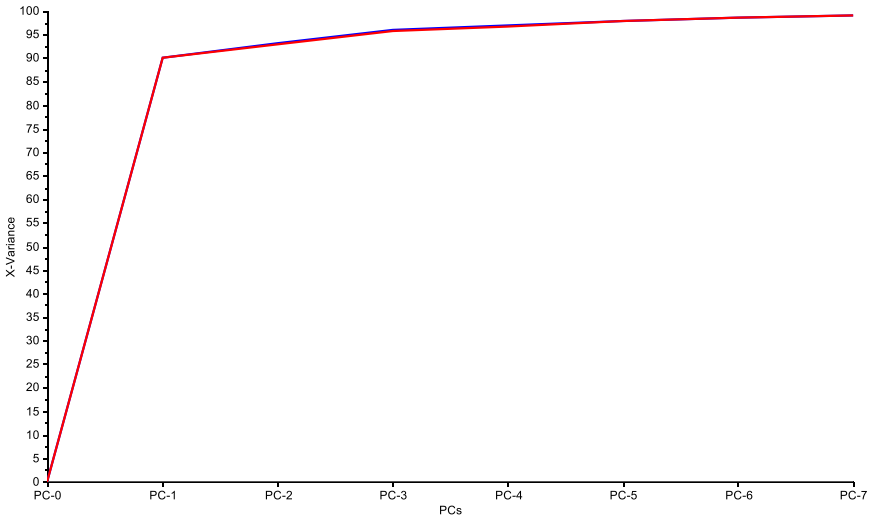


Figure 10 : Scree plot obtained for the PCA performed on the overall data set

Spectra from the 166 samples were plotted along the first three PCs (Figure 11). Although additional PCs did explain variation (Figure 10), they did not help distinguishing more similar samples. Indeed, as they only explain 0.5% of the variance, they are probably describing noise, and thus are not helpful.

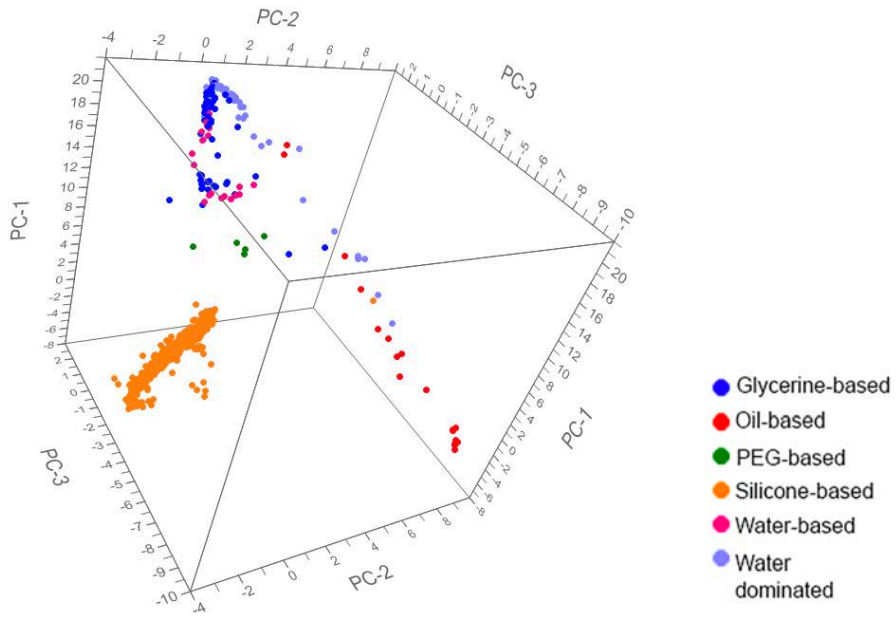


Figure 11: 3-dimensional PCA scores plot of the overall dataset, generated using the first three PCs, highlighting the distribution of the chemical profiles of the population based on their ATR-FTIR profile.

As shown in Figure 11, samples are separated primarily along PC1. Silicone-based samples were separated from the water-based samples along PC1 and the oil-based samples along PC2. This clustering pattern reinforces the main categories identified during the qualitative examination of the spectra. Water-based samples were found to offer a strong variation along PC1 and PC2, revealing at least 2 sub-clusters. The use of PC3 showed that water-based samples were clustered together, but PEG containing samples were separated from the rest of the water-based samples.

Interestingly, visually distinguished samples were clustered in the same groups along these PC1 and PC2, and samples that were qualitatively indistinguishable based on their spectra were found to be visually clustered in different groups in the scores plot. Additionally, different sub-groups could be observed compared to the visual examination. The minor peaks enabling visual differentiation between specific groupings were likely dismissed by PCA as having low significance and thus incorporated into later PCs that weren't examined. These observations demonstrate the importance of visually examining the data, and not just relying on chemometric interpretation.

The factor loadings for these PCs (Figure 12) were used to identify the spectral regions, and thus specific chemical components, contributing to sample discrimination. PC1 was found to be negatively correlated with peaks at ~790, 1020, 1090, 1260 and 2963 cm^{-1}, which are characteristic of the silicone backbone from PDMS. A highly positively correlated peak at ~ 3000-3700 cm^{-1} was also observed, consistent with the O-H stretching bond attributed to water

and water-based components. Consequently, the discrimination between classes across PC1 is due to the presence or absence of silicones and water containing components. The samples containing silicone bonds attain large negative values on PC1 while the remaining samples attain significant positive score on PC1. These observations match the clustering that was observed in Figure 11.

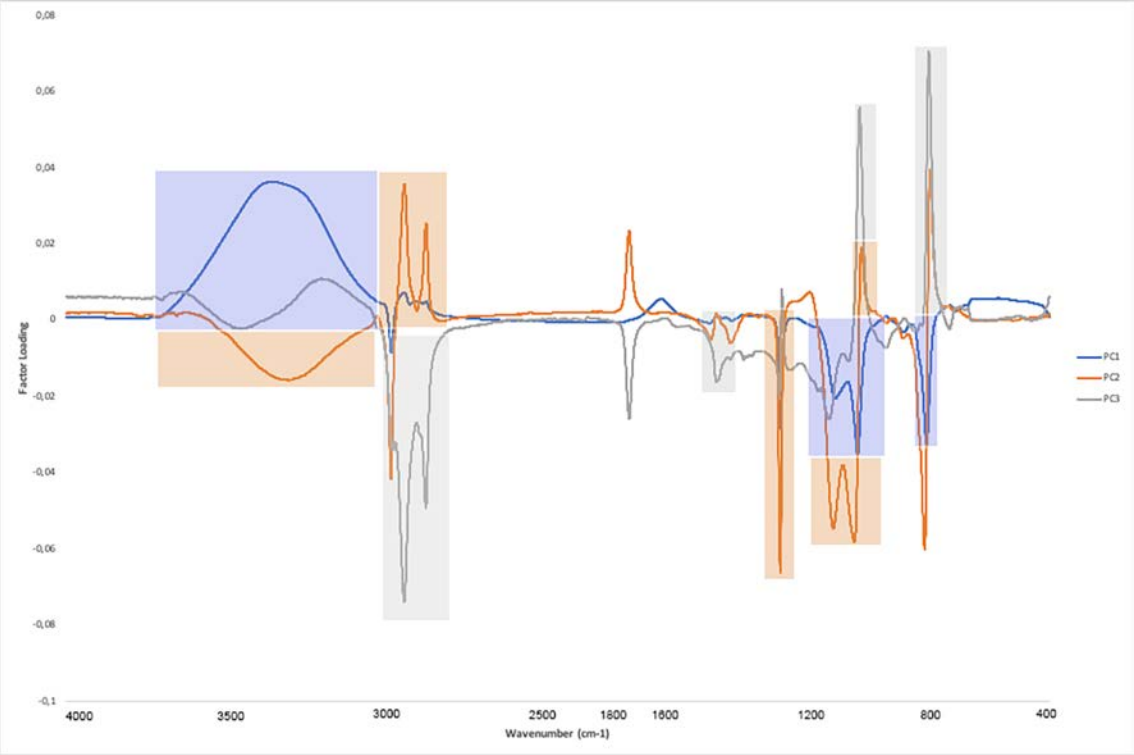


Figure 12: Factor loadings of PCs 1-3 for PCA conducted on the entire dataset, based on their ATR-FTIR spectra

The same correlations appeared along PC2 (Figure 12), suggesting that variations were detected within the silicone content of the different samples. Several other peaks were found to contribute to separation along PC2. A peak at 1000 cm^{-1} was linked to glycerine, while those at 3700-3000 cm^{-1} (O-H stretching), 2920 cm^{-1} (CH_2 asymmetric stretching), and 2850 cm^{-1} (CH_2 symmetric stretching) could be attributed to PEG, glycerine and other water-based components. This explains why water-based containing samples are all within the same interval along PC2 in Figure 13. The peak at 1740 cm^{-1} (C=O stretching) could not be attributed to any of the major compounds or other water-based components, suggesting it could be coming from an oily compound in some of the oil-based samples, as this vibration is usually associated with a carboxylic acid. This explains the strong variability of the water-based and oily samples. Finally, along PC3, peaks at 1254 cm^{-1} (CH_2 twisting) and around 1360 and 1460 cm^{-1} attributed to CH_2 wagging and CH_2 scissoring, allowed the discrimination of the PEG and glycerine containing samples on Figure 11.

As shown by the loading plots (Figure 12) and the PCA scores plot, excessive leverage on the overall model was obtained due to silicone or non-silicone content, potentially reducing discrimination between the rest of the samples. Therefore, other possible clusters might be hidden, dominated by this major grouping. To ensure proper detection of further clusters and be able to understand the diversity within a given population, it was deemed more suitable to generate a first separation based on the presence or absence of silicones (Figure 13A) and then replot each subpopulation separately. Results are shown in Figure 13B1 for silicone-containing chemical profiles, and Figure 13B2 for water-based chemical profiles.

The silicone-based cluster was found to incorporate three classes of samples, including condoms, lubricants and personal hygiene products. This observation highlights that silicones can be used in various other products that might be found in the vaginal matrix. Despite the improved separation of the silicone-containing samples, some overlap was observed between lubricants and condoms with a non-differentiated silicone chemical profile (Figure 13B1). The present observations confirm previous assessments on the separation of condom lubricant types (see **Chapter 2**) as most of the condoms used in this study were found to share the same qualitative profile, i.e. silicone based. Although lubricants were found to mainly contain water-based compounds, 18% (5 of 27 samples) of the lubricant batch (*Astroglide Waterproof Silicone Liquid, Ansell Skyn Maximum performance, Ansell Lifestyles Luxe Silicone Based Lubricant, Astroglide Diamond Silicone Gel Personal Lubricant, Durex Play Perfect Glide*) presented a silicone-based profile that was clustered with the condom profiles. This was expected as these products specifically indicated the presence of silicone in their composition. Coming back to the context of the *R. v. Andrew Nicholas Malkinson* case [22,24], this suggest that the silicone profile could originate from at least 2 different classes, and condom class would not be the only possibility for the present profile.

Except for two of the silicone-based lubricants (i.e. *Durex Perfect Play Glide*, (Composition: dimethicone) and *Astroglide Diamond Silicone Gel Personal Lubricant,* (Composition: dimethicone, cyclomethicone, dimethicone/vinyl dimethicone crosspolymer, coconut oil)), whose replicates could not be entirely separated from the condom population nor distinguished based on their chemical profile (Figure 14), intimate products and lubricants were found to cluster separately from condoms when focusing only on the silicone content (Figure 13B1).

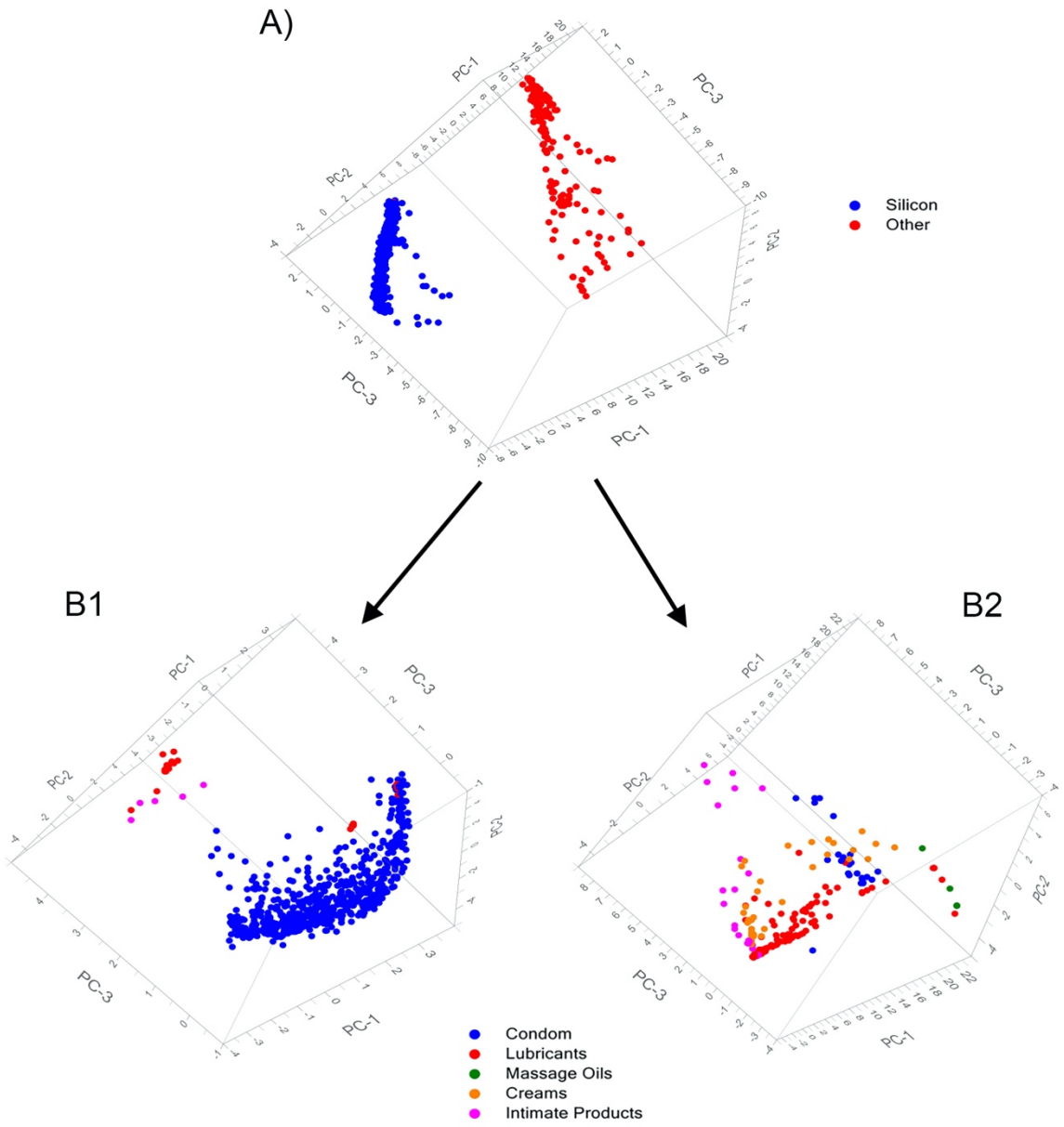


Figure 13: 3-dimensional PCA scores plot showing the distribution of the samples A) according to the chemical profile (i.e. silicone vs other), B1) within the silicone-containing cluster, B2) within the water-based containing cluster.

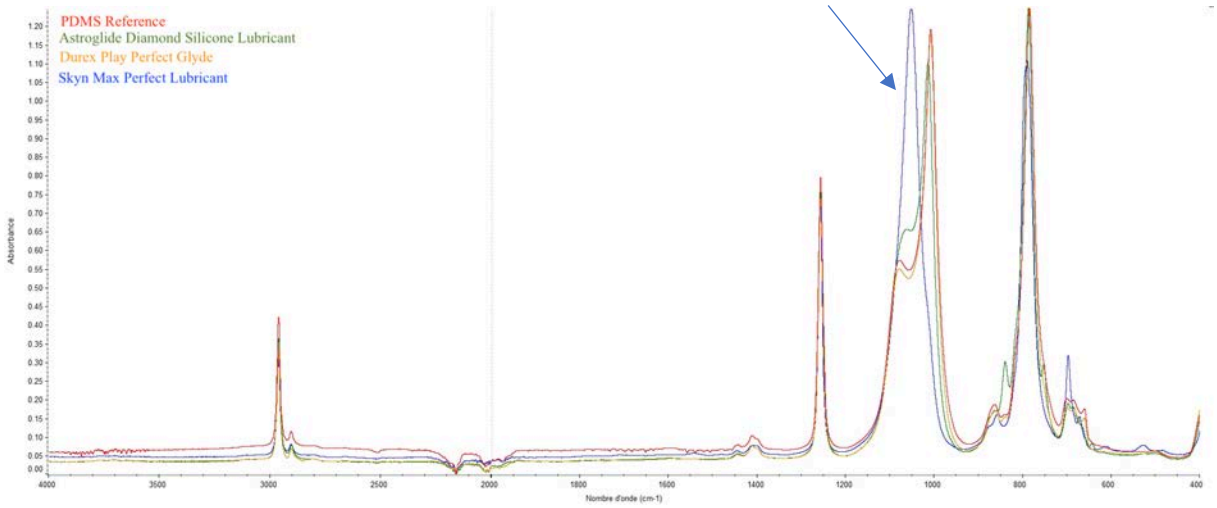


Figure 14 : ATR-FTIR spectra of PDMS standard (red), statistically non distinguishable lubricants Durex Perfect Play Glide (orange) and Astroglide Diamond Silicone Gel Personal Lubricant (green), and statistically distinguishable lubricant Skyn Max Perfect Lubricant (blue) for comparison. The difference resides in the shape and number of peak observed for the Si-O vibration (blue arrow).

Replicates of the *FairSquared Sensitive Dry* condom, which was found to be a strongly powdered and poorly lubricated condom were found to be clustered within the silicone population, increasing slightly the variability of the population. This highlights that silicone is present, but in smaller relative amounts than for other condoms, and the spectra were very noisy. This suggest that even though condoms are not obviously lubricated, there is always a small amount of lubricant applied on the latex. Therefore, lubricants may not only be present for sexual penetration, as mentioned in, but also to prevent the latex from sticking to itself.

It was also observed that 3.9% of the whole condom batch (5 of 126 samples) presented a significantly different chemical profile that clustered with water-based samples (Figure 13B2). These condoms (*Ansell- Lifestyles Party Variety Warm/Cool*, *Ansell- Lifestyles Party Mix Warm Smooth*, *Manix OrgazMax Plus*, *Manix Endurance* and *Ceylor Gold*) represented 'warming' condoms from a single supplier (Manix being manufactured by Ansell) and potentially contained nonoxynol-9 (a spermicide) or other water-based additives, explaining their similarity to other water-based products. The other lubricants clustering with those special condom types were not warming lubricants.

Replicates from *Ceylor Gold* sample were projected separately from the rest, which was expected as this condom contains nonoxynol (see **Chapter 6**), which may be a significant factor in the discrimination of this sample. Ceylor Gold presented a pattern different from the other silicone-based condoms (Figure 15), as it showed traces of peaks consistent with PDMS. This lubricant is expected to be an OH-PDMS, as presented in [118], given the presence of water-based components (i.e. PEG, nonoxynol). The OH-PDMS are however not distinguishable from

CH_3-terminated PDMS. No nonoxynol-9 standard was run to confirm its presence in the spectra.

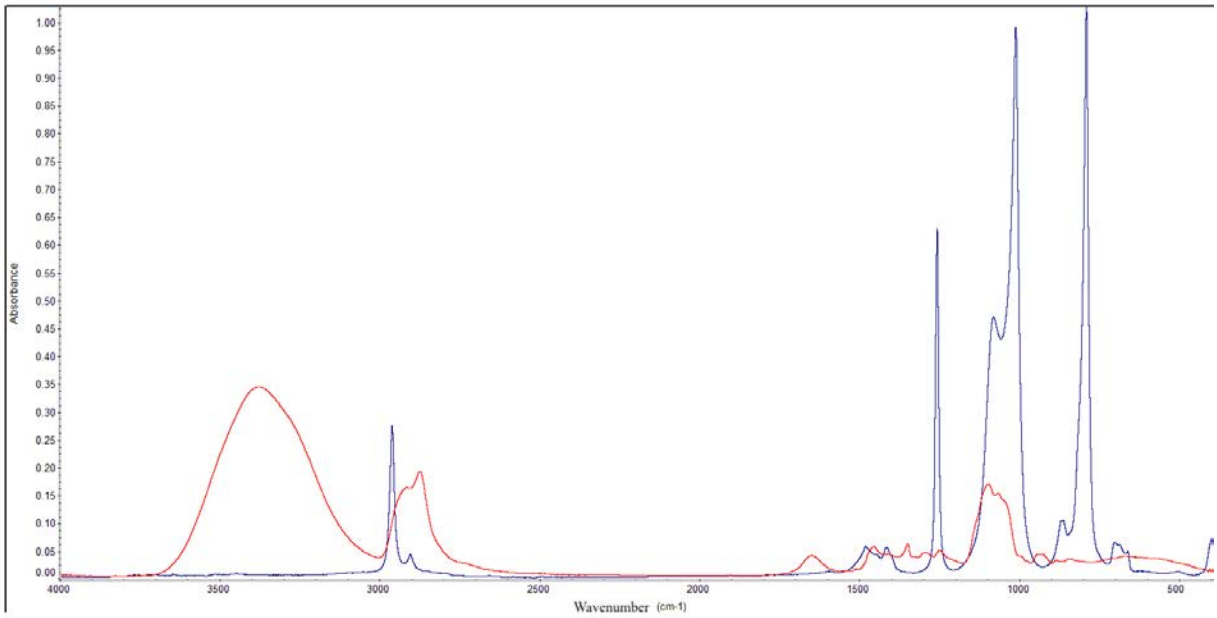


Figure 15: ATR-FTIR spectra of Ceylor Gold (silicone lubricated condom with water-based components) sample (in red), and a purely silicone-lubricated sample (in blue) for comparison.

Regarding the largest cluster of non-silicone-based products identified in Figure 13B2, the separation could not be fully assessed and significant overlap of chemical profiles from the different sample types were found. These observations suggest that manufacturers use the same types of components, or components presenting same chemical characteristics. Discrimination between these samples was found to be possible with more complex techniques such as DART-TOF-MS as reported by [16,17,44].

Massage oils and personal hygiene products were found to present different chemical profiles (Figure 13B1 and Figure 13B2), clearly separated from the other samples. It was interesting to note that a cluster was observed with other water-based lubricants (dotted circle on Figure 13B1, and 13B2). It appeared that the lubricant in question was named as a Personal Lubricant and Massage Oil. It can be therefore assessed that massage oils, named as such, present a separate and specific profile, which can be separated from other types of samples.

65% of the creams (4/6) reported silicone in their composition but were not found to cluster close to silicone-based condoms. This may be explained by the complete composition being a mixture of glycerine and silicone-based components, with a predominance of glycerine, water, wax or paraffin wax leading to a specific profile. The two samples reporting no silicone in their composition were found to cluster very closely to the main lubricants class. This observation was expected, as the discrimination of the samples generated in the PCA was mainly dictated by the silicone composition.

Finally, concerning personal hygiene products, classified in Figure 13 as intimate products, a highly significant dispersion was observed, although most of the profiles were clustered close together and close to cream and lubricant products. One of the samples was found to be silicone-based, and the initial composition of the product stated dimethicone as the most important component of the product, dimethicone being another denomination for polydimethylsiloxane. Such differentiation is therefore not surprising. The dispersion on the water-based side of the clusters was due to only one sample (*FemFresh Daily Intimate Wash*), whose profile was found to be very highly variable.

4.2.3 Market structure

The aim of studying the structure of the market is to determine whether the clusters observed can be associated with particular characteristics of the samples. This is useful to generate investigative leads from a questioned sample by predicting its origin.

Some brands, such as *Durex*, were found to be present in all the studied markets, as well as in previous studies [9,10,17,41,42,44]. However, the presence of similar brands within different marketplaces does not mean they are part of the same distribution network. It also appeared that some condom types were linked to different brands in the different countries although being from a same generic company. For example, in the Australian and New Zealand markets, *Skyn* condoms were attributed to Ansell company, whereas in the Swiss market these products are sold under the *Manix* label (*Manix* being part of the Ansell group since 1995; http://www.manix.net). All the samples from *Manix* or *Skyn* brands were therefore assessed to Ansell company, independently of their country of origin. It was also found that some brands were exclusively to a single country's market (e.g. *Migros* or *Coop* are specifically Swiss brands). Statistics run on the condom profile obtained using ATR-FTIR exclusively revealed no major compositional differences between the different markets (Figure 16).

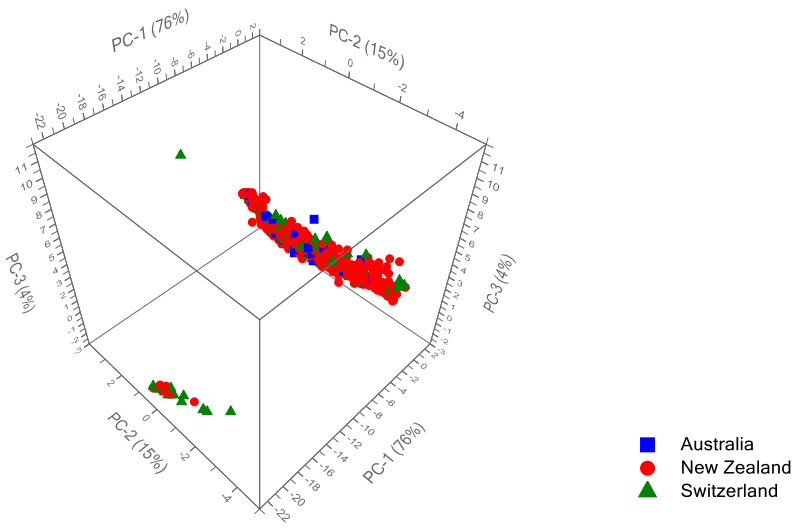


Figure 16 : Illustration of the score plots obtained on condom samples, as a function of the purchasing localisation. The cluster along PC1 is due to the water-based content of the condom samples.

These observations are not surprising given the international regulations [5,30–32]. Consequently, although products can be distributed in different countries, the same chemicals are used at the production level. Therefore, between and within brands discrimination should be investigated to understand whether products coming from different brands can share the same qualitative profile as well as whether different brands use the same type of chemicals for the same type of sample.

Previous research highlighted that most of condoms from different brands could be classified as coming from a same lubricant type, i.e. 200 cSt viscosity PDMS for silicone-based lubricants [9]. In the forensic context, the source of the recovered evidence is usually questioned, and specific source is targeted. In the case of condom evidence, one of the possible scenario is that a specific source may refer to the brand or a model of condom that could potentially be recovered, either from the crime scene or a suspect. Variation between different brands was investigated using a smaller dataset containing only silicone-based condoms, but no evident cluster resulted from the statistical investigation (Figure 17). This observation highlights that products from different brands present the same qualitative profile and that different brands do apparently use the same type of chemicals for the same type of samples. These findings are in total agreement with a study conducted in 1995 [9] assessing that most of the condoms present on the market, when lubricated, present the same type of lubricant, with chemical properties being too similar to allow visual or statistical differentiation.

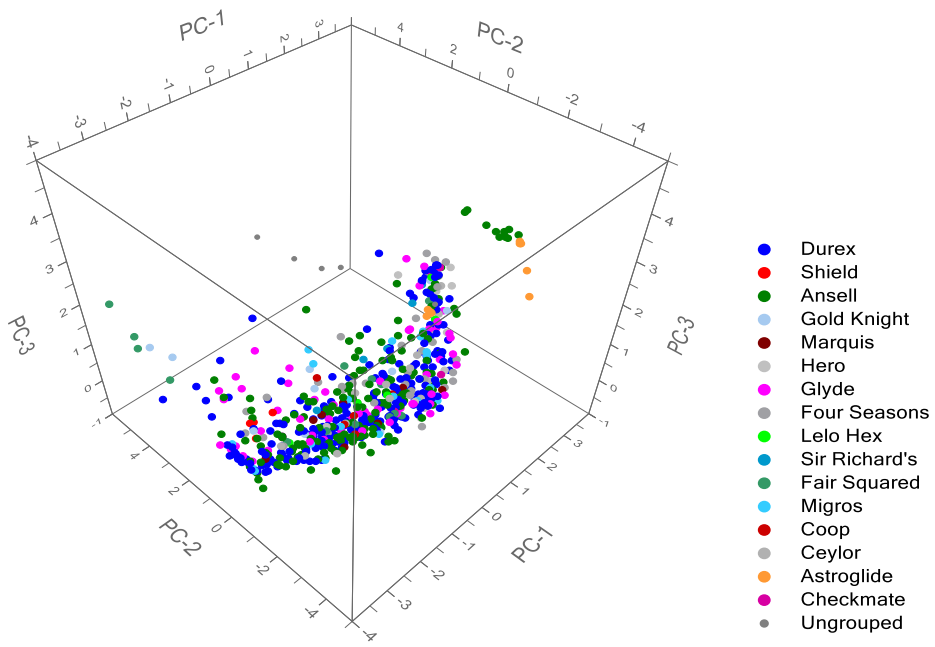


Figure 17 : Separation of silicone-based condoms according to their brands

The absence of clusters in Figure 17 appears to confirm the hypothesis that that all brands use the same lubricant type and apply it on all their products. However, it was thought possible that different chemical profiles could be obtained from classic and flavoured condoms, generating two different statistical clusters. Figure 18 illustrates the unsupervised classification obtained for condom samples from *Durex* brands, labelled according to their model type, with no evident clustering. Examination of the IR spectra showed a significant consistency across all the chemical profiles, with no evident differences in the chemical patterns.

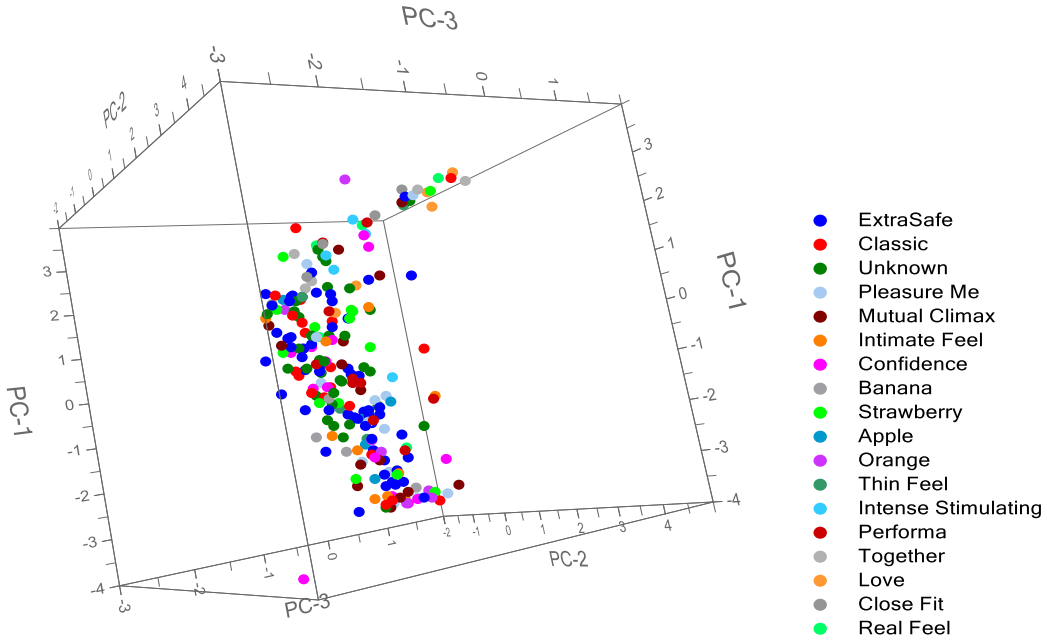


Figure 18: Condom samples from Durex brand, classified according to the different models

4.3 Classification model and prediction procedure

4.3.1 Enhancing sample discrimination

As discrimination of the samples was observed, classification is now needed to evaluate the accuracy of the model when predicting the origin of an unknown chemical profile, as might be obtained from a case work swab (although other parameters need to be considered). The first question was to evaluate whether the sample discrimination presented in section 4.2 could be improved to enable more precise prediction of external samples and real samples. Therefore, 12 different pre-processing methods were applied to the whole dataset and were visually compared using PCA to see which combination allowed the best discrimination of the samples (Table 5) reports the explained variance within the whole dataset. Most of the pre-processing methods enabled over 80% of the variance of the data to be explained by the first 3 PCs, which was found to be quite good.

Table 5: Explained variance for the 13 different data preprocessing methods applied on the whole dataset.

	Percentage of explained variance			
Pre-processing	**PC1**	**PC2**	**PC3**	**Total**
Raw data	90	3	3	96
SNV	84	6	5	95
MSC	91	8	0	99
Der1	63	19	5	87
Der2	46	17	12	75
SNV + Der1	61	21	6	88
Der1 + SNV	59	13	8	80
SNV + Der2	45	19	10	74
Der2 + SNV	53	13	6	72
MSC + Der1	95	2	1	98
Der1 + MSC	82	14	2	98
MSC + Der2	78	10	3	91
Der2 + MSC	95	4	0	99

Second derivative preprocessing significantly affected the explained variance. It must be remembered that second derivative is employed to correct a linear baseline, by eliminating the constant linked to the slope present in the first derivative function. The data were already baseline corrected, which could be the source of the lower level of explained variance. However, as previously mentioned tests on pure raw data (i.e. truncated but not baseline corrected, nor range normalized) did not produce any better results. Although the use of MSC was found to significantly improve the percentage of explained variance when PCA was performed, the 3D scores plot for these preprocessing didn't show improve separation of the samples compared to the raw data (Figure 19). First derivative allowed the best separation of the silicone-based samples but did not adequately separate the rest of the samples. However, coupling first derivative to SNV avoided a complete cluster of the non-silicone-based samples, and overall good separation of the samples was achieved, especially along PC1, which was a significant improvement compared to the raw data. None of the other preprocessing methods tested enabled improved separation of the different samples.

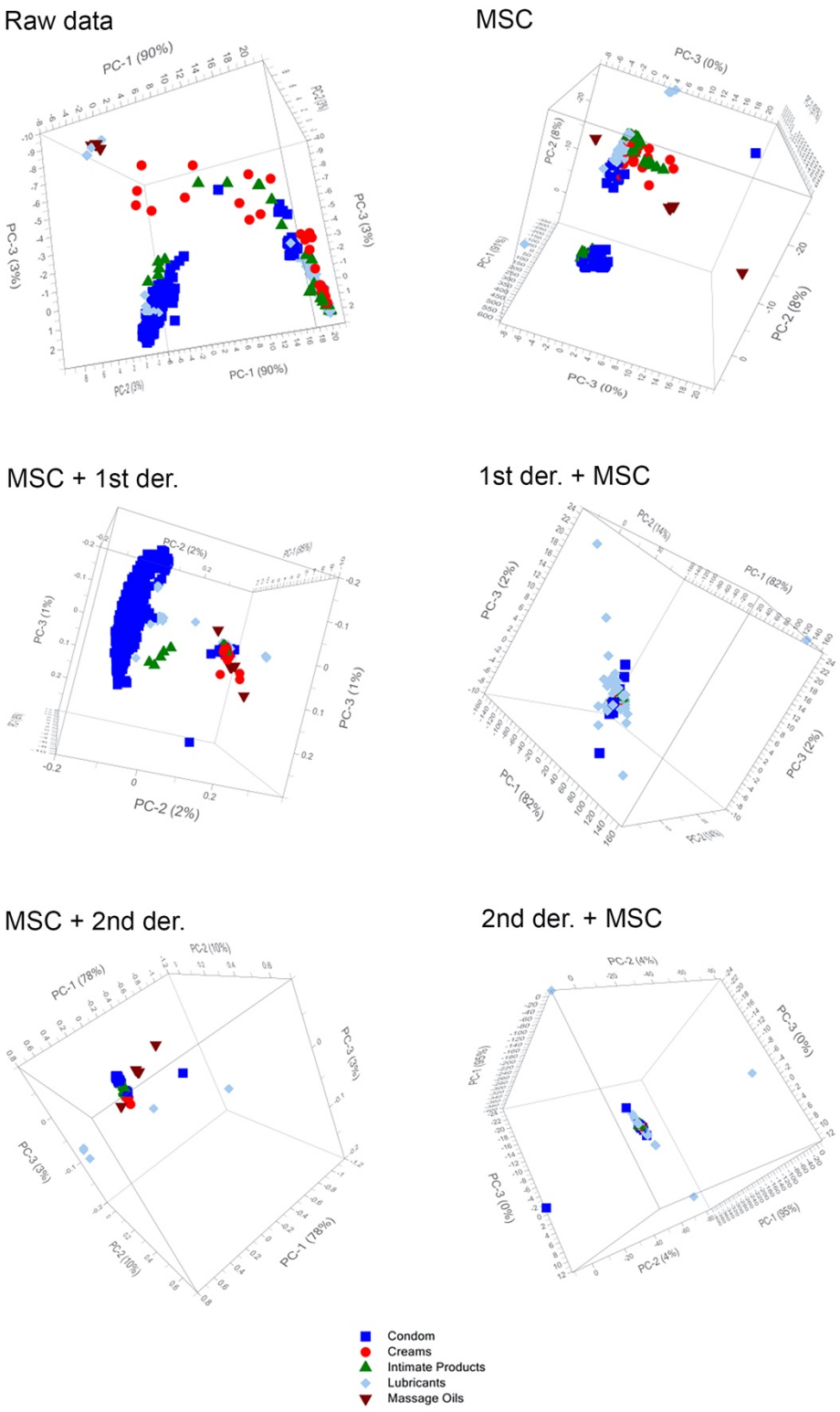


Figure 19 : Comparison of the cluster of the raw data and MSC corrected data

4.3.2 Classification model - Creation

Five different discrimination models (LDA, QDA, and SVM with three different algorithms: linear, 3rd degree polynomic and radial basis function) were constructed based on the sample class. SVM analysis with a radial basis function kernel (RBF), γ (kernel parameter) set at 0.01 and C (soft margin parameter) set at 0.001 was found to present the highest discrimination (calibration) accuracy (92.3%) and predictive (validation) accuracy (92.05%), as well as the best classification regarding sample content. Details of the different creation models are gathered in Appendix IX. The model was constructed on the entire dataset using the training set containing 2/3 of the dataset, and then the validation set (1/3 of the dataset) was predicted to provide a more realistic estimate of model performance. Only 3 samples out of 101 were misclassified: 2 false negatives (6 % rate) and 1 false positive (13.3% rate).

Regarding false positives and false negatives, a false positive indicates that a profile from a personal hygiene product has been associated with a condom profile. In contrast, a false negative indicates that a profile from a condom has been associated with a profile from another intimate product. The interpretation of these two results in a real case is important. Observations of the classification quality showed that samples are easily classified according to their chemical profile, but that the identification of the membership of a sample class is difficult, has a high error rate and is certainly related to the initial classification by chemical profile. This suggests that classification should be undertaken in a two-step approach to limit errors:

1. Identify the sample category according to the chemical profile of silicone or non-silicone type (Figure 13A). This step aims to determine whether silicones are present in the sample.
2. Identify the class of origin separately within each population, i.e. within the silicone profile population or the other profile population (Figure 13B1, Figure 13B2)

Discriminant models were built using the two-step approach to classify the samples, with SVM again found to give the most accurate classification. This discriminant model gave 100 % accuracies for both the calibration and validation sets. The model was then used to predict external test sets, including both samples present in the model and samples not yet represented in the model, analysed using different instruments and analysts. Finally, a blind validation test was carried out to validate and evaluate the robustness of the developed approach. From a forensic point of view, blind validations are mandatory before considering any application to casework, as it is representative of the simulation of a real scenario, where the source and parameters of the specimen are unknown. Also, it can minimise potential confirmation bias from a classic validation procedure.

4.3.4 Classification model - Validation

The models were then tested on three external sample batches: a *known-matching samples* set, which was acquired in New Zealand on a different instrument, a *known non-matching samples* set, which was acquired in Switzerland on a different instrument, and a blind validation performed on random samples, unknown to the experimenter. Samples were subjected to the same data pre-processing as the samples used to build the model and were then projected and classified using each model. Therefore, performance on an external sample set is crucial to assessing a proper discrimination model. Therefore, the different models were evaluated using external sample sets and performances were calculated.

Known-Match Samples

The known-match samples validation consists of projecting in the model samples that are known to be in the model. In this study, two sets of known-match samples were used. The first set consisted in 101 unique analysis of 101 products analysed in New Zealand, with the same analytical conditions than the samples present in the model, i.e., 32 scans at a resolution of 4 cm^{-1}. The second set was made of 10 individual spectra acquired *in situ*. These samples were designed to be representative of a potential real sample. To proceed, cotton swabs were individually rubbed on 10 different condoms and were then stored in the same manner as DNA case work samples, i.e., left to dry 1 hour then sealed and stored in the freezer until analysis. For the analysis, the cotton swab was squashed on the ATR crystal, creating strong contact between the sample and the crystal. Live view was activated and pressure on the sample was adjusted to get rid of the noise and obtain a profile.

Regarding the classification according to the type of chemical profile (silicone or non-silicone), 100% of the samples were correctly classified in the model, in agreement with the operator observation. Thus, the model represents the reality of the samples and is adequate for the prediction of real samples.

With regard to the classification according to the original class of the samples, the results show, on the sample population analysed directly on the crystal, that 2 condom samples were classified as "other" type. Additionally, one "other" type sample was been classified as a condom. Thus, this confirms the false positives and false negatives rates obtained within the classification model, which are 6% false negatives and 13.3% false positives respectively. These values are very high for the forensic area, especially considering that usual acceptation

error rate would be within 1 to 5 %, depending on the analytes and the question. In this case, these values are not acceptable.

Known Non-Match Samples

The known-non-match samples validation consists in projecting in the model samples whose origin is known but that were not used to build the model. Here again, two sets of known-non-match samples were used. The first set consisted in 6 products analysed in New Zealand and 11 products analysed in Switzerland, with the same analytical conditions than the samples present in the model, i.e. 32 scans at a resolution of 4 cm^{-1}. The second set was made of 4 individual spectra acquired *in situ*. These samples were designed to be representative of a potential real sample.

Regarding the classification according to the type of chemical profile (silicone or non-silicone) 100% of the samples were correctly classified in the model and were in agreement with operator observations. Thus, the model represents the reality of the samples and is adequate for the prediction of real samples. Results of the classification according to the original class of the samples showed that 1 sample of *a priori* lubricant type was classified as condom. The chemical profile observed for this profile was a silicone-type profile. As previously highlighted with the known-match samples, lubricants or other type of evidence containing silicones are very likely to be classified as condoms, as the vast majority of condoms contained silicones. Some very noisy spectra were not attributed clearly to any of the classes, suggesting that a variability within the spectra can be induced, due to different instrumentation or different operators.

Blind Validation

The ability to associate an unknown sample with the correct sample type validates this method and model, as it has replicated a situation where the analyser is unaware of the class and is void of inherent bias towards association. This method of validation addresses the prevalent issue of bias and subjectivity in the analysis of results. 195 spectra were collected for the blind validation and were labelled with the operator name followed by the number of the sample (e.g. SWL01.SPA). All the spectra were first opened in Omnic® and processed through ATR correction. Spectra were then extracted and uploaded in The Unscrambler X and processed performed as described for the data used to build the model.

While the blind validation was successful, there were detectable differences at the time of the projection of the samples in the model. These differences could be associated either the operator or the analytical conditions on the analysis. Indeed, when observing the patterns coming out of

the sample projection, silicone-based clusters obtained when building the model and when projecting the samples were slightly separated from each other, although they were clustered together. This highlights a certain lack of robustness which was not further investigated in this study. Observation of the spectra highlighted differences between the baselines, which were not flat on the predicted sample. This could be the reason behind such a variation. However, more examples of other operators should be investigated to determine the significance of this hypothesis. The results of the blind sample prediction highlighted that all the replicates were correctly associated to their sample class, using the two-step approach suggested in this chapter. Considering that a positive result is a silicone profile classified as a condom, the error related to this evidence is lower than 2.5%, which is the error rate obtained on the overall prediction set. This demonstrates the reproducibility of the technique of analysing condom and lubricants by ATR-FTIR and chemometrics in a real-world forensic context.

Highlights

ATR-FTIR was used for the market study, with the following observations: . The main results are the following:

- At least 4 main groups could be visually observed amongst the 166 samples analysed
- Up to 10 populations could be distinguished when considering presence and absence of peaks, as well as peak position and shape
- Discrimination of the samples is led by the silicone, water and oil content of the samples
- No differences were observed between the profiles found on international market
- No differences were observed between different silicone-based condom brands, nor between different models coming from a same brand
- When considering the identification of the origin of a silicone chemical profile on a one step model, false positive were found to be 13.3% and false negative 6%, which are too high for forensic use.
- Using a two-step approach for classification, 100% good classification rate was obtained to separate samples according to their content (silicone or water based) and their class (condom or lubricant).
- No silicone based personal lubricant were statistically classified in the condom category, although visual examination would have clustered it as a condom.

Chapter 5: Py-GC/MS Study of Silicone-Based Products

This chapter is based on the following articles (Appendix I) :

Article 3: Maurer J, Buffaz K, Massonnet G, Roussel C, Burnier C (2020) *Optimization of a py-GC/MS method for silicone-based lubricants analysis*, Journal of Analytical and Applied Pyrolysis

Book Chapter: Challinor J. M., DeTata D. A., Pitts K. M., Burnier C., *Chapter 8: Examination of Forensic Evidence*, Handbook of Applied Pyrolysis, Third Edition, *in press*

Article 7: Burnier C., Massonnet G, Coulson S, DeTata D, Pitts K, (2021) *Characterization and classification of silicone-based compounds in condoms and personal hygiene products using pyrolysis-GC/MS*, Forensic Science International

Article 11: Burnier C., Maurer J., DeTata D, Pitts K, (2020) *Outlining the importance of adequate analytical instrumentation: an example of pyrolysis-GC/MS*, Manuscript under revision by the coauthors

Forensic analysis of condom lubricants and their discrimination is usually led in the litterature using MALDI-MS or DART-MS as previously highlighted in **Chapter 2**. These are the only three existing models for the discrimination of sample classes present in the literature. However, the two techniques have not been applied to diluted samples, case simulations or real cases.

In contrast to these two techniques, pyrolysis gas chromatography mass spectrometry (py-GC/MS) was applied in multiple studies for the detection of condom traces in simulated cases, as well as to study the persistence in different matrices [15, 18, 50]. This is a proven technique, along with Fourier transform infrared (FTIR) spectroscopy, more specifically with DRIFTS-FTIR [41], and has demonstrated potential applicability to extracted traces and real cases [15]. Given the potential offered by py-GC-MS, it is relevant to investigate its discriminatory power, in order to determine if the technique is able to differentiate samples from different classes that have indistinguishable profiles by infrared spectroscopy. Indeed, as demonstrated by [41,42], FTIR spectroscopy is successful at identifying silicone and non-silicone-based samples, which in casework would be important to know, in order to use the most relevant method for the analysis of the evidence. Therefore, in casework, the analytical sequence should be constituted of FTIR analysis prior to any chromatographic or mass spectrometry technique [41]

The advantage of using py-GC/MS is that large and non-volatile polysiloxanes, which cannot reasonably be analysed by GC/MS otherwise, can be analysed using this instrument. Silicone lubricants, such as PDMS, are the first target of this type of analysis, especially as

more than 95% of condoms found on the international market contain PDMS [9,11]. It has also been established that glycerol or PEG-type water-based lubricants are easily analysed using GC/MS without requiring pyrolysis [18]. Moreover, the pyrolysis of these water-based lubricants does not result in characteristic profiles, probably due to the decomposition to CO_2 and H_2O of the molecules.

During the pyrolysis, PDMS is degraded into cyclic oligomers of low molecular weight called DMS (dimethylsiloxanes) [18,119]. These DMS oligomers are usually labelled according to the number of silica atoms in them: the cyclic hexamer (IUPAC hexamethylcyclotrisiloxane) is called D3 and is the preferred conformation, as it is the most stable one [79]. Pyrolysis generates a range of cyclic oligomers of increasing molecular weight, based on their chain lengths. Their passage through the capillary column is therefore no longer a problem. Pyrolysis is therefore often used for the analysis of polysiloxanes [99,100,120,121]. It turns out that the viscosity of the siloxanes, given by the number of repeated [$Si(CH_2)$-O] units, affects the size of the area of the peaks in the pyrogram: the lower the viscosity, the lower the area [120]. The effect of the pyrolysis temperature is not negligible because the number of pyrolysis products varies if the temperature increases or decreases [98,121–123]. A fairly high temperature must be applied to obtain the degradation of PDMS [18,79], but not too high otherwise the degradation of PDMS is altered [99].

5.1 Optimization of analytical conditions

5.1.1 Samples and preparation

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 0.1mg/ml and 1 mg/ml. Quartz tubes for pyrolysis and glass wool both come from CDS Analytical (USA). A 5µl syringe eVol XR ® from SGE Analytical Science was used to deposit the samples into the quartz tubes.

5.1.2 Separation conditions

The GC was equipped with an Agilent HP5-MS column (30m x 0.25mm x 0.25µm). Data acquisition and processing was performed on Agilent's ChemStation® software. Initial analysis parameters were taken from the literature [15,18,41]. It has been shown in several cases [91,124] that the amount of sample deposited in the quartz tube has an impact on the chemical profile obtained. It was therefore mandatory to determine the most appropriate sample quantity

for the analyses. For a quartz tube containing glass wool (CDS Analytical Instrument) [91,98,123], the 3µL was the maximum possible sample limit to be deposited to reach the maximum retention capacity of the glass wool. For a stainless-steel cup (Frontier Lab Instrument), 10µl was found to be a more adequate amount of sample to obtain repeatable and stable pyrograms.

The concentration of the sample must also be investigated, in order to obtain repeatable and adequate profiles for the realization of the experimental plans. At first, a concentration of 0.1 mg/mL was used, and split was set at 1:100. This avoids saturation of the column. Three analyses were first performed but will not be presented here as several sample preparation problems were encountered. Instead, the method used a 1:100 split, but with a 2-minute solvent delay added to protect the detector. The analyses carried out with a concentration of 0.1 mg / mL show a peak of D3 very present while the other cycles D4-D7 are very small. Despite the modification of the analysis parameters, it was not possible to observe the oligomeric degradation of siloxanes up to at least D8 even when using splitless instead of split injection. The increase of the concentration by a factor of 10 made it possible to obtain very large abundances but also to ensure the presence of the oligomers D3-D8. Thus, a concentration of 1 mg / mL was used to carry out the experimental plans.

Regarding the analytical parameters, the initial method lasted 32 minutes. The pyrogram obtained showed that peaks eluted until about 20 minutes, with no additional compounds appearing in the last 12 minutes. It therefore seemed reasonable to modify the oven temperature program, so as to reduce the analysis time. Various parameters were changed, i.e. oven parameters, split/splitless injection as well as insertion of a solvent delay in the method. The final parameters used for subsequent experiments are therefore presented in Table 6 below.

Table 6 : Final GC/MS analysis parameters (Method: py-GCMS_CB_02.M)

Pyrolysis parameters	
Temperature of the filament	620°C
Time	20 sec
Ramp	20°C/ms
Interface Parameters	
Resting temperature (interface)	275°C
Initial temperature (interface)	275°C
Ramp	100°C/min
Final temperature	375°C for 1 minute
Valve oven	300°C, continuously
Transfer line	300°C continuously
GC parameters	
Injection	
Temperature of injector	280°C
Injection mode	Splitless
Separation parameters	
Gaz	Helium
Flow	1.0ml/min
Analysis time	32 minutes
Temperature program	50° C for 2 minutes
	10°C/min to 230°C
	20°C/min to300°C
	Keep 5 min at 300°C.
MS parameters	
Transfer line temperature	250°C
Ionisation source temperature	250°C
Quadrupole temperature	150°C
Acquisition mode	Scan
Range m/z	30-450 m/z

5.1.3 Pyrolysis conditions

The goal of optimization was to identify the parameters that influence the variability of results. This involved modelling the response surface, in order to identify the experimental conditions that produce the lowest variability. This surface is limited but unknown, despite the literature. It was therefore necessary to identify the appropriate response factor to measure the variability of the results. At first, the variability was measured qualitatively, with respect to the composition of the chemical profile obtained. Subsequently, a semi-quantitative evaluation was then proposed based on some of the compounds detected. Variability was measured using the total variance, standard deviations and coefficients of variation obtained from the relative abundances of the selected compounds.

Based on [91,93,98,125] it is known that four factors affect the response surface in the case of pyrolysis: the pyrolysis temperature, the pyrolysis time, the heating ramp and the type of sample. Three of these parameters are directly related to pyrolysis and can vary over a wide

range of times (1s - several minutes), temperatures (1-1400 ° C) and pyrolysis rates (0.01-20.00 ° C/ms). The ramp was set at its maximum level so that the pyrolysis temperature was reached as quickly as possible, thus giving rise to flash pyrolysis. The minimum temperature was accepted as that of the laboratory, i.e. 25 ° C. The type of sample used for optimization is important and depends on the purpose of the research. In the context of analysis of PDMS or lubricants from different sources, the optimized method should be applicable to as wide range as possible of the lubricants of the population of interest. It would therefore be consistent to work on two different types of condoms to optimize the conditions of pyrolysis. Campbell *et al*. (2007) showed that pyrograms obtained on 10 samples of different makes and models were all similar, and that PDMS could be detected in all cases. In the context of traces, the PDMS was also clearly identified [15]. The PDMS whose viscosity is most frequently found in condoms [9,11,34] will be used for the optimization of the pyrolysis parameters. Finally, an application of the optimised method to real samples will demonstrate whether the parameters are adequate for discrimination of the various samples.

5.1.3.1 Surface Response Screening

A first Full Factorial Design (FFD) experiment plan [102] was generated to observe the response surface. The chosen FFD plan used two replicas of each point and three replicas for the central point. Maxima and minima were 420 and 920°C for the temperature, and 10 and 30 secs for the time of pyrolysis. The central point was defined at 620 °C and 20 seconds as it was the temperature obtained in the literature. This plan made it possible to conduct a broad screening of the response surface.

The parameters of the transfer line and the interface were not changed during the experiments and were set at 300 °C and 275 °C respectively. Indeed, as previously shown by Gueissaz (2013) [91], too low temperature of these two instrument parts leads to recondensation of the analytes, and the entirety of the pyrolyzed compounds does not enter the GC. In a second step, eight additional analyses were added to the plan. The latter come from the desire to study the variability of the extreme points, namely the couples 420°C /10s, 920°C /30s and 620 °C/20s.

Variability of the pyrograms

All analyses performed under the same experimental conditions were first superimposed to assess their qualitative variability, both in terms of peak presence and number, before further analysis. The visual comparison of the analyses conducted at **420 °C** and 10 seconds and 30 seconds found 6 major peaks, with good abundances (i.e. above a threshold value of 3,000 AU,

which corresponds to approximately 3x the background noise). (Figure 20). These were the only peaks repeatedly present among all these analyses. The analyses also highlighted several non-repeatable peaks present only on certain pyrograms. As a result, these conditions were judged to be poor, and analyses at 420 °C were discontinued for further work.

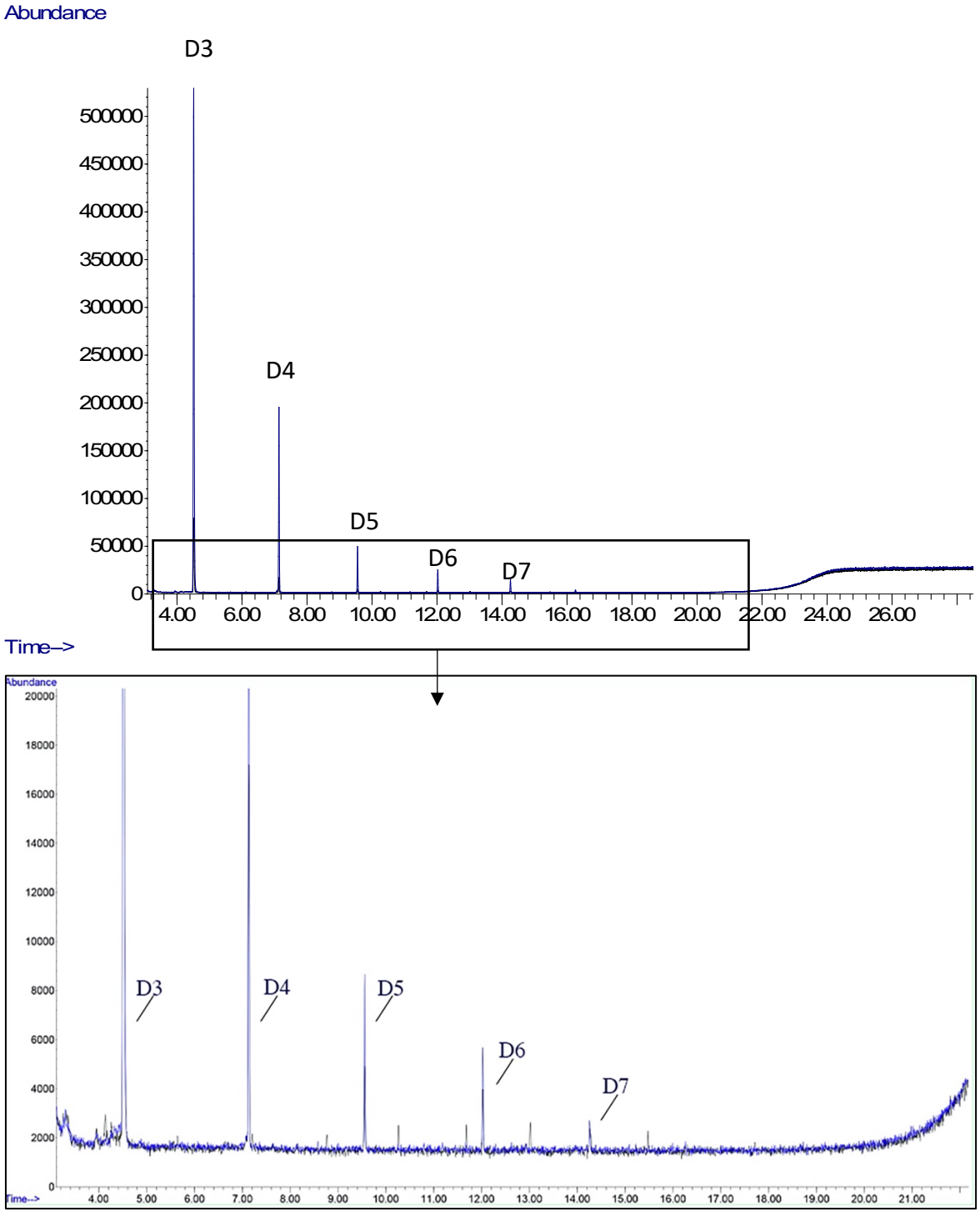


Figure 20: Illustration of pyrograms acquired at 420°C/10 sec (temperature/pyrolysis time), two replicates per design point are presented. Cyclic oligomers D3-D9 are indicated after identification using NIST database. *Reproduced from Maurer J, Buffaz K, Massonnet G, Roussel C, Burnier C (2020) Optimization of a py-GC/MS method for silicone-based lubricants analysis, Journal of Analytical and Applied Pyrolysis*

The visual comparison of the analyses carried out at **620** °C and 20 seconds still contain the 6 major peaks, corresponding to the D3-D8 oligomers. An enlargement of the abundance zone between 0 and 20,000 AU reveals about 10 other peaks, which separate well from noise and have good abundance (Figure 21). Overlay of the replicates for the 620 °C and 20 second analyses show that the pyrograms are repeatable in terms of the number of peaks observed and their retention time. On the other hand, the relative abundances sometimes seemed to vary between the different replicas.

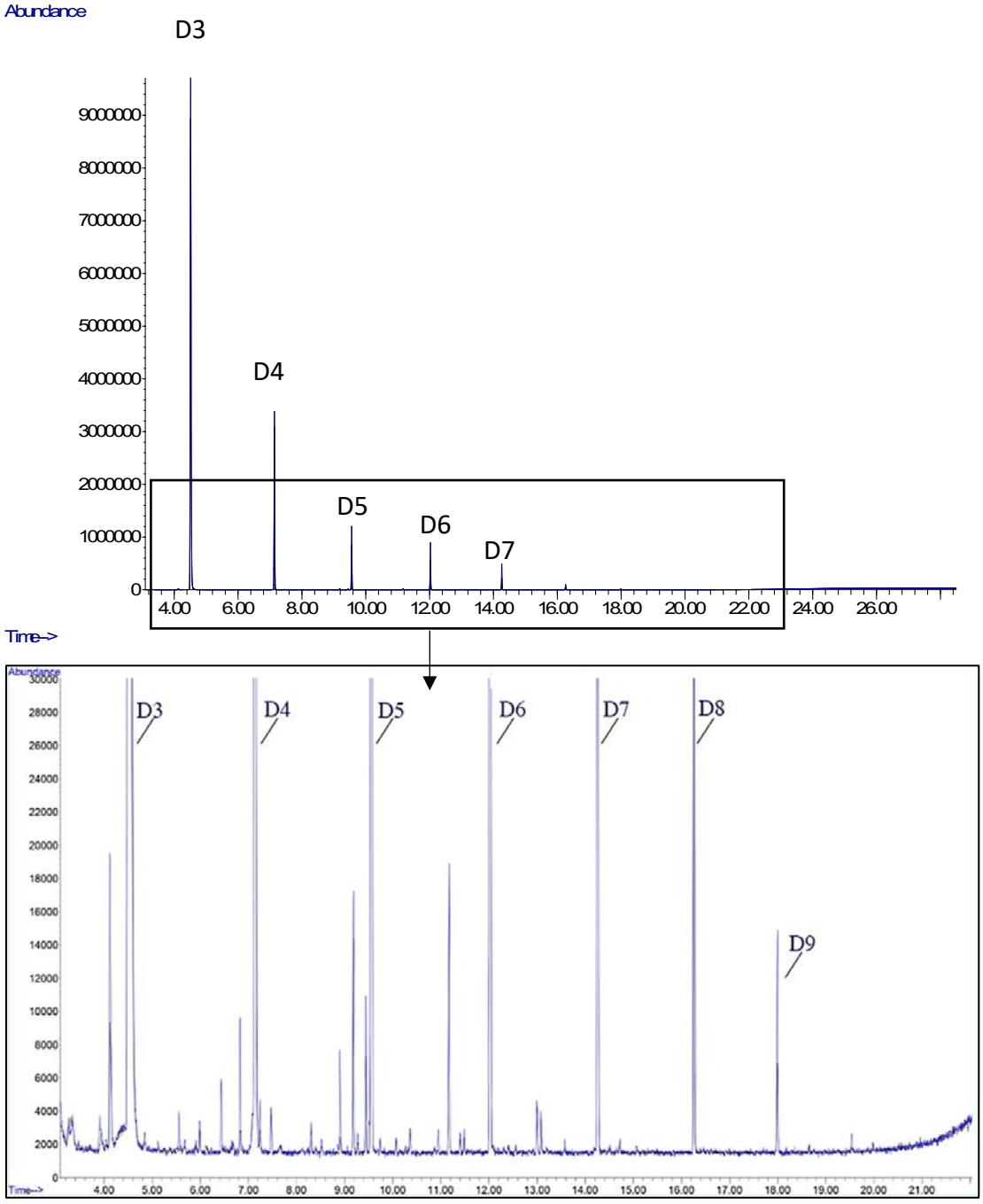


Figure 21: Illustration of pyrograms acquired at 620°C/20 sec (temperature/pyrolysis time), two replicates per design point are presented. Cyclic oligomers D3-D9 are indicated after identification using NIST database. *Reproduced from Maurer J, Buffaz K, Massonnet G, Roussel C, Burnier C (2020) Optimization of a py-GC/MS method for silicone-based lubricants analysis, Journal of Analytical and Applied Pyrolysis*

The pyrograms at **920** °C with a pyrolysis time of 10 seconds showed good repeatability in the number of peaks observed and their retention time. Many peaks were present, and their respective abundance was satisfactory, since for the most part it is above 30,000 AU (Figure 22). Some relative peak abundances seemed to be less repeatable between different analyses. The same findings were made for analyses with a pyrolysis time of 30 seconds. Extraction of the target ion area and mathematical observations may confirm these observations.

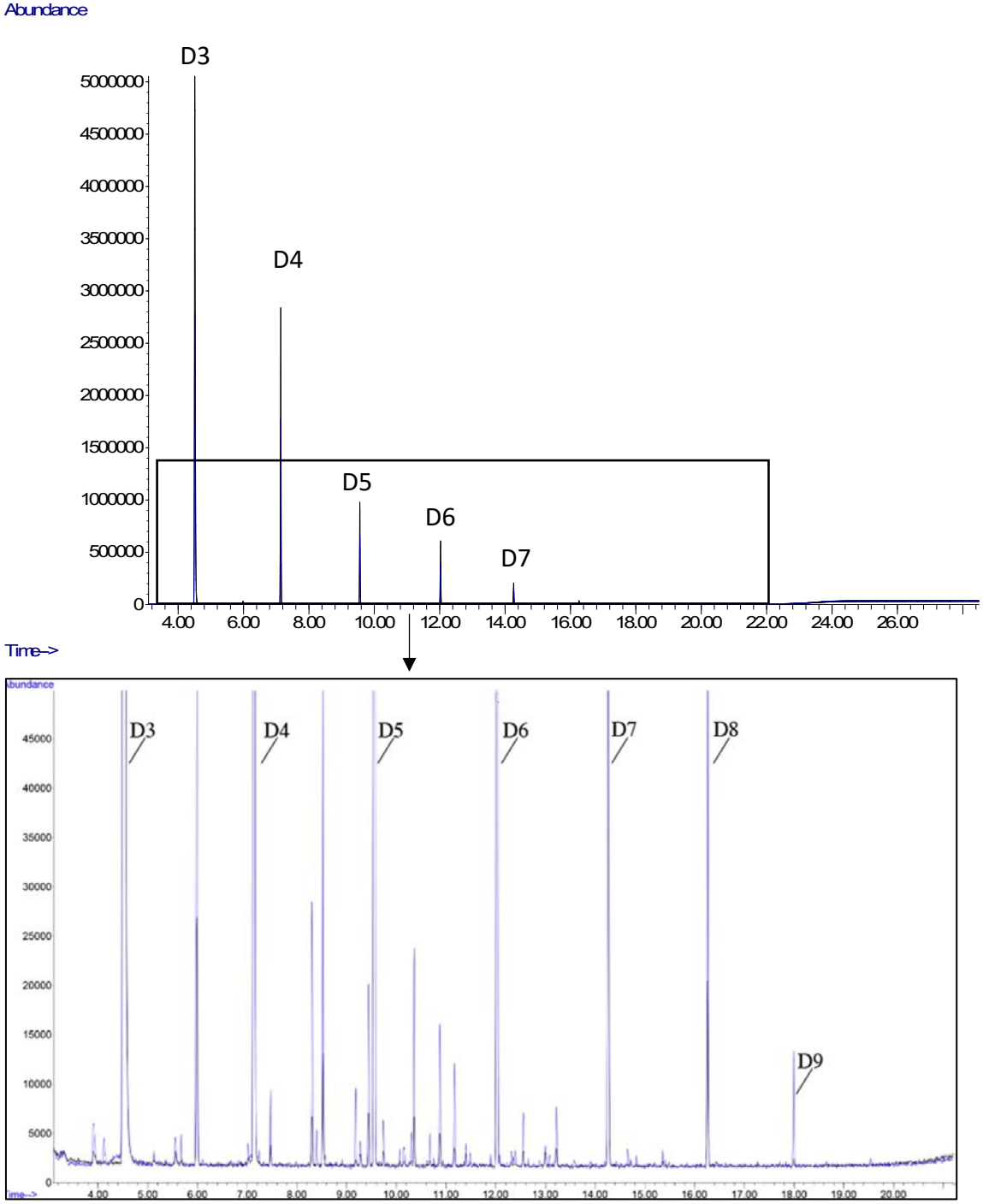


Figure 22: Illustration of pyrograms acquired at 920°C/20 sec (temperature/pyrolysis time), two replicates per design point are presented. Cyclic oligomers D3-D9 are indicated after identification using NIST database. *Reproduced from Maurer J, Buffaz K, Massonnet G, Roussel C, Burnier C (2020) Optimization of a py-GC/MS method for silicone-based lubricants analysis, Journal of Analytical and Applied Pyrolysis*

Semi-Quantitative variability

To study the variability of the relative abundances observed during the study of visual variability, only the major oligomers D3 to D8 were integrated into the data extraction macro. Their parameters are shown in Table 7.

Table 7 : Name, retention time, extraction ion for the selected compounds. *Reproduced from Maurer J, Buffaz K, Massonnet G, Roussel C, Burnier C (2020) Optimization of a py-GC/MS method for silicone-based lubricants analysis, Journal of Analytical and Applied Pyrolysis*

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

All the pyrograms were subjected to this macro, which was based firstly on the search for a definite time interval (RT ± 0.5 min) then on the search for the target and qualitative ions necessary to determine the presence of the compound and in integrate the area. This area therefore represents the abundance of the ion for a given compound at a given retention time.

Data were exported to an Excel® file. As indicated by Gueissaz (2013) [91], pre-treatment by normalization at the sum of the areas is a pre-treatment suitable for py-GC/MS analyses because the sample quantity can be variable between the depositions. This pre-treatment allows the quantitative comparison of the various analyses. Following normalisation of the data, other pre-treatments can be applied. Several pre-treatments were tested. Finally, the means and standard deviations for each compound were calculated on the basis of standardized and pre-processed data.

Figure 23 shows the distribution of the coefficients of variation obtained from the raw data. Almost all coefficients of variation were greater than a limit value of 10%. Figure 23A also shows a tendency for CV to increase as a function of the analysis time, which is particularly apparent at 620°C/20sec and 920°C/30sec. The points of the 420°C/10sec and 420°C/30sec plans have the highest coefficients of variation. Pre-treatment of the data is therefore necessary to correct these very high coefficients of variation. The first mandatory pre-treatment when considering py-GC/MS data is normalization to the sum of peak areas. This pre-treatment makes it possible to standardize the samples without the use of an internal standard, which is not possible in the case of these analyses. Figure 23B illustrates the effect of normalization on the sum. The dispersion of the coefficients of variation is homogeneous between the analyses of the various points of the plane and the coefficients were found to be, for a small number of

them, lower than 10%. The trend of increasing variation along the elution time was confirmed and the trend is very clearly visible for all analyses in Figure 23.

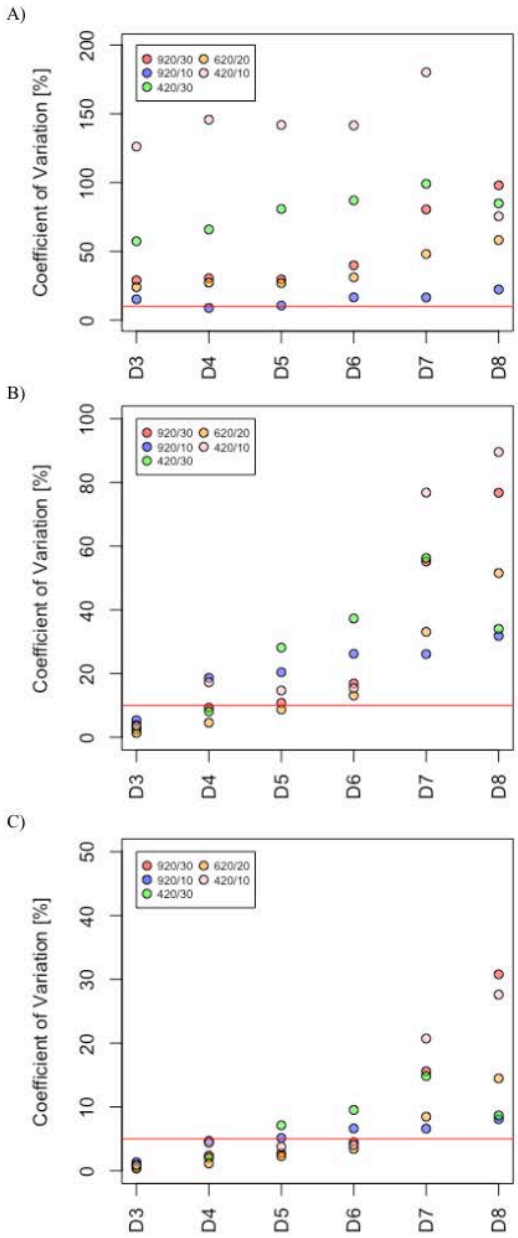


Figure 23 : Distribution of variation coefficient for all the experiments, A) raw data, B) areasum normalisation (red line = 10% limit), C) areasum normalisation followed by double square root. Red line is the 5% limit. D3 to D8 represent the cyclic oligomers from the PDMS degradation.

Several pre-treatments were tested, including logarithm, square root, and double square root. The latter was selected as the most suitable pre-treatment for the data because it has the smallest number of CV > 10%, as shown in Table 8.

Table 8 : Comparison of different preprocessing and number of coefficient of variation over the 10% limit for each point of the plan.

Pre-processing	Log	Square Root	Double Square Root
920/30	5	2	2
920/10	5	3	0
620/20	5	2	1
420/30	5	4	1
420/10	5	2	2

Figure 23C shows the distribution of the coefficients of variation after pre-treatment at the double square root. An increase in variation was observed with the elution time (D7 and D8 more variable than the other oligomers). The CV% variation limit was lowered from 10% to 5% because it is a generally accepted value for GC/MS analyses. This distribution has many values above the 5% limit, including compounds D7 and D8. The CVs of the compounds eluting towards the end of the pyrograms were on average higher than those at the beginning. The results for each point of the plan should be compared to obtain as much knowledge as possible and to adapt the target zone to investigate of the subsequent work. To estimate the total variability of the relative abundances of the compounds, the variances of each compound were calculated and summed (Table 14). The experiments carried out at 420 ° C gave usable pyrograms but the obtained pyrograms were not repeatable which led to these analysis parameters being abandoned. It can be seen from Table 9 that the central point causes about four times less variability than for the 920°C points, although the number of CVs greater than 5% is not significantly different. The distributions are all different, with a much lower dispersion of values for the point at 920°C and 10s. The central point has an undifferentiable median from those obtained at the other points. CVs were mostly below 3% for the central point, apart from oligomers D7 and D8, which remained above the limit of 5%. Compounds processed under these experimental conditions were judged to be repeatable in their relative abundance. The results obtained here indicate that the temperature influences the analysis results but also that an increase in the pyrolysis time generates an increase in the variability of the relative abundance of the compounds.

Table 9 : Comparison of the results of different points of the plan for D3-D8 oligomers.

Point of the plan	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

This first series of experiments made it possible to scan the response surface over a very wide area. A temperature of 420°C made it possible to obtain usable results in terms of cyclic oligomers characteristic of PDMS, but this was not repeatable for the rest of the qualitative analysis. This 420°C temperature is also present in the plateau zone of the temperature degradation curve presented by [99]. The various TG and DTG curves presented in this article [99] do not show an interesting peak around 420°C. It was therefore not judged to be a suitable temperature for analysis for either identification or discrimination purposes. The central point presented the most repeatable results in relation to the different points of the plan studied. The variance of this point was lower than that of the other points and most of the compounds exhibited an overall variation of less than 3%. The study of the variability of these analyses shows good repeatability. In addition, a temperature of 620°C recurrently appears in the literature as for the pyrolysis of PDMS from condoms. According to the TG and DTG curves by Camino [99,100] a temperature around 600-620°C would produce maximum degradation of PDMS. A slightly higher temperature would ensure the complete degradation of the PDMS by decreasing the total variance of the compounds. The points at 920°C showed that the temperature is a bit high for the sample type. The compounds are certainly repeatable, but the variance obtained for the various compounds is as important as that obtained for the points at 420°C. Variability increases during the analysis, and consequently the coefficients of variation increase with time. The point at 920°C and 30 seconds has the greatest variability but its variance is equal to that of the 920°C point and 10 seconds. However, the coefficients of variation are always greater than 5% for the 920°C point and 10 seconds, whereas the oligomers D3 to D5 have coefficients of variation of less than 5% for the 920°C point and 30 seconds.
The conclusions of these experiments are the same as those drawn by Gueissaz [91,98,123] which are that too low pyrolysis temperature results in usable but non-repeatable results and a significant overall variance whereas a too high pyrolysis temperature leads to an increase in the variability of the compounds produced. Similarly, a short pyrolysis time generates an increase in the overall variance of the compounds produced and a long pyrolysis time tends to decrease the variability of the compounds produced.

5.1.3.2 Effect calculation and surface response modelisation

The knowledge obtained during the first cycle of experiment made it possible to reduce the zone of experimentation regarding the temperature. The new temperature range was chosen within ± 100 ° C of the central point (520-620-720°C). The time variables were not modified but correspond to a variation of ± 10 seconds around the value of the central point. A new FFD-

type plan was therefore drawn with 7 experiments. The purpose of these experiments was to estimate the effects of the factors. The first experimental planning required several point analyses of the experimental design as well as the integration of several compound. Not all of them were found to be relevant in casework practice, and low concentration level samples might not present all the different compounds. Therefore, compound D3 was chosen as a new response factor since the increase in peak area of this compound was related to a decrease in the coefficients of variation. Although the interaction model obtained under these experimental conditions was very good, with over 90% of the variance covered, the response surface obtained never reached its extremums (Figure 24). In addition, the adjustment for the previous models is absolutely not adequate and therefore does not model the response surface well. Thus, it was necessary to modify the plan by increasing the temperatures including the central point and extreme temperature points.

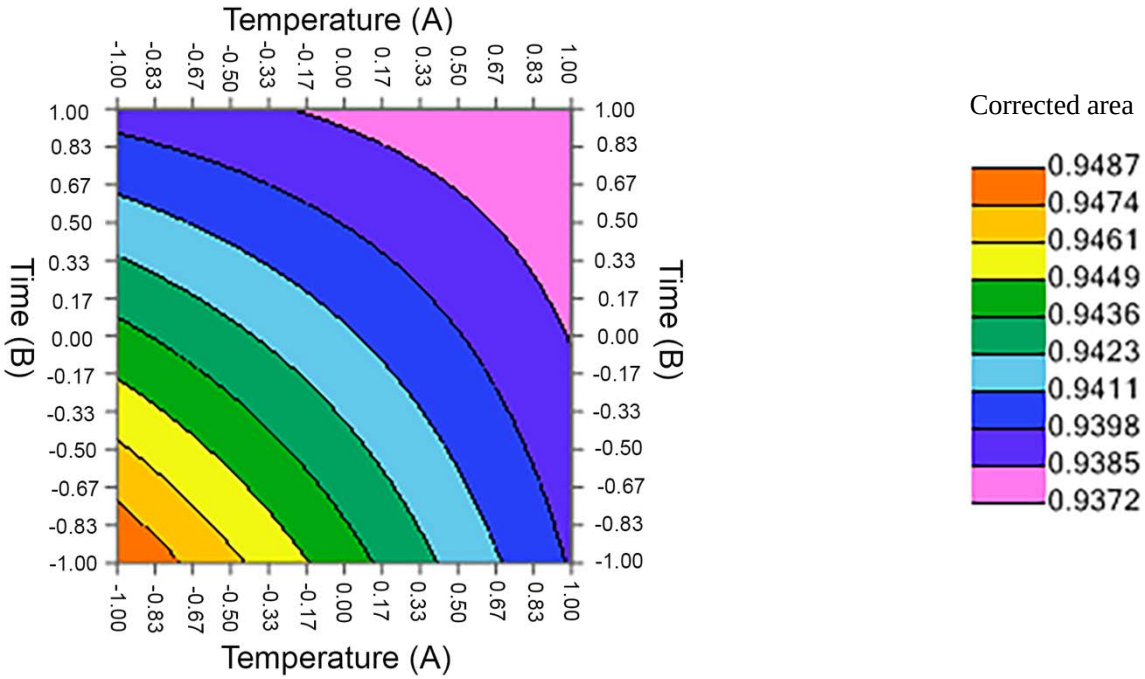


Figure 24: Response surface for the interaction model, as a function of the corrected D3 area (normalised and preprocessed).

New analyses were carried out to recreate an FFD plan by considering the analyses already carried out. The new centre point was set at 720 ° C and 20 seconds of pyrolysis. It was found that the total variance observed on the replicas made for this time/temperature pairing was smaller than the variance of pyrolysis at 620 ° C for 20 seconds. Thus it seems that the most suitable temperature to obtain a minimum variability is greater than the point of degradation

obtained by DTG [99,100]. A new FFD plan was therefore developed, and the first two previous models were re-evaluated in the light of new experiments.

The calculation of the main effects of this new plan allowed to obtain respective effects of ~ **-0.0126** for the temperature and~ **-0.0157** or the time. These results show that the effects are almost equivalent and equally influence the relative abundance of D3. Both factors always have negative effects and therefore result in a decrease in the relative abundance of D3. This observation supports the hypotheses that there is a threshold above which the increase in temperature and time parameters affects the variability of the results. The effect of the interaction has also been calculated and it is ~ **-0.0126,** almost as much as the effects of the main factors. This interaction is therefore important for the model because its effect is as important as that of the main effects. The parameters obtained for the interaction regression model $Y = a_0 + a_1X1 + a_2X2 + a_3X1X2$ (Eq. 2) are given in Table 10.

Table 10: Full statistics for the regression model $Y = a_0 + a_1X1 + a_2X2 + a_3X1X2$

Regression statistics	
Multiple determination Coefficient	0.9445
Adjusted R^2 Coefficient	0.889
F-Statistics	17.01
Model significance (p-value)	0.02183

	DF	Sum square	Mean square	F	F-critic value
Variable X1	1	1.595e-04	1.595e-04	14.32	0.0324
Variable X2	1	2.492e-04	2.492e-04	22.37	0.0179
Variable X1X2	1	1.600e-04	1.600e-04	14.36	0.0322
Residues	3	3.342e-05	1.114e-05		
Total	6				

	Coefficient	Error	T-Statistics	Probability
Intercept	0.942060	0.001262	746.714	5.3e-09
Variable X1	-0.006315	0.001669	-3.784	0.0324
Variable X2	-0.007893	0.001669	-4.729	0.0179
Variable X1X2	-0.006324	0.001669	-3.789	0.0322

Here, the temperature and time factors and their interaction are all significant (Table 10). The R^2 is 0.9445, this model better captures the variability of the response than the previous model. The adjusted R^2 must also be considered since it determined the best regression model given the number of variables used. As the adjusted coefficient varies from 0.51 (for the first model) to 0.889 for the second model, the latter is preferred. Other models are gathered in Appendix VI.

5.1.3.3 Surface response optimisation

The previously presented FFD plan was supplemented by a CCD (Central Composite Design) plan, which makes it possible to add axial points and thus to better investigate the interactions between the pyrolysis parameters. This type of plan makes it possible to measure the quadratic and cubic effects of the variables [126]. New analyses were carried out for the purpose of optimization, completing the new FFD plan. The chosen optimization plan will not be a star plan but an FCC (Faced Central Composite) plan that will act as an extension of the FFD plan already used. The acquisition of these new data made it possible to build second degree models. The first model followed the equation $Y = a_0 + a_1X1 + a_2X2 + a_3X1X2 + a_4X1^2 + a_5X2^2$ (Eq. 3). Temperature and time variables as well as their interaction were found to be significant with a 95% confidence level. The squared temperature and squared time variables were not significant. Removing the squared parameters reduced the model equation to $Y = a_0 + a_1X1 + a_2X2 + a_3X1X2$, which was found to be the more relevant models for the data obtained. Verification of the quality of the model requires checking the condition of linearity of the model and the conditions of normality of the errors. Figure 25 illustrates the relationship between the theoretical quantiles and the quantiles of the data. The residuals are normally distributed if they follow the line y = x. The observations in Figure 25 highlight three points that stand out from this line. These were found to be linked to the highest and lowest point of the dataset, which were presenting significant variability within the pyrogram.

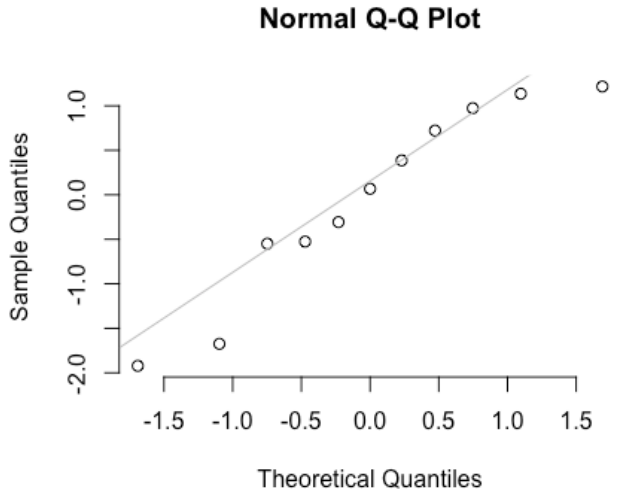


Figure 25 : QQ plot of studentised residues for the chosen model

It is necessary to illustrate the relationship between the residuals and the predictive variables used in the model. Indeed, point distributions should be random for the model to be accepted

as such. If a trend is visible, there is a violation of the model that suggests that some points significantly affect the latter. Figure 26 illustrate the relationship between the studentized residuals and the model variables. Although there are few points, on any of the three graphs a trend is not highlighted. No points stand out clearly from the rest of the residues.

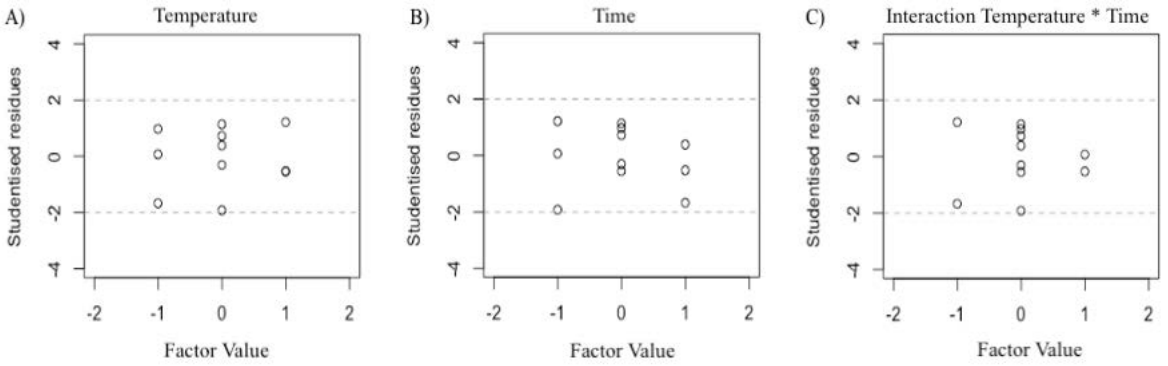


Figure 26: Studentised residues A) vs Temperature, B) vs Time, C) vs Interaction Temperature * Time

In conclusion, the quality of the chosen model is good, and it is not sensitive to fluctuations. However, it is expected that the data will vary little, as can be seen by the central point, whose maximum relative abundance difference for compound D3 is 0.003 between replicas, and the total variance for 6 cyclic oligomers of PDMS is less than 0.0005. The observation of the response surface of Figure 27 shows that the optimum lies in the zone X1 = -1 and X2 = -1 (orange zone of Figure 27), which would correspond to a temperature / time pair of 520 ° C. and 10 seconds of pyrolysis, to obtain a relative abundance of the D3 oligomer of 94.8%. It turns out that the area between 520 and 720 ° C (i.e. for X1 between -1 and 0, orange to yellow zone in Figure 27) is close to this value of 94%. This zone can therefore be considered as a local maximum. The study of the response surface also makes it possible to demonstrate a significant decrease in the abundance of D3 as the temperature and the time increase.

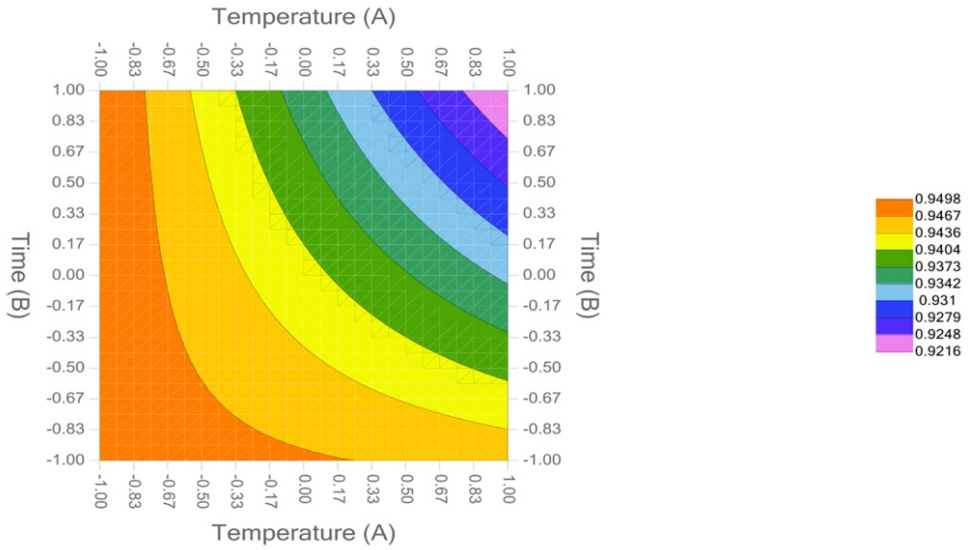


Figure 27: Surface response for the chosen model. *Reproduced from Maurer J, Buffaz K, Massonnet G, Roussel C, Burnier C (2020) Optimization of a py-GC/MS method for silicone-based lubricants analysis, Journal of Analytical and Applied Pyrolysis*

This confirms the hypotheses that there was a threshold above which the increase in parameters increased the variability of the relative abundances of the compounds produced. The threshold would therefore be the local optimum.

According to the 3D observation of the response surface (Figure 28), it appears that if the pyrolysis time is too high (X2 = 1) or too low (X2 = -1), the abundance of oligomer D3 decreases. There is a maximum zone at around 20 seconds of pyrolysis time which maximizes the relative abundance of the target oligomer. This confirms the hypothesis that too high or too low pyrolysis times increase the variability of the target compounds. However, there seems to be a minimum and maximum time threshold between which the variability is minimal, around a central point (X2 = 0). With regard to temperature, Figure 28 clearly shows a maximum in low temperatures (X1 = -1) and a minimum in high temperatures (X1 = 1). This confirms the hypothesis that there is a temperature threshold at which the variability of the relative abundances of the produced compounds is increased. The inflection point seems to be around X1 = -0.5, which corresponds to a pyrolysis temperature of about 620°C, which is in agreement with the literature [15,18,50,99,100].

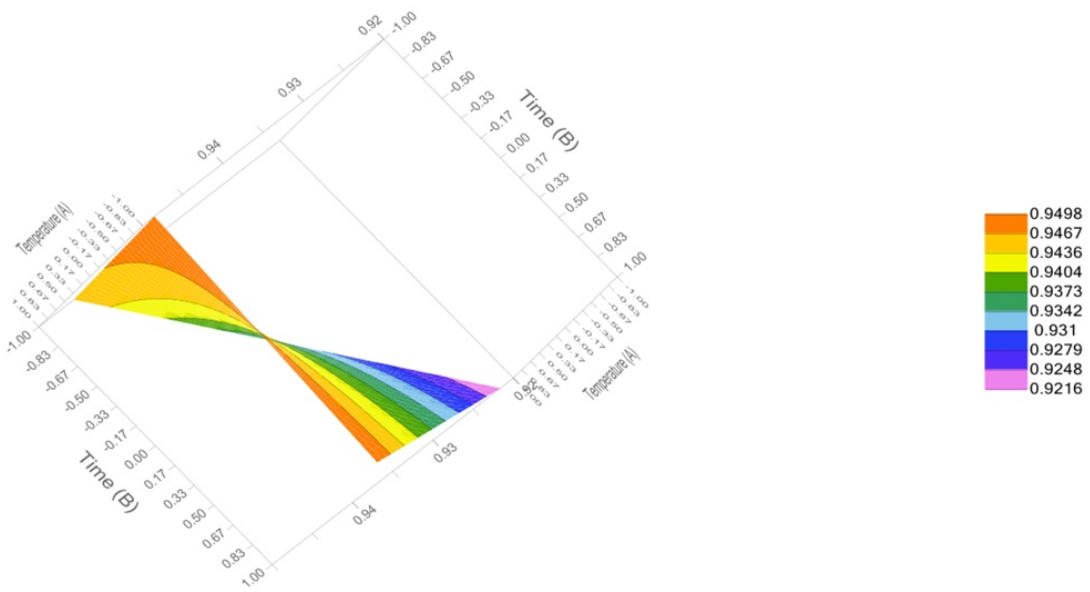


Figure 28: Observation of 3D surface response. *Reproduced from Maurer J, Buffaz K, Massonnet G, Roussel C, Burnier C (2020) Optimization of a py-GC/MS method for silicone-based lubricants analysis, Journal of Analytical and Applied Pyrolysis*

Although oligomer D3 is the target compound for optimization, it should not be overlooked that it is not the only criterion for selecting the conditions of analysis. The repeatability of the results as well as the evaluation of the intravariability of at least six cyclic oligomers characteristic of PDMS are to be taken into consideration. On this basis, the point at 620°C and 20 seconds of pyrolysis has a higher overall variance over 5 replicas than the 720°C point and 20 seconds of pyrolysis. The relative abundance of D3 allowed for a better understanding of how temperature and time factors influenced the response and to define an optimal temperature zone between 620 and 720°C and 20 seconds of pyrolysis.

It is however necessary to keep in mind that the statistical model drawn here makes it possible to process the information that it has available without considering all the complex chemical interactions that can occur in the context of pyrolysis. The choice of a pyrolysis temperature at 620°C makes sense following the various studies listed in the literature [15,18,50,99,100]. The choice of a 720°C pyrolysis temperature also makes sense with respect to the minimum variance as shown in the above experiments. Only an application to real samples will be able to highlight if a temperature of 720°C or 620°C is more adequate, and this by analysing several replicas (see section 5.2.4).

5.2 Variability reduction – Instrumental Impact

There are various types of pyrolysis device that can be used to conduct py-GC/MS analysis. The three most common ones can be categorized as either isothermal oven pyrolyzers or

filament pyrolyzers (Curie point pyrolyzer and resistively heated filament pyrolyzer). Following descriptions of the pyrolyzer comes from [91,125].

Isothermal oven pyrolyzers are designed to be set up directly over the GC injector. The sample is introduced to the pyrolysis furnace using a sliding piston system. The carrier gas enters the furnace from above and sweeps the analysis chamber to rapidly drive the produced volatiles into the injection port of the chromatograph. This is done for the purpose of avoiding or minimizing secondary pyrolysis, which may happen if the products come into contact with the walls of the furnace. These pyrolyzers operate at constant temperature. Since the pyrolysis chamber is already at the desired temperature, this type of pyrolyzer is particularly suitable for the analysis of liquids or gases. Small cups are used to deposit the sample into the pyrolyzer, which makes it easy to analyse any type of sample. This type of pyrolyzer can be illustrated by the **Frontier Lab 3030S Single Shot Pyrolyzer**, which is the device used for the analysis of condom residues reported in the literature [15,18,50] and available for research in ChemCentre (Perth, Western Australia).

The principle of filament pyrolyzers is that the sample is placed on or inside a room temperature filament, which is then inserted into an interface, called the pyrolysis chamber, where it is rapidly heated to the desired temperature. The filament is attached to a probe that is inserted into the pyrolysis chamber, which must be heated separately to avoid condensation of the pyrolysates. This chamber is connected to the injection port and a valve system ensures the continuity of the carrier gas flow, which sweeps the pyrolysates into the GC injector. These pyrolyzers provide the option of varying the temperature and the filament heating rate, the only restriction being the maximum temperature a filament can reach. This type of pyrolyzer thus makes it possible to analyze a sample at various pyrolysis temperatures without changing the filament. The main disadvantages of resistance-heated filaments are their fragility and in some cases the need to use a transfer line to introduce pyrolysates into the GC. Quartz tubes are usually used to deposit the sample, which is quite a challenge as the samples should not touch the tube walls at any point. While this is not much of an issue for solid samples, it becomes more challenging when it comes to liquid or more viscous samples. This type of instrument can typically be exemplified by the **CDS Analytical 5050 Pyrolyzer**, available within the School of Criminal Justice (University of Lausanne).

The aim here was to compare the two instruments above to see if there are any significant differences between the chemical profiles, and to assess the qualitative and semi-quantitative repeatability in order to choose the most suitable device for condom residues analysis. To begin with, the two devices were compared on a set of 2 samples analysed on the same column type,

to identify which device offered the best overall results in terms of presence of peaks, resolution and overall variance for a given set of interesting peaks.

5.2.1 Instrumentation and samples

The first instrument used was a resistively heated filament Pyroprobe 5150 from CDS Analytical Inc. The pyrolysis device was coupled to an Agilent GC 6890N GC system interface with an Agilent 5975C mass spectrometer. Software used were respectively Pyroprobe 3.21 from CDS and ChemStation v. D00.01.27 from Agilent. The second instrument was an isothermal oven Frontier Lab py-3030S single shot pyrolyzer device coupled to an Agilent GC 7890B system, interfaced with an Agilent 5977N mass spectrometer. Software used were respectively Py3030S Control (v. 1.77) from Frontier Laboratories and ChemStation v. F.01.03.2357 from Agilent. The exact same separation conditions were used on both instruments to observe any variation. In the same way, two common samples were used to evaluate instrumental variation.

The comparison was performed from both a qualitative and semi-quantitative perspective. First, within-sample variation was individually evaluated based on the numbers of peaks observed and their retention times for each sample. From a qualitative point of view, the presence or absence of peaks, the number of peaks, their position (relative retention time) and the pattern in terms of relative abundances was noted. Peaks over 10,000 AU found in all chromatograms were identified and their areas integrated so that semi-quantitative analysis could be performed. A semi-quantitative comparison was investigated after data processing by normalization to the sum of the peak areas, followed by double square root. Mean, standard deviation and coefficient of variation were calculated. For the qualitative analysis, ChemStation software (v. F.01.03.2357) from Agilent was used. Microsoft Excel and R software were used for data processing and semi-quantitative evaluation.

5.2.2 Visual interpretation of the pyrograms

All the pyrograms obtained exhibited the different oligomers coming from the degradation of polydimethylsiloxane (PDMS), as described by other studies [15,18,50]. Therefore, these compounds were used as known reference points to evaluate and identify minor components in the different pyrograms. As differences were observed in terms of retention time, due to different columns of both the same and different types, it was important to find a way to compare the position of the peaks in the chromatograms. Therefore, relative retention times

were used. The first peak used was the D3 peak, whose RRT was set at 0. All other peaks' positions were measured relative to D3 by subtracting the initial D3 RT.

The overlay of all the pyrograms obtained for a single sample showed good repeatability in terms of peak number and retention time in the region between the D3 oligomer (RT between 4.00 and 5.00 min) and 23.00 min. This is illustrated in Figures 29-32 for both the Manix_Strawberry and Manix_OrgazmaxPlus samples acquired on the two instruments. After 23.00 min, no further peaks were present. Differences were observed in the results obtained with the Frontier instrument in the region before the D3 oligomer, as one poorly resolved peak was present and found to not be repeatable. The chromatograms acquired on both instruments highlighted a strong qualitative variation between the chemical profiles obtained. The general pattern observed was rather similar, as the cyclic oligomers were predominant in the pyrograms. However, in terms of minor compounds originating either from the extraction of other residues from the condom, or from the pyrolysis itself (recombination), the latter were found to be variably present in the samples, especially between two instruments. Samples acquired on the CDS instrument presented more peaks than the ones acquired on the Frontier Lab instrument, which suggest a better sensitivity. Another marked difference was that the patterns of the same minor peaks found in both samples were visibly different (Figure 33).

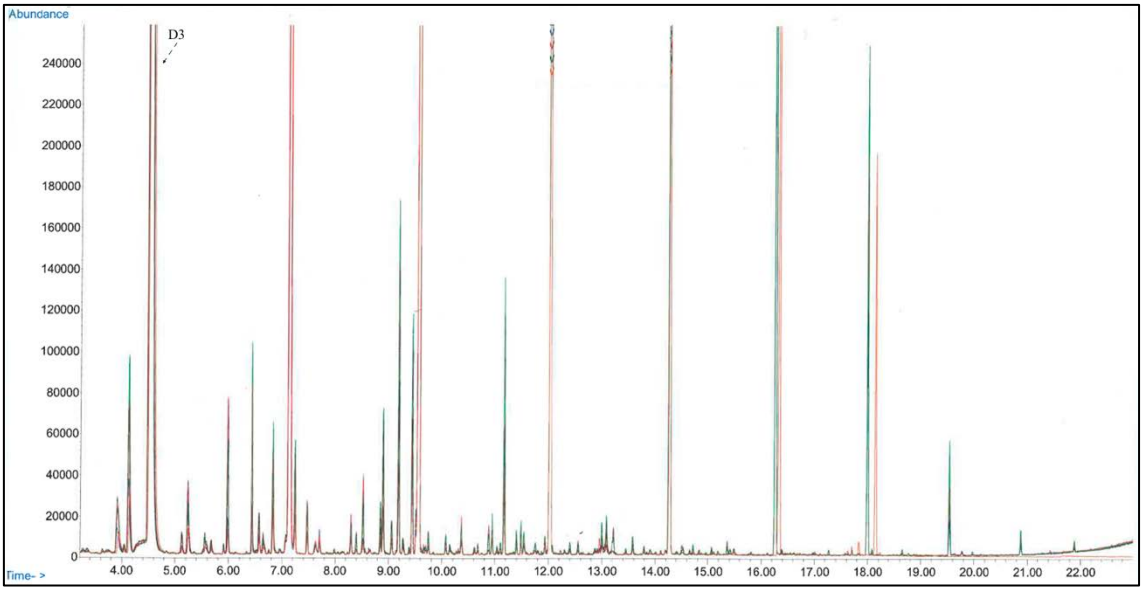


Figure 29: Illustration of the replicates for *Manix Strawberry* sample, acquired on the CDS Analytical Instrument

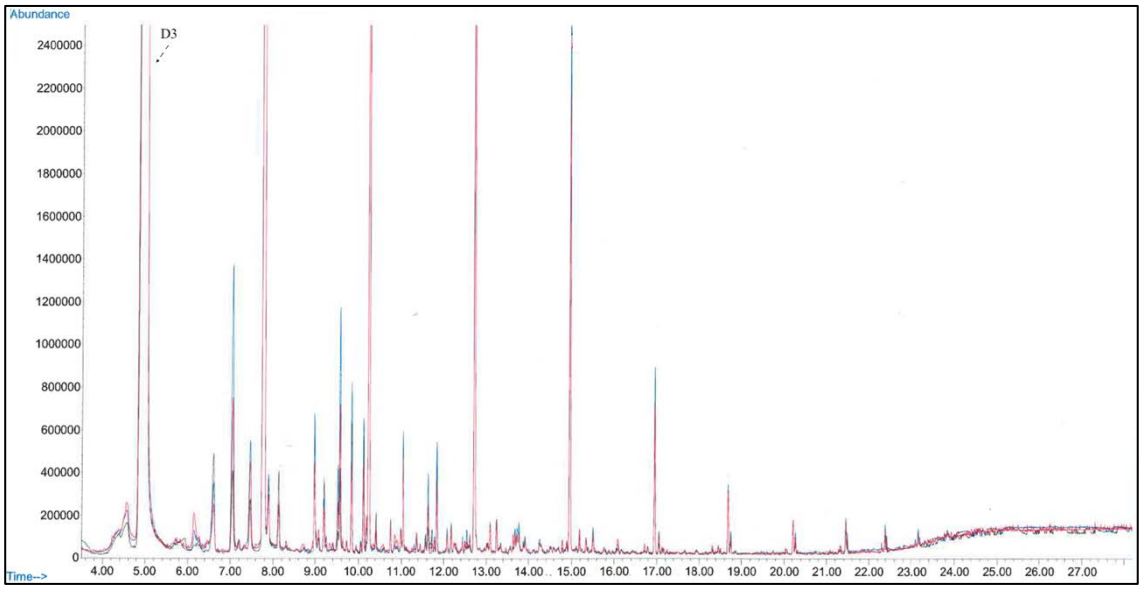


Figure 30: Illustration of the replicates for *Manix Strawberry* sample, acquired on the Frontier Labs Instrument

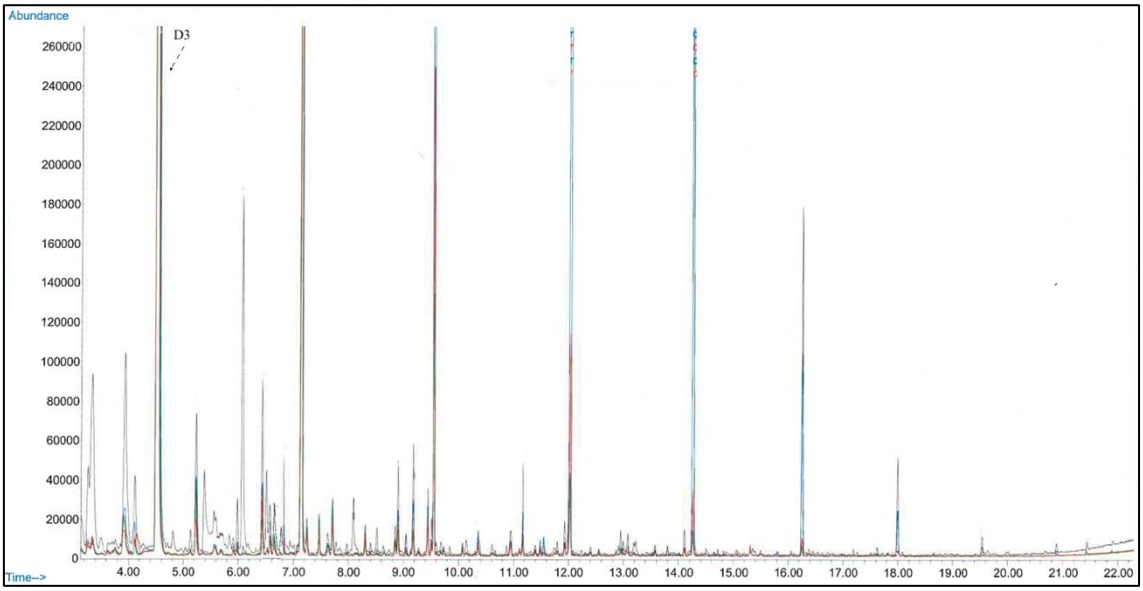


Figure 31: Illustration of the replicates for *Manix Orgazmax Plus* sample, acquired on the CDS Analytical Instrument

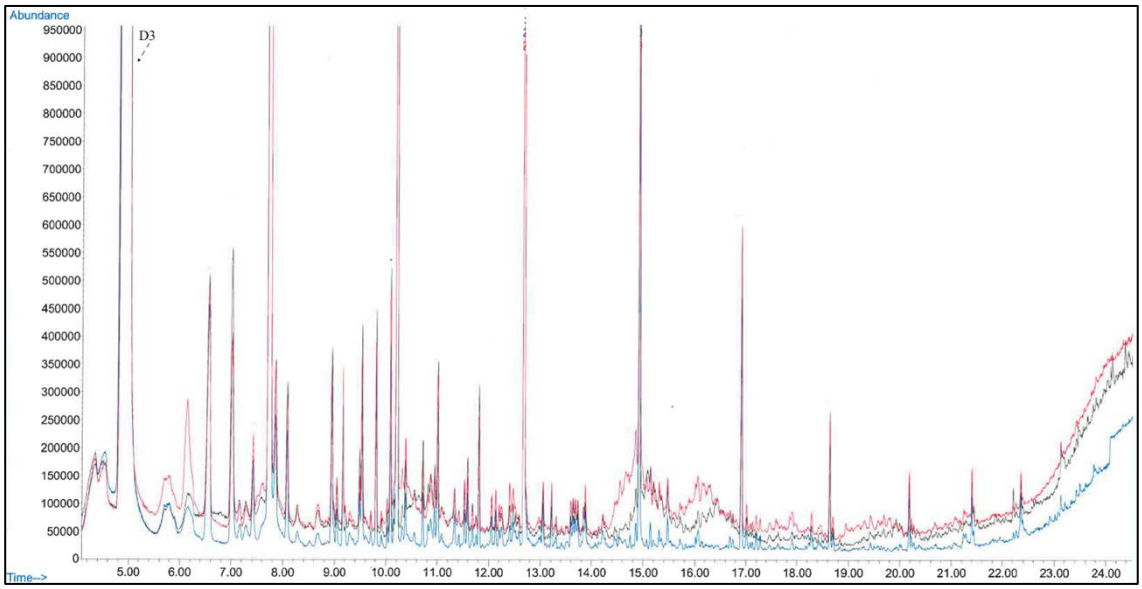


Figure 32: Illustration of the replicates for *Manix Orgazmax Plus* sample, acquired on the Frontier Labs Instrument

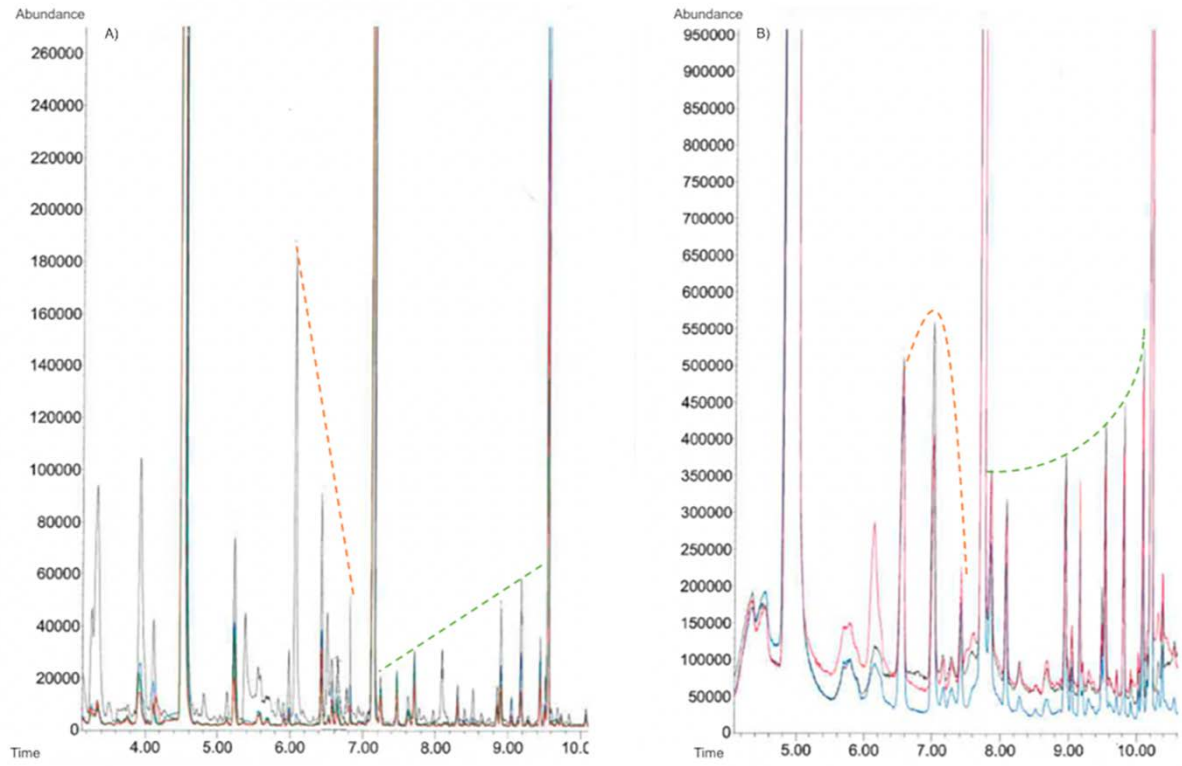


Figure 33: Illustration of the qualitative pattern differences between sample acquired on CDS instrument (A) and on Frontier instrument (B). Green and orange dotted lines illustrate the patterns of the peaks in each sample (*Manix Orgazmax Plus)*

5.2.3 Semi-quantitative comparison of the pyrograms

The compositions of all samples were slightly different, as certain peaks were not identifiable in all the pyrograms. Compound selection for the semi-quantitative comparison was limited to RRT between 0 and 16.00 min (Figure 34), present in all of the pyrograms, with an abundance equivalent to 3 times the background noise (Table 11).

Data extraction and processing was performed using the extraction macro built. Integrated compounds were then verified and exported into an Excel sheet. Data were normalized to area sum and processed using double square root. Relative abundances of these compounds represent the *chemical profile* of the analysed condom.

Table 11 : Compounds selected to compare the analysis.

Peak nº	RRT	Compound name	Target Ion	Qualifiers
1	0	D3	207	191, 133, 96
2	1.53	Unknown5.9	207	192, 221, 176
3	1.985	Unknown6.4	192	208, 96, 135
4	2.38	Unknown 6.8	207	223, 190, 133
5	2.695	D4	281	265, 193, 133
6	2.805	Unknown7.2	266	126, 250, 192
7	3.88	Unknown7.28	264	248, 190, 125
8	4.475	Unknown8.8	266	248, 190, 125
9	4.755	Unknown9.1	341	324, 163, 73
10	5.02	Unknown9.4	341	334, 162, 73
11	5.14	D5	355	266, 73
12	6.745	Unknown11.7	326	415, 73, 398
13	7.61	D6	341	429, 324, 147
14	9.845	D7	281	503, 415, 147
15	11.845	D8	355	401, 281, 221
16	13.575	D9	429	236, 196, 146
17	15.115	D10	503	146, 280, 355

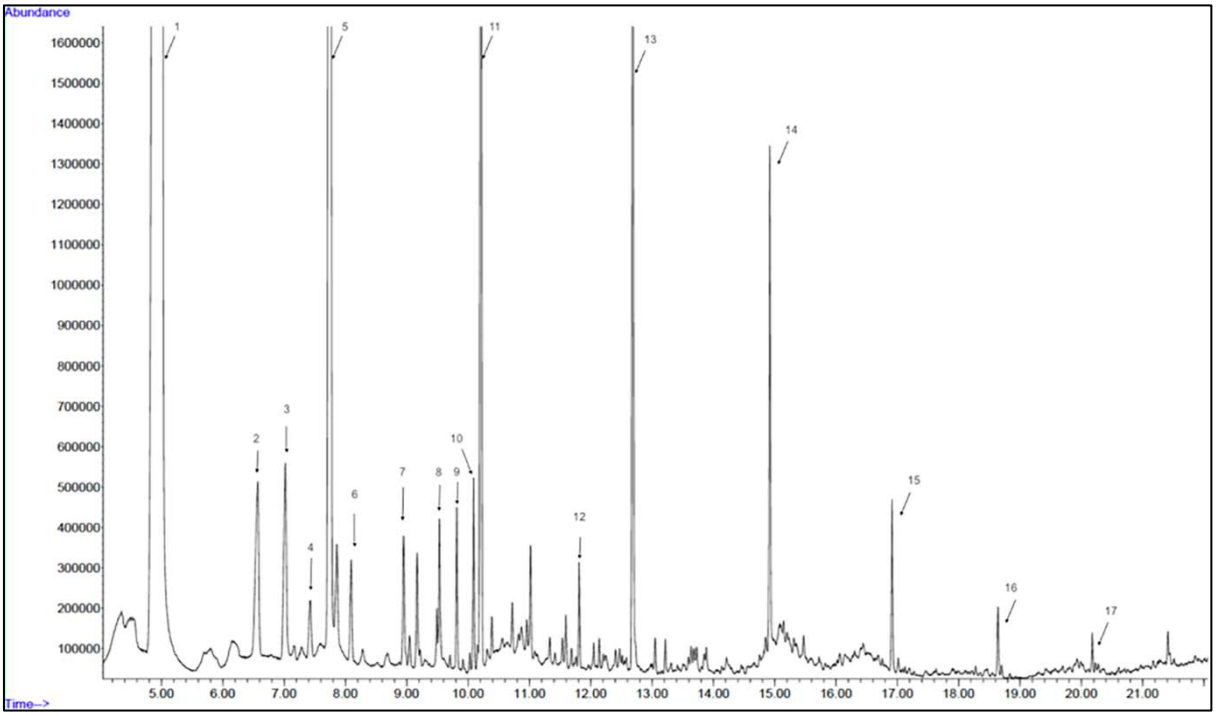


Figure 34: Compounds selected for the extraction macro for comparison purposes, TIC mode representation, CDS Analytical Instrument.

Figure 35 illustrates the coefficient of variation obtained on the pre-processed data, on all the replicates of each sample considered.

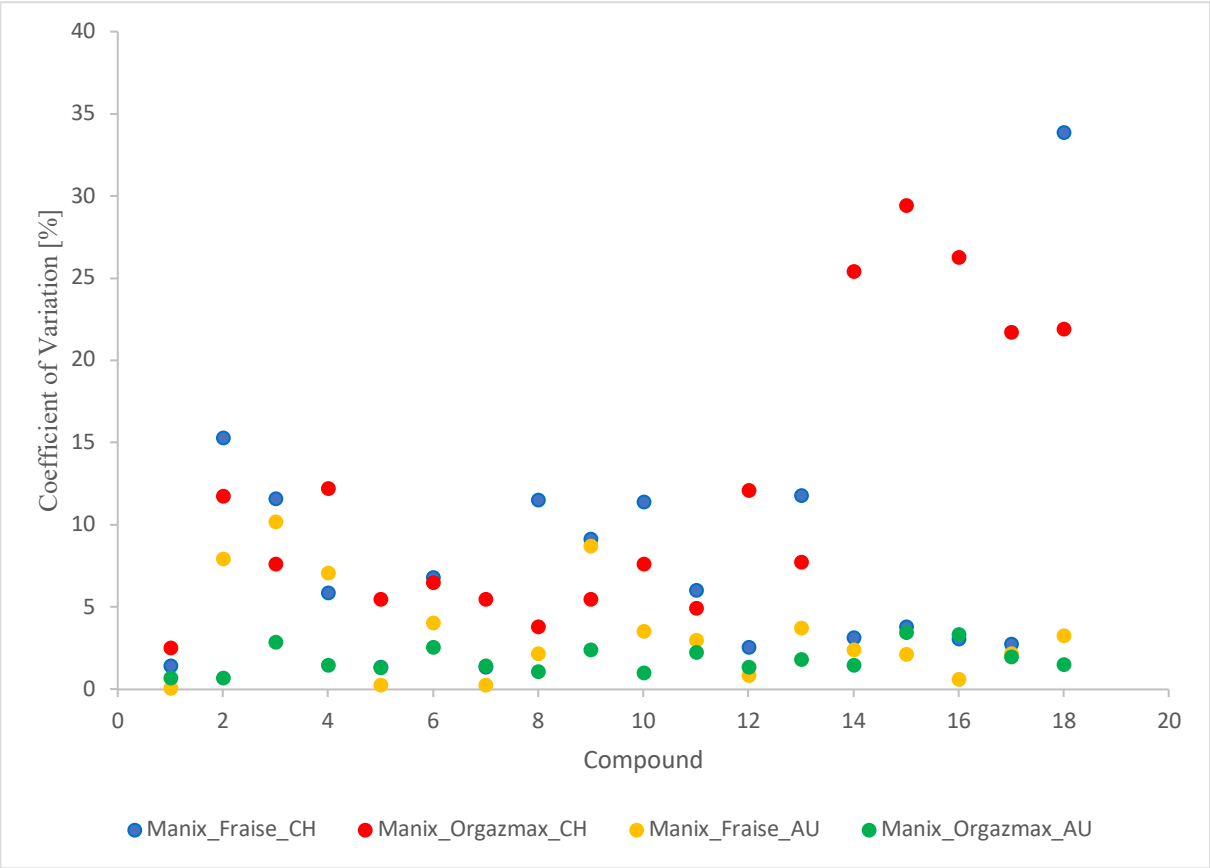


Figure 35 : Distribution of the coefficient of variation of the 18 compounds selected for each sample (data pre-processed), as a function of the instrument used. CH stands for the CDS Analytical Instrument, while AU stands for the Frontier Labs Instrument

As illustrated in Figure 35, the different compounds were highly variable. The coefficient of variation was over the acceptable limits, which were set at 20 % and 10 % for the normalized and preprocessed data respectively. Samples analysed with the CDS Instrument tend to offer a higher variability, especially in sample Manix_Orgazmax_CH, where the cyclic oligomers D6 to D10, which were very small peaks in the pyrogram, display a variability far over 20 %. Generally, samples presented significantly lower coefficients of variation when acquired on the Frontier instrument.

The qualitative observation of the chromatograms acquired on different instruments highlighted a strong variation between the chemical profiles obtained, and semi-quantitative analysis of common compounds showed better repeatability of the samples acquired with the Frontier instrument. Two possible reasons are offered to explain these differences.

The first is that the methodology for the CDS instrument involved spiking the sample onto quartz wool manually inserted into the middle of the quartz sampling tubes. The amount of quartz wool in the tube is likely to be variable (but can be weighed if needed), which would affect the adsorption or desorption of the residues spiked for the analysis. This is not an issue

with the Frontier instrument, as metallic cups are used. This is reinforced by the properties of PDMS, which is known to complex with glass. Even though the replicates acquired with this method all showed the exact same pattern and a very good qualitative repeatability, the semi-quantitative results support this hypothesis, as the variation for the same sample was more important for these samples with this instrumentation.

Additionally, the CDS instrument is a resistively heated filament pyrolysis device, connected to the GC injector using a transfer line. The pathway to the injector is therefore quite long and recombination can happen between the time of the pyrolysis and the time of the analysis. This recombination process is very likely to be responsible for the additional compounds found in the samples acquired on the CDS instrument only, as well as the variation in terms of abundance patterns. Although the transfer line was heated and maintained at 300 ºC, the presence of the transfer line itself may generate a negative influence on the reproducibility of the results, with some compounds not reaching the GC injector. An hypothesis was that compounds become recondensed as they leave the pyrolysis furnace, therefore, they are not separated on the column and not detected, but no evidence was observed.

5.2.4 Selection of pyrolysis conditions – Repeatability study

The overlaid replicates obtained for the Durex Strawberry sample at conditions of 620°C and 20 sec of pyrolysis are presented in Figure 36.

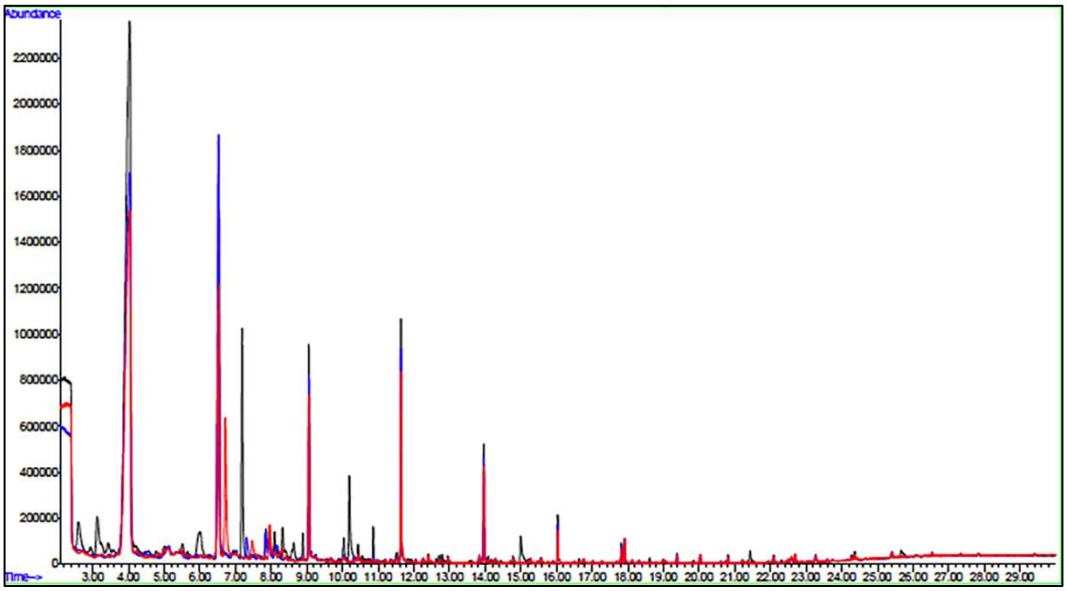


Figure 36 : Illustration of the repeatability of the pyrograms for *Durex Strawberry* condom (TIC mode) between 3 and 28 minutes, at 620°C and 20 sec of pyrolysis, Frontier Labs Instrument

The oligomers produced from PDMS degradation, from D3 to D9, presented an excellent reproducibility in terms of peak shape and retention time, and this was found not to be

dependent on the sample or on the temperature. This reproducibility was observed for all the other analysis of the present work. However, a strong difference between the replicates was observed for Durex Extra Safe especially between 3.00 and 9.00 minutes. Before 4.00 minutes, small peaks were eluted that were not regularly found in the pyrograms. Characterization with the databases showed that they were 5-hexen-2-ol, 2-hexanone and 5-hexen-2-one. It is expected that these come from hexane degradation and pyrolysis recombination. This reinforces the importance of allowing sufficient time for solvent evaporation to ensure that only the polymeric material extracted is pyrolyzed. Peaks were observed between 7 – 8 mins that did not completely overlay between the replicates and appeared to vary randomly. It was found that their mass spectra were always the same and could be attributed to 2,5-Hexanedione (NIST18). The shifts in retention time may be due to the polarity of the column. The overlaid replicates obtained for one sample at conditions of 720°C and 20 sec of pyrolysis are presented in Figure 37.

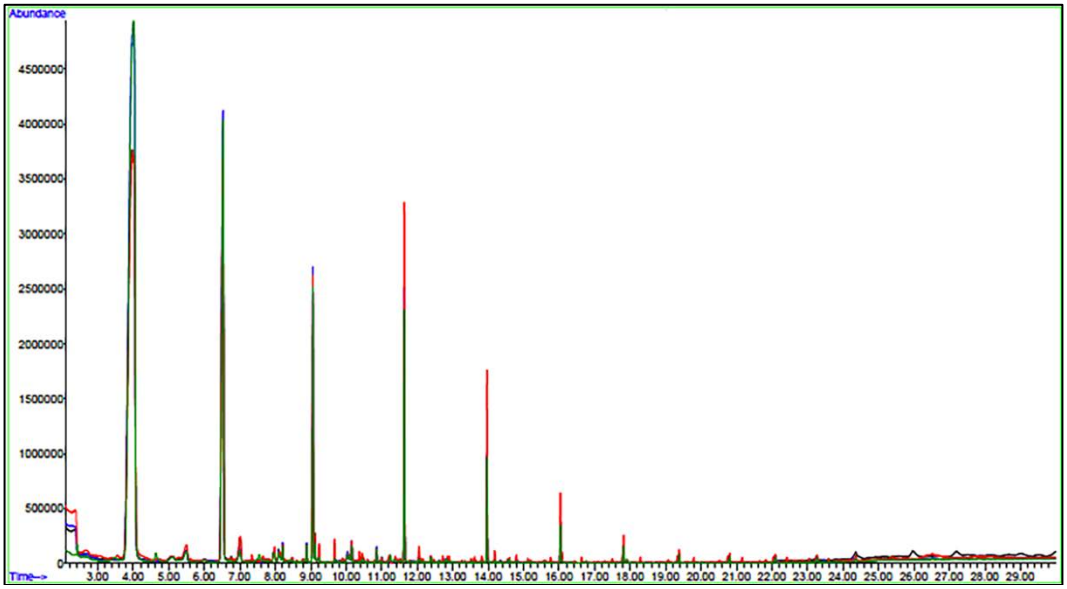


Figure 37 : Illustration of the repeatability of the pyrograms for Durex Strawberry condom (TIC mode) between 3 and 28 minutes, at 720°C and 20 sec of pyrolysis, Frontier Labs Instrument.

Chemical profiles obtained with these parameters offered better reproducibility and more consistency when considering the whole profile. Cyclic oligomers from PDMS degradation were highly reproducible and there were no variable peaks as shown in Sample 01 with 620°C. 2,5-Hexanedione was no longer present in the chromatogram. Only one replicate from Sample 01 presented extra peaks before 4.00 minutes. According to NIST18 database, the compounds are respectively 2 and 3-Hexanone. Therefore, this suggests that the solvent had not completely evaporated before the analysis.

The two-pyrolysis temperature obtained from the experimental design (FFD followed by CCD) realized on CDS Instrument showed that there was a range of temperature, between 620 and

720°C where the profiles were all consistent and a rather low variance was observed. Both temperatures were then tested to see which one is the more adequate for the purposes of this research. Reported studies generally use 600°C based on Camino's observations from TGA and DTG curves of PDMS [99,100]. As the main target component is PDMS, the degradation of the latest in oligomers was targeted, and oligomers D3-D9 were used for semi-quantification. After pre-processing of the data (normalization to area sum and double square root), coefficients of variation were obtained and are shown in Table 12. All coefficients of variation were lower than 5% except for the analysis of Sample 2 at 720°C. Total variance are both very low.

Table 12 : Coefficient of variation for 6 oligomers coming from PDMS degradation and total variance of each analysis set, n=5.

Pyrolsis Device	CDS Analytical		Frontier Laboratories	
Compound	Sample 01 620°	Sample 01 720°	Sample 02 620°	Sample 02 720°
D3	0,24%	0,16%	0,28%	0,66%
D4	0,36%	0,54%	0,47%	1,31%
D5	0,63%	0,46%	1,70%	1,41%
D6	2,32%	1,08%	3,05%	1,17%
D7	2,49%	1,50%	2,87%	1,05%
D8	3,41%	1,64%	2,02%	1,25%
D9	3,36%	2,84%	1,67%	2,75%
Total Variance	3,17E-04	9,15E-05	3,21E-04	1,61E-05

Based on these considerations, pyrolysis conditions at 720°C for 20 seconds were found to be the more adequate conditions and will be used for the rest of the study.

5.3 Market Study – Qualitative analysis

5.3.1 Material and methods

5.3.1.1 Material

Hexane (AR grade, Sigma Aldrich, USA) was used as a solvent. Pyrolysis sample holders were Eco-Cups SF and Eco-Sticks SF purchased from Frontier Laboratories. PDMS 200 centiStokes (cSt), with a molecular weight ~9430, obtained from Sigma Aldrich (USA) was diluted in hexane at a concentration of 1 mg/mL

Samples were obtained from commercially available distributers in Australia, New Zealand and Switzerland. The samples obtained were considered representative of the markets, as they covered major condom brands and sub-brands and were available to consumers. The dataset includes 2 personal hygiene products (PHP), 7 lubricants and 61 condoms, that are all known to contain silicone lubricants. These samples were taken from the larger dataset used in Chapter 4, which contained multiple samples from the same brand and model. Replicate samples were

removed. Only silicone-based samples were analysed, as water-based samples are known not to provide any profile due to degradation of the molecules in CO_2 and H_2O, not in the mass range to be detected by the instrument.

5.3.1.2 Sample preparation and analysis

For py-GC/MS analysis, condoms were opened, unrolled, deposited in a 40 mL glass bottle and covered with 25 mL of hexane. The bottles were then capped and ultrasonicated for 15 minutes. The extracts were then diluted 10-fold prior to analysis. Liquid samples, such as personal lubricants, were weighed and diluted in hexane to the approximate concentration of the diluted, extracted condom, between 1.5 and 2.5 mg/mL

For each sample, 10 µL of the hexane solution was spiked in the stainless-steel cups and left to evaporate prior to analysis. Three replicates were prepared from each condom extract, to account for sample variability, as well as any variation due to the instrumentation and sample preparation. Blanks were run between each analysis to avoid cross contaminations.

All the analyses were carried out using an isothermal oven Frontier Lab py-3030S single shot pyrolyzer device coupled to an Agilent GC 7890B system, interfaced with an Agilent 5977N mass spectrum detector using the parameters aforementioned in Table 23, with pyrolysis parameters being 720°C for 20 seconds.

5.3.1.3 Data processing

Qualitative analysis

Identification of the compounds was undertaken using three different mass spectral databases, i.e. NIST18 (*National Institute of Standards and Technology*, available in ChemCentre), PP (*Pyrolysis Products*, provided by FrontierLab, in-house database from ChemCentre [127–131]) and TOX3 (*Wiley 138 Drug and Pesticides*, available in ChemCentre and in ESC), and comparisons with retention time and mass spectra obtained from the analysis of bulk PDMS, as well as referring to literature [93,94,125,132]. However, identification of pyrolysates is a difficult task as pyrograms are usually composed of a large number of peaks. Some compounds could not be identified using the aforementioned methodology and therefore were named "Unknown" followed by their retention time. Most of the compounds detected in the chromatographic profile could not be assessed using the databases, as the latest do not contain pyrolysis products.

Using Agilent ChemStation® software, areas of the target ions within all the acquired pyrograms were integrated for each peak. Peaks were selected as to be repeatable, and distinguishable from the background, over a threshold value of three times the noise, which led

to an threshold at 30,000 AU Table 13 references the peaks and their parameters. Data were exported to Microsoft Excel, normalised to the area sum, and the double square root calculated prior to multivariate statistical processing.

Chemometrics

Principal component analysis (PCA) was undertaken on the normalised data, using the non-iterative partial least squares (NIPALS) algorithm. Three dimensional scores plots were used to visually explore the data structure and to assess the loadings of the main components. Firstly, groupings related to class (i.e. condom, lubricant, PHP) were examined, to determine if separations were clear or if overlaps between classes existed. The loadings plots related to these sample scores were evaluated to understand the variables most important for sample discrimination and to investigate the potential reduction in the number of variables. Finally, quadratic discriminant analysis (QDA) was undertaken on the entire dataset to build the classification model. Each variable was assumed to have equal a priori probabilities, with a variable weight of 1.0 for each variable. Both PCA and QDA were performed using the Unscrambler X v. 10.5 (Camo Software, Norway).

5.3.2 Repeatability

Samples were visually classified in different groups according to their chemical profiles. Overlaid chromatograms were assessed to evaluate repeatability and to compare the sample profiles as well as, when indistinguishable patterns were observed, to compare retention time and mass spectra obtained between the different samples. The superposition of all the pyrograms obtained for the same condom showed excellent repeatability in terms of the number of peaks and their retention time, as illustrated in Figure 38 for sample *Durex Strawberry* and in Figure 39 for sample *Manix Contact*.

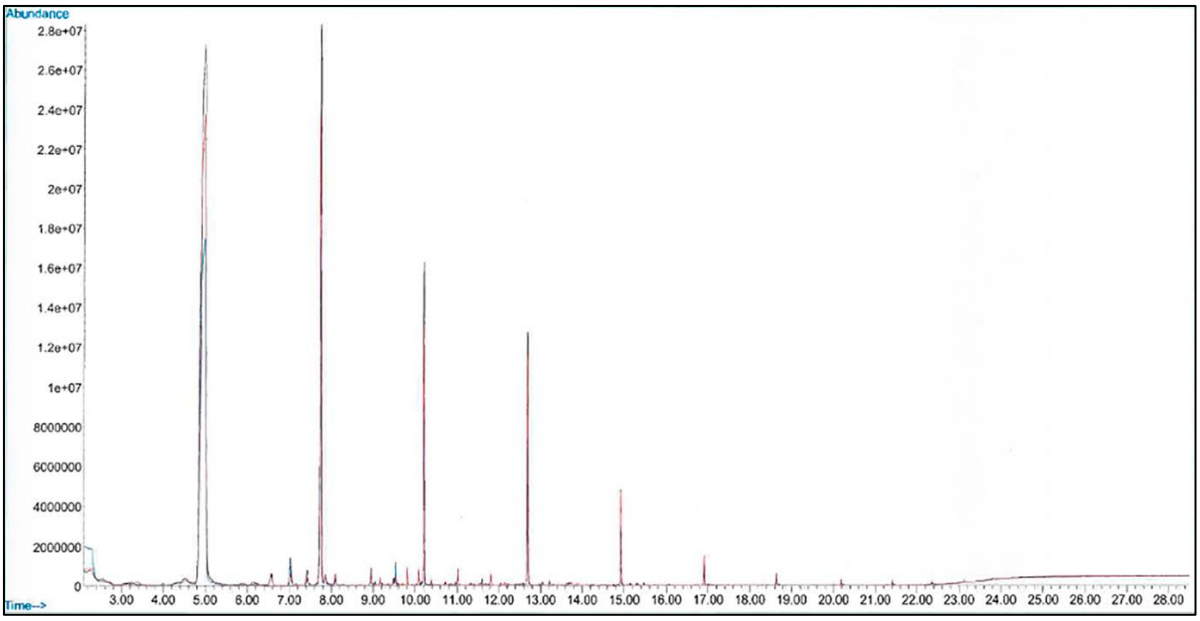


Figure 38: Illustration of the repeatability of the pyrograms for *Durex Strawberry* condom (TIC mode) between 3 and 28 minutes.

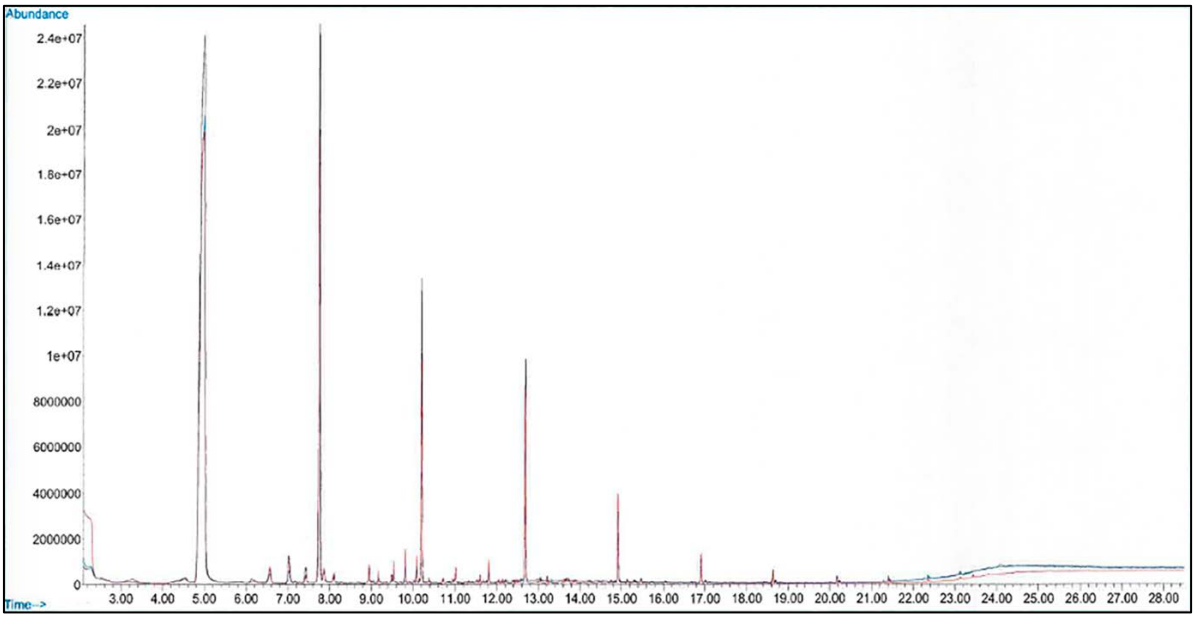


Figure 39: Illustration of the repeatability of the pyrograms for *Manix Contact* condom (TIC mode) between 3 and 28 minutes.

No major visual differences were noted in the pyrograms of the condom extracts. However, before the appearance of compound D3, i.e. before 4.9 minutes, peaks of hexane-2,5-dione were identified with a high-quality ranking in the databases. They were found not to be reproducible between replicates. Non-evaporated samples were run and their chemical profile was compared to one of the evaporated samples to investigate if this compound could originate from the solvent. Chemical profiles were found to be affected if the evaporation was incomplete (data shown in the Supplementary Information). It is therefore possible that this compound is more likely derived from the pyrolysis and recombination of hexane and hence, was likely due to the

solvent not sufficiently evaporating prior to pyrolysis, rather than from the pyrolysis of any other compounds. Recommendation to avoid these issues to be recurrent is to let the sample evaporate long enough before putting the cups into the pyrolysis unit.

5.3.2 Qualitative Characterization

Approximately 50 compounds were characterized in the pyrograms. They are ordered according to their increasing retention time in Table 13 and their positions in the chromatogram are shown in Figure 40. Of these 50 compounds, 10 could be identified as originating from the cyclic oligomers generated during the PDMS pyrolysis, i.e. D3-D13, based on comparisons with the databases as well as the literature. The remaining 40 compounds could not be identified.

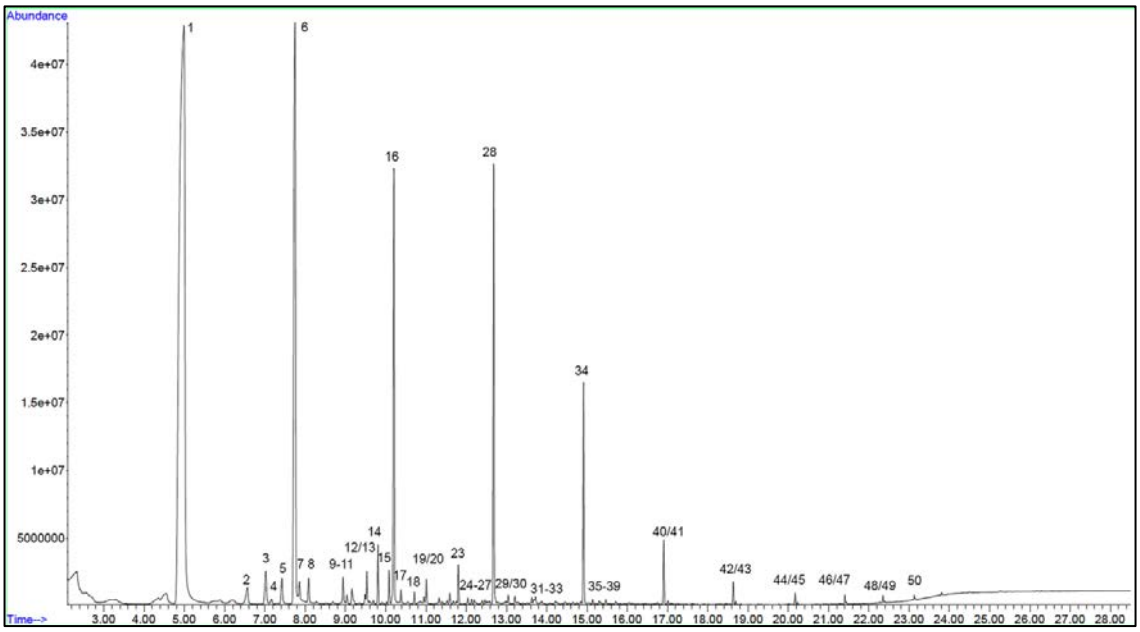


Figure 40: Chromatographic pattern of PDMS 200cSt reference, analysed under optimized conditions. Compounds selected to create the extraction macro. Compound numbers are related to Table 30.

Table 13: Compounds selected to compare the analysis. Names in brackets are suggestions for compounds that were not identified using databases or literature.

Nº	Name	RT [min]	Target ion (*m/z)*	Qualifiers (*m/z)*
1	D3	4.93	207	191, 133, 96
2	(Linear D3)	6.58	207	193, 221, 177
3	Unknown 7.02	7.02	192	209, 97, 134
4	Unknown 7.16	7.16	267	193, 207, 281
5	Unknown 7.41	7.41	207	223, 191, 133
6	D4	7.73	281	265, 191, 249
7	Unknown 7.86	7.86	267	281, 250, 126
8	(linear D4)	8.08	281	265, 207, 133
9	Unknown 8.94	8.94	265	125, 249, 191
10	Unknown 9.03	9.03	207	193, 247, 176
11	Unknown 9.16	9.16	281	295, 233, 193
12	Unknown 9.48	9.48	267	250, 192, 126
13	Unknown 9.53	9.53	267	126, 250, 283
14	Unknown 9.80	9.80	341	325, 163, 73
15	Unknown 10.08	10.08	341	325, 163, 73
16	D5	10.21	355	267, 73, 251
17	(linear D5)	10.37	355	267, 250, 73
18	Unknown 10.71	10.71	355	267, 250, 73
19	Unknown 10.95	10.95	339	323, 162, 128
20	Unknown 11.00	11.00	339	323, 162, 154
21	Unknown 11.32	11.32	281	339, 267, 321
22	Unknown 11.59	11.59	341	324,163,73
23	Unknown 11.80	11.80	326	415, 73, 399
24	Unknown 12.04	12.04	326	415, 73, 398
25	Unknown 12.19	12.19	326	399, 415, 73
26	Unknown 12.40	12.40	326	415,269, 253
27	Unknown 12.46	12.46	401	341, 429, 73
28	D6	12.67	341	429, 325, 147
29	(linear D6)	13.04	341	324, 429, 147
30	Unknown 13.21	13.21	413	324, 399, 73
31	Unknown 13.63	13.63	400	489, 326, 384
32	Unknown 13.67	13.67	324	413, 207, 190
33	Unknown 13.72	13.72	400	489, 326, 384
34	D7	14.90	415	281, 147, 326
35	(linear D7)	15.14	503	415, 147, 281
36	Unknown 15.29	15.29	399	487, 325, 147
37	Unknown 15.46	15.46	487	399, 147, 281
38	Unknown 15.71	15.71	475	147, 73, 400
39	Unknown 16.74	16.74	73	147, 221,281
40	D8	16.90	355	401, 281, 221
41	(linear D8)	17.00	221	147, 281, 355
42	D9	18.62	429	355, 221, 147
43	(linear D9)	18.70	221	355, 147, 429
44	D10	20.17	503	281, 355, 147
45	(linear D10)	20.23	533	281, 221, 147
46	D11	21.40	355	535, 147, 281
47	(linear D11)	21.44	429	355, 207, 281
48	D12	22.35	429	355, 207, 147
49	(linear D12)	22.38	207	281, 355, 429
50	D13	23.12	207	281, 355, 429

It could be determined that all of these compounds were produced from the degradation of PDMS, according to their mass spectra. This necessitated further study into PDMS degradation

mechanisms as well as the potential chemical structures that could explain two compounds with the same mass spectrum but different retention times.

PDMS consists initially of the $SiOC_2H_6$ monomer with a molecular weight of 74.1553 a.m.u. The diagnostic ion produced by electronic impact (EI) ionisation is *m/z* 73. The specific extraction of this ion in the chromatogram is illustrated in Figure 41. The observed pattern is very close to that observed in the raw chromatograms obtained for the various samples, from 10 minutes. The pattern of degradation is very obvious, with a sharp decrease in the presence of this ion over time, suggesting a lower presence in terms of the amount of these heavier compounds in the sample.

Other degradation mechanisms were also observed due to the ionization. There is a series of degradation $(R\text{-}58)^+$ which results from the degradation by loss of a radical $CH_3^{\bullet}$ coming from ions $(R + 73)^+$. This series is not always detected. The *m/z* associated with this series in the mass spectrum are, among others, 131, 205, 279, 353, 427. Another more well-known series of degradation is the $(R\text{-}59)^+$ series that comes from the loss of a $SiOCH_3$ unit. This series produces ions *m/z* 131, 207, 281, 355, 429, 503 in the mass spectrum. The ion *m/z* 207 is the characteristic ion of the cyclic oligomer D3, and the pattern related to the presence of this ion in the chromatogram is shown in Figure 42.

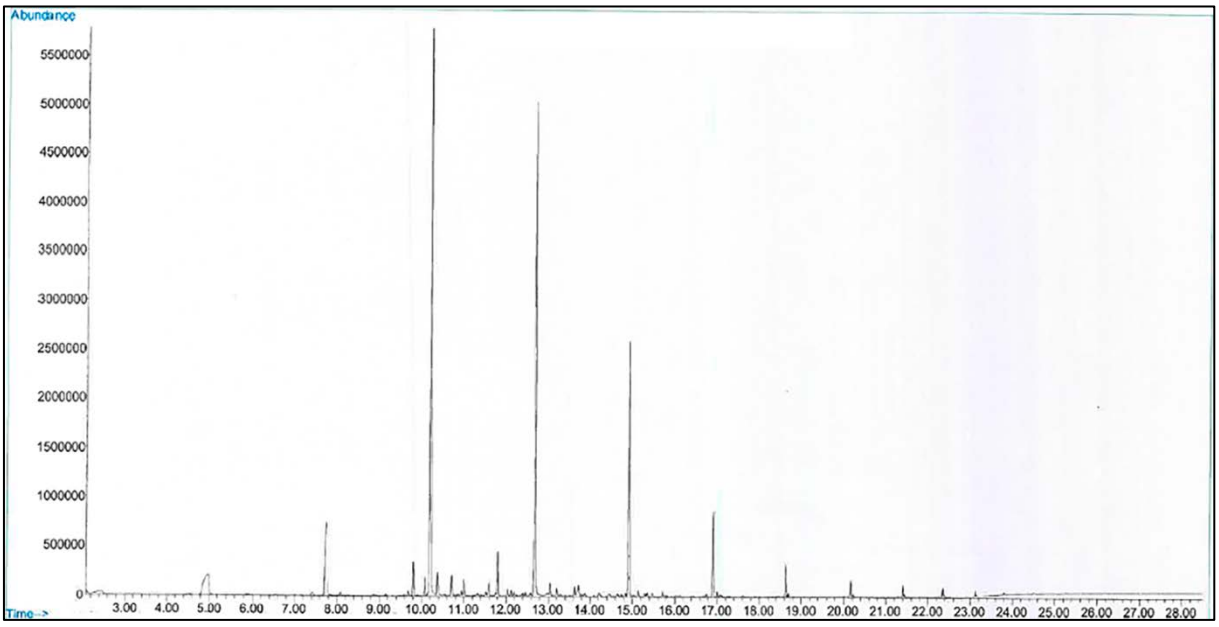


Figure 41: Extracted Ion Chromatogram (EIC) for the monomer ion at *m/z* 73

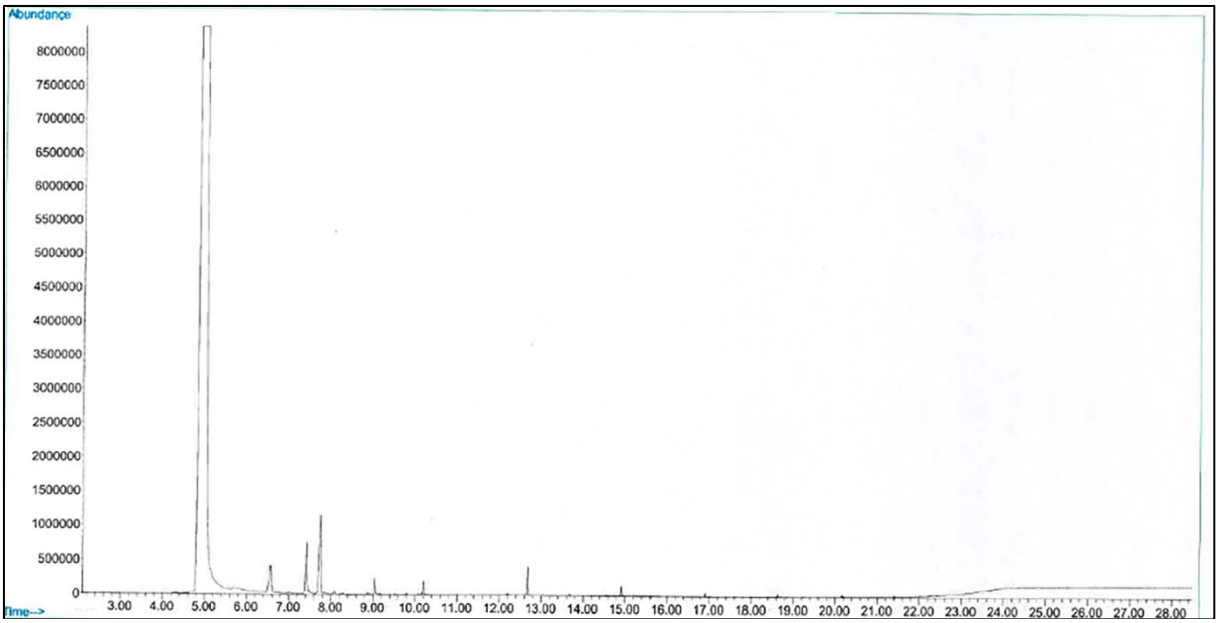


Figure 42: Extracted Ion Chromatogram (EIC) for the monomer ion at *m/z* 207

5.3.3 Qualitative Comparison of samples

70 silicone containing samples (here after: the sample set) coming from the bigger dataset presented in **Chapter 4** were analysed. The study of the pyrograms obtained for the 70 samples (containing condom, lubricants and personal hygiene products) revealed at least 6 different profiles, which are illustrated in Figure 43-48 respectively, which appeared to be indistinguishable when analysed using ATR-FTIR. With the exception of the profile in Figure 45, all profiles show the characteristic peaks of PDMS, or at least silicone residues. Most condoms presented a pattern as illustrated in Figure 43 with the exception of two condoms, Ceylor Gold and FairSquared Sensitive Dry, which presented slightly different chemical profiles, including the presence of PDMS oligomers (Figure 44). As these condoms were however already differentiated using their ATR-FTIR spectra, it is more likely that these profiles come from hydroxy-terminated PDMS. Profiles similar to those in Figure 45 were observed on FemFresh Sample, which indicates that they contain no silicone lubricant, or very few quantities that are not able to be detected. These condoms had already been differentiated based on their FTIR spectra.

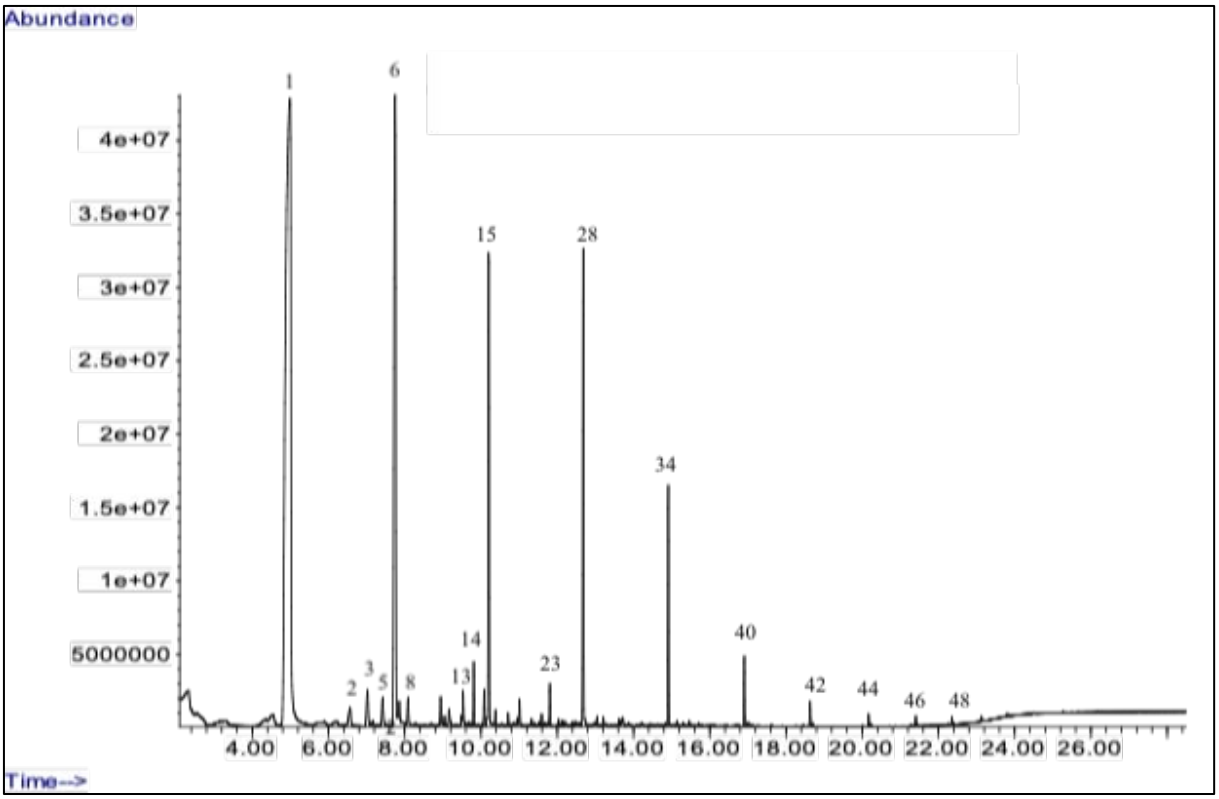


Figure 43: Chemical profile of silicones extracted from condoms samples, methylterminated PDMS. Reproduced from *Burnier C., Massonnet G, Coulson S, DeTata D, Pitts K, (2020) Characterization and classification of silicone-based compounds in condoms and personal hygiene products using pyrolysis-GC/MS, Manuscript submitted to Forensic Science International*

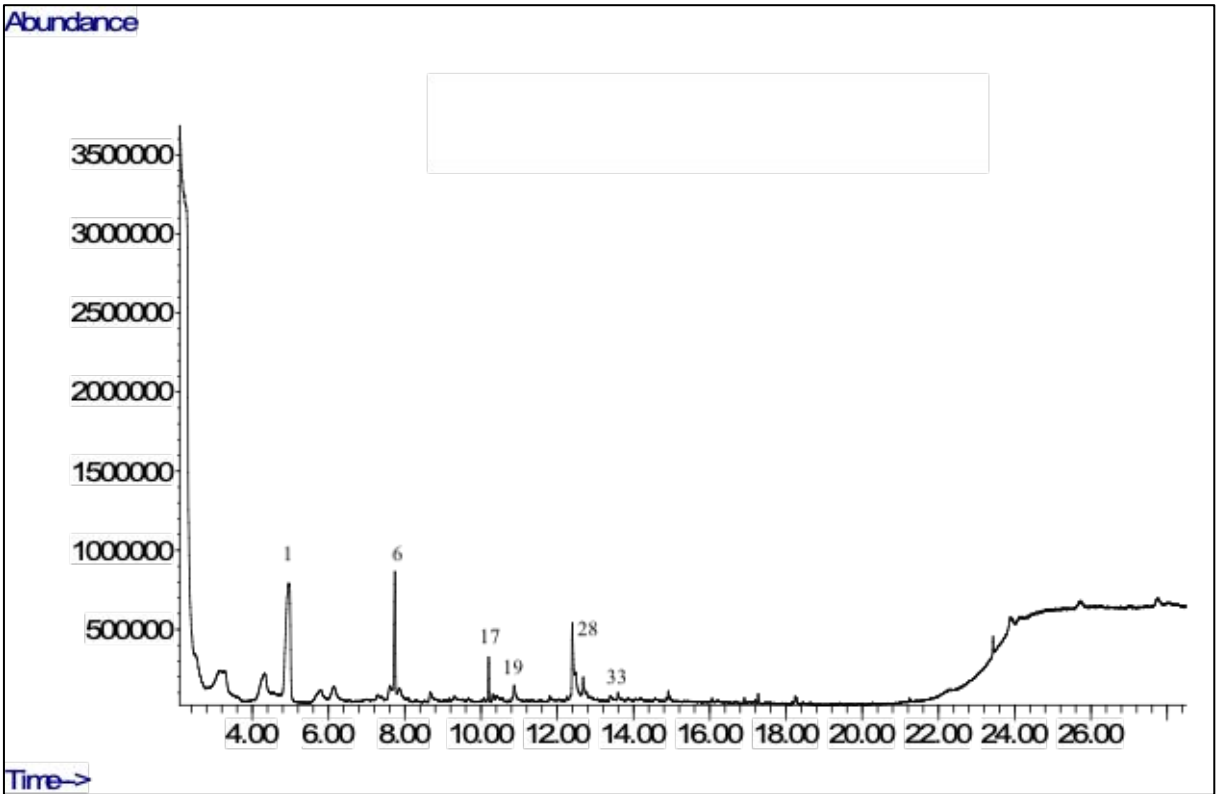


Figure 44*:* Chemical profile of silicones extracted from condom samples, hydroxy-terminated PDMS. Reproduced from *Burnier C., Massonnet G, Coulson S, DeTata D, Pitts K, (2020) Characterization and classification of silicone-based compounds in condoms and personal hygiene products using pyrolysis-GC/MS, Manuscript submitted to Forensic Science International*

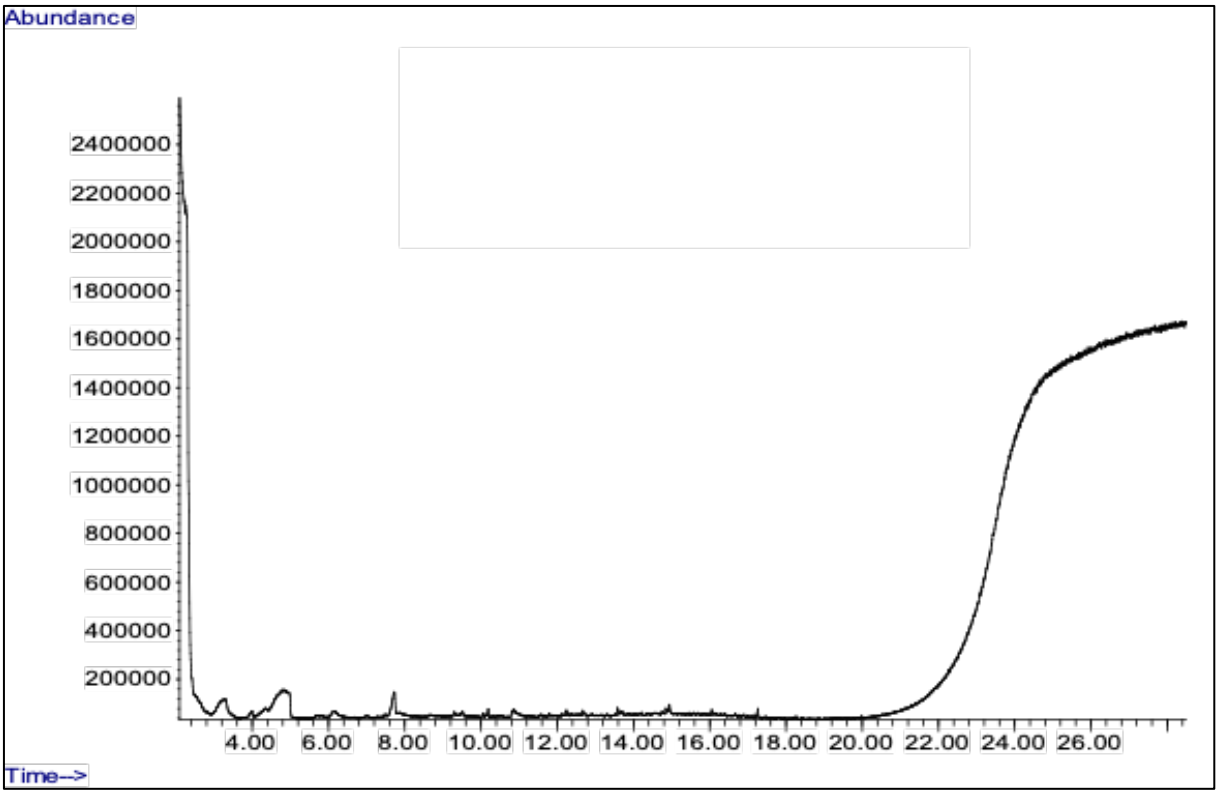


Figure 45*:* Chemical profile obtained for FemFresh Feminine deodorant. Reproduced from *Burnier C., Massonnet G, Coulson S, DeTata D, Pitts K, (2020) Characterization and classification of silicone-based compounds in condoms and personal hygiene products using pyrolysis-GC/MS, Manuscript submitted to Forensic Science International*

Amongst the silicone-containing personal lubricant populations, four different profiles were observed, one of them being indistinguishable to condom pyrograms (Figure 43) and the three others being visually discriminated (Figures 46-48) based on the presence or absence of peaks in the pyrograms. Lubricants indicate very different profiles from those found in condom extracts, notably by the absence of significant peaks used in the ion extraction macro to compare samples. Some of the observed peaks, particularly in Figure 47, between 17.00 and 24.00 minutes, are not present in Table 30 as they occurred in only one sample and hence were not included. These peaks presented similar mass spectra and different retention time. Comparison of the mass spectra with the NIST database suggested they were long chain silicones: respectively hexa- (15.712 min), hepta- (17.632 min), octa- (19.270 min), nona- (20.712 min), deca-(21.810 min) and undeca- (22.683 min) siloxanes. This indicates that silicones of different chain lengths, and therefore of different viscosities, were used for different products

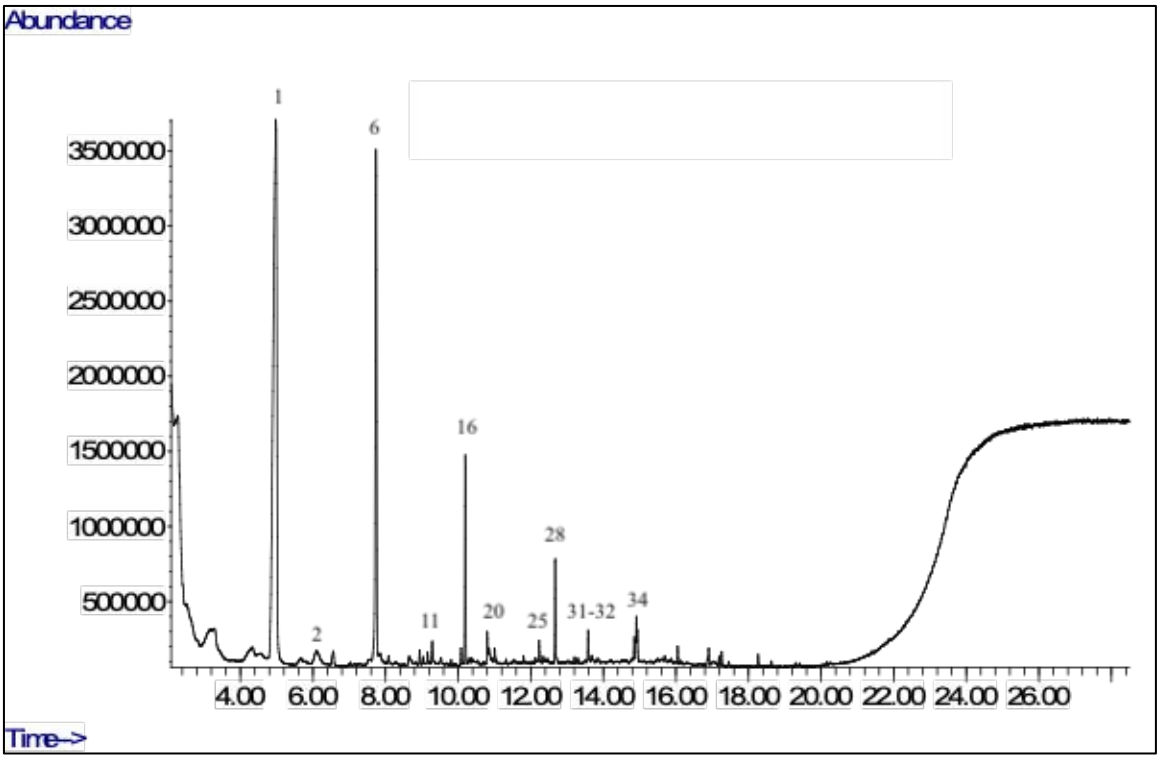


Figure 46: Chemical profile obtained for silicone lubricant, Ansell LifeStyles Luxe Silicone-based lubricant. Reproduced from *Burnier C., Massonnet G, Coulson S, DeTata D, Pitts K, (2020) Characterization and classification of silicone-based compounds in condoms and personal hygiene products using pyrolysis-GC/MS, Manuscript submitted to Forensic Science International*

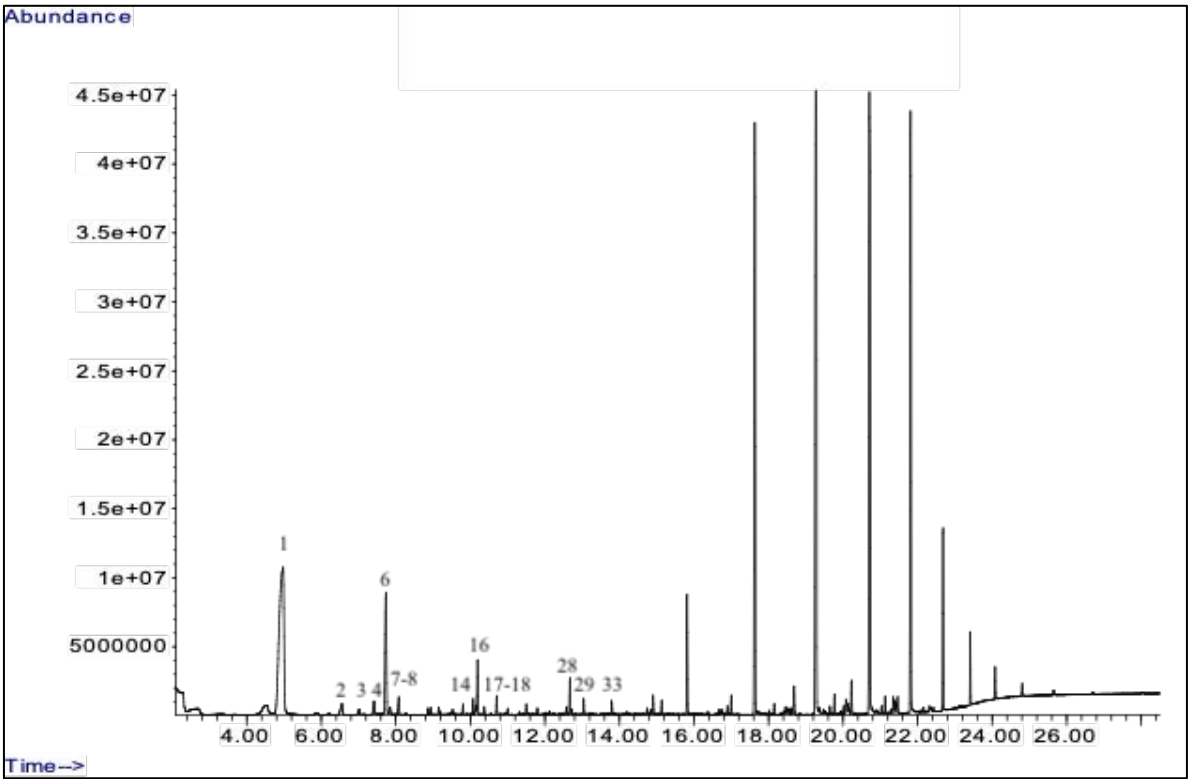


Figure 47: Chemical Profile obtained for silicone lubricant, Astroglide Diamond Silicone Gel Personal Lubricant. . Reproduced from *Burnier C., Massonnet G, Coulson S, DeTata D, Pitts K, (2020) Characterization and classification of silicone-based compounds in condoms and personal hygiene products using pyrolysis-GC/MS, Manuscript submitted to Forensic Science International*

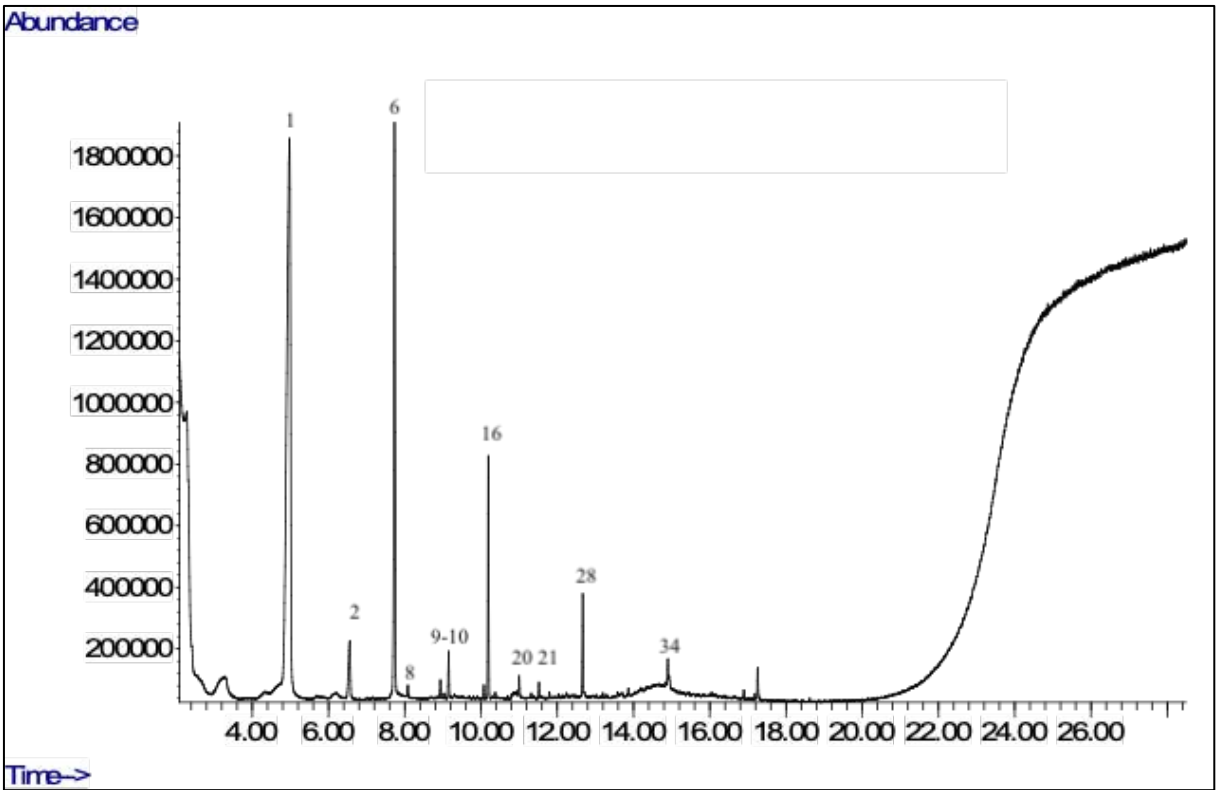


Figure 48: Chemical profile obtained for silicone lubricant, Astroglide Waterproof Silicone Liquid. . Reproduced from *Burnier C., Massonnet G, Coulson S, DeTata D, Pitts K, (2020) Characterization and classification of silicone-based compounds in condoms and personal hygiene products using pyrolysis-GC/MS, Manuscript submitted to Forensic Science International*

The observation of these different profiles within a population of silicone-based products with undistinguishable infrared spectra is a positive point. Indeed, the chromatographic profiles obtained in extracts from condoms are all indistinguishable, while those from other sources have very different visual profiles.

Chromatograms for multiple condom brands are presented in the Appendix X. Some condoms presented hydroxy-terminated silicone lubricants, which allowed the distinction due to a different distribution pattern. Since the silicone-containing condoms all resulted in indistinguishable pyrograms, regardless of brand or type, it may be assumed that minor compounds, such as flavours or dyes, were not extracted by hexane, due to their chemical properties. This was expected, due to the polarity of these compounds, and indicates that a polar solvent extraction followed by conventional GC/MS analysis should be considered in order to understand the exact composition of the sample. This may also further isolate compounds of interest for the discrimination of samples within classes. However, this must be handled with caution, as the likelihood of the transfer and persistence of these minor compounds is unknown, and they may potentially not be found in trace swabs. In addition, there is a risk that other compound coming from the vaginal matrix, the cotton swabs or other products would be extracted and could not be distinguished from the condom composition. This will be

investigated in **Chapter 9, 10** and **11**. Condoms are a mass-produced product in a regulated industry. Observations of visually indistinguishable profiles confirm the hypothesis that either very similar formulations are used by manufacturers or that all the PDMS used may come from one chemical manufacturer. Both hypotheses are possible as the number of PDMS suppliers around the world is unknown.

Although DNA is the most common evidence to be collected from condoms, lubricants can also be used for both investigative and evaluative purposes. If no DNA is detected, the presence of a condom lubricant can infer the use of a condom and provide a possible explanation for the lack of any DNA. In these cases, the py-GC technique can answer questions of interest as the presence of diagnostic patterns from condom lubricants can infer the use of a condom and potentially the profile can be linked to one of the condom profiles in the database. The chemical profiles obtained from condoms were indistinguishable between condom manufacturers, and the chemical profiles obtained from personal lubricants were, in most cases, distinguishable from the condom ones (Figures 46-48). However, the initial target molecule was found to be the same, i.e. PDMS. Therefore, the hypothesis that the manufacturers use the same source of PDMS for condoms may be true. These observations also suggest that the PDMS used in lubricants is different to that used on condoms, in terms of viscosity and chain length, which results in the generation of different chemical profiles. Also, pyrolysis is affected by the concentration of the sample, it is known that higher viscosity lubricants produced more higher molecular weight cyclic oligomers (such as D9-D13) than lower viscosity lubricants. In addition, it is known that peak area is linked to viscosity, the higher the viscosity, the higher the peak area of the cyclic oligomers, especially for the D3 oligomer. This is a very interesting point to consider when it comes to potential discrimination of the samples. The use of chemometrics and statistics was applied to evaluate the potential discrimination and classification of the samples constituting the dataset. Therefore, descriptive statistics and principal component analysis will be applied to the data and their results are presented in the next sections.

5.4 Market Study - Discrimination of the samples

Data gathered from samples acquired in section 5.3 will now be used to study the discrimination of the samples.

5.4.1 Extraction of the data

In order to compare the pyrograms obtained on the basis of semi-quantitative data, peak areas of the compounds found in the pyrograms were integrated and normalized. Semi-quantitative

evaluation was based on the coefficient of variation of the relative abundances of the target compounds, calculated on the whole set of replicates acquired for each sample. Normalized data as well as normalized and double square root pre-processed data were used. A threshold was set to decide if the repeatability was acceptable. If the coefficient of variation was lower than the threshold, the compound was considered as repeatable.

As it was not possible to know in advance which compounds would offer a high discrimination potential between the condoms analysed, it was decided to consider and therefore integrate the area of a maximum of compounds, covering the entire pyrogram. However, selection criteria were set up and the following information were taken into account:

1. Compounds should be present in all the replicates from a same sample.
2. Selection of the compounds to consider for area integration was previously reported but was mainly targeting the cyclic oligomers coming from PDMS degradation. Other compounds can be found in the pyrograms and can be of significant interest for the discrimination of samples.
3. Difficulties in the integration procedure can be encountered, specifically with compounds presenting very similar mass spectra and a close retention time. Manual integration will be used to correct integration errors. Nevertheless, the macro built for ion extraction was enhanced to limit as much as possible integrating compounds whose close neighbours present fairly similar mass spectra.
4. Targeted compounds should be easily distinguishable from the noise and present different mass spectra in terms of target ions and qualifiers or abundances in the main ions.

The macro developed with the method *2019_pyGC_CB_02.M* was used to integrate the 50 target compounds in the samples.

Most compounds had coefficients of variation above a threshold of 20%, with 48 out of 50 compounds being off-limits (over the 20% threshold) for standardized data and 40 out of 50 compounds for pre-treated data. The compounds which met the acceptability criteria were D3 and D4 oligomers for standardized data and the sequence of cyclic oligomers D3 to D9, respectively, as well as some minor compounds found in all samples. These observations suggest that some of the integrated compounds are not repeatable. Lack of repeatability can come from two main sources:

1. The absence of the compound in some samples could generate these variations.

2. The difficulty of integrating a peak linked to a compound, because the latter would be very close to background noise and therefore could be poorly integrated.

The second hypothesis is unlikely, as a manual correction procedure for the integration of each compound was been put in place during data extraction. Confirmation of the first hypothesis can be achieved by examining the distribution of coefficients of variation according to the sample rather than the compound. The majority of the coefficients of variation obtained for the different compounds were found to be higher than 20% for both normalised and pre-processed data. The variation in the proportion suggests that these compounds are not repeatable. However, before removing them from the model, it is strongly advised to evaluate if these compounds are helpful for discrimination purposes.

5.4.2 Variable selection for sample discrimination

A first PCA was run to investigate sample discrimination (data not shown). A selection process was undertaken because it was noted that a large proportion of the variables did not seem to play a major role in the separation of samples. The loading plots coefficient enabled identification of the compounds which had the greatest influence on sample discrimination. A reduction of variables was investigated on a small dataset made of 38 samples, to evaluate if the number of compounds to use for sample discrimination could be reduced.

Selection No. 1 was based on the consideration of only the cyclic oligomers of the PDMS. Since these compounds are present in virtually all samples and easily detectable, it is therefore interesting to understand if these compounds can be sufficient to discriminate between samples. Selection No. 1 thus contains 10 variables related to cyclic oligomers D3-D13.

Unlike the first selection, selection No 2 only takes into account the minor peaks in order to observe their impact in the discrimination of the samples. Selection No. 2 thus contains 40 variables related to these peaks present in different samples and which may be a source of the discrimination of the various samples.

Selection No. 3 was based on the removal of variables with a relative abundance of less than 1%, and therefore could easily be confused with background noise. These compounds are not recommended in the context of routine application, considering the need for a dedicated expert for this type of analysis. Thus, 22 variables were removed from the model, thus limiting the potential errors that may arise from poor integration. Selection No. 3 thus contains 28 variables in total.

Selection No. 4 was based on the loadings of the first three principal components. Only variables whose loadings had values greater than 0.1 or less than -0.1 for were retained.

Retained variables were D3, D4, D5, D6, Unknown 6.56, Unknown 7.02, Unknown 7.4, Unknown 7.86, Unknown 8.08, Unknown 9.80.

Table 14 shows the percentages of explained and cumulative variance for each of the four variable selections. The first three models failed to classify the samples separately with the replicas of a sample belonging to the same class grouped together. The first two models suggest that discrimination is based on a mixture between the major compounds and minor observed in the pyrograms. The groups formed using selections 1 to 3 are presented in Appendix VII. A replica of sample 109 (*Ansell LifeStyles Luxury Silicone-based lubricant*) was systematically separated from other samples, suggesting an outlier due to a problem during injection into the instrument. The model obtained with selection No 4 was found to be the most adequate considering the knowledge of the samples analysed. Not only was the entire variance fully explained by the first 7 principal components, but the classes are projected separately along the first three principal components, as shown in Figure 49.

Table 14*:* Percent of explained variance (EV) and cumulative variance (CV) for the four different variable selection

	Selection No 1		Selection No 2		Selection No3		Selection No4	
PC	%EV	%CV	%EV	%CV	%EV	%CV	%EV	%CV
1	70	70	41	41	43	43	56	56
2	14	84	21	62	18	61	21	77
3	6	90	13	75	10	71	10	87
4	4	94	7	82	7	78	7	94
5	2	96	5	87	5	83	4	98
6	1	97	4	91	4	87	2	100
7	1	98	2	93	3	90	0	100

Figure 49 shows that the model obtained with Selection No. 4 separates the samples into five major groups, each formed by replicas of the same type of samples. For 2 out of 33 condoms, the chemical profiles obtained were significantly different and were projected separately from the replicas obtained for the other condoms. Replicates of lubricants are all classified into two groups, mainly based on the presence of additional peaks: in fact, sample 171 was very strongly separated from the other lubricants analysed. Among condoms not containing silicone, two subgroups were observed, and labelled according to the sample analysed. The *Ceylor Gold* sample has less variability than the *FairSquared* sample. This is not unexpected knowing that the FairSquared sample already had a large dispersion during infrared analysis, due to its slightly different composition, as it is a non-lubricated condom.

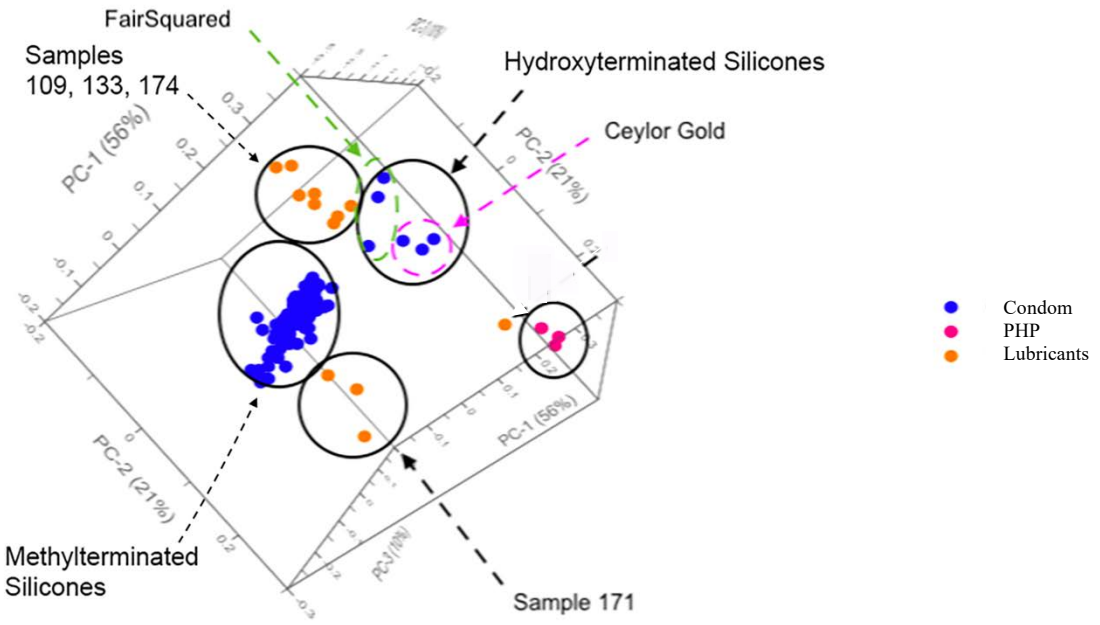


Figure 49: 3-dimensional scores plot showing the distribution of the data collected from the 37 samples constituting the dataset. along PC1, PC2 and PC3, after variable selection No 4. In blue are the condoms, pink the personal hygiene products and orange the lubricants.

5.4.3 Sample discrimination using extended dataset

A principal component analysis was undertaken on all the preprocessed data of the samples present in the data set and the variable selection no 4 (section 5.4.2). Only the preprocessed data (sum of the areas followed by the double square root) were considered, since these are the data that allow the best exploitation of the results. The goal was to visually explore the data structure to assess whether the samples in the dataset could be separated from each other. Since there are several samples belonging to the same class (i.e. condom, lubricant, cream), it is interesting to see whether the samples are grouped by class, or if, on the contrary, each sample is distinguished from the others, or even if samples from one class may be confused with others. The same procedure as that described in the previous chapter was used.

The percentage of variance explained as well as the percentage of cumulative variance for each of the principal components are presented in Table 15.

Table 15: Percentage of explained variance and cumulative variance for the processed data from the 70 samples in the dataset

Principal Component	Explained variance (%)	Cumulative variance (%)
1	57	57
2	18	75
3	11	86
4	6	92
5	3	95
6	3	98
7	1	99

Since the explained variances of calibration and validation are close (Figure 50), only the values for the calibration are presented.

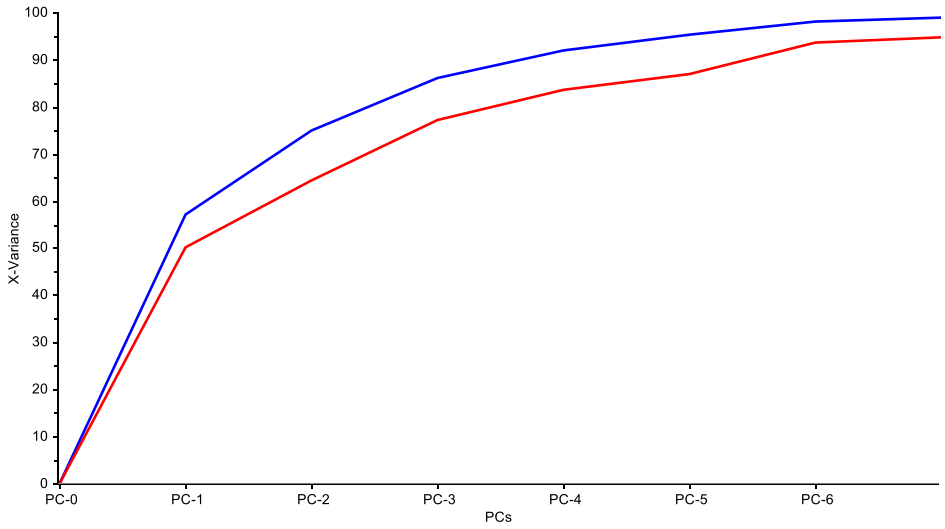
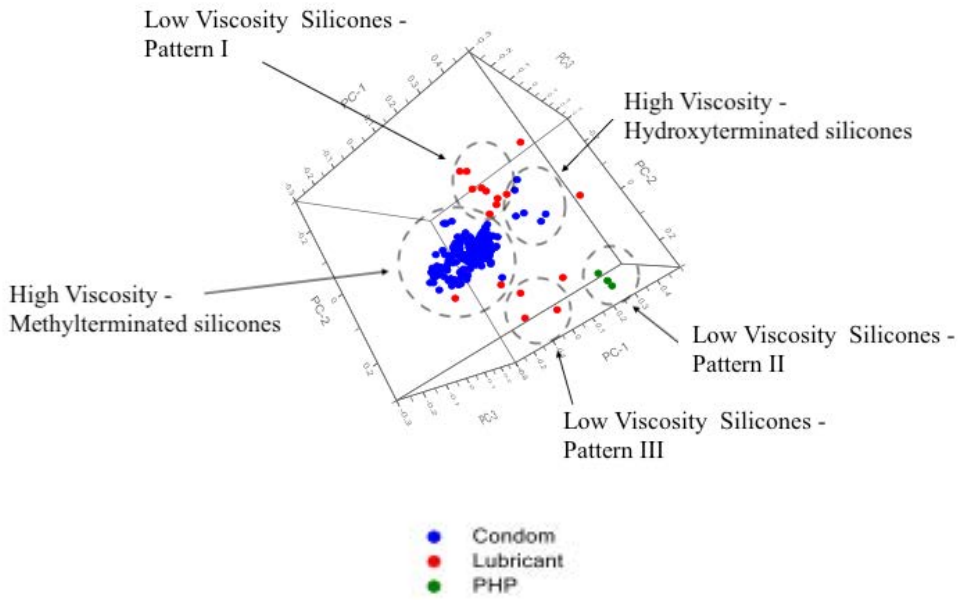


Figure 50: Scree plot depicting the cumulative variance in the dataset retained by each PC

The first four main components explain more than 92% of the variance in the data. This result is considered good. Figure 51 shows the scores for PC1-2-3 (Figure 51A) and PC 1-2-4 (Figure 51B). Discrimination was not enhanced using other principal components.

Note the presence of several groups in Figure 51. At least 5 groups can be seen in Figure 51A and at least 4 in Figure 51B. The clusters will be discussed here according to the sample classes.

A)



B)

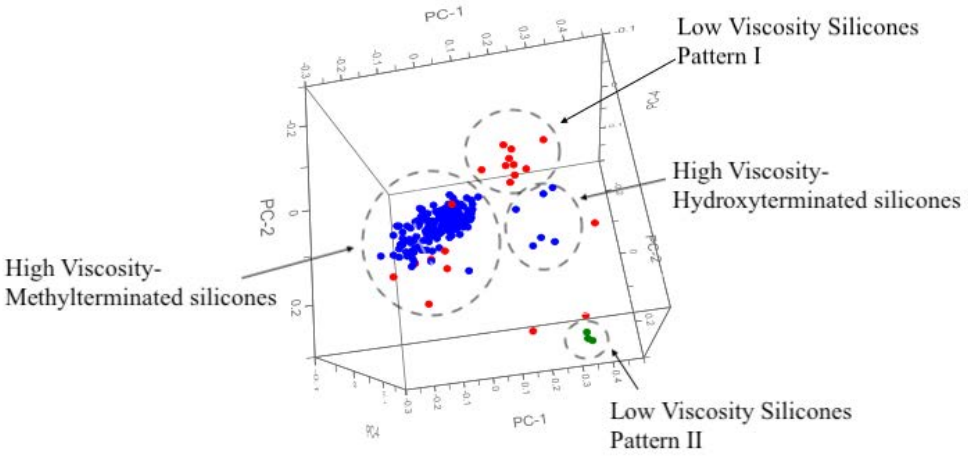


Figure 51: 3-dimensional scores plot showing the distribution of the data collected from the 70 samples constituting the dataset. A) Along PC1, PC2 and PC3, B) along PC1, PC2 and PC4. In blue are the condoms, pink the personal hygiene products and orange the lubricants. Reproduced from *Burnier C., Massonnet G, Coulson S, DeTata D, Pitts K, (2020) Characterization and classification of silicone-based compounds in condoms and personal hygiene products using pyrolysis-GC/MS, Manuscript submitted to Forensic Science International*

5.4.3.1 Sample Class – Condom

As illustrated in Figure 51, condom samples were found to form two clusters: the first one from samples presenting the clear diagnostic pattern of siloxane degradation, and the second containing only 2 samples (FairSquared Sensitive Dry and Ceylor Gold) presenting unclear patterns of siloxane degradation, with significant variations of peak concentrations (see Figures

44 and 45). In FTIR analysis, FairSquared Sensitive Dry did not present any evidence of the presence of silicone compounds, whereas Ceylor Gold presented a pattern different from the CH_3-PDMS (Figure 52), showed peaks consistent with OH-PDMS as presented in [118] as well as possible PEG contributions.

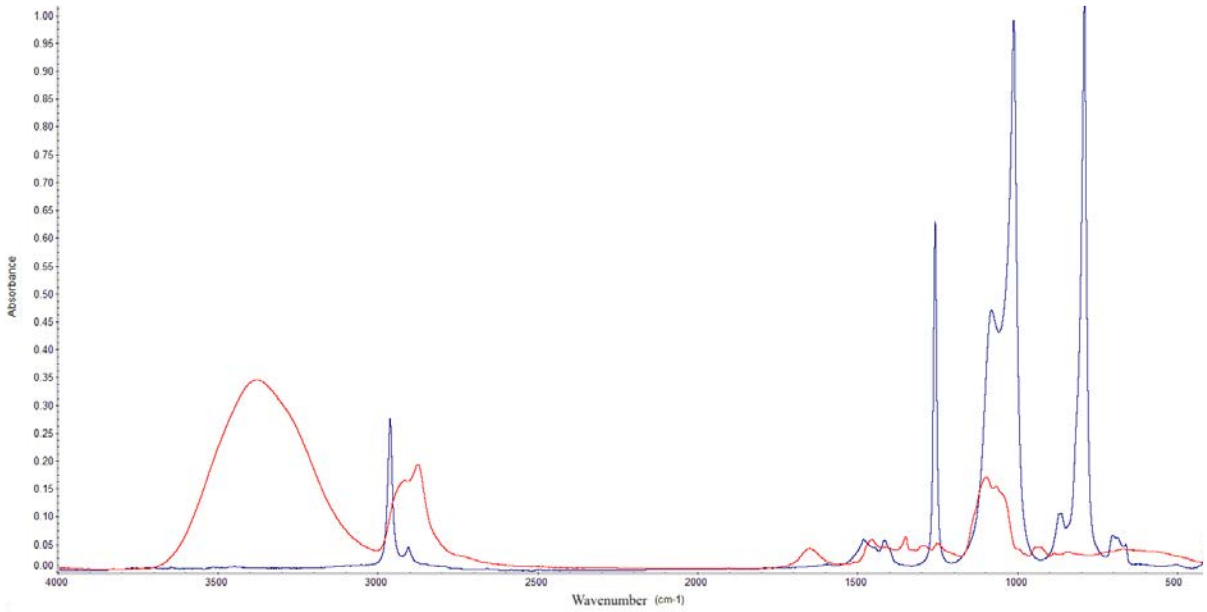


Figure 52: ATR-FTIR spectra of Ceylor Gold sample (in red), and a silicone-based sample (in blue) for comparison.

However, traces of cyclic oligomers D3-D6 were observed, in very low concentrations. No traces of solid particles, such as furfural, furaldehyde or furane derivatives, were found in the pyrolysis patterns. In the eventuality of the use of a dry condom in an alleged assault, stereomicroscopy and microscopy should be used to help detect these particles [14,34,59,133,134], as they are not being specifically extracted and/or detected using the hexane/Py-GC/MS protocol. These two samples are challenging, as their profiles might be misinterpreted as a "negative" profile when present in real world samples, given the low concentration of silicone lubricants. Hence, the absence of chemical residues should be carefully evaluated in the forensic context, with other techniques used to detect other types of compounds, or an evaluation of the factors affecting transfer and persistence of the samples in the matrix.

Within the cluster containing the majority of the condom samples, it was determined that one replicate from sample 40 (Durex Confidence) was slightly separated from the rest of the dataset, but not enough to be clustered with the other samples. It was found to be very close to lubricant sample 171 (Astroglide Diamond Silicone Gel Personal Lubricant). This replicate can be considered an outlier, possibly due to a cross contamination and was removed from the

discrimination models when using the classification algorithm (LDA, QDA, SVM). Replicates from sample 64 (Lifestyles Assorted Choc Ripple Ribbed) were found to be slightly separated from the major condom group, as their scores along PC1, 2 and 3 were observably different. Two replicates presented a positive value along PC1, whereas the rest of the condoms were found to have negative values along this PC. Similarly, values along PC2 for most condoms were around 0.002 and was over 0.1 for sample 64. The PC3 value for sample 64 was negative, whereas all other condoms had positive values. As the pyrogram for sample 64 was visually similar to all the other condoms, flavourings were not considered as the source of the observed classification difference. A possible hypothesis is that changes in the structure of the latex (i.e. ribbed condoms) generated variations in the amount of lubricant that can be added, resulting in this condom being distinguished from the rest of the sample set. This would not be surprising as py-GC/MS is known to be sensitive to the amount of sample used for analysis [94]. However, the other ribbed condom present (sample 184 – Ansell LifeStyles Ribbed Condoms) was not found to be distinguished from the rest of the dataset.

Therefore, except for condoms presenting visual distinguishable patterns, it was not possible to differentiate silicone-based lubricated condoms.

5.4.3.2 Sample Class – Lubricants

Lubricants initially presented several visually different patterns. The statistical model confirmed that it was possible to differentiate the samples, mainly into 3 groups. One lubricant, sample 181 (Durex Perfect Play Glide), was found to cluster with condom samples, and could not be separated with subsequent PCs. When examining the chemical profile, there was no difference noted between this profile and the ones coming from the condoms (Figure 53). It was the only lubricant that was classified with condom samples.

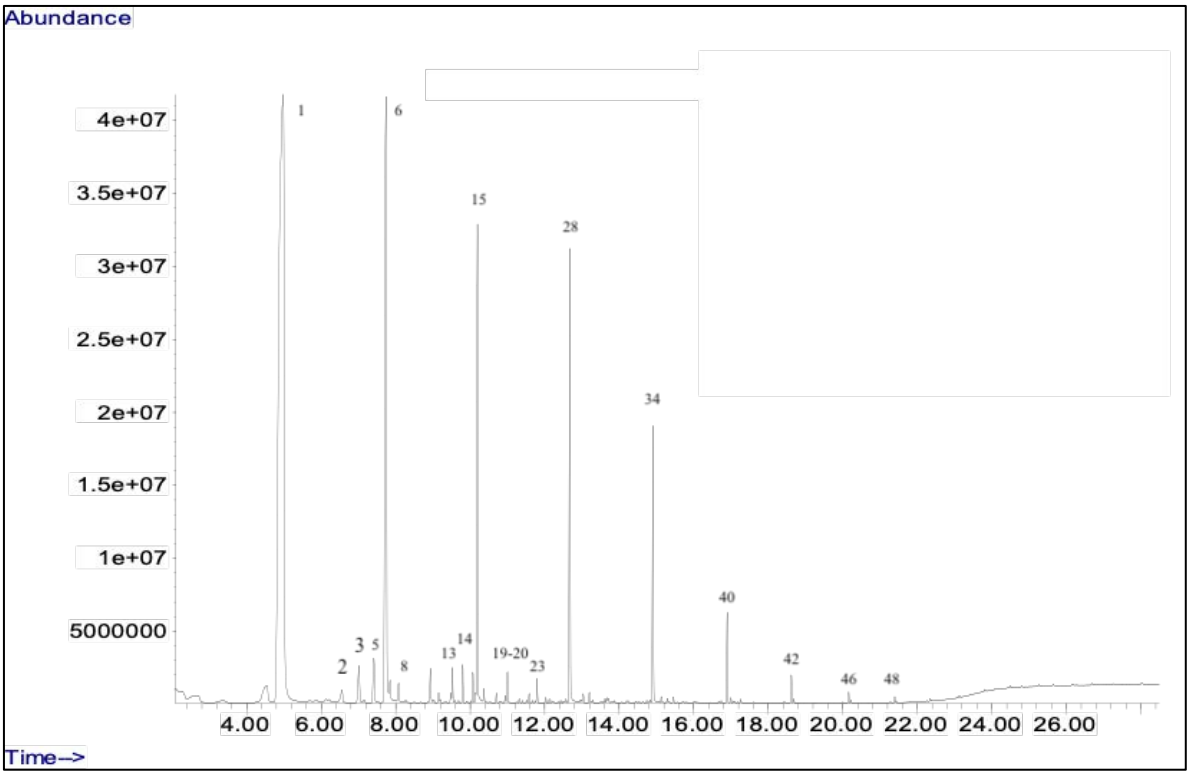


Figure 53: Illustration of the pyrogram of Sample 181. Overlay of the pyrogram with the ones obtained in Figure 43 did not highlight any significant differences.

Sample 171 (Astroglide Diamond Silicone Gel Personal Lubricant) was found to separate to an isolated cluster, close to the condom samples. However, PC2 and PC4 helped separate this sample from the condom sample set.

Three outliers from the groupings were noted, which were replicate 2 from sample 172 (Astroglide Gel Personal Lubricant), replicate 3 from sample 109 (Ansell LifeStyles Luxe Silicone-based Lubricant) and replicate 2 from sample 168 (Ansell Skyn Intimate Moments). These replicates were significantly spread and plotted away from the other replicates of the same samples, thus indicating that there may have been some variation, either at the acquisition of the chromatogram or during the extraction of the data procedure. These samples were not considered in the classification steps. Regarding sample 172, the pattern of silicone peaks was close to the background and hence, may not be detected in case work. In addition, sample 172 was a water-based lubricant, thus GC/MS analysis may be more appropriate for analysis and interpretation than py-GC/MS. Therefore, it was removed from the sample set when performing the classification process.

5.4.3.3 Sample Class – Personal Hygiene Products

The smallest class of the dataset, personal hygiene products, was under-represented in this model. Indeed, only one of the 70 samples available in the initial dataset presented a silicone-

based chemical profile when analysed with the screening method. The chemical profile obtained for this sample 156 (FemFresh Intimate Deodorant) was inconclusive, resulting in it being clustered significantly separately to the rest of the dataset. Replicates showed a higher variability than for the rest of the dataset, and the detection of silicones was found to be inconsistent between analyses. This might be due to its aerosol nature, which made it quite challenging to collect sufficient residues for analysis. However, from the chemical and statistical point of view, the residues from this sample were found to be distinguishable from the rest of the dataset.

5.4.3.4 Dataset Reduction

It was previously highlighted that 2 condom samples were previously identified as non-silicone-based products and easily distinguishable using ATR-FTIR spectroscopy, 3 replicates were outliers and personal hygiene products were not representative enough to be used when building a classification models, these samples were removed and PCA was rerun, to determine if other clusters could be observed. Results of the new PCA are presented in Figure 54. Good separation was achieved using the four first PCs accounting for 91% of explained variance. Further principal components did not enhance discrimination of the samples. The same pattern as the one observed on Figure 51 was observed. The lubricant clustered in Group 1 was Sample 181 – *Durex Play Perfect Glide*. Its chemical profile was found to be qualitatively indistinguishable from the ones obtained for methylterminated-PDMS detected on condom extracts (Figure 43).

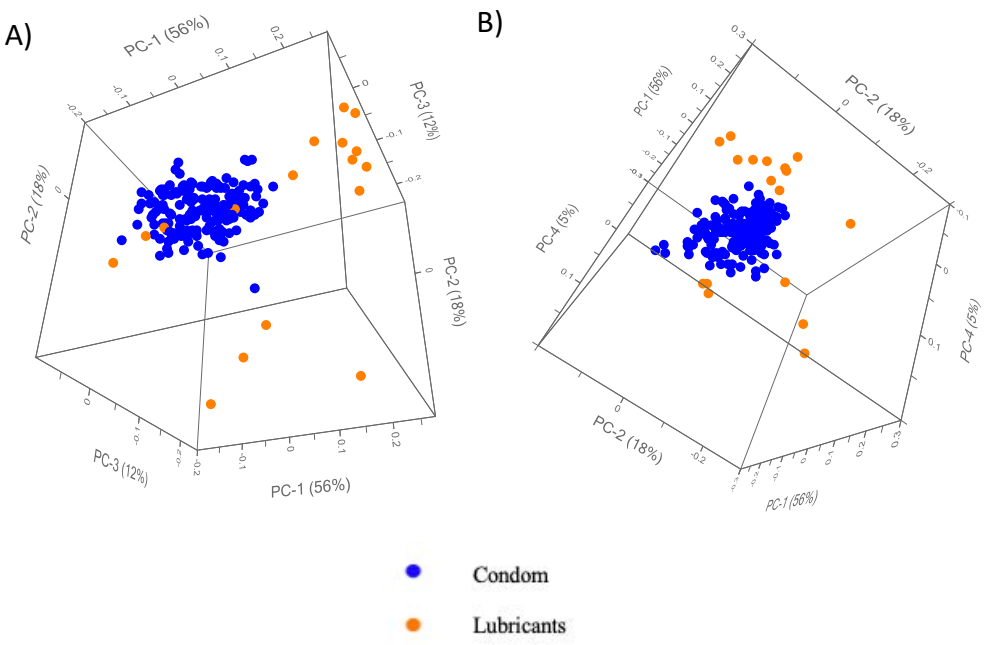


Figure 54: 3-dimensional scores plot showing the distribution of the data collected from the 70 samples constituting the dataset. A) Along PC1, PC2 and PC3, B) along PC1, PC2 and PC4. In blue are the condoms and orange the lubricants.

Finally, all the seventy samples constituting the dataset were grouped in the five categories that were finally observed on the overall dataset and the lists are gathered in Table 16.

Table 16: Summary of the sample(s) comprised in each grouping

Group	Source of the sample	Samples in the group
Group 1	Condom (59) Lubricants (1)	Samples 1, 10, 11, 12, 13, 14, 20, 23, 24, 33, 34, 36, 38, 40-50, 53-56, 58-66, 68, 69, 79, 110-115, 117-123, 125-127, 130, 178 - 184
Group 2	Lubricants (5)	Samples 109, 133, 168, 172, 174
Group 3	Condom (2)	Samples 116, 124
Group 4	Lubricants (1)	Sample 171
Group 5	PHP (2)	Sample 156, 158

5.5 Classification and prediction models

Since it has previously been demonstrated that it was not possible to separate the samples on the basis of brands and/or models, the classification models were built on the basis of only the classes of the samples, i.e. condom, lubricant, cream, oils and intimate hygiene products. The parameters selected for the production of discriminant analyses are presented in Table 17. Two models of discriminant analysis were tested (LDA, QDA) as well as an SVM classification

Table 17 : Parameters used for LDA

Variable weight	1.0 for each variable
A priori probabilities	Equality assumed
Method	Linear Quadratic

The replicates of the seventy samples analysed for the discrimination purposes were classified according to the class to which they belong. Thus, the samples are grouped into three categories. LDA was applied to the scores of the first four principal components[‡], since these were necessary for the proper separation of the samples. All the discriminant analyses were processed assuming equal probabilities for each sample. The training and validation sets were automatically defined by The Unscrambler X. The groups were created using the replicates of each samples, considering 7 lubricants (17 replicates removing the outliers), and 61 condoms (173 replicates after removing the outliers). Personal Hygiene Products were not used in the models.

The application of linear discriminant analysis to the whole of the replicas made it possible to obtain a good classification rate of around 92.11%. The application of the discriminant analysis to the entirety of the replicas obtained a good classification rate of around 97.37 % and the SVM analysis gave a classification rate of around 96.32% on the calibration set and 96.31% on the validation set. The confusion matrices linked to each of these respective models are presented in Table 18.

Table 18:Confusion matrix for discriminant analysis applied on the integrality of the replicates of the 70 samples, classification based on the class A) using LDA algorithm, B) using QDA algorithm, C) using SVM algorithm

A)	Condom	Lubricant
Condom	163	5
Lubricant	10	12

B)	Condom	Lubricant
Condom	169	1
Lubricant	4	16

C)	Condom	Lubricant
Condom	171	7
Lubricant	2	10

Quadratic discriminant analysis provided the best results in terms of sample classification. The misclassified replicates were studied, and the following observations were made:

- One replica of Sample 40 (Durex Confidence) was classified in the lubricant category. The PCA results indicated a very close proximity between the chemical profile of this replica and that of sample 171. The replicate of sample 40 which was misclassified was the closest to the replicates of sample 171.

[‡] This is due to the use of The Unscrambler, which needs the PCA to proceed to LDA. The choice was made as this project aimed to be used by ChemCentre (Perth, WA), a laboratory that use The Unscrambler X for data processing.

- Two replicates of sample 64 (Ansell Lifestyles - Assorted - Choc Ripple Ribbed) were classified in the lubricant category instead of the condom category. These samples were not outliers. The results of the PCA showed that the chemical profiles of these condoms were slightly separated from the condom population to which they were expected to belong, although it was not possible to assign them to another class. The centroid of the condom group was found to be located around 0.005 along PC1 and -0.018 along PC2, whereas the lubricant group was located around 0.17 along PC1 and -0.06 along PC2. Classification values obtained for the two replicates were found to be negative for clustering to the condom group and were found to be positive for clustering with the lubricant group, the distance being 0.05 to the condom group, and 0.11 to the lubricant group for the first replicate, and 0.04 and 0.12 for the second. The third replicate presented eigenvalues of -0.007 and 0.03 along PC1 and PC2, making this sample closely clustered with the condom class. Given the difference in coefficient of distance to the centroid, this suggests that an additional class should be suspected. As previously stated, such a class could be due to changes in the structure of the latex (i.e. ribbed condoms), which would generate variations in the amount of lubricant that can be added to it, thus they could be distinguished from the rest of the sample set.
- A replicate of Sample 60 (Ansell Lifestyles - Assorted - Banana Bump Studded) was classified into the lubricant category, instead of the condom category. Visual examination of the chemical profile did not allow it to be distinguished from the rest of the condom population. The results of the PCA showed that the chemical profile of this condom was slightly separated from the condom population to which it was expected to belong. However, the other 2 replicates were clustered appropriately within the condom population. These observations correspond to the previous ribbed sample that also had classification issues, reinforcing the hypothesis that changes in the structure of the latex (i.e. ribbed condoms), could generate variations in the amount of lubricant and therefore in the discrimination and classification patterns, due to the sensitivity of py-GC/MS to changes in sample concentrations. Visual observations of the pyrograms did not allow to detect any differences in the data that would explain such false classifications.
- One replicate of Sample 168 (Ansell Skyn Intimate Moments) was classified in the condom category, instead of the lubricant category. The PCA results indicated proximity between the chemical profile of this replicate and that of the condom population. The classification results are thus compatible with what was observed for

the PCA, and it was not surprising that the classification model was not able to correctly classify this sample.

The three replicates of sample 181 (Durex Perfect Play Glide), which presented a chemical profile indistinguishable to the condom ones, were correctly classified in the lubricant category. Visual analysis and observation of the PCA scores plot indicated that the chemical profile of this silicone-based lubricant was indistinguishable to the chemical profiles obtained for condom-type samples. However, the QDA algorithm correctly clustered these samples in the lubricant classes. Evaluation of the eigenvalues showed out that the separation was led along PC4, with the condom groups presenting an average value of 0.00029 and the lubricants one of 0.023. The sample eigenvalue was 0.04 (±0.01) which makes it cluster to the lubricants class. This is surprising, since it would have been reasonable to expect these samples to be misclassified. The algorithm was able to differentiate samples that were very close during the principal component analysis, however this may not be the case when additional samples are added to the dataset. In addition, the distance between this sample and the centroid of the lubricant samples is smaller than the distance between the sample and the centroid of the condom samples, which is not surprising considering the variability coming out of the condom cluster. These observations reveal that QDA is reinforcing the quality of the classification procedure, as visual analysis of the data, or investigation of the eigenvalues might not be sufficient to classify the samples in the correct classes.

The results previously discussed show that the quadratic discriminant analysis provided results corresponding to what had been observed for PCA. Most false classifications were one of the replicates of a sample having slightly variable characteristics, leading to a correlation to samples within the population of another class. The study of misclassifications, supported by the results of the PCA, indicates that these samples generally differ not in terms of their visual chemical profile but in semi-quantitative terms. These differences can generally be explained by analytical and operator variations, such as spiking reproducibility or manual integration of the peaks, especially considering the great variability that occurs in pyrolysis events. Variations in the quantitative amounts present in the various samples may also explain the observed variations, although the chemical profiles do not differ significantly.

The classification model based on classes of silicone-based samples can thus be validly used to predict the class (i.e. condom or lubricant) of a trace whose origin is unknown. Two limitations to the use of this model can be encountered. The main limitation is that the detection of the fifty peaks used to build the model may not be present in real cases, since the interaction with the vaginal matrix has not, at present, been fully examined. A focus on the major cyclic

oligomers is recommended, and this is the reason why the presented model was built with only 10 out of 50 compounds. The second limitation is that the proportion of lubricants and intimate hygiene products based on silicones is relatively low and consequently, other samples present on the market may contain different chemical profiles. On the contrary, the condom population included different brands and types, flavoured and coloured samples, those containing specific additives, with or without latex and a range of prices to be the most representative. However, a comprehensive model may not be feasible, the number of brands and types on the market being relatively large (more than 200 products on the Swiss market, and almost as many on the Australian market). In addition, new products are frequently released on the market and hence a continuous update of the model may prove necessary, although the list of authorised lubricants is not constantly changing.

The discriminant analysis models were used to assess the possibility of statistically differentiating the samples from the dataset, based on their chemical profiles. The main conclusion from these models is that condoms of different brands and types, the lubricant of which is based on silicones, are generally not differentiable. Samples of different classes that do not differ qualitatively can be differentiated. Samples of different sources (condom, lubricants, PHP) that show slight differences in the level of minor compounds can generally be differentiated, but only if these compounds are detected. Application to a couple of casework samples were analysed and revealed that these minor compounds were also observed when trace evidence was analysed (see Chapter 7).

Condoms constituting the data set came from different brands and models, thus including samples that were flavored, colored, contained specific additives, with or without latex, more or less expensive or being as classic as possible. In view of international regulations concerning the manufacture and production of condoms, it is therefore understandable that the classification model is not able to construct rules which would allow samples to be classified according to these various parameters.

Although the results of the qualitative analysis did not reveal any profiles specific to a type of condom or a brand, it was of interest to investigate potential brand discriminations. The results of the PCA did not reveal any features that would allow separation by brand or type (Figure 55). The chemical profiles of different condoms do not differ significantly between brands, nor do the chemical profiles of condoms of different types within the same brand. These findings indicate that the variation between the different condom manufacturers is very small. This is

likely due to the very high level of control and international regulations for the production of condoms and limited PDMS suppliers.

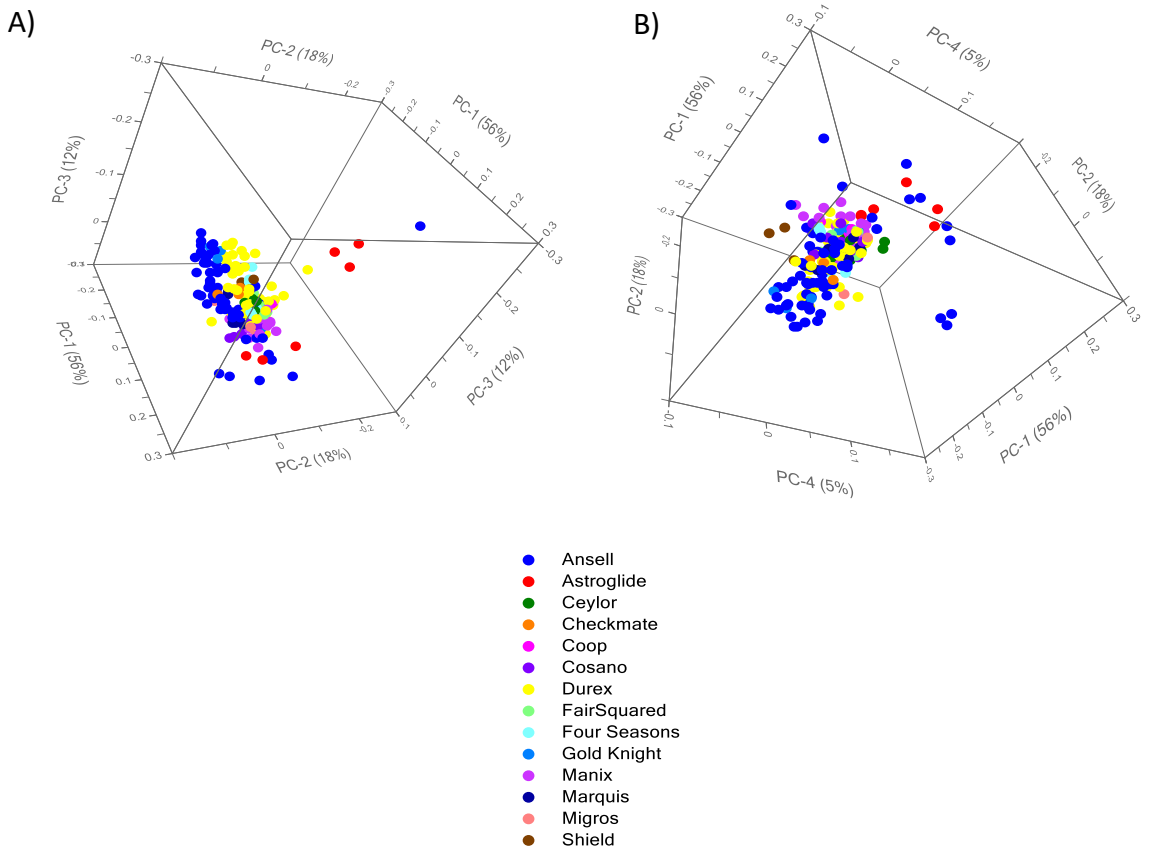


Figure 55: 3-dimensional scores plot showing the distribution of the data collected from the 70 samples constituting the dataset. A) Along PC1, PC2 and PC3, B) along PC1, PC2 and PC4. Classification based on the brand.

Under these conditions, the classification model has 14 categories. LDA and QDA were not possible because some classes ended up with significantly too few samples. The classification performed with an SVM model gave a good classification rate of approximately 54.74%, respectively 51.05% for cross validation. The classification rate here is not satisfactory but it is not surprising in view of the PCA.

5.6 Considerations on sample dilution

The model presented above was constructed considering complete profiles, with distinct peaks of background noise and easily integrable. However, it must be considered that traces collected in a real case do not have such a clear profile, due to, among other factors, persistence in the vaginal matrix and the time elapsed between the moment of the report and the sampling. Traces will, therefore, by definition be fragmentary and the main problem encountered will be the impact of dilution on the potential for assignment to a sample class.

In order to evaluate the impact of the dilution on the chemical profile and consequently on the classification potential, 6 samples were arbitrarily selected among the different classes and then diluted until the profiles were so close to the background that the integration of the peaks became difficult. In total, 1 condom (sample 01) and 5 lubricants (samples 109, 133, 171, 174 and 181) were analyzed. Considering that the initial analysis used for the model was 1: 10 (estimated concentration of 1-2 mg/mL for condoms and 1 mg / mL for lubricants), the dilution steps were 1: 50, 1: 100, 1: 500 and 1: 1000, which corresponds to estimated concentrations of 0.5, 0.1, 0.05 and 0.01 mg/mL respectively.

5.6.1 Visual Evaluation

The pyrograms obtained from the 6 aforementioned samples were observed and compared with pyrograms previously acquired with the 1:10 dilution, which was necessary to avoid overloading the column. The acquired pyrograms are available in Appendix VIII.

The chemical profiles obtained from Sample-01 (condom) and Sample-181 (lubricant), which are high viscosity methylterminated silicones, were not affected by the 1:50 dilution. The profiles were complete, all the expected peaks were present, and the relative abundances not significantly modified.

The profiles obtained from the 1:100 dilutions did not seem to present any major differences vis-à-vis the profile obtained for the previous dilution. However using the chromatogram overlay one can observe that several minor peaks are no longer detected. The profiles obtained for the 1:500 dilutions of these same samples showed a very strong impact of the dilution, with a significant decrease in the abundance of cyclic oligomers, where only oligomers D3 to D8 still detectable (Figure 56). Minor peaks from 11 minutes disappeared in the background noise and were no longer integrable. It is also observed that the pattern is slightly modified, with for sample 181 more minor peaks than for sample 01.

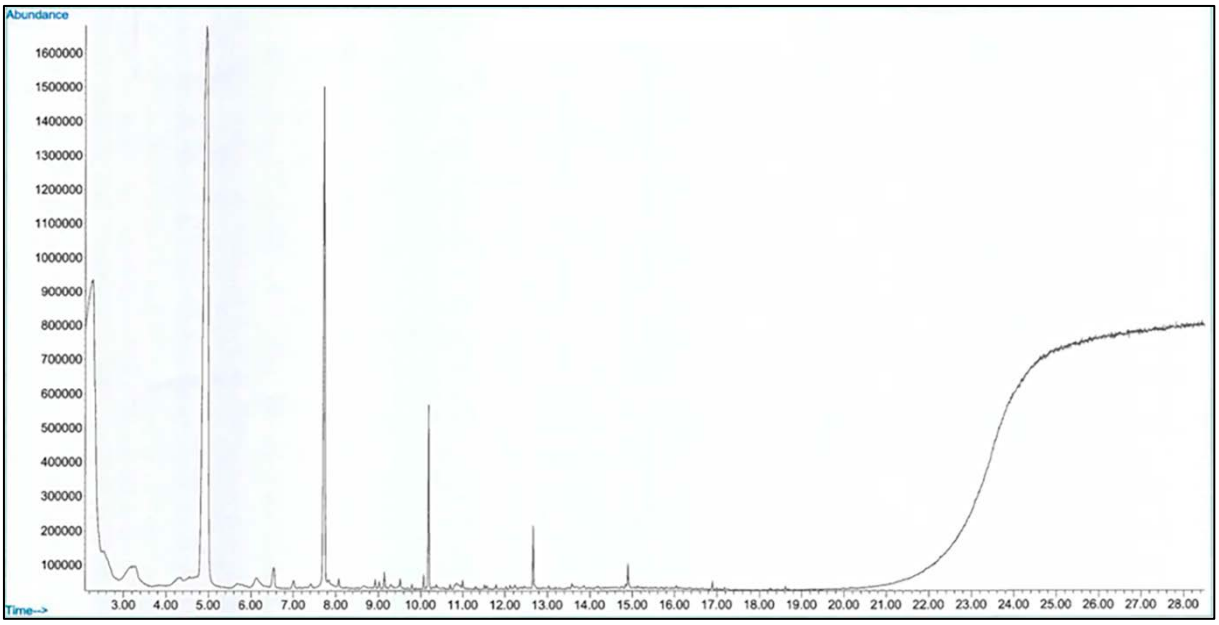


Figure 56: Illustration of the chemical profile of a condom sample (Sample-01) after 1:500 dilution

Finally, the profiles obtained from the 1:1000 dilutions showed only 3 distinct peaks, with relative abundances of about 90,000 AU. These peaks were D3-D5 cyclic oligomers (Figure 57).

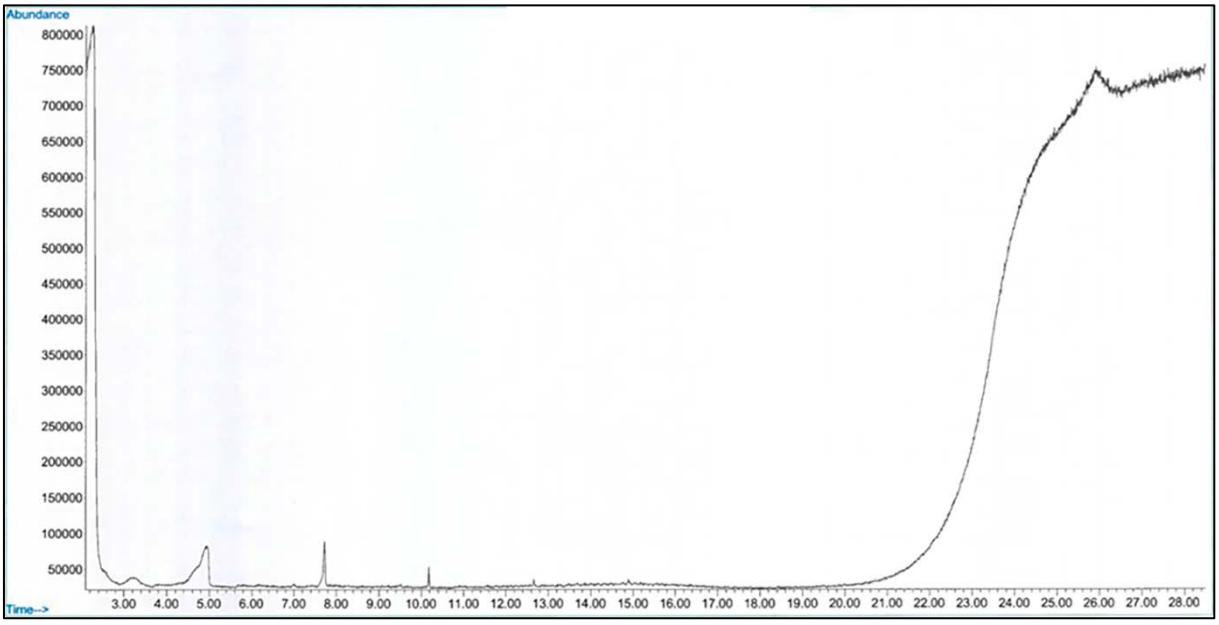


Figure 57: Illustration of the chemical profile of a condom sample (Sample-01), after 1:1000 dilution

The abundance threshold to define that a peak is distinguishable from the background noise is 30'000 AU. At this dilution level, the peaks were still distinguishable from the background noise. Further dilution would make the peaks completely undetectable. The condom type samples therefore have a detectable profile when diluted up to 1000x (i.e. at an estimated concentration of about **0.01 mg/mL**). At this stage, only three characteristic peaks were still

visible and the classification of these profiles as belonging to the condom samples has yet to be evaluated.

With regards to sample 171 (lubricant), the same findings can be made as for samples 01 and 181. In fact, the profile is not significantly affected by the 1:50 dilution, but the 1: 100 dilution exhibited a large difference, due to loss of the distinctive elements of this sample, especially at the end of the chromatogram (Figure 58). Indeed, the 1:10 dilution chemical profile obtained contained 8 peaks between 16 and 24 minutes, whereas the 1:100 dilution chemical profile only presents 4 peaks between 19 and 23 minutes.

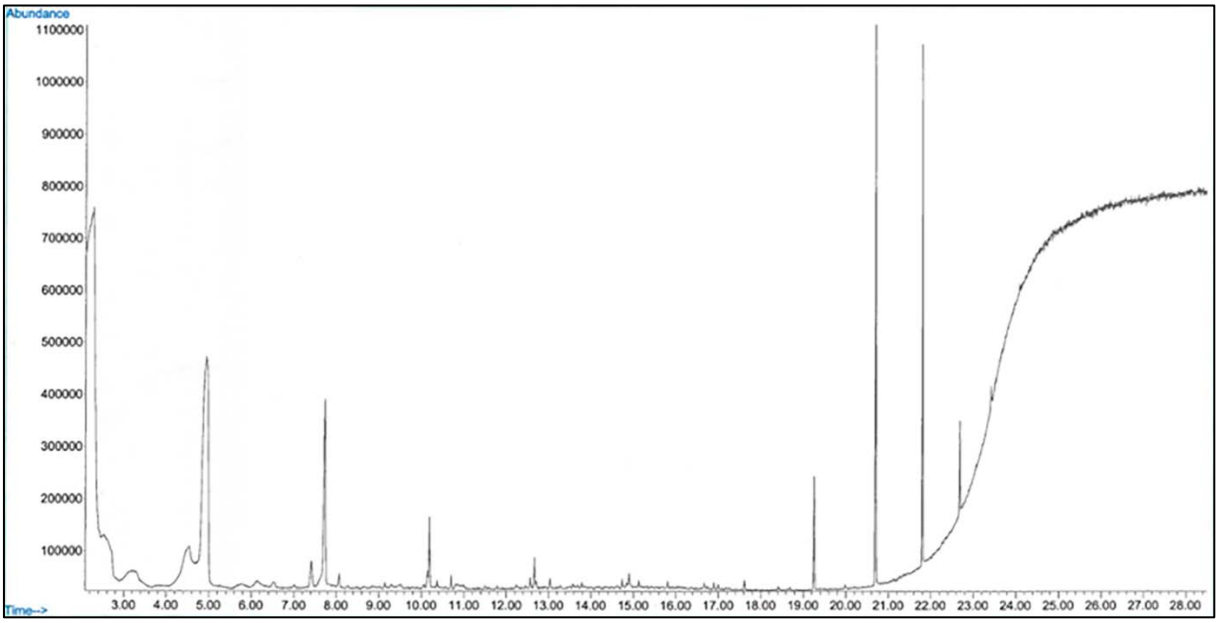


Figure 58: Chemical profile obtained for Sample 171 after 1:100 dilution.

The 1: 500 dilution revealed an even greater loss of the minor components, and the profile had only 3 cyclic oligomers and 3 characteristic peaks at the end of the chromatograms, between 19 and 23 minutes. Finally, with a 1: 1000 dilution, the D3-D5 oligomers were still present, as were the 3 peaks at the end of the chromatograms (Figure 59).

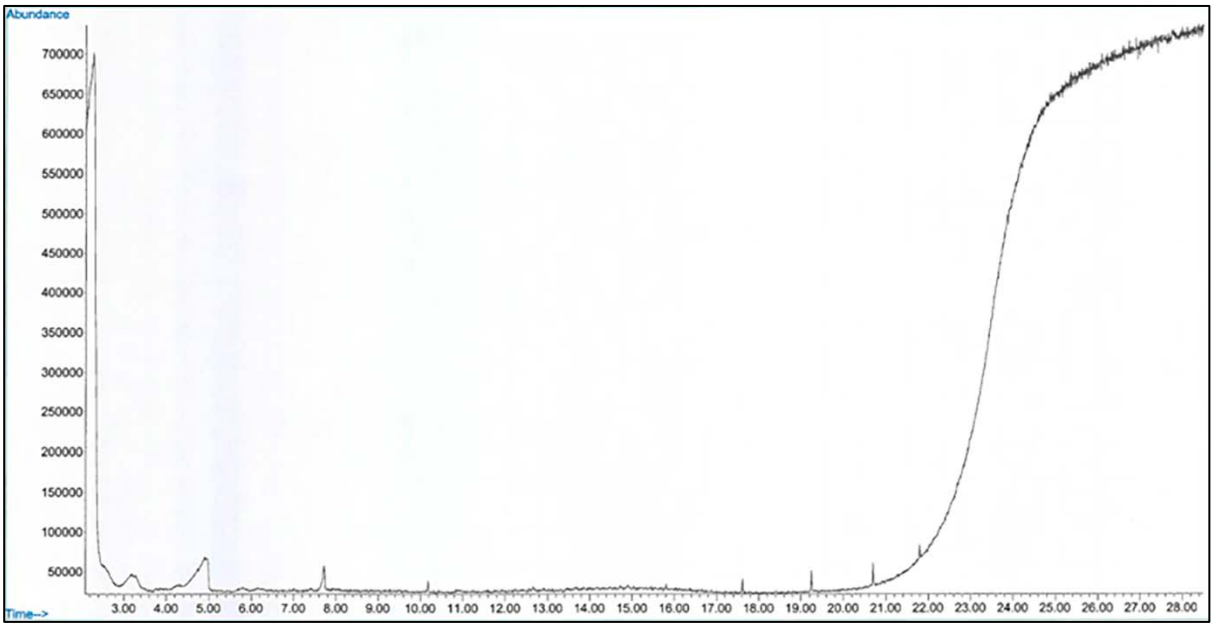


Figure 59: Chemical profile obtained for Sample 171 after 1:1000 dilution

The only major difference was their relative abundance, which was less than 90,000 AU, which indicates a closer approximation of the assigned limit for considering peaks as background noise.

Finally, the 1:50 dilution very strongly impacted the profiles obtained for the samples 109, 133 and 174. Indeed, the relative abundance of the peaks was almost reduced by 10, whereas the dilution was only 5x compared to the previous analysis. The profiles obtained with the dilution 1:100 only show traces of the peak D4 in the samples and the 1:500 and 1:1000 dilutions present the characteristic profiles obtained in the blanks of the analyses. Indeed, the relative abundances of the peaks present, if any, were lower than 50,000 AU.

Thus, observations indicate that lubricants and personal care products are more readily affected by dilution than lubricants extracted from condoms. In addition, very diluted samples all look fairly similar visually (Figure 60), except for samples with distinct features, such as Sample 171.

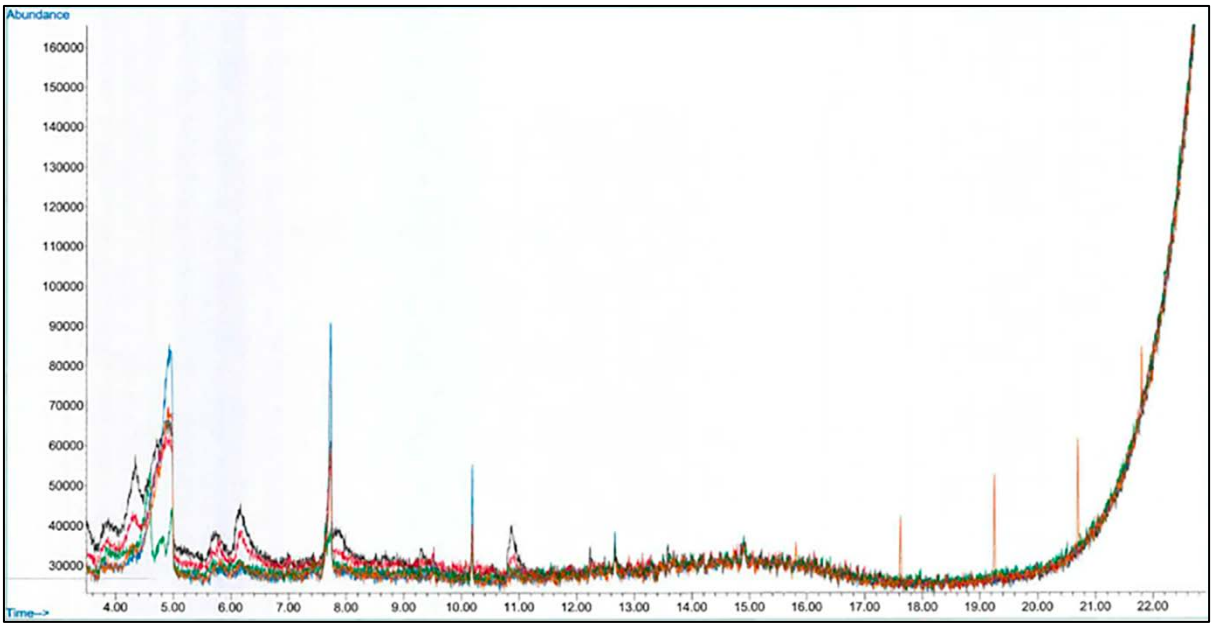


Figure 60: Comparison of the chemical profile for 5 different samples after 1:1000 dilution

Dilution questions are also relevant when questioning which pyrolysis compound(s) need to be detected or a sample to be identified as containing PDMS. This is a particularly important question when considering casework samples which are very likely to be weak samples. As it has been illustrated in Figures 56 and 57, condom samples keep being very abundant even after strong dilutions: the important peaks from cyclic oligomers D3, D4 and D5 are still present, and well defined from the background of the instrument. D3 being the smallest oligomer obtained from the degradation of silicone oil, it is the most likely compound to be recovered. However, it does not inform on the type of silicone. It would just be used to assess the presence of silicone in a sample. Further investigation of different types of silicones should be led to ensure that D3 would be specific, and that it can not be obtained from the degradation of other chemicals.

As illustration in figures 56-60, there are strong modifications of the chemical profiles, which suggest that classification errors might occur with very diluted samples in the previously constructed model. The compounds used to create the sample discrimination model were integrated into the set of pyrograms obtained and a semi-quantitative evaluation by projection into the model is discussed in the next section.

5.6.2 Semi-Quantitative evaluation

To investigate the limit of detection of the instrument on given samples, dilutions were used and modeled using the peak area of D3 as a function of the dilution and the sample, also considering the initial dilution used for the construction of the statistical model, i.e. 1:10. Figure

61 illustrates the decrease in peak area as a function of dilution. As noted during the visual evaluation of the chromatograms, samples that do not come from condoms are more affected by the dilution than the others, a 1:50 dilution already generating very large losses. Condom-type samples were significantly affected by the 1:1000 dilution, at which sample differentiation was no longer possible. Therefore, it would be recommended, if a blank profile is observed, to reanalyze the sample without dilution, or with a lower dilution so as to be able to confirm the observed profile and the absence of peaks.

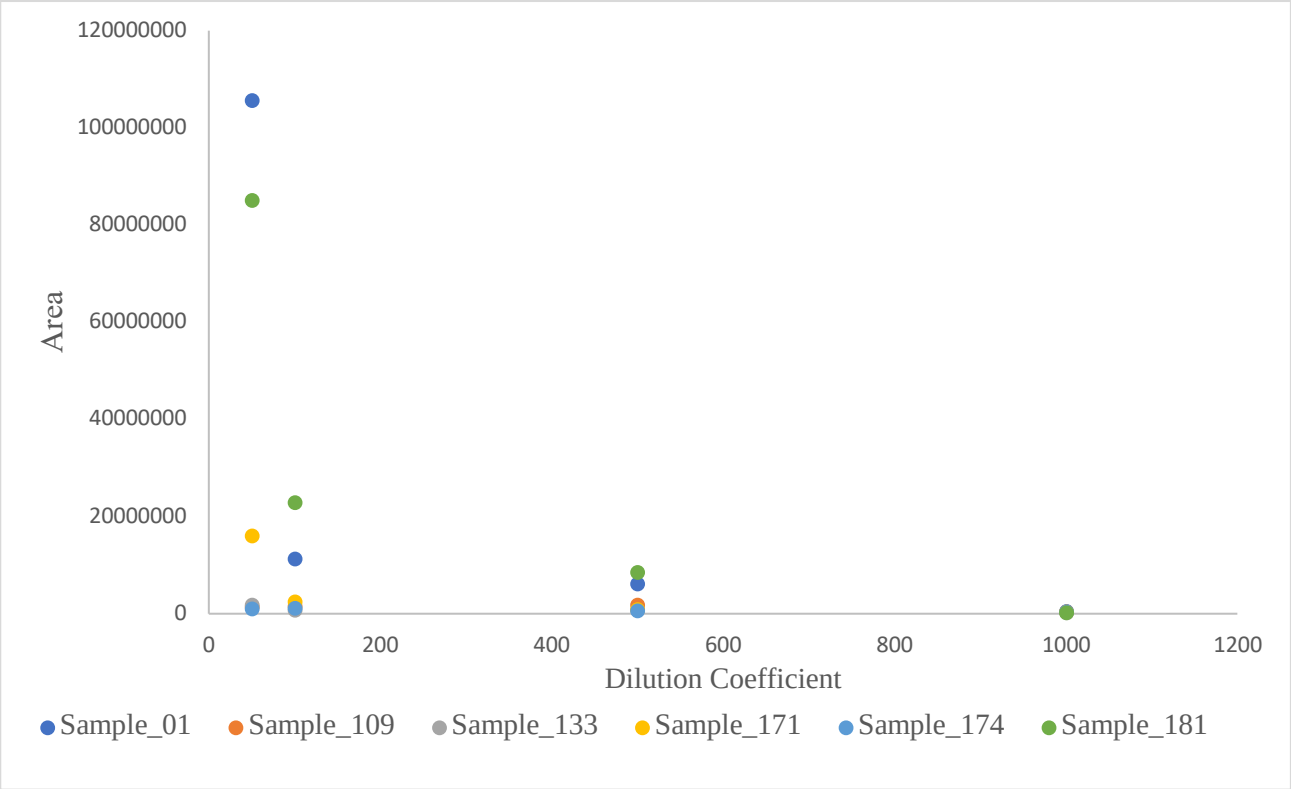


Figure 61: Observation of D3 loss due to the dilution

In order to determine whether dilution can impact classification in the previously created statistical model, the 50 compounds sought in the condom-type samples were extracted from the chromatograms, then the data were normalized and pretreated before carrying out the chemometric analysis. First, PCA was performed on the diluted samples in order to observe whether clusters might be formed as a function of the dilution. As illustrated in Figure 62, it was not possible to highlight clusters due to dilutions. No characteristic pattern of the samples could be observed. PCA does not seem to take into account the dilution itself, but rather the type sample.

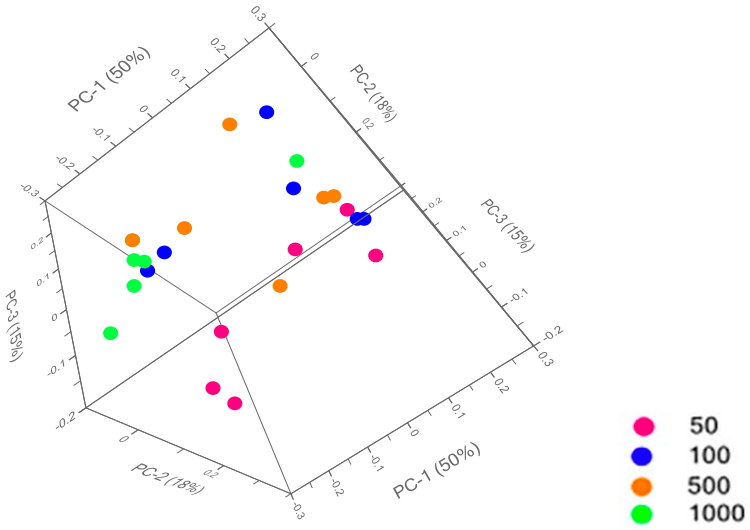


Figure 62:3D Scores plot from PCA obtained on the diluted samples, colors representing the different dilutions

The samples were then projected into the discrimination model, in order to locate at best where they could be located and if confusion could be observed. The result of the projection is illustrated in Figure 63.

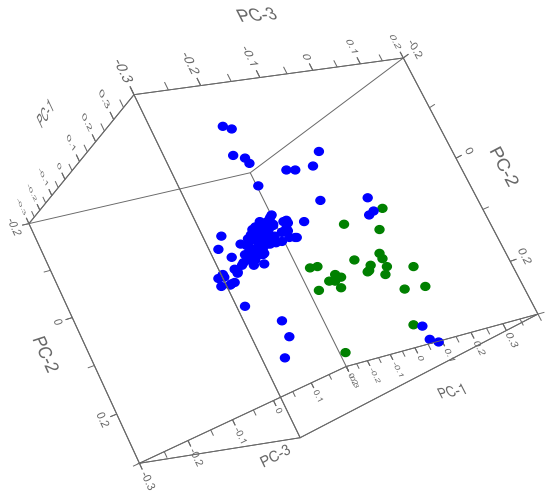


Figure 63: 3D-Scores plots of the projection of the diluted samples (green dots) in the discrimination model (blue).

Based on the 2D scores plots as well as the 3D scores plots, it is possible to demonstrate that the diluted samples form a group practically by themselves, located at the intersection between the other groups. Only a few 1:50 dilution samples were projected with the groups from which they originally came. The very specific localization in the model as well as the observation of the modification of the chemical profiles makes it possible to conclude that the diluted samples are difficult to differentiate, and that other methods could be necessary to bolster the discrimination of the samples. One could reasonably think that if diluted samples are not clustered with group they originate from, then the model does not work on traces. However, it

is to be reminded that PDMS is not expected to be diluted in vaginal secretions but rather to be deposited as a film on the matrix. The concentration would therefore be affected by sampling, and might not generate similar clusters, but this has not been investigated. A negative profile which would be grouped with the samples is explained as follows: the sample does not contain compounds soluble in hexane and analyzable by py-GC/MS. Such a result also suggests the need for methanol extraction followed by GC/MS analysis to observe the rest of the chemical profile.

Highlights

This chapter investigated py-GC/MS analysis for condom residues. The main results are the following:

- Using design of experiments, optimal pyrolysis conditions were set at 720°C and 20 seconds.
- The optimized conditions allow the analysis of extracted condom lubricants.
- Major compounds produced by the pyrolysis of PDMS were identified.
- Qualitative analysis highlighted at least 6 different chromatographic patterns in the population.
- 10 variables coming from the chromatographic pattern enable the discrimination of samples with 94% of variance explained on 4 PC.
- Most condoms present the same chemical profile and are not statistically different.
- Only one lubricant (Durex Play Glide) was found to be indistinguishable from condoms.
- Good classification rate was obtained with QDA algorithm.
- Real samples were not analysed in this chapter and the clustering with the correct population has not been investigated. Some examples will be presented in Chapter 7.

Chapter 6: GC/MS Analysis of Water-Based Components

This chapter is based on the following article (Appendix I) :

Article 5: Burnier C., Massonnet G, Coulson S, DeTata D, Pitts K, (2020) *Characterization and classification of water-based compounds in condoms and personal hygiene products using GC/MS*, Forensic Science International, Vol. 317

The previous chapter illustrated the potential of py-GC/MS analysis of silicone compounds and more generally of compounds soluble in hexane. It was shown that silicone-based condoms had a characteristic chemical profile, which only contained compounds resulting from the degradation of silicones following pyrolysis.

However, the FTIR analysis carried out during the market study demonstrated that certain condoms are likely to contain other products, in addition to PDMS. Indeed, other lubricants such as glycerin or polyethylene glycol are the second category of lubricants that can be applied to condoms [10,12,14,18,41]. In the same way, minor compounds such as flavorings, colors, anesthetics or preservatives can be used as reported in [10,14,34,40]. These compounds both have two major common points, which are their volatility in GC/MS and their polarity, implying their low solubility in an apolar solvent such as hexane, previously used for py-GC/MS analyses. In contrast, Maynard *et al.* (2001) have shown that these compounds can be completely extracted with polar solvents, specifically methanol [41]. Thus, based on observations from the literature, a procedure was implemented for the analysis of these compounds.

6.1 Material and Methods

6.1.1 Material and solutions

Methanol (Optima® LC-MS AR grade, Fisher Chemical) and diphenylmethane (99% Sigma-Aldrich) were used as the solvent and internal standard respectively. A solution of 0.1% (v/v) diphenylmethane in methanol was prepared as the extraction solvent.

6.1.2 Sample preparation and analysis

One hundred and sixty-five (165) samples, consisting of 26 personal lubricants (i.e. bottled lubricants), 5 personal hygiene products, 6 creams, 1 massage oil (vegetable oil) and 127 water-based, silicone-based and non-lubricated condoms were purchased for this study from multiple shops in Australia, Switzerland and New Zealand. The sample set is the same as the one used for **Chapter 4** market study.

Lubricants, creams, personal hygiene products and massage oils were diluted at a concentration of approximately 1 mg/mL in the extraction solvent of methanol containing 0.1% diphenylmethane as internal standard (IS). Condoms were unrolled and soaked in 20 mL of extraction solvent. All extracts and dilutions were diluted 1:10 (v/v) in methanol before analysis. Each sample was analysed on the GC/MS twice from the same extract to account for repeatability. 5 lubricants and 1 personal hygiene products could not be solubilized in methanol and therefore could not be analysed using GC/MS. A total of one hundred and fifty-nine (159) products are used in this study.

6.1.3 Instrumental conditions

Analyses were carried out on an Agilent GC 7890B system, interfaced with an Agilent 5977N mass spectrum detector, utilising Agilent ChemStation v. F.01.03.2357. Separation was achieved on an HP-5MS capillary column (30m x 0.25mm x 0.25µm) using helium as a carrier gas, at a flow rate of 1mL/min. Injections were carried out in splitless mode, the injector temperature being set at 280°C. The chromatographic program was as follows: held at 50° C for 2 minutes, then ramped at 10°C/min to 230°C and 20°C/min to 300°C, and a final hold for 5 min at 300°C (total run time 28.5 mins). Considering 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 8 (2^3).

6.2 Qualitative analysis

The overlay of the two chromatograms obtained by GC/MS for each of the 157 samples shows good repeatability in terms of the number of peaks present as well as their retention time. Slight variations were observed at the start of chromatograms, up to around 4 minutes, with a first peak whose position varies slightly in the analyses. This peak is present in the blanks, due to a column shared for multiple different evidence analysis, which implies that it is not linked to the

samples but is present repeatedly in all the chromatograms obtained. It will therefore not be considered in further analysis.

Water-based compound were detected in 46 (28.9%) of the 165 samples constituting the dataset. The most common compounds were glycerin, propylene glycol, phenoxyethanol and PEG. Amongst these samples, 56.25% of the samples contained glycerin, 37.5% propylene glycol, 6.25% PEG, 25% phenoxyethanol and 21.8% were found not to contain any of these compounds. Glycerin and propylene glycol were often present together, this mixture accounting for 31.25% of the samples.

No compounds linked to aromas or colorants were detected in any of the samples. This suggests that if any were present, they were either not extracted by the solvent, or not separated and/or detected by the GC/MS method or were present in quantities below the detection limit. Therefore, further research into methods for the extraction and detection of these compounds is necessary using other instrumentation or extraction techniques.

Most of the analytes detected were listed as ingredients on the packaging for most of the lubricants and personal hygiene products. Interestingly, PEG was only found in 6% of the samples, mostly in condom samples. This is surprising as literature reports that besides PDMS, one common lubricant that might be found on condoms is PEG. However, the non-lubricated condoms that were analysed in this dataset had a high glycerin content and a small amount of propylene glycol. PEG was only detected on condoms that were also lubricated with silicone. Campbell et al. (2007) [18] indicated that PEG found on condoms was often used with a spermicide. However, only one of the samples in the dataset revealed traces of nonoxynol-9, the only spermicide permitted for use on condoms. This difference may relate to changes in condom regulations, in particular nonoxynol-9 is no longer permitted on condoms present on the New Zealand market, due to its effect on the endometrium [135,136].

6.1.1 Sample Type - Condom

In general, the chemical profiles obtained for most of the “condom” type samples showed no peak differentiable from background noise or absent from the blanks.

Among the results obtained for the condoms that presented a chemical profile different from the background of the instrument, 4 types of compounds could be detected in certain samples and are presented in Table 19 below.

Table 19: Compounds detected in polar extracts from condom samples

Compound	RT [min]	Target ion (*m/z*)	Qualifiers (*m/z*)
Propylene Glycol	4.167	45	61, 76,29
Glycerin	9.802	61	43, 31, 15
Benzocaine	17.015	120	165,92, 137, 65
PEG Traces			
Hexaethylene glycol	19.415	45	89, 133
Heptaethylene glycol	22.147	45	89, 133, 207
Octaethylene glycol	24.078	45	89, 133, 283

Extracted ion chromatogram (EIC) showed that only the three oligomers of the PEG mentioned in table 1 above are present in the chromatograms.

The study of the different profiles and of the composition of the different samples analyzed made it possible to determine that:

- 120/127 (94%) condoms exhibit a silicone profile when analysed using py-GC/MS and do not demonstrate any other compounds in the GC/MS profile.
- 3/127 (2.3%) condoms exhibit a silicone profile and also contain benzocaine and/or traces of PEG as polar components.
- 2/127 (1.5%) condoms exhibit a silicone profile also contain propylene glycol. These were found to be samples promoting extra pleasure or long-lasting pleasure.
- 2/127 (1.5%) condoms exhibit a non-silicone profile but contain glycerin and / or propylene glycol.

These observations suggest that PEG or Propylene Glycol are used are used together with PDMS as a lubricant and that condoms that do not contain PDMS are more likely to contain glycerin. Benzocaine was also detected only from condoms containing PDMS.

It should also be noted that part of the samples analyzed in the context of this chapter (exclusively condoms) had previously been extracted with hexane for analysis by py-GC/MS. Most of these condoms were present in a single exemplar. Condoms extracted with hexane were previously removed from the hexane solution in which they were stored and were placed in a new vial for methanol extraction. It was noted that the hexane extraction did not a priori affect the polar composition of the samples, since various polar compounds could be observed in the GC/MS profiles. Thus, in the case where an actual sample has been tested negative for silicone residues in pyrolysis, that is to say that no peak characteristic of the degradation of silicone lubricants has been observed in the chemical profile of the sample, a re-extraction of the sample with methanol is therefore possible and can also be carried out in sequence on the same cotton swab. These observations support those made by New Zealand researchers who have

demonstrated that the extraction of silicone compounds was not affected by a prior DNA extraction procedure [15].

Nonoxynol-9 was only detected in one condom sample (Ceylor Gold) in the dataset. In addition, some of the samples in the dataset were known to contain flavorings and/or aromas, as well as colorants, evident by the strong scents or unusual colors of the product. None of the chemical profiles obtained in the various samples analysed showed any diagnostic peaks that could be associated with these components. These products might be present in very small quantities, as well as being volatile organic compounds (VOC). Other techniques, such as SPME might therefore be more relevant to analyse these compounds. However, if they are not detected in an extract or a dilution of a sample obtained in controlled conditions, it seems very unlikely that they would be transferred and detected in case work. Their forensic relevance can thus be questioned.

6.1.2 Sample Type – Personal Hygiene Products

Among the 38 samples constituting the set of intimate hygiene/lubricant/cream products, sample 156 (*FemFresh Feminine Deodorant Spray*) could not be analyzed because it was not possible to dissolve it in methanol, because of its initial aerosolised form. Similarly, it was not possible to dissolve the samples 109 (*Ansell LifeStyles Luxe Silicone-based Lubricant*), 133 (*Ansell Skyn Maximum Performance Lubricant*), 171 (*Astroglide Diamond Silicone Gel Personal Lubricant*), 174 (*Astroglide Waterproof Silicone Liquid*) and 181 (*Durex Play Perfect Glide*) in methanol. Consequently, these six samples will not be discussed in this chapter, as they could not be analyzed by GC/MS. The set of samples analyzed therefore contains 32 samples, the content of which had been previously identified as being aqueous-based, and therefore not eligible for py-GC/MS. Observation of the various chromatograms characterized 25 compounds found in the 32 left samples. These compounds, as well as their retention times and their target ions used for the extraction of the data, are presented in Table 20.

Among the set of samples analyzed, the following observations could be made:

- The majority of the samples contained propylene glycol (12 / 32) or glycerin (18 / 32)
- 10/32 samples contained **both** propylene glycol and glycerin.
- 10/32 samples contained **either** propylene glycol **or** glycerin, but not both.
- PEG was found in 2 / 32 samples
- Some samples did not contain any of the markers sought, either PEG, glycerin or propylene glycol (7/32)

It has also been observed that the quantities seemed to vary significantly in view of the abundance of the peaks and their shape. This should be taken into account during the semi-quantitative analysis. The compounds presented in Table 20 were found in some samples, but not in all. The most common were 2-phenoxyethanol, found in 8 / 32 samples, and octanediol detected in 3 / 32 samples. PEG was in only 2 condoms, and 2 lubricant samples. This therefore suggests that it is not a lubricant very frequently used by manufacturers.

Table 20 *:* Compounds detected in polar extracts from PHP samples

Compound	RT [min]	Target ion (*m/z*)	Qualifiers (*m/z*)
Propylene Glycol	4.167	45	61, 76,29
1,2-Pentanediol	7.042	55	73
1,2-Hexanediol	8.718	69	87, 41
Glycerin	9.802	61	43, 31, 15
2-Phenoxyethanol	11.76	94	138, 77, 66
Triethylcitrate	17.75	157	115, 203, 43
Octane, 1,1'-oxybis	17.792	57	71, 112
1-chlorotetradecane	17.95	57	43, 91, 71
Xylitol	18.157	61	43,74,103
Cyclodecane	18.711	57	43, 168, 199
Hexaethylene glycol	19.415	45	89, 133
Oxalic Acid	19.918	83	139, 55, 95
Cyclododecane	20.249	83	111, 196, 224
Pentadecanoic acid, 14-methyl-methylester	20.684	74	87, 143, 55
2.Tetradecyloxyethanol	21.515	57	166, 125, 85
1-Octadecene	22.072	83	97, 111, 125
Heptaethylene glycol	22.147	45	89, 133, 207
8,11-octadecadienoic acid methyl ester	22.192	67	81, 109, 294
Lauryl Acetate	22.748	145	168, 213, 127
Nonoxynol-9	23.930	45	89, 133, 206
Cyclotetradecane	23.978	173	168, 83, 43
Octaethylene glycol	24.078	45	89, 133, 283
Glycerol-1-Palmitate	24.865	239	299, 98, 134
Cyclohexadecane	25.223	145	173, 57
Glycerol Tricaprylate	28.134	127	327, 57, 201

6.3 Discrimination and classification modelisation

The qualitative analysis highlighted that samples could be categorized into two broad classes: those which have a positive profile to a polar compound, whatever its nature, and those presenting a negative profile to any polar compound. It is clear that in the eventuality that the entire sample set would be used to create a statistical model of discrimination, the first discrimination would be based on the presence or absence of these compounds. Since this discrimination can easily be carried out in a qualitative way, samples not containing any polar compounds (i.e., practically all the samples of the condom type) will not be considered in the discrimination model created using semi-quantitative analysis.

6.3.1 Qualitative Variability

Although the variability of the composition of water-based samples has already been noted by researchers using advanced spectrometry methods (DART-MS, MALDI-MS) [10,16,17,20], it has never been evaluated in GC/MS or on extracts or dilutions of products, which would represent a situation of real cases, where the target compounds would be extracted from a cotton swab before carrying out an analysis. The present study therefore examined this using the chemical profiles acquired from the 32 lubricants/personal hygiene products/creams water-based samples present in the database.

The distribution of the profiles in the population was observed and the distribution of the variation of the compounds in the population is presented in Figure 64. Glycerin and propylene glycol are very clearly the compounds which have the greatest impact on the distribution of the data.

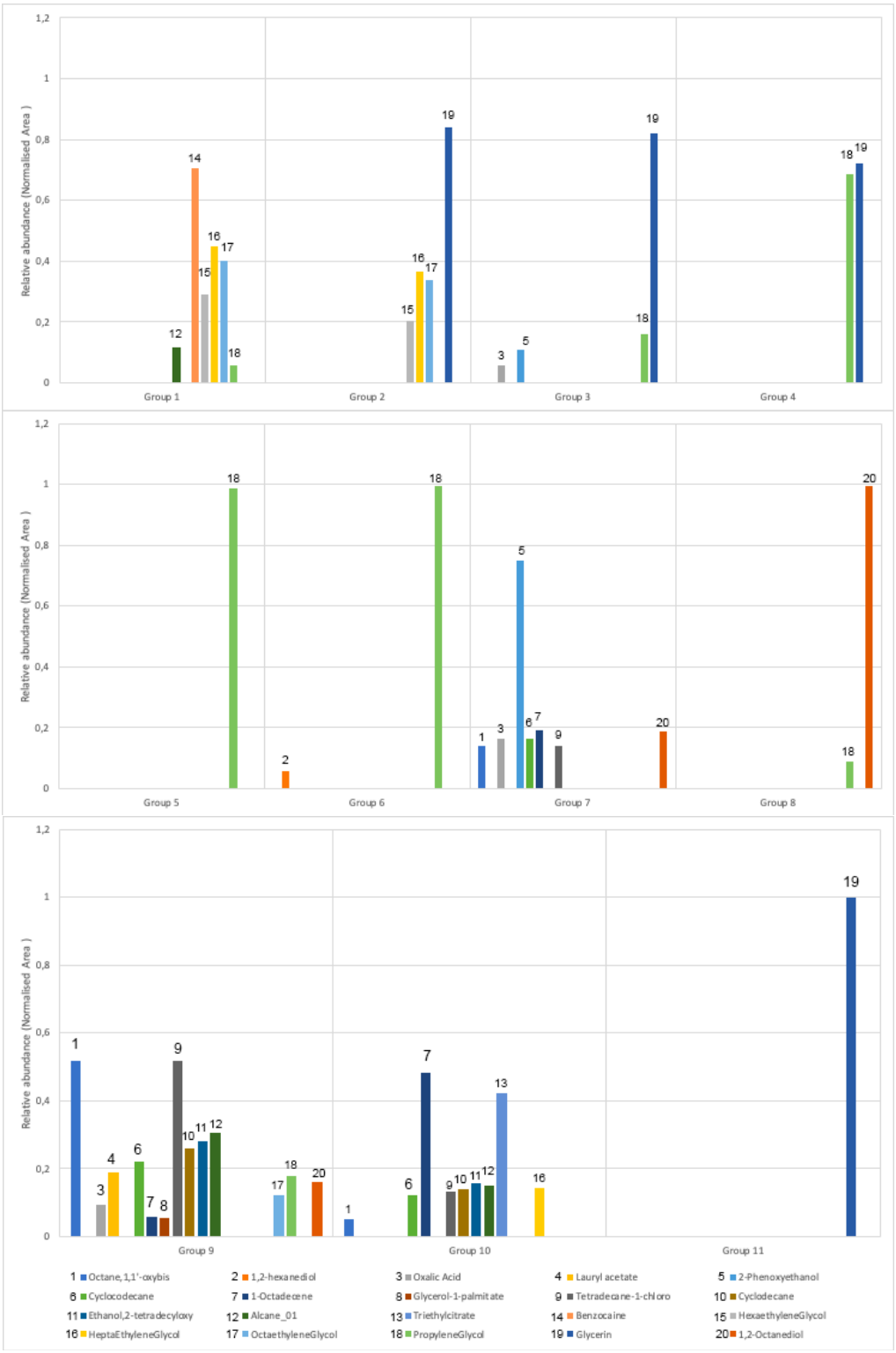


Figure 64: Qualitative data obtained on the mean of the data present in each group (Table 22), highlighting the disparity of the groups. Only compounds that were presenting a relative abundance over 0.05 are presented. Reproduced from *Burnier C., Massonnet G, Coulson S, DeTata D, Pitts K, (2020) Characterization and classification of water-based compounds in condoms and personal hygiene products using GC/MS, Forensic Science International*

In order to be able to observe the impact of each variable, glycerin and propylene glycol were removed. Data are gathered in Figure 65.

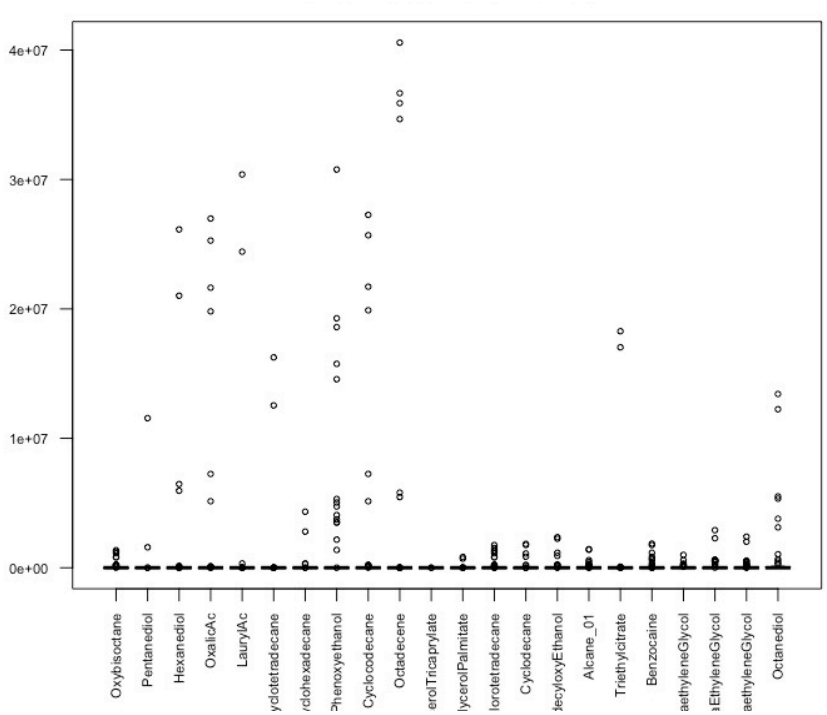


Figure 65: Boxplot of the variability of the various components in the data set, internal standard, glycerin and propylene glycol removed.

Significant variability is highlighted in Figure 65, but none of the compounds presented is clearly distinguished from the rest. The following parameters were therefore considered to target compounds that could potentially discriminate between samples:

- Presence in all (or in the majority) of the products studied
- Absence or minimal presence in the vaginal matrix
- Reasonable abundance in all samples
- Good resolution of peaks in the chromatograms

The compounds selected were therefore propylene glycol (PG), glycerin (GLY), phenoxyethanol (PE), polyethylene glycol (PEG) and octanediol (OCT). These are the major compounds presenting a relative abundance high enough to be able to be detected in the samples, and potentially in case work samples.

The coefficients of variation (CV%) were calculated for within sample variability (intravariation) of three randomly selected samples (Sample 177, Sample 70, Sample 136) and between samples variation (intervariability) of all the samples, following the normalization of the areas of the selected compounds by the area of the internal standard (Figure 66A). For all compounds, the intravariability was found to average around values around 35%, which is fairly high. A few values above 100% have been observed, especially for compounds whose presence in all samples was not guaranteed. On the other hand, intervariability was found to be significantly higher, since it reached more than 150% for practically all the compounds, and up

to 450% in some cases. The use of a different pretreatment, i.e. normalization by the sum of the areas of the compounds (all compounds included except internal standard), was tested and found to reduce the general variability, significantly in particular for propylene glycol. and glycerin (Figure 66B). The CV% may have been reduced for certain compounds but others have seen their CV increase, such as for example octanediol. These observations regarding variability are very important, for the development of a method of discrimination but also from the perspective of the development of a transfer and persistence study, since the variability of the initial composition had been identified as a significant factor of variation [14].

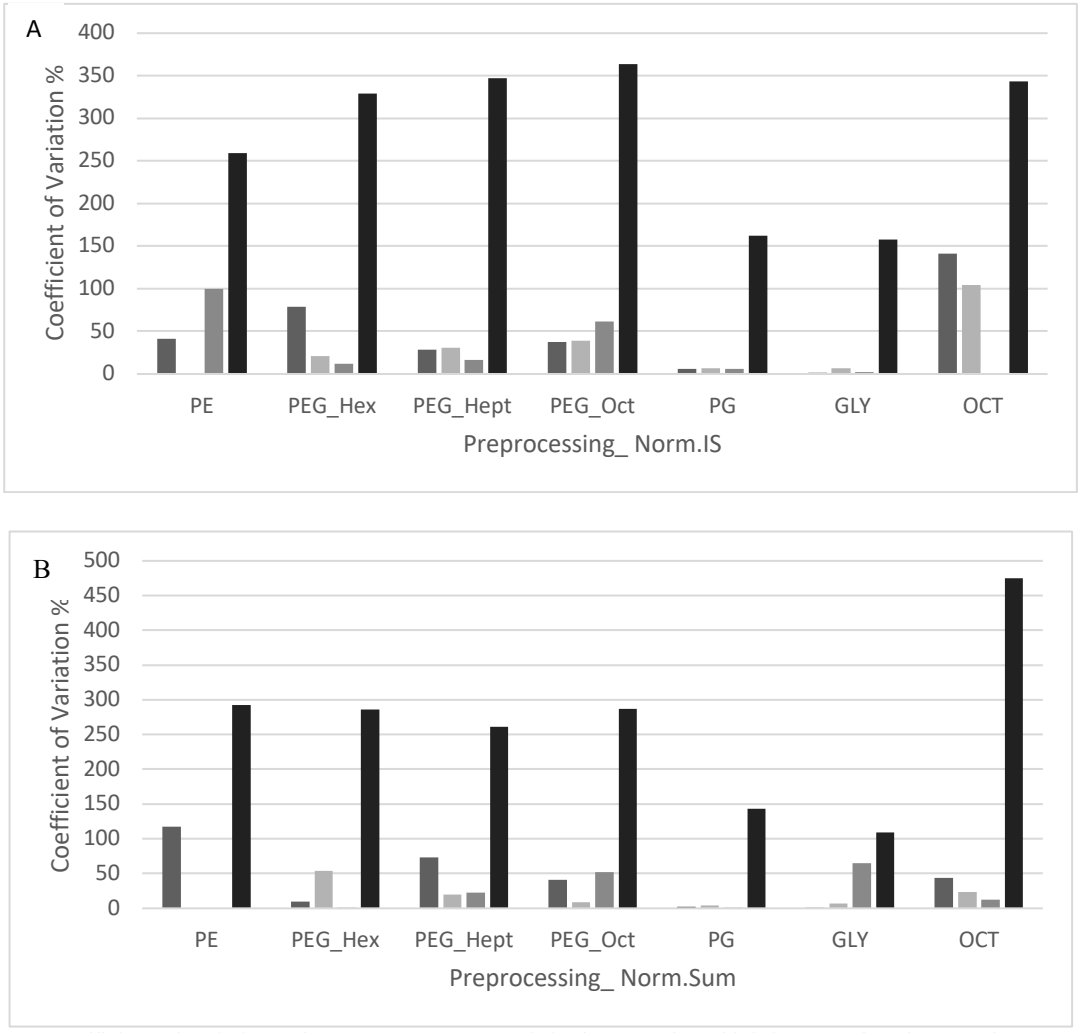


Figure 66: Coefficient of variations of seven target compounds in the water-based lubricant market, for Sample 177 (Astroglide Strawberry Liquid Personal Lubricant), Sample 70 (Ansell Lifestyles – Party Mix – Warm Smooth) and Sample 136 (Four Seasons Nature Lubricant vegan friendly). Within sample variation is shown in grey tones and between all the samples in black. A) Normalization to internal standard (Norm.IS), B) Normalization to area sum (Norm.Sum)

6.3.2 Semi-Quantitative discrimination

Finally, eight different pretreatments were tested in order to study the possibility of developing an objective classification of samples based on pretreated compounds, using PCA. The choice of the best pretreatment was based on the quality of the separation between samples belonging

to different classes. Among the pretreatments tested were normalization to the internal standard, normalization to the sum of the areas, the logarithm, the square root, the normalization to the internal standard followed by the square root, the normalization to the internal standard followed by the logarithm, the normalization to the sum of areas followed by the square root and normalization to the sum of areas followed by the logarithm.

Normalizing to the sum of the areas followed by the square root was found to achieve the best separation, and 73% of the total variance was described by the first three PCs (Figure 67). The other PCs did not allow to enhance sample discrimination, therefore only the first three PCs were retained for subsequent discriminant analysis. Table 21 presents the percentage of variance explained as well as the percentage of cumulative variance for each of the principal components.

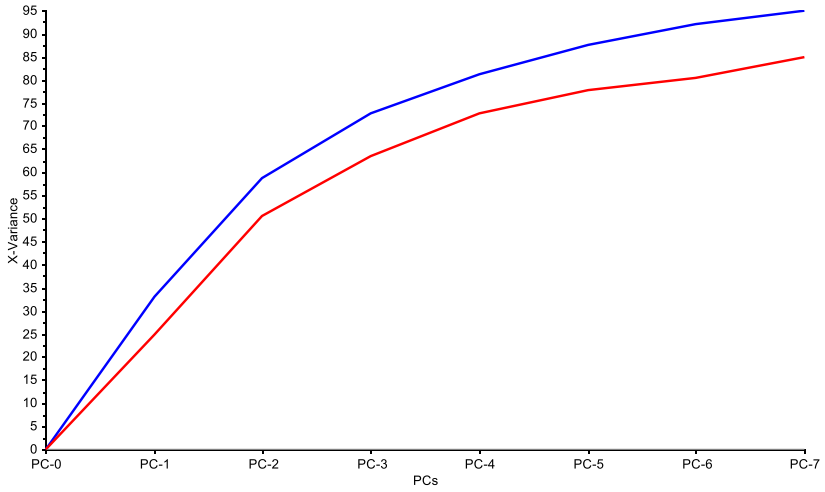


Figure 67 : Scree plot illustrating the cumulative variance in the GC/MS dataset retained by each PC.

Table 21: Percentage of explained variance and cumulative variance for the processed data from the entire dataset

	Processed Data	
Principal Component	Explained variance (%)	Cumulative variance (%)
1	33	33
2	26	59
3	14	73
4	8	81
5	6	87
6	5	92
7	3	95

The PCA was therefore carried out on the compounds pretreated in this way and scores plot, produced using combinations of the result of the PCA, are shown in Figure 68. Replicates from each sample were generally clustered together, indicating reasonable measurement reproducibility. The overall clustering patterns observed were found to be very unclear. Indeed, several small groups are visible, but samples are very closely clustered between the different

classes. The observation of PCA loadings highlighted that the separation of the samples was mainly based on the GLY, PG and PE content.

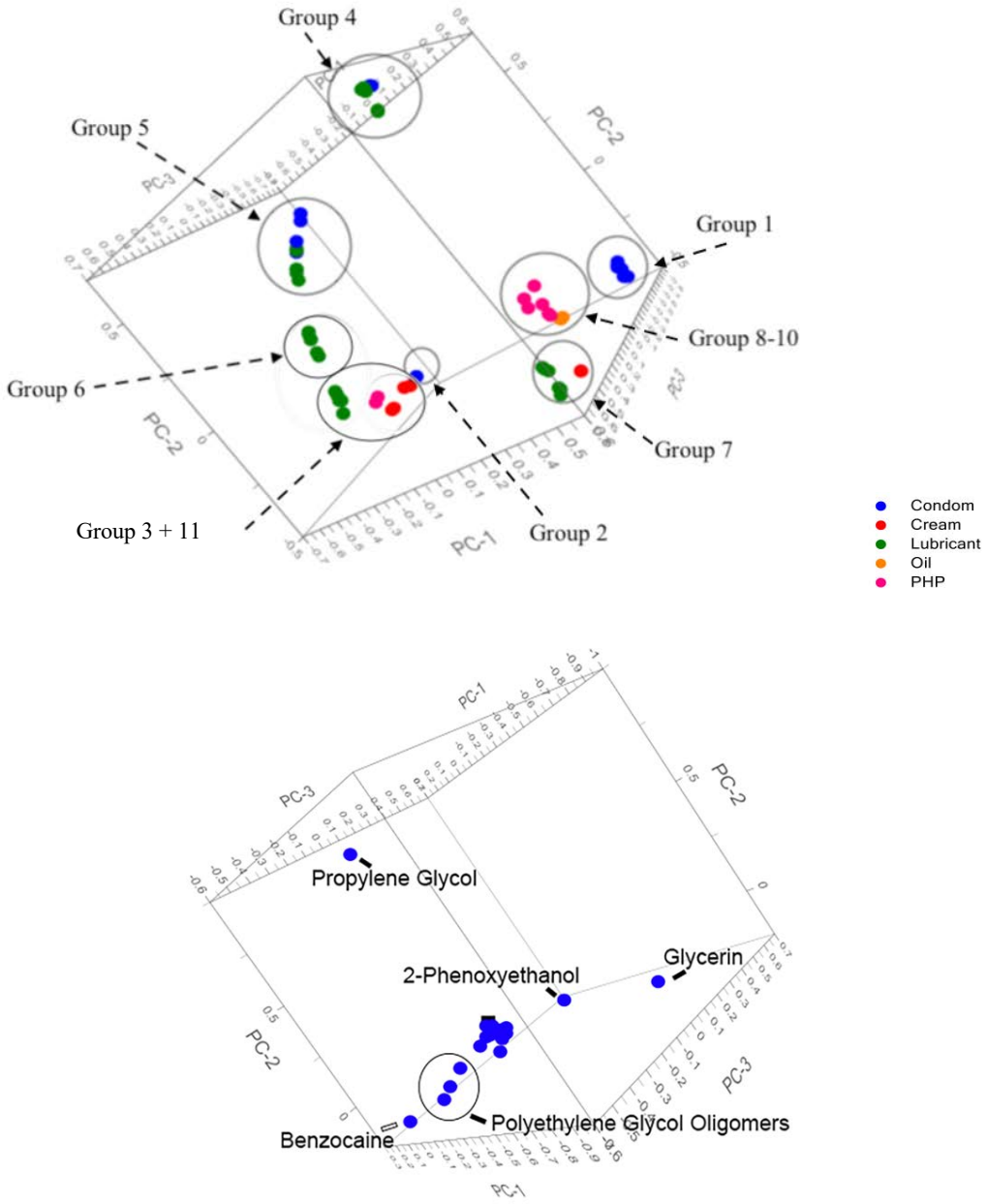


Figure 68: 3D Scores Plot of the dataset along PC1, PC2 and PC3 highlighting the distribution of the 32 samples based upon their corresponding chemical content. Data were processed using normalization to areasum followed by square root (Norm.Sum + SQRT). Samples are classified following their initial class, and loading plots associated. Black circles indicate visual clusters observed when modelling the three-dimensional space. *Reproduced from Burnier C., Massonnet G, Coulson S, DeTata D, Pitts K, (2020) Characterization and classification of water-based compounds in condoms and personal hygiene products using GC/MS, Forensic Science International*

Several clusters could therefore be identified in the separation of the dataset, based on the sample composition, as illustrated in Figure 68. Significant variability within the lubricant class was observed, as some contained PG + GLY, some GLY + PE, some PE and other compounds, and more rarely PG only. Certain lubricants and condoms were found to form overlapping clusters together, especially in the case of PG+ GLY containing samples. Indeed, as these

components were found in most of the samples, and some samples contained exclusively these two components, such confusion is not surprising. However, it is then quite challenging to infer the initial class of the sample in these conditions. Clusters containing Benzocaine + PEG or PEG + GLY were found to exclusively belong to condom classes. Finally, regarding personal hygiene products, creams and oils, various contents were observed, and it could be observed that these samples are more likely to be clustered together, with a very high proximity between the samples coming from the same class but from different products.

In examination of scores and loadings for PC4 and PC5 as presented in Figure 69, there was a positive correlation along PC4 of benzocaine and polyethylene glycol. Alkane patterns were found to generate a negative correlation along PC4. Finally, separation of samples along PC5 was found to be mainly due to differences in minor components. These separations were found to assist strongly in separating groups that were not distinguished on the first three PCs, as shown in Figure 68 but may be limited in abundance in case work samples.

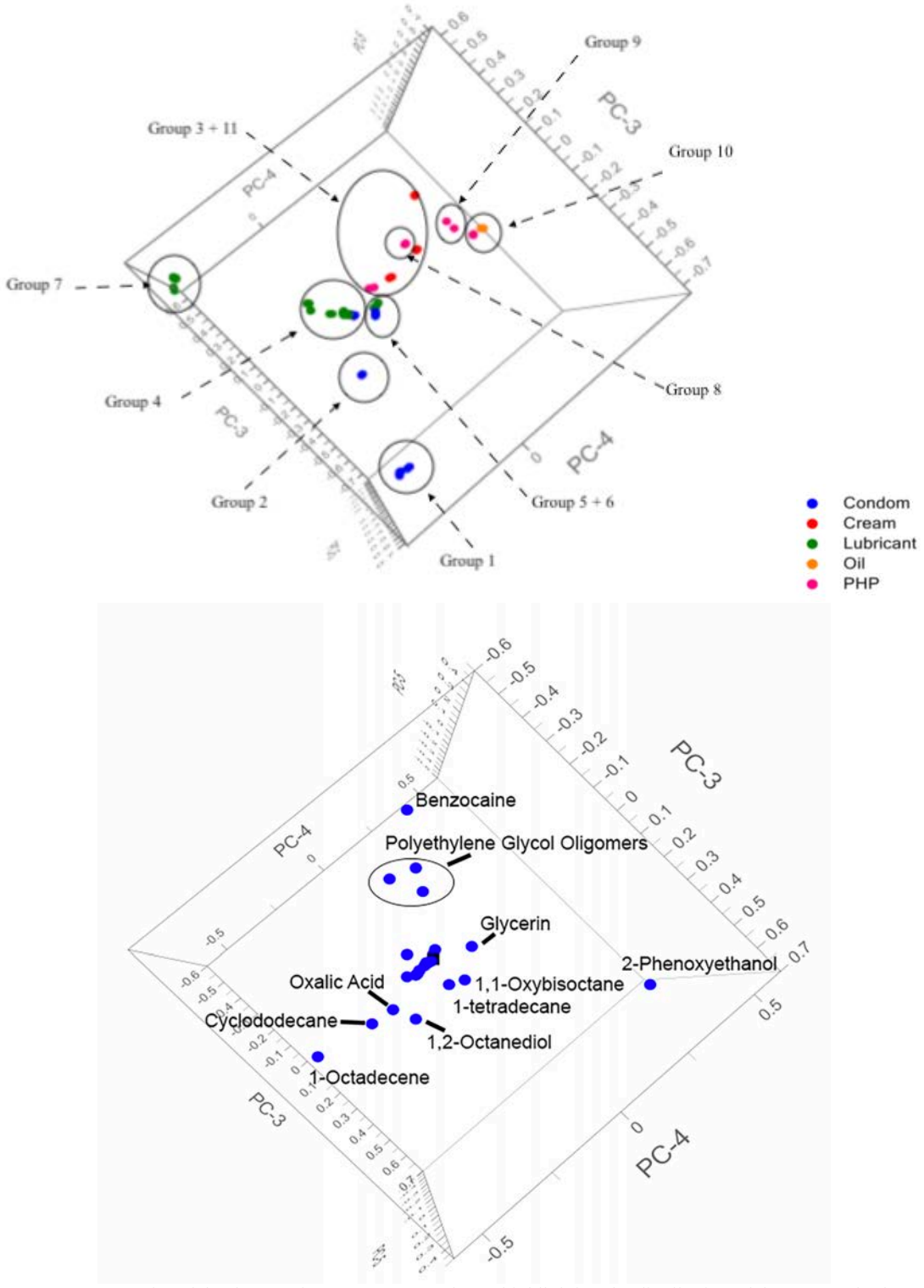


Figure 69 : 3D Scores Plot of the dataset along PC3, PC4 and PC5 highlighting the distribution of the 32 samples based upon their corresponding chemical content. Data were processed using normalization to areasum followed by square root (Norm.Sum + SQRT). Samples are classified following their initial class, and loading plots associated. Black circles indicate visual clusters observed when modelling the three-dimensional space. *Reproduced from Burnier C., Massonnet G, Coulson S, DeTata D, Pitts K, (2020) Characterization and classification of water-based compounds in condoms and personal hygiene products using GC/MS, Forensic Science International*

Finally, all the samples constituting the dataset were grouped in the five categories that were finally observed on the overall dataset and the lists are gathered in Table 22.

Table 22: Summary of the sample(s) comprised in each grouping. PEG = Polyethylene glycol oligomers; GLY = glycerin; PE = phenoxyethanol; PG = propylene glycol; OCT = 1,2 octanediol; other = minor compounds in the chromatograms. 6 lubricants could not be analysed as they could not be solubilized in methanol, and 1 water-based lubricant is missing as there was no sample left for GC/MS analysis. Total number of samples is 159. *Reproduced from Burnier C., Massonnet G, Coulson S, DeTata D, Pitts K, (2020) Characterization and classification of water-based compounds in condoms and personal hygiene products using GC/MS, Forensic Science International*

Group	Source of the sample	Content	Samples in the group
Group 1	Condom (3)	Benzocaine + PEG	Samples 14, 19, 22
Group 2	Condom (1)	GLY + PEG	Sample 124
Group 3	Lubricants (1) Creams (5) PHP (1)	GLY + PE + PG + other	Samples 140, 159, 160, 161, 162, 163, 164
Group 4	Condom (2) Lubricants (8)	GLY + PG	Samples 57, 70, 108, 135, 141, 165, 175, 176,177, 182
Group 5	Condom (2) Lubricants (2)	PG only	Samples 112, 113, 134, 136
Group 6	Lubricants (2)	PG + other	Samples 168, 170
Group 7	Lubricants (3) Creams (1)	OCT + other	Samples 142, 158 166, 167
Group 8	PHP (1)	OCT + PG	Sample 154
Group 9	PHP (1)	PEG + other	Sample 155
Group 10	Massage oil (1) PHP (1)	Other water-based	Sample 138, 157
Group 11	Lubricants (2)	GLY only	Sample 137,172
Group 12	Condoms (119)	No detectable compounds	All condoms with no observable pattern

It is now interesting to understand if the groups obtained in the PCA are significantly different from the ones obtained in Table 22. Groups 1, 2, 4, 5 and 7 from Table 22 were found to be clustered together in the PCA provided along PC 1, 2 and 3, as well as along PC 3, 4 and 5. Groups 8, 9 and 10 of Table 22 were only found to be separated in the PCA provided along PC 3, 4 and 5, suggesting that PC 4 and 5 were using other minor compounds for the separation of the groups, assisting in creating extra sub-groups. Group 3 was found to contain all the left-over samples that could not be clustered with other groups, which leads to a considerable inhomogeneity, confirmed by the spread of the data belonging to this group. However, groupings were obtained based on a certain number and amount of compounds detected, and some subclasses using additional accurate visual comparison in group 3 might lead to further subgroupings.

6.4 Classification model and prediction

The replicas of the seventy samples were classified according to the class to which they were known to belong. Thus, the samples were grouped into five categories. Linear discriminant analysis was applied to the scores of the first three main components, since these were necessary for adequate separation of the samples. All the discriminant analyses were processed assuming

equal probabilities for each sample. The efficacy of the model was then evaluated using a separate validation set of 5 samples, one water-based lubricant, three creams and one oil-based lubricant, that were not present in the model.

LDA, QDA and SVM discrimination models were tested. The resultant models yielded a classification accuracy of 43.18% for LDA, 64.18% for QDA and 82.09% for SVM on the original model for the calibration set. The confusion matrices for each of the models are gathered in Table 23.

Significant misclassification was observed with PHP and cream samples using LDA and QDA, as the latter were found to be classified as lubricants. SVM reduced this error rate, and only condom and creams were found to be misclassified with lubricants and PHP classes respectively. This in addition to the higher percentage of correct classification led to the choice of SVM for classification purposes and its application to the validation set. Validation accuracy on an internal set randomly chosen by the algorithm for SVM classification was found to be 79.10%.

Table 23: Confusion matrix for A) LDA classification model, B) QDA classification model C) SVM classification model. Column name refers to the real class, line name refers to the classified class.

LDA	Lubricant	Condom	PHP	Cream	Oil
Lubricant	29	10	8	4	2
Condom	0	0	0	0	0
PHP	6	6	0	2	0
Cream	0	0	0	0	0
Oil	0	0	0	0	0

QDA	Lubricant	Condom	PHP	Cream	Oil
Lubricant	27	8	0	0	0
Condom	4	4	0	0	0
PHP	2	0	6	2	0
Cream	2	4	2	4	0
Oil	0	0	0	0	2

SVM	Lubricant	Condom	PHP	Cream	Oil
Lubricant	35	8	0	0	0
Condom	0	8	0	0	0
PHP	0	0	8	2	2
Cream	0	0	0	4	0
Oil	0	0	0	0	0

SVM analysis provided the best results in terms of sample classification. The misclassified replicates were studied, and observations were the following:

- The replicates of Sample 112 (Manix Orgazmax Plus) and 113 (Manix Endurance) were classified as lubricants instead of condoms. The PCA results indicated a very close proximity between the chemical profile of these replicates and other samples that were

containing PG + GLY. However, these samples were also found to contain silicones and were easily clustered as condoms in the silicone model. This coupled information between py-GC/MS and GC/MS results might be helpful following recovery of a condom sample from a crime scene.

- The replicates of Sample 158 (Vagisil Oatmeal Cream) were classified as personal hygiene products rather than creams. The PCA results indicated a close proximity between the chemical profile of this sample and a PHP also containing PE and other products. The classification results are thus compatible with what had been observed for the PCA and it is not surprising that the classification model is not able to correctly classify this sample.
- The replicates of Sample 138 (Four Seasons Massage Oils) were classified as personal hygiene products rather than oils. The PCA results indicated a close proximity between the chemical profile of this sample and the one of the PHP population also containing PE and other products. The classification results are thus compatible with what had been observed for the PCA and it is not surprising that the classification model is not able to correctly classify this sample.
- The replicates of Sample 57 (Ansell Lifestyles – Party Variety -Warm/Cool) and 70 (Ansell Lifestyles – Party Mix – Warm Smooth) were found to be classified as lubricants rather than condoms. These two samples were found to be water-based lubricated condoms containing PG + GLY only, and no silicone traces were observed in their py-GC/MS profiles. Based on the observation of their chemical profile as well as the PCA results, it is not surprising that these sample were misclassified, given their similarities to the lubricant population. This misclassification can be an issue for the interpretation of the evidence, as it is therefore impossible to discriminate a condom and a lubricant both containing PG + GLY. This factor has to be taken into account under the form of the error rate, with 5% of the samples that were found to be misclassified.

External validation using five samples not present in the model was performed to validate the current procedure. Results of the classification are gathered in Table 24. Classification was found to be accurate for the cream and lubricant classes. However, the oil-based sample was clustered with personal hygiene products. The same observation was made with sample 138 previously. These samples were found to present no significant traces of GLY or PG, but mainly alkanoic patterns that were easily spotted in the chromatograms. This observation coupled to the specific spectrum that can be gathered when analysing the sample using FTIR spectroscopy would help correctly classify the sample as a massage oil sample.

Table 24: Results of the classification for 5 external samples using SVM classification.

Sample	Real Class	Predicted Class
139	Lubricant	Lubricant
161	Cream	Cream
162	Cream	Cream
164	Cream	Cream
173	Oil	PHP

The results previously discussed show that the SVM classification provided results compatible with what had been observed with PCA. The vast majority of false classifications were found to be replicates of a sample having very slightly variable characteristics. The study of misclassifications, supported by the results of the PCA, indicates that these samples generally differ not in terms of their visual chemical profile but in semi-quantitative terms.

The samples constituting the dataset came from different brands and models, covering a whole swathe of the market in terms of personal hygiene products and lubricants. In regard to their composition as written on the packaging, consistent observations were made. The major components found in general in the samples were glycerin, propylene glycol and phenoxyethanol, in various concentrations. These variables were taken into account to build up the classification model, but both discrimination and classification were not found to be very specific to a certain type of sample. It was possible to differentiate samples based on the presence or absence of certain components, but it was not possible to properly distinguish sample classes within a cluster. However, in the qualitative analysis, some of the peaks observed were found to be specific to some types of samples. Unfortunately, these variables were not relevant enough to enhance the discrimination of the samples.

Highlights

This chapter investigated GC/MS analysis for condom residues. The main results are the following:

- 157 condoms and personal hygiene products were analysed after extraction or dilution in methanol.
- 1.5% of the condoms contained water-based lubricants; most of the condoms contain exclusively silicone lubricants (as illustrated with py-GC/MS in Chapter 5)and thus do not present any GC/MS chemical profile.
- Major compounds present in the different samples were identified
- Chemometrics coupled to visual analysis revealed 12 different groups.
- Classification of massage oils, creams and personal hygiene products was found to be less accurate than for other groups of samples.
- Real samples (i.e., traces) were not analysed in this chapter. Some examples will be presented in Chapter 7.

Chapter 7: Forensic Application of the framework

This chapter is based on the following article (Appendix I) :

Article 8: Burnier C., Kelly M., DeTata D, Pitts K, (2021) *Investigation of condom evidence in cases of sexual assault: a case study* Manuscript accepted in Forensic Science International: Reports

ChemCentre (Perth, WA) in collaboration with the Sexual Assault Resource Centre (SARC) and the Western Australia Police Force, has recently implemented advancements, in how to collect and analyse condom evidence. This procedure involves forensic clinicians, as well as police officers and forensic scientists, as they are all part of the forensic investigation. The analytical and statistical framework developed in **Chapter 4 to 6**, using FTIR, py-GC/MS and GC/MS will be tested on proficiency trials before being used on case work samples. Unfortunately, at the time of the analysis, the FTIR instrument in ChemCentre was out of order. Therefore, FTIR will not be tested, and only py-GC/MS and GC/MS will be used.

The application of the method to known casework simulants in the form of proficiency trials is necessary to validate the framework, before proceeding to casework analysis. The implementation of this new framework in Western Australia has generated an increase in case numbers in which condom evidence is submitted and analysed. Case samples were processed, and gaps that affect the interpretation of this type of evidence highlighted within the current framework.

7.1 Proficiency trials

The following cases come from four proficiency trials carried out by ChemCentre between 2015 and 2019. These cases had already been analysed beforehand by the laboratory's *physical evidence* team. The specimens to be processed were sent by Forensic Testing Services (Williamston, USA). The 4 proficiency trials systematically contain one recovered cotton swab, one blank cotton swab and three questioned samples, which can be either lubricants or condoms. Case scenarios usually entail a female sexual assault victim who claims that the assailant used a lubricant when assaulting her. Swabs were collected after the assault for examination. A suspect was identified, and bottles of lubricants or condoms were seized. The case investigator orders a comparison of these items to any lubricant present on the swabs

collected from the victim. The task requested is to identify which questioned sample is the source of the profile observed on the recovered swab[§].

7.1.1 Analysis procedure

The recovered and blank cotton swabs were cut and individually deposited in a 2mL glass vial. 1mL of hexane was added to the sample. Vials were vortexed for 1 min and placed in an ultrasonic bath for 15 min. The extract was analysed using pyrolysis-gas chromatography- mass spectrometry (py-GC/MS). In the eventuality of a negative result or the impossibility to differentiate samples based on their apolar content, the same extraction procedure was repeated using a polar solvent. Recovered and blank cotton swabs were individually deposited in a 2mL glass vial. 1mL of methanol was added to the sample. Vials were vortexed for 1 min and placed in an ultrasonic bath for 15 min. The extract was analysed using gas chromatography- mass spectrometry (GC/MS)

Condom samples were opened, unrolled and deposited in a 40mL glass bottle and covered with 25mL of hexane. The bottles were then closed and put in the ultrasonic bath for 15 minutes. Before analysis, samples were aliquoted and diluted 10 times. Liquid samples, such as lubricants, were weighed and diluted in hexane to reach an approximate concentration close to that expected on the condom, which was estimated, after dilution, to be 1.5 – 2.5 mg/mL. The same concentration was used for the other samples to be able to compare the results. For each sample, 10µL aliquots of the hexane solution were spiked in the stainless-steel cups and left to evaporate to dryness before the analysis. For all samples, if judged necessary following py-GC/MS results, the same procedure was repeated using methanol as extraction solvent or dissolution solvent. Analytical conditions were as presented in the previous chapters.

7.1.2 Preliminary considerations

Condoms provided for the proficiency trials were found to be exclusive to the American market. Therefore, their profiles were not included in the database, which was based on products commercially available on Australian, New Zealand and Swiss markets. However, the samples were projected in the discrimination model and were found to cluster with the samples that contain silicone. Therefore, there do not seem to be major differences between condom samples that come from different markets and countries.

[§] With all the bias that it implies, as there is at least one matching material in the comparison material provided.

Another important consideration is the fact that some samples, more specifically the cotton swabs, were previously extracted using dichloromethane (DCM) as part of the previously used methodology. There was no indication whether only one or both cotton swabs had been used for the extraction. Only one of the swabs was used for hexane extraction, the other being reserved for methanol extraction if needed. Hexane extraction was thought to be possible as Maynard *et al*. (2001) showed that DCM extractions were incomplete [41], suggesting that residues could still be present on the swabs. Chemical profiles were obtained from these hexane extracts, confirming previous observations. A solvent blank was also analysed to ensure that there was no contamination.

The final consideration involves instrumental limitations. The current analysis framework includes FTIR analysis as a screening method. Unfortunately, at the time of the analysis of these samples, no FTIR instrument was available. Therefore, py-GC/MS and GC/MS analyses were conducted, without any prior indication of the possible sample content.

7.1.3 Results

FTS-15-LUB

This proficiency test provided 1 condom (I003) and 2 lubricants (I002 and I004) that were potential sources of the chemical profile observed from the recovered swab (item I001). A blank cotton swab was provided (I005).

From a qualitative point of view, pyrograms obtained from item I001 (recovered swab) presented at least 6 visible peaks characteristic of siloxane degradation (Figure 70A). Degradation of siloxane patterns including D3-D7 oligomers was observed. Pyrograms resulting from items I002 and I004 did not present any relevant chemical patterns. These observations suggest that neither items contained any silicones and GC/MS analysis would be recommended to evaluate the profile of the samples (Figure 70B and 70D). The pyrogram obtained from item I003 presented at least 6 visible peaks characteristic of siloxane degradation (Figure 70C). Degradation of siloxane patterns including D3-D7 oligomers was observed. The pyrogram obtained from item I005 did not present any pattern.

Based on these observations, samples can be compared to each other to assess, which of the collected items is a potential source of the profile observed on item I001. These comparisons indicated the following:

- Comparison of the pyrograms obtained from items I001 and I005 showed that all the peaks observed in the profile of item I001 come from the trace, and not from the cotton swab itself.
- Comparison of the pyrograms of I001 and I002 showed visually different chemical profiles. Extraction of target ions confirmed the observations. It can be stated that Item I002 is not the source of the chemical profile observed from I001.
- Comparison of the pyrograms of I001 and I003 showed consistent chemical profiles in terms of number of peaks and position. The relative abundance of D5 and D6 peaks were different. This was previously observed in the practice (Personal communication Dr. Sally Coulson, ESR, NZ) and therefore, a semi-quantitative analysis with extraction of the target ions is required. Extraction of target ions showed indistinguishable patterns.
- Comparison of the pyrograms of I001 and I004 showed visually different chemical profiles. Extraction of target ions confirmed the observations. It can be stated that Item I004 is not the source of the chemical profile observed from I001.

From a semi-quantitative point of view, projection of the results in the discrimination model built for py-GC/MS (Chapter 5) outlined that items I001 and I003 were clustered together, whereas items I002 and I004 clustered together but strongly distinguished from the other samples. Therefore, based on the visual and statistical observation of the results, it appears more likely that item I003 is the source of the chemical profile observed from I001. I002 and I004 can be excluded as sources of the chemical profile. This evidence makes it more likely that item I003 is the source of the chemical profile observed from I001, rather than if item I002 or item I004 are the source of the chemical profile observed.

The manufacturer-supplied result of the proficiency test was that the questioned swabs (Fisherbrand Cotton-Tipped Swabs) from Item 1 were prepared by sampling a package of Astroglide X brand lubricant directly with the swabs. The swabs were then sealed in capped test tubes (BD Flacon 5mL polystyrene round-bottom tubes) for shipping. Item 2 and Item 4 were correctly eliminated as a source for Item 1. From the three items provided, only Item 3 presented a profile that could be *identified* as a source for Item 1.

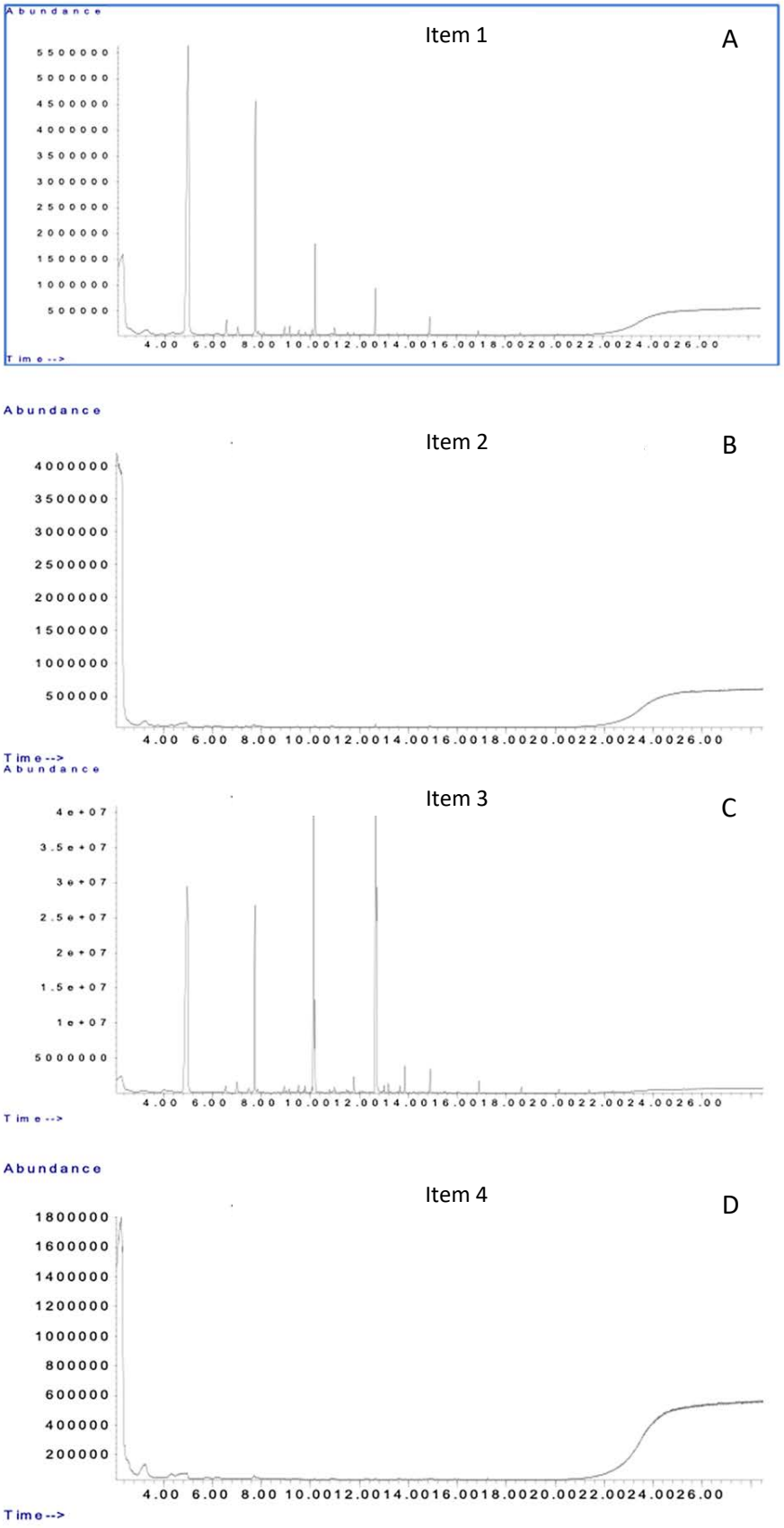


Figure 70: Chemical profiles obtained for the questioned sample (in blue) and the comparison samples – py-GC/MS analysis. 70A) pyrogram of item I001(questioned swab) extract; 70B) pyrogram of item I002 Sliquid Organics® lubricant; 70C) pyrogram of item I003 Astroglide X lubricant; 70D) pyrogram of item I004 Lifestyles Warm Lovin lubricant.

The same procedure was performed on FTS-18-LUB and FTS-19-LUB. In all cases, the source for the swabs' profile was correctly identified and the other profiles could be easily excluded, based on the observation of the chemical profiles.

FTS-16-LUB

This proficiency test provided 3 condoms that could be potential source/s of the chemical profile observed from item II001.

From the qualitative point of view, pyrograms obtained from Item II001 (Figure 71A) presented at least 8 visible peaks characteristic of siloxane degradation. Significant peaks were also observed after 18 minutes. Siloxane pattern D3-D10 was visible in the pyrograms. The pyrogram obtained from Item II002 (Blank swab) did not present any pattern.

The pyrograms obtained from items II003 (Figure 71B), II004 (Figure 71C) and II005 (Figure 71D) presented at least 8 peaks characteristic of siloxane degradation. II003 also presented small peaks in the region after 18 minutes. No significant peaks were noted in the region 14 - 28 minutes for II004 and II005. Siloxane pattern D3-D10 was visible in the pyrograms for all samples.

Comparison of the pyrograms obtained from item II001 and II002 determined that all the peaks observed in the profile of sample I001 come from the trace to be investigated, and not from the cotton swab itself.

As items II003, II004 and II005 were all condoms, the first step was to see if the items could be qualitatively differentiated by assessing the three chromatograms acquired using py-GC/MS. No visual differences in terms of siloxane pattern were observed with D3 – D10 detected in any of the items. Small variations within the chromatographic content were observed on item II003, compared to the other two items, as it presented different minor compounds, especially at the end of the chromatograms.

These observations illustrate the different chemical profiles obtained from items II003, II004 and II005. II004 and II005 could not be visually distinguished. Therefore, the visual comparison with II001 was based mainly on the patterns observed at the end of the chromatogram. Overlay of the chromatograms of II001 with II004 and II005 showed generally corresponding visual chemical profiles. The number of peaks and their positions could not be differentiated in the region of up to 18 minutes. After 18 minutes, differences were observed, as II004 and II005

had no peaks whilst II001 presented several important peaks, being assessed to D10 to D13 oligomers coming from the pyrolysis degradation. Overlay of the chromatograms of II001 and II003 again showed generally corresponding visual chemical profiles. The number of peaks and their positions could not be differentiated in the region of up to 22 minutes of elution. After 22 minutes, differences were observed, as II003 did not present any peaks and II001 presented several important peaks. Visual observations of the chromatograms did not allow any conclusions to be drawn at this point. Statistical evaluation was required to evaluate the potential discrimination between the samples.

Projection of all the samples in the database showed that all the items, including II001, were clustered together within the high viscosity silicone pattern, known to be from condoms. Classification indicated all the samples were from a condom.

Therefore, it was planned to re-extract the samples using a polar solvent (i.e. methanol) to perform GC/MS. As this proficiency trial had previously been analysed using dichloromethane extraction and GC/MS analysis, data were collected and studied (Figure 72). Please note that these chromatograms are significantly different from the ones presented in Chapter 6, as a different solvent was used. Chromatograms of II001 (Figure 72A), II004 (Figure 72C) and II005 (Figure 72D) were overlaid and showed significant differences in the chemical pattern, especially between 10 and 20 minutes, with the absence of the major, specific peaks in the chromatograms of II004 and II005, that were present in II001. Overlay of II001 and II003 (Figure 72B) showed indistinguishable chemical profiles along the entire chromatogram. Therefore, the chromatographic evidence makes it more likely that II003 is at the source of the chemical profile observed on II001, rather than if II004 and II005 are the source of the chemical profile observed.

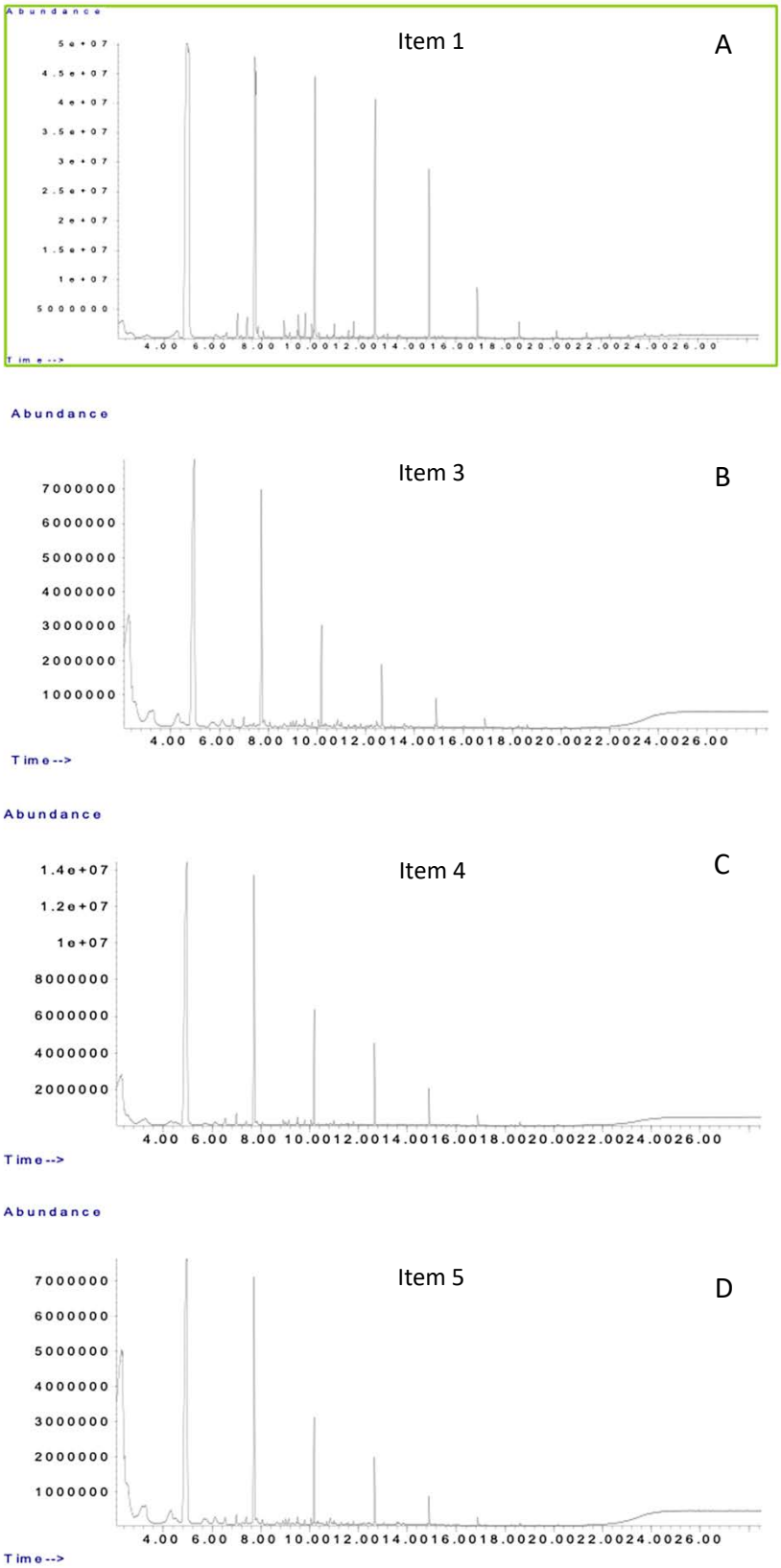


Figure 71: Chemical Profiles obtained for the questioned sample (in green) and the comparison samples – py-GC/MS analysis. 71A) pyrogram of item I001(questioned swab) extract; 71B) pyrogram of item I002 Durex Intense Sensation latex condom; 71C) pyrogram of item I003 LifeStyles Ultra Sensitive latex condom; 71D) pyrogram of item I004 Trojan Ultrathin Lubricated latex condom.

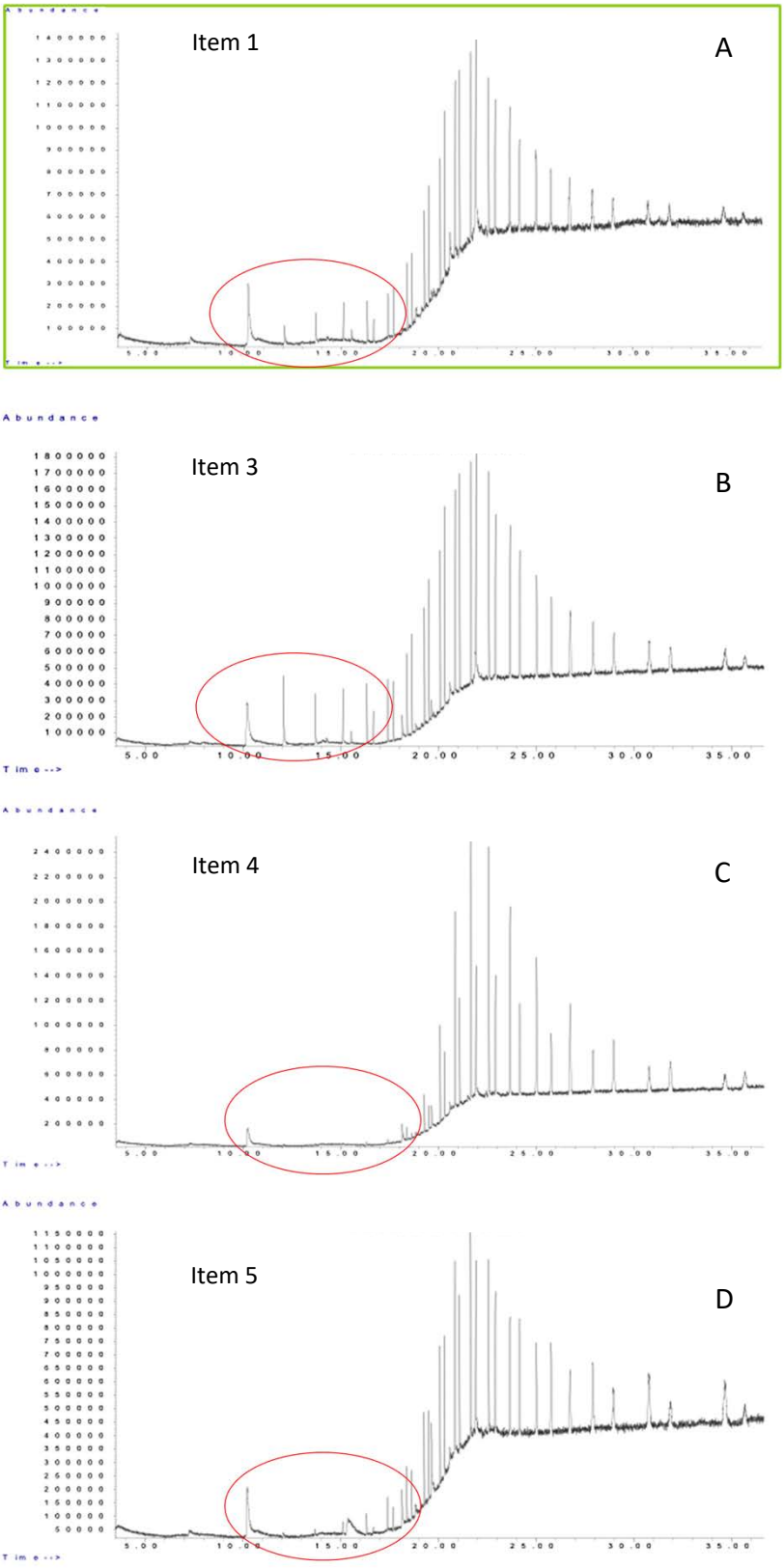


Figure 72: Chemical Profiles obtained for the questioned sample (in green) and the comparison samples – GC/MS analysis. 72A) pyrogram of item I001(questioned swab) extract; 72B) pyrogram of item I002 Durex Intense Sensation latex condom; 72C) pyrogram of item I003 LifeStyles Ultra Sensitive latex condom; 72D) pyrogram of item I004 Trojan Ultrathin Lubricated latex condom.

Proficiency trials FTS-16-LUB answer:
The questioned swabs (Fisherbrand Cotton-tipped Swabs) from Item 1 were prepared by sampling the lubricant from a package of a Durex® condom of the same product (Natural Feeling or Intense Sensation) and lot number as distributed with item 3, directly with the swabs. The swabs were then sealed in capped test tubes (BD Falcon 5mL polystyrene round bottom tubes) and further sealed in a manila envelope.
Item 4 and Item 5 correctly eliminated as a source for item 1. From the three items provided, only Item 3 presented a profile that could be *identified* as a source for Item 1.

7.2 Case studies

The work presented in this section are real case items processed at ChemCentre (Perth, WA) between December 2019 and January 2020. The results of the initial expert reports are presented in the first part and then compared with the procedure implemented in this research.

7.2.1 Case #1

Case context

Non-consensual penile-vaginal intercourse was alleged. The assailant wore a condom, which was not recovered. It is unknown whether the suspect ejaculated inside the condom or intravaginally without a condom. The complainant and suspect were known to each other, had not had prior consensual sexual contact and were not in a domestic relationship at the time.
A high vaginal swab only was submitted for analysis. A blank cotton swab was used as a control to ensure the absence of contamination. Samples were collected on Dec. 2. 2019, 02.00PM and sent for analysis on Dec.11 2019.

Mission

An RFA is requested to forensically examine SARC forensic biology specimens for the presence of trace DNA, spermatozoa and condom lubricants.

Forensic specimen collection & additional information

One bag containing 1 item was received on Dec. 11, 2019, at 10.00AM. The bag contained 1 cotton swab in a sealed packaging. The analysis was run on Dec. 14-16, 2019.
No other information regarding sample collection, time elapsed between activity and sample collection or victim's activity was available in the case file.

Analysis procedure

The analysis procedure used in this case was the following:

1. Extraction of trace evidence was run by Dr. DeTata following the procedure available in ChemCentre for condom lubricants trace evidence. The recovered cotton swab was cut into a vial and then soaked in 1mL hexane and placed in an ultrasonic bath for 15 minutes. One blank cotton swab was extracted at the same time to obtain the matrix chemical profile, and thus being able to subtract non-pertinent peaks from the questioned sample. Extracts were stored in the fridge until analysis time.
2. Analysis measurements using pyrolysis-GC/MS to determine the chemical profiles of the recovered swab and the blank swab. Overlay of the chromatograms enabled identification of similarities and differences.
3. Sample reconcentration was performed by evaporating all the extraction solvent under N_2. The residues were then dissolved in 100 µl hexane.
4. Confirmation analysis was performed by analysing the concentrated sample.

Results

The overlay of the blank swab and the recovered swab chromatograms acquired is presented in Figure 73. Observation of the chromatograms revealed the presence of D3 oligomer (black arrow on Figure 73) in the recovered item. This peak was absent from the blank swabs and from the solvent analysis. Therefore, it can be determined that it comes from the sample itself.

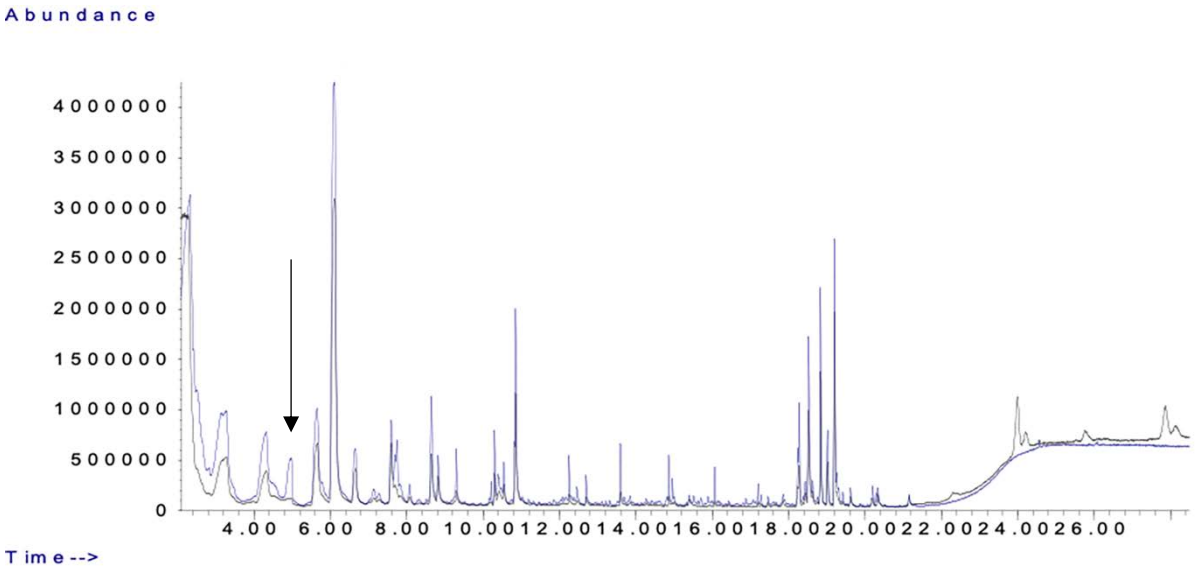


Figure 73: Overlay of TIC chromatograms of pyrolyzed residues. Black arrow points out D3 oligomer. In blue is the sample chromatographic pattern, in black is the blank swab.

The blank swabs presented a lot of peaks in the pyrogram. Given that the cotton swabs used were mounted on a plastic stick (COPAN Interpath ServicesH043N), and after careful identification of the peaks in the blank, it was determined that the peaks were coming from the plastic stick. This highlights the importance of cutting the cotton swab right at its base with the stick, or to use cotton swabs mounted on wooden sticks (such as COPAN 150C)

An Extracted Ion Chromatogram procedure was run on both concentrated samples (blank swab and recovered item) to confirm the presence of siloxanes degradation residues. Figure 74 presents the EIC for the recovered item. Extracted ions were *m/z* 73, 147, 221 (siloxane monomer, dimer and trimer respectively), 207, 281 and 355 (D3, D4 and D5 indicators). As illustrated in Figure 74, all these ions were found to be present in the recovered item. In addition, extraction of the 50 peak areas used to build the discrimination model database revealed that all the 50 compounds were present in the sample.

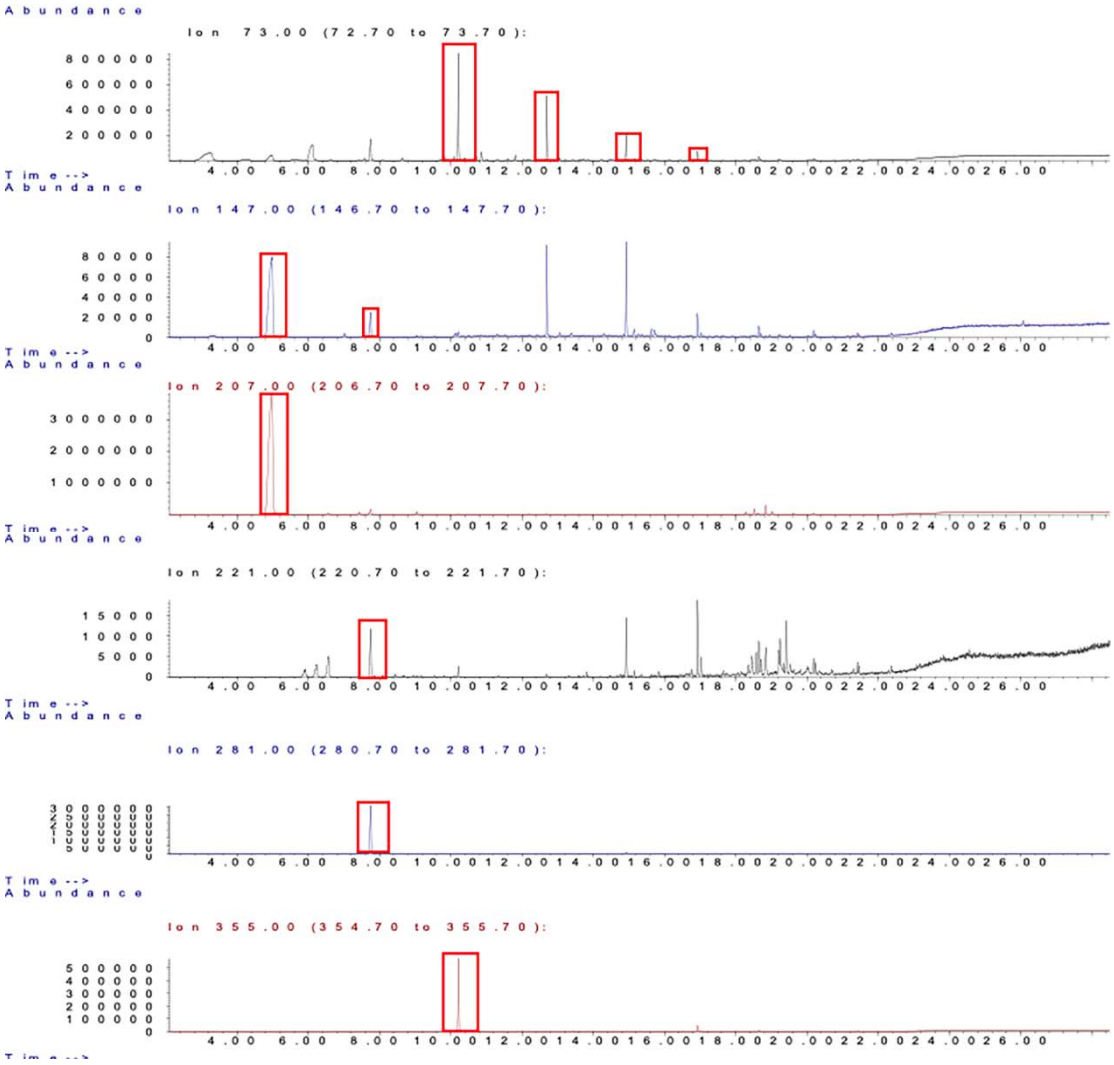


Figure 74: Extracted Ion Chromatogram (EIC) of siloxanes degradation m/z in recovered sample. Red boxes indicate peaks characteristics from siloxanes degradation.

These observations are consistent with those made during the analysis of condom lubricant extracts. However, a projection in the discrimination model would help to assess the dedicated class to which the present sample belonged. Therefore, the areas of the 50 target compounds were extracted, and normalized to the sum followed by double square root preprocessing, before projecting the sample in the discrimination model built. Figure 75 illustrates the projection of the sample in the discrimination model built for py-GC/MS acquired samples.

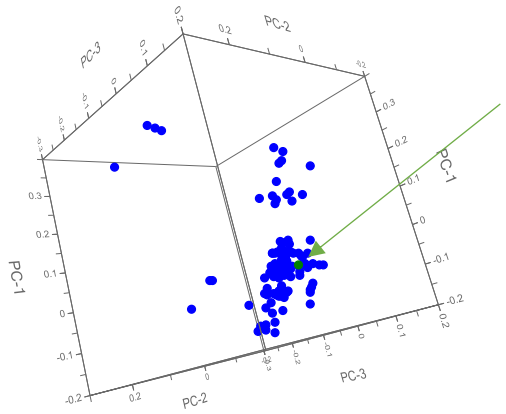


Figure 75: 3D Score plot of the projection of the sample in the discrimination model. The green arrow helps locating the sample in the clusters.

As illustrated in Figure 75, the chemical profile collected from the recovered evidence is clustered with condom samples. Therefore, it can be stated that the chemical profile collected from the recovered evidence is consistent with that of condom lubricants. The sample is therefore more likely to originate from a condom than from any other silicone-based sample present in the discrimination model. GC/MS analysis was not performed on the samples.

7.2.2 Case #2

Case Context

At 22h30 on Aug. 13th 2019, St John's Ambulance (SJA) were called to respond to a female patient that was allegedly suffering a drug overdose. SJA units attended the incident location, located an unresponsive female in an office area and commenced CPR. During the visual inspection, SJA observed on the female patient what appeared to be small wound to her rear rib cage below the shoulder blade and deemed the incident suspicious, thus calling for police attendance at 22h48. The female was the conveyed to the Royal Perth Hospital where paramedics observed a small wound to the centre of her chest. The female was worked on at Royal Perth Hospital where she was declared life extinct at 23h13.

An X-Ray of the deceased identified what is believed to be a projectile in the deceased's torso. The entry wound for the projectile indicated the deceased was shot from behind with an upward trajectory consistent with the offender positioned to her rear. It was possible the female was subjected to a sexual assault. It was known the deceased was alone with two males.

Mission

Examine forensic biology specimens for the presence of lubricants or condom evidence.

Forensic Specimen Collected

On Aug. 15th 2019, 09.15-09.40AM/10.20AM-03.10PM**,** SARC doctors collected labial swabs, perineal swabs, low vaginal swabs, high vaginal swabs, endocervix swabs, vaginal swabs using lubricated speculum, mons pubis skin swabs, inner thigh skin swabs. One swab was collected for testing for condom or lubricant residues. 1 black fibre was collected, and 1 hair was retrieved from cervix.

On Aug. 16th 2019, forensic pathologists collected urine, liver, stomach content, vitreous humour, 1 nasal swab and 2 oral swabs. Lubricant samples were not collected, and pubic hair not combed.

Aggression time is unknown, condom use, ejaculation, activity, menstruation or intercourse are unknown. The victim was fully dressed when SJA found her. Elapsed time between death and investigation is **around 10 hours**.

Analysis procedure

The analysis procedure used in this case was the following:

1. Extraction of trace evidence was run by Dr. DeTata following the procedure available in ChemCentre for condom lubricants trace evidence. The recovered cotton swab was cut into a vial and then soaked in 1 mL hexane and placed in an ultrasonic bath for 15 minutes. One blank cotton swab was extracted at the same time to obtain the matrix chemical profile, and thus being able to subtract non pertinent peaks from the questioned sample. Extracts were stored in the fridge until analysis time.
2. Analysis using pyrolysis-GC/MS to determine the chemical profiles of the recovered swab and the blank swabs. Overlay of the chromatograms enabled identification of similarities and differences.

3. A second extraction for polar compounds was conducted on the same swabs. The swabs were taken out of the hexane solvent, put in new individual clean vials and soaked in 1 mL methanol.
4. GC/MS was run to determine the chemical profiles of the recovered and blank swabs. Overlay of the chromatograms enabled identification of similarities and differences.

Results

Pyrolysis-GC/MS analysis did not detect any silicone-based residues after subtracting the blank swab peaks, nor after extracting relevant ions from the mass spectrum. No peaks were present and were therefore not integrated for projection in the model. These observations are consistent with the absence of condom lubricants or silicone-based lubricants products in the swabs. However, the initial mission was to find out whether lubricants were used. Knowing that polar lubricants might be present, a methanol extraction was analysed with GC/MS.

GC/MS results did not show evidence of glycerin, polyethylene glycol, propylene glycol or benzocaine, after subtracting blank swab peaks nor after extracting relevant ions from the mass spectrum. These results do not reveal indicators of the presence of PDMS nor of water-based compounds linked to any of condom, lubricant, oil and/or personal hygiene product use. These results are to be considered under the light of the possibilities to interpret the absence of evidence (see section 7.3 and **Chapter 12**).

7.2.3 Case #3

Case Context

On 17.12.2019 the complainant was at a family member's house with family. The suspect arrived and both spent a few hours drinking with other family members. At some point the complainant and suspect walked to a secluded bush area under a bridge. The suspect physically assaulted the complainant and choked her causing her to lose consciousness several times and urinate on herself. The suspect threatened to kill the victim and rape the victim. The complainant was able to crawl away from the suspect and make her way back to the family member's house. The complainant's sister conveyed the complainant to hospital. On police attendance a used condom with visible blood was located in the complainant's jacket pocket. The condom was seized, but was not sent to ChemCentre for analysis. The complainant was unsure whether she had been sexually penetrated by the suspect due to passing out and losing consciousness.

Mission

ChemCentre analysis was requested to forensically examine SARC specimens for the presence of condom lubricants.

Forensic Specimen Collected

One bag containing 1 item was received on Jan. 01, 2020, at 09.29AM. The bag contained 1 cotton swab in a sealed packaging. The analysis was run on Jan. 03, 2020.

Aggression suspected time was Dec. 16-17, 2019 between 10PM and 5AM. Evidence collection time was Dec. 18, 2019, 01.05PM. Elapsed time for persistence interpretation is **at least 20 hours**.

No other information regarding sample collection or victim's activity was available in the case file.

Analysis Procedure

The analysis procedure used in this case was the same as the one for the Case #2

Results

Pyrolysis-GC/MS analysis did not detect any silicone-based residues after subtracting the blank swab peaks, nor after extracting relevant ions from the mass spectrum. No peaks were present and were therefore not integrated for projection in the model. These observations are consistent with the absence of condom lubricants or silicone-based lubricants products in the swabs. However, the initial mission was to find out whether lubricants were used. Knowing that polar lubricants might also be present, methanol extracts were analysed with GC/MS.

GC/MS results did not show evidence of glycerin, polyethylene glycol, propylene glycol or benzocaine, after subtracting blank swab peaks nor after extracting relevant ions from the mass spectrum. These results do not reveal indicators of the presence of PDMS nor of water-based compounds linked to any of condom, lubricant, oil and/or personal hygiene product use. These results are to be considered under the light of the possibilities to interpret the absence of evidence (see section 7.3 and **Chapter 12**).

7.2.4 Case #4

Case Context

Between 17h00 on 22.12.19 and 03h00 on 23.12.19 the complainant was at a work Christmas function where she consumed a significant amount of alcohol. Due to this, the complainant has

a loss of memory from approx. 22h30 on 22.12.19 until 04h30 on 23.12.19 (no drugs consumed, nil indication of drink spiking). The complainant believes she was sexually assaulted during the period she cannot recall. SARC medical and forensic examination detail the complainant has injuries which could be consistent with sexual penetration. It is unknown what type of vaginal penetration occurred or if there was ejaculation or a condom used.

Mission

ChemCentre analysis is requested to forensically examine SARC specimens for the presence of condom lubricants.

Forensic Specimen Collected

One bag containing 1 item was received on Jan. 15, 2020. The bag contained 1 cotton swab in a sealed packaging. The analysis was run on Jan. 15, 2020.

Aggression suspected time is Dec. 22-23, 2019 between 10.30PM and 0.304AM. Evidence collection time is Dec. 23, 2019, 04.00PM. Elapsed time for persistence interpretation is **at least 12 hours**.

No other information regarding sample collection or victim's activity was available in the case file.

Analysis procedure

The analysis procedure used in this case was the same as the one for the Case #2

Results

Pyrolysis-GC/MS analysis did not detect any silicone-based residues after subtracting the blank swab peaks, nor after extracting relevant ions from the mass spectrum. No peaks were present and were therefore not integrated for projection in the model. These observations are consistent with the absence of condom lubricants or silicone-based lubricants products in the swabs. However, the initial mission was to find out whether lubricants were used. Knowing that lubricants can also be under a polar form, and therefore extractable with methanol, methanol extraction was processed and analysed with GC/MS.

GC/MS results did not show evidence of glycerin, polyethylene glycol, propylene glycol or benzocaine, after subtracting blank swab peaks nor after extracting relevant ions from the mass spectrum. These results do not reveal indicators of the presence of PDMS nor of water-based compounds linked to any of condom, lubricant, oil and/or personal hygiene product use. These

results are to be considered under the light of the possibilities to interpret the absence of evidence (see section 7.3 and **Chapter 12**).

7.3 Challenges

The four real case items processed within the framework of this thesis have highlighted that the main question of interest is whether a trace is detected or not. There is rarely any talk of comparative examinations that could lead to a Bayesian assessment in order to interpret scientific evidence. In addition, all the cases turned out to be investigative, with no proposal. According to the data from ENFSI guideline [137], it is therefore not possible to ask for an evaluation level under these conditions. From an investigative point of view, only the presence or absence of traces are evaluated.

Regarding the interpretation of the presence of traces, it is possible to say that there has been use of a product whose content is silicone-based. The use of the discrimination and classification model also makes it possible to determine the origin of the samples, and thus to provide investigative information. On the other hand, in the case where the victim would have had a consensual protected relationship with their partner for example, it would not be possible to differentiate different protected relationships on the basis of the chemical profile, since all condoms are not statistically differentiable. The relevance of the trace must however be studied. This is usually done using background noise studies (i.e. presence for legitimate reasons), studies which have not yet been published. The preliminary results presented in the publication of [76] have highlighted that there is a priori no trace of silicone-based residues in the vaginal matrices. However, this is a preliminary study that only targeted silicone-based residues. Regarding the interpretation of the absence of traces, several parameters, described by [14] must be considered:

- Characteristics of the donor (i.e. condoms): it has been shown in the previous chapters that most condoms on the market have a silicone-based or aqueous-based profile. These two types of samples have a variable persistence according to the literature, between 12-48 hours for silicone-based samples, and between 8-24 hours for water-based samples. However, there is a third type of sample, which are non-lubricated condoms, which have a composition mainly based on solid particles (i.e. cornstarch, polyethylene powder). These samples present a blank profile regardless of the analytical technique used. These condoms constitute less than 1% of the population of condom samples found in the three markets studied. Thus, a negative profile can be explained by the

absence of lubricant on the condom used, for example in the case of a dry condom (e.g. FairSquared Sensitive Dry).

- Characteristics of the receiver: these data are unknown and have not been referenced in the literature at present. Variations in the vaginal matrix can be observed, but they do not seem to significantly affect the profiles of the silicone-based samples [15]. The impact on the profiles of water-based samples is not known. The period of menstruation, however, can significantly affect the profiles, generating significant losses of traces.
- Contact: as noted by Locard's exchange principle [138], contact with a condom would leave a trace. The absence of a trace can be explained by the absence of contact with a condom as well as by the aforementioned criteria.
- Elapsed time: a key parameter influencing the detection of a trace is the time elapsed between the time of the aggression and the time of evidence collection. It is important to know, in order to be able to assess the persistence of the traces. Studies such as Tottey *et al*. [15] help to interpret the persistence of traces. However, as noted by [14], persistence times reported in the literature vary significantly. A silicone compound such as PDMS could thus be detected between 12- 48 hours post-coitus, while aqueous-based compounds, such as glycerin or propylene glycol type, could be detected between 8 and 24 hours in the vaginal matrix.
- Receiver's activity: it is a common reaction of victims of sexual assault to wash and get rid of their clothes or to wash them at very high temperatures. While the study by Monzò (2017) showed that washing underwear, even at 90ºC, did not affect the recovery of silicone-based compounds [25], the effect of victims' activity on the persistence of traces has not yet been extensively studied. The study by Tottey *et al*. which relates to the persistence of traces in the vaginal matrix unfortunately did not record the activities undertaken by the volunteers within the studied time intervals [15]. Based on the physico-chemical considerations of the compounds, it seems likely that the water-based compounds are most affected by washing activity. Monzò (2017) recorded activities, namely having a shower, performing a dynamic activity (sport) or a passive activity (sleep) or going to the toilet [25]. No significant results were obtained, and it was observed that the most important factor to affect the transfer and the persistence of the traces was the receiver itself.
- Sampling: there are currently no standardized and publicly available protocols for sampling of condom evidence. Variability can be introduced by the operator. However, the main consideration is to evaluate whether sampling is adequately processed, i.e.

using appropriate protection to avoid contamination or loss of evidence. Considering evidence is usually collected by doctors (e.g. Sexual Assault Resource Centre doctors), sampling can be considered as representative and adequate for condom evidence.

In the three negative cases noted, the following observations can be made:

- In the second case, as the victim had died, activity was considered to be null. The samples were collected directly following death, and so there is no lag time that can explain any loss of traces. Therefore, if a trace was present, it should be found, since it is known that these traces transfer in relatively high quantities [76]. The absence of traces thus is better explained under the assumption that there was no use of condom during sexual intercourse rather than under the assumption that there was use of condom during of sexual intercourse. This statement obviously depends on the time of the intercourse, which is likely to have happened prior to death (which is an assumption and is not verified), but the time interval is unknown.
- In the third case, the time between the attack and the time the trace was collected was at least 20 hours. The persistence interval was therefore within that defined by the various authors, which is 12-48 hours. The activity of the victim between these two moments is unknown. It is therefore difficult to interpret the absence of a trace in this case, even if we are at the investigative level.
- In the fourth case, the time between the assault and the time of collecting the trace was at least 12 hours; the lower limit of the time interval defined in the literature. The activity of the victim between these two moments is unknown. It is therefore difficult to interpret the absence of traces in this case. However, the context of the case indicates that a condom was seized from the victim's pockets. A complementarity with DNA could be investigated, but we currently have no information on further analysis. Analysis of this sample would make it possible to determine the evidence type to focus on, in order to be able to infer on the absence of traces. Indeed, in a case where the condom does not contain any lubricant, the absence of traces could be explained.

This chapter presented the application of the methodology developed on examples of practical cases. Several elements were highlighted and noted in the various examples treated:

1. The analysis of residues from condoms by py-GC/MS makes it possible to obtain a distinctive characteristic chemical profile when silicone products are present. When no

silicone profile was detected, GC/MS analysis was able to provide complementary information. This underlines the importance of an approach combining the two instrumental techniques.

2. The various cases encountered in practice have shown that these traces are essentially sought for an investigative purpose. A statistical assessment under two mutually exclusive assumptions was not developed, because in any case no assumption was provided, and it was considered too dangerous to position oneself as an expert under these conditions.
3. Furthermore, it was also shown that the methodology was applicable to trace detection in real cases. However, in the typical cases that can be encountered, i.e. a victim who has lost consciousness, the profiles obtained are more likely to be negative, given that condoms are only found in 20% of the rape or sexual assault cases, all the more so if there is no additional contextual information. In these cases, and in particular those including unknown perpetrators, DNA analysis should be prioritized and the analysis of traces of condoms considered in the case of a negative DNA profile. The analysis thus becomes incorporated in a process involving not only forensic scientists but also experts in forensic genetics and toxicologists, or even doctors. This confirms the importance of the collaboration between different domains of expertise when it comes to casework.
4. Concerning the interpretation of the results, more specifically negative results, in this work, only an investigative use was highlighted. It has been shown that trace detection can be affected by persistence and that this information is often missing when collected by doctors before the forensic process. However, parameters such as the activity of the victim or a recent protected intercourse should be incorporated during the transmission of the samples since these parameters allow the pre-evaluation of the evidence and therefore determine whether it is worthwhile to proceed with analysis of the sample.
5. Finally, the last point concerns transfer and persistence studies which can help in the interpretation of the results. The time interval between the suspected time of the assault and the time of the collection of the trace is generally less than 24 hours. However, according to the information reported in the literature, significant variations can occur, making the interpretation of the trace complex and requiring more investigation in terms of transfer and persistence, but also in terms in background.

The casework and the interpretation questions presented outlined some needs in terms of condom evidence analysis, but also illustrated some recommendations for practitioners.
Firstly, it is highly recommended that condom evidence is considered when Forensic Physicians examine a complainant alleging a recent sexual assault. The dedicated specimen should be collected with a cotton swab and sent for chemical analysis. Medical examiners should also ask the victim specific questions relating to the use of condoms, lubricants or personal hygiene products in the past week, and if the victim took a shower prior to evidence collection.
Once the cotton swabs is received by the forensic laboratory, the flowchart previously described in the litterature should be followed. Infrared spectroscopy may be added in the sequence as a screening method, which would indicate the appropriate instrumental analysis (i.e. water-based or PDMS focussed analysis), but the recommended FTIR technique, DRIFTS, might not be available in all laboratories. The flowchart (Graph 1) is proposed from the present study, and is recommended for condom evidence analysis.

Further questions on how to analyse swabs for both DNA and lubricants are still pending. Indeed, 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. This question has been investigated in a Master's Thesis led at the School of Criminal Justice in Spring 2021 (See Chloé Mbo's Master Thesis) and therefore will not be addressed in this PhD research.

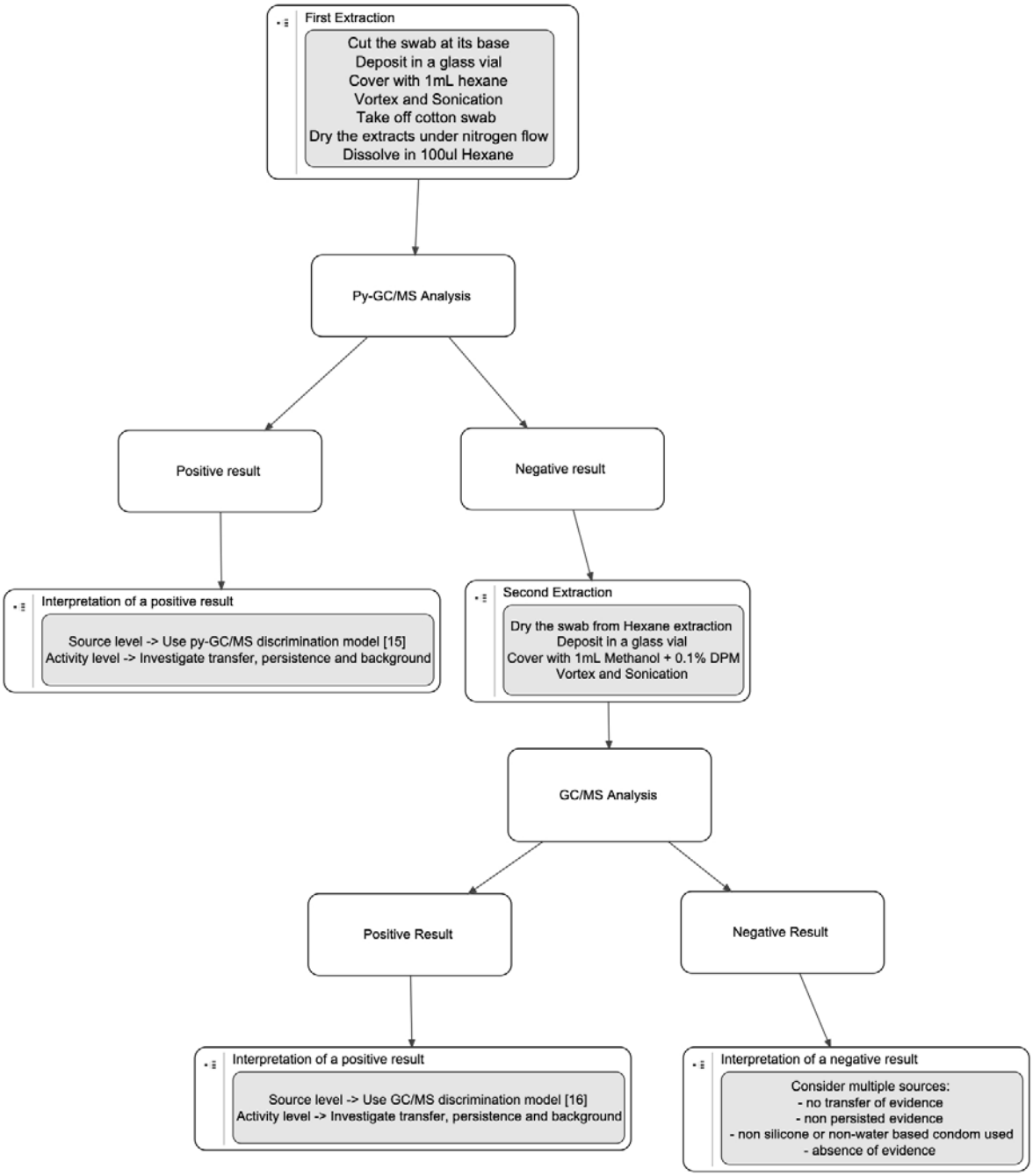


Chart 1 : Flowchart of the procedure recommended for condom evidence analysis in casework

Highlights

This chapter investigated the applicability of the framework on proficiency trials and case work samples. The main results are the following:

- The analytical framework was found to be applicable to proficiency test and to real situations
- FTIR was not tested, only py-GC/MS and GC/MS were
- Positive results are easy to interpret within the statistical framework presented.
- Interpretation of a negative result is more complex due to all the influence factors to consider
- There is a need for further research regarding background, transfer and persistence to assist the interpretation of the evidence.

As case work interpretation has highlighted which parameters were specifically needed in order to be able to interpret the evidence, the following chapters (**Chapter 8, Chapter 9** and **Chapter 10**) will be presenting first the optimization of the FTIR parameters for casework samples, followed by a prevalence study to represent the background parameters and preliminary studies of transfer and persistence. **Chapter 11** will eventually discuss interpretation of the evidence from a purely theoretical point of view.

Chapter 8 : FTIR analysis – Traces Oriented Questions

Selection, optimization and quantitative evaluation for silicone-lubricated condom traces analysis

This chapter is based on the following articles (Appendix I) :

Article 4: Burnier C., Van Bronswijk W., Massonnet G. (2020) *Comparison of spectroscopic methods in detection of silicone-based condom lubricants evidence,* Analytical Methods

Article 10: Burnier C., Monzò M., Massonnet G, Lewis S.W, Sauzier G. (2020) *Reporting negative results on quantification of PDMS using optimized DRIFTS analysis*, Manuscript in redaction

Article 14: Burnier C., Favre V., Massonnet. (2021) *The use of an optimized DRIFTS-FTIR method for the forensic analysis and classification of silicone condom lubricants*, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

Spectroscopic techniques, such as infrared and Raman spectroscopy, are very powerful and valuable tools for analysing evidence in forensic sciences. In sexual assault cases, vibrational spectroscopy has been reported as a useful screening method to detect condom residues. However, there has been no study to identify which specific method could be the most suitable technique for the analysis of silicone-based lubricants as forensic evidence.

8.1 Selection of the FTIR technique

Several modes of infrared analysis (micro-transmission, DRIFTS, micro-ATR and ATR) have been tested in the literature, but the conditions vary according to the authors, as presented in Table 25. It is clear from the information provided that there is no single established method for the analysis of PDMS-type silicone lubricants using infrared spectroscopy. It is also surprising to observe that the number of scans and the resolution vary significantly between studies, knowing that these are two factors influencing the reproducibility of the measurements, as well as the presence of artefacts [139]. The results obtained in the literature are therefore not comparable here. In order to be able to choose the most suitable method for the analyses that will follow, a standardised comparison of all the methods is necessary.

Table 25 : FTIR analysis parameters found in the literature. NR = not reported. Reproduced from *Burnier C Van Bronswijk W., Massonnet G. (2020) Comparison of spectroscopic methods in detection of silicone-based condom lubricants evidence, Analytical Methods*

Ref.	Mode	Resolution	Scans	Range of measurement	Notes
[11]	Micro-transmission	4 cm^{-1}	100	NR	Use of a 3M IR card
[11]	DRIFTS	4 cm^{-1}	100	NR	
[9]	DRIFTS	2 cm^{-1}	500	NR	
[41]	Micro-ATR	NR	256	650-4000 cm^{-1}	
[41]	DRIFTS	NR	128	650-4000 cm^{-1}	
[42]	ATR	4 cm^{-1}	256	600-4000 cm^{-1}	Germanium Crystal used
[71]	ATR	4 cm^{-1}	256	950-3600 cm^{-1}	Imaging used

Regarding the use of Raman spectroscopy, three articles have reported its use for PDMS analysis. The analysis settings used in these articles are as follows (Table 26):

Table 26 : Raman analysis parameters found in the literature NR = Not reported. Reproduced from *Burnier C Van Bronswijk W., Massonnet G. (2020) Comparison of spectroscopic methods in detection of silicone-based condom lubricants evidence, Analytical Methods*

Ref.	Mode	Laser	Power	Scans	Resolution	Spot Size
[71]	Imaging	788nm	10mW	NR	1 cm^{-1}	1 x 10 µm
[69]	Imaging	532nm	NR	NR	NR	NR
[13]	FT-Raman	1024nm	NR	128	8 cm^{-1}	50 µm

Despite their common application to forensic case work, few studies have been carried out into IR and Raman spectroscopy of condom traces, and no strategic choice of parameters by the authors was clearly stated. This lack of data can be confusing and would generates variable and unreliable analyses in real-life cases. Therefore, the first step is to identify which spectroscopic technique is the most relevant.

8.1.1 Material and sample preparation

Chemicals and samples

Hexane (Sigma-Aldrich), ethanol 99% for GC (Sigma Aldrich), ethyl acetate (Sigma Aldrich), polydimethylsiloxane 100cSt (Acros Organics), dimethylpolysiloxane 200cSt (Sigma-Aldrich) and polydimethylsiloxane-hydroxyterminated 750cSt (Sigma-Aldrich) were used as received. Microscope glass slides from VWR International were used.

Solutions and extractions

Because of the viscous consistency of all the reference materials (100, 200 and 750cSt), a solution of each reference material is required for DRIFTS. Moreover, this is also more representative of real case challenges, considering that most often an extraction procedure will be needed to remove the lubricant from swabs in case work. Solutions of PDMS in hexane were

prepared at concentration of 100 mg/ml, 10 mg/ml and 1 mg/ml. All the solutions were vortexed 1 minute before the analyses, to ensure a uniform dilution of the polymers in hexane. All solutions were prepared fresh and stored in a freezer at -18 ° C.

Instrumental conditions

The following FTIR and Raman instrumentation and accessories were used for the analyses (Table 27 and Table 28). FTIR acquisition parameters were fixed at 64 scans, a resolution of 4 cm^{-1} and a gain of 1 for all analyses, as a good compromise between the spectral quality and spectrum acquisition time.

Table 27 : FTIR Instrumentation and accessories

FTIR	Digilab FT 3000 Excalibur Series
Microscope	Digilab UMA600, Objective 16x
Compartment Accessories	*Transmission:* Diamond Compression Cell Kit (Thermo-Spectra-Tech) *ATR*: Golden Gate Single Reflection Diamond ATR System (Specac) *DRIFTS*: Spectra-Tech 0030-05 Collector II Diffuse Reflectance Accessory (Spectra Tech)
Micro-FTIR	*Transmission:* Micro Single Diamond Plate Cell (Thermo-Spectra-Tech) *ATR*: Slide-On ATR Objective [Germanium Crystal] (Thermo Nicolet)

Table 28: Raman instrumentation

Raman	Renishaw RM1000
Microscope	Leica DML
	Objectives 5x, 20x, 20x LWD, 50x, 100x
Lasers	488, 514, 633 and 785nm

Methodology

Transmission and Micro-Transmission - FTIR

A blank of the support (half diamond cell windows) was determined before spiking one droplet of the bulk material with a Pasteur pipette on to one diamond cell window and in the case of the compression cell the second window was then pressed on and the assembly finger tightened. Once spectral acquisition of the sample was completed, the support was cleaned with ethanol (as recommended by the suppliers), and a new blank was taken before the next analysis. Solutions were analysed with both methods with 5 minutes drying time before the analysis so that all the solvent had evaporated. For each bulk PDMS or solution a series of analysis consisting of 5 replicates analysed the same day by each analysis method was carried out.

ATR and Micro-ATR- FTIR

The same procedure as for transmission was used (see 2.4.1). Once the bulk reference material was deposited on the support (i.e. glass microscope slide for micro-ATR and diamond cell window for ATR), the crystal was then brought into contact. The contact pressure could not be standardised, but real-time visualization of the acquired spectrum as the pressure was increased ensured that sufficient pressure was applied to obtain a spectrum. For each reference standard, a series of analysis made up of 5 replicates analysed the same day by each analysis method was carried out. No solution samples were analysed with these methods.

DRIFTS- FTIR

For DRIFTS analyses, KBr was finely ground for about 15 minutes using an electric mechanical grinder. The finely mixed KBr was placed in sample cups and dried for 15 minutes at 100 ° C. When the bulk material was deposited on the KBr it was observed that it could not be absorbed on to it. Hence solution samples of each PDMS were used and individually deposited on the dry KBr filled cups using an eVol® syringe. Four cups filled with just KBr were kept for blanks. After spiking with 10µl of solution, samples were put in an oven for 15 minutes at 100 ° C to ensure solvent evaporation. Samples were left to cool down before analysis. A blank was taken every 3 analyses. For each reference standard solution, a series of analysis made up of 5 replicates analysed the same day was carried out.

Raman

Bulk PDMS was deposited on a microscope slide covered with aluminium foil. Blank measurements were made just next to the droplet. Diluted solutions were also analysed. For these analyses, 5 minutes drying time was used before the analysis, so that all the solvent had evaporated. For each reference standard or solution, a series of analysis made up of 5 replicates analysed the same day on each analysis method was carried out.

8.1.2 Chemical profiles – Observations

Preliminary considerations

Reference material was used based on previous research from [9,11,25]. According to [9,11,14,41,42,76], PDMS exhibits 4 major peaks linked to Si-C stretching at 1263 cm^{-1}, Si-O-Si asymmetric stretching at 1090 and 1020 cm^{-1}, and dimethyl and trimethyl deformation close to 807 cm^{-1}. Typical reference PDMS spectrum is illustrated on Figure 76. Vibrations coming from Si-O and Si-C bonds are the most relevant ones as they are directly linked to the PDMS

backbone, and therefore are diagnostic peaks to attest presence of silicone-based compounds in a sample. Interesting wavelength are therefore mainly in the zone between 1500 and 500 cm^{-1}.

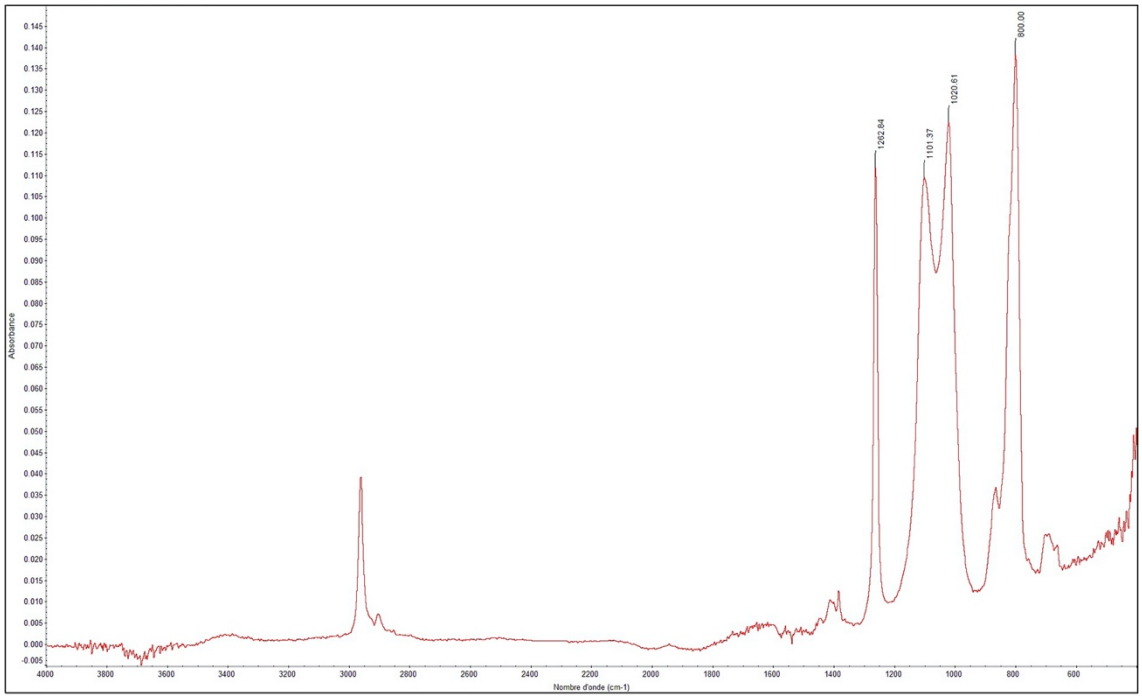


Figure 76 : Reference spectrum for PDMS 200 cSt viscosity, acquired with DRIFTS. Reproduced from *Burnier C Van Bronswijk W., Massonnet G. (2020) Comparison of spectroscopic methods in detection of silicone-based condom lubricants evidence, Analytical Methods*

Transmission (diamond cell) and Micro-transmission

The visual evaluation of the 15 analyses of pure PDMS samples showed that the 4 expected peaks for the PDMS are all present in each of the samples analysed. The quality of the spectra is good, with well-defined peaks, an absence of spectral saturation and a good resolution (Figure 76). A significant visual variation is shown on Figure 77, with absorbance intensities varying between 0.2 and 0.5 for the same sample. This is due to the sample preparation, as the amount of sample spiked on the diamond cell was not always the same between the replicates. Finally in terms of preparation time and analysis time, the latter two are very short (less than 2 minutes, about 5 minutes of preparation), but it is necessary to clean the diamond cell between each analysis, which implies making a blank measurement immediately afterwards to make sure there is no contamination. Thus, the number of analysis is doubled, and this can be a considerable disadvantage when the number of samples to be analysed is significant. Moreover, in view of the vertical holding of the cell and the design of the cell, which is not made to contain liquids, the analysis of liquid samples from solid-liquid extractions of the sample swabs seems unideal.

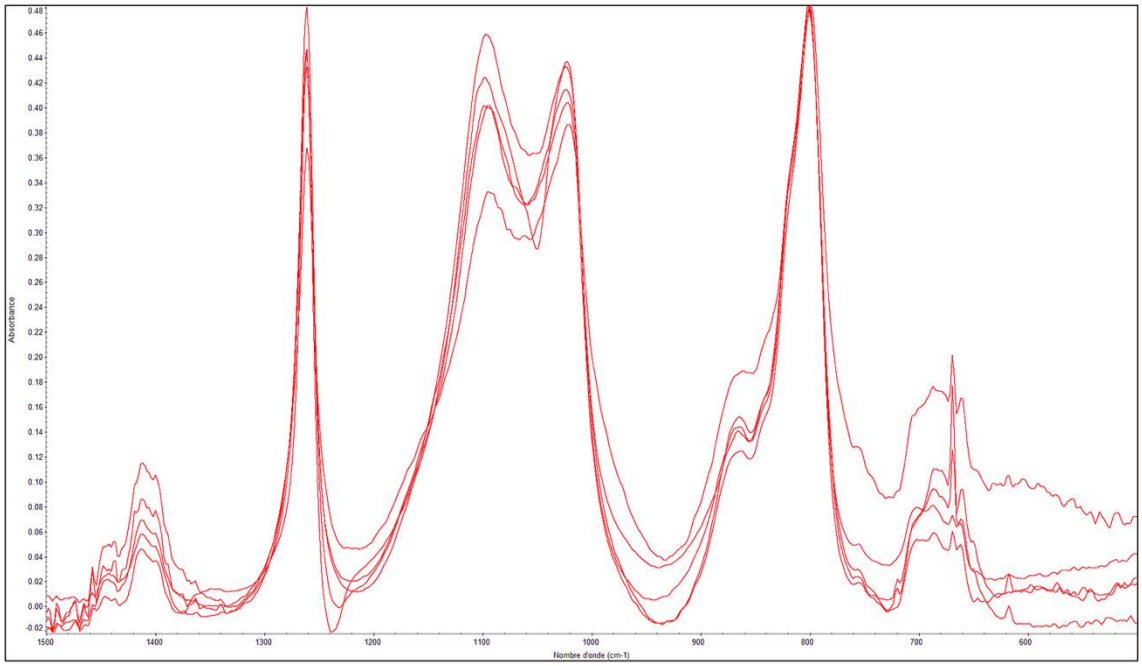


Figure 77: Illustration of the variability of compression cell transmission PDMS IR spectra, between 500 and 1500cm^{-1}, on a 200cSt viscosity PDMS. 100cSt and 750 cSt viscosity PDMS are shown in Appendix V. Reproduced from *Burnier C Van Bronswijk W., Massonnet G. (2020) Comparison of spectroscopic methods in detection of silicone-based condom lubricants evidence, Analytical Methods*

Micro-transmission was only run on 4 analyses as saturation of the detector could be observed (Figure 78). This resulted in the peaks appearing flattened or simply not visible, as highlighted on Figure 78. Despite the saturation, two spectra presented a semblance of profiles presenting the expected 4 peaks characteristic of PDMS. Thus, it was not considered useful to continue the analyses on pure standards, but an evaluation on diluted standards would be interesting to determine if this method is sensitive enough for trace analyses, as indicated by other researchers[**].

[**] Personal Communication, J. Dake, Navy Criminal Investigation service (november 2017)

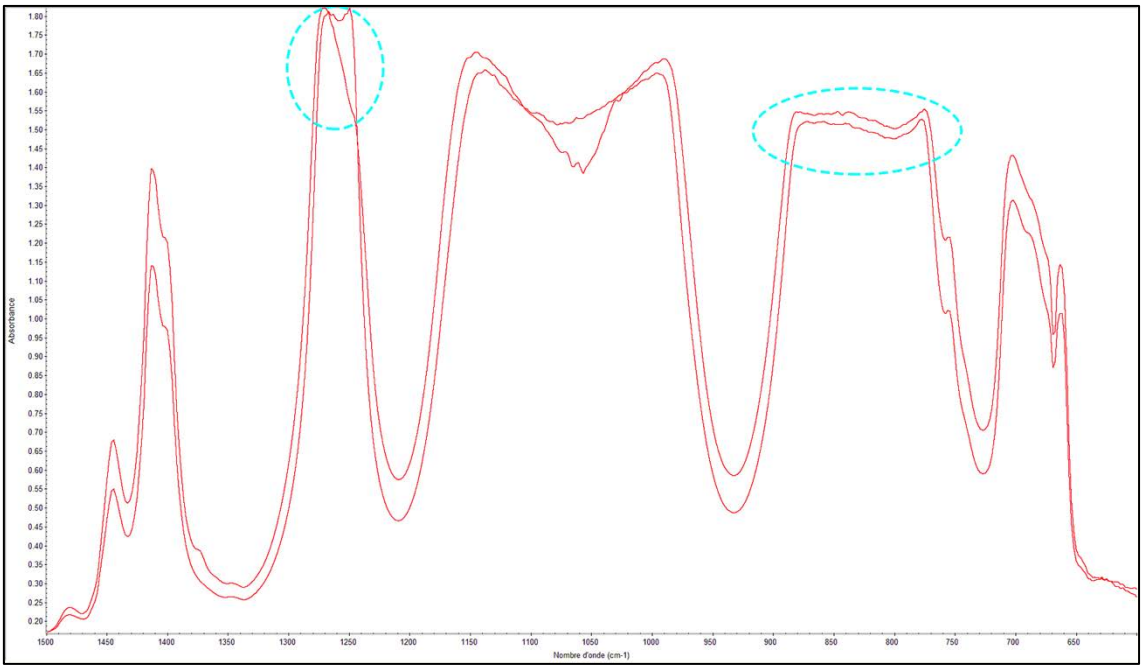


Figure 78: Illustration of the saturation of micro-transmission spectra, between 500 and 1500cm^{-1}, on a 200cSt viscosity PDMS Reproduced from *Burnier C Van Bronswijk W., Massonnet G. (2020) Comparison of spectroscopic methods in detection of silicone-based condom lubricants evidence, Analytical Methods*

At the time of deposition of the solution on a microscope slide, the solution is homogeneous and the PDMS is uniformly distributed in the solvent. Due to the effect of the sol-gel transition linked to the dissolution of a polymer in a given solvent, as the solvent begins to evaporate the capillary forces that come into play generate irregular agglomerates that are randomly distributed on the surface of the slide. At the time of analysis, all the solvent had evaporated and only the PDMS aggregates remained (Figure 79). The aggregates can be difficult to find on the microscopy slide, especially if the concentration is low. Indeed, it is necessary to carefully examine the entire deposition zone using very high magnification, in order to find one of these polymeric aggregates. Spectra were acquired on three aggregates and the profiles were found to be similar to that obtained for the bulk material. However, the time penalty when using micro-transmission as a screening method for the analysis of condom lubricant residues limits its usefulness.

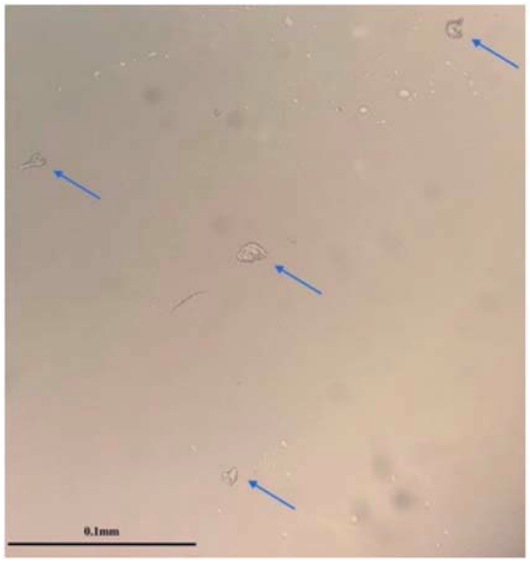


Figure 79: Aggregates illustration, transmission mode, Leica DMRX-P Microscope, 40x objective, bright field. Reproduced from *Burnier C Van Bronswijk W., Massonnet G. (2020) Comparison of spectroscopic methods in detection of silicone-based condom lubricants evidence, Analytical Methods*

ATR and Micro-ATR

Visual evaluation of the spectra obtained for the ATR-FTIR and micro-ATR-FTIR analyses showed undistinguishable spectral profiles for all the samples (Figure 80 and 81). Although Si-C elongations (1260 cm^{-1}) and CH deformations (800 cm^{-1}) are well defined, the doublet of the Si-O-Si bond is not clearly defined, which is the main difference with transmission spectra. Only the 1020 cm^{-1} peak of the doublet is distinctly present, while the second at 1090 cm^{-1} appears more as a shoulder, highlighted by the blue arrows on Figures 80 and 81.

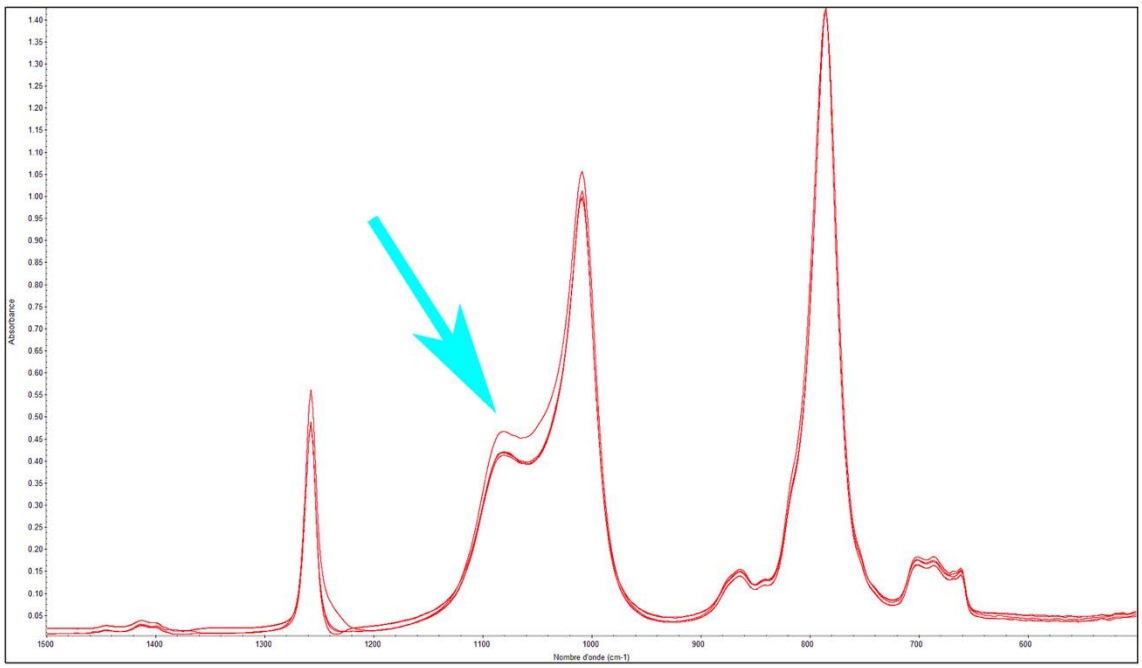


Figure 80: ATR-FTIR spectra obtained between 500 and 1500 cm^{-1}, on a 200cSt viscosity PDMS. 100cSt and 750 cSt viscosity PDMS are shown in Appendix V. Reproduced from *Burnier C Van Bronswijk W., Massonnet G. (2020) Comparison of spectroscopic methods in detection of silicone-based condom lubricants evidence, Analytical Methods*

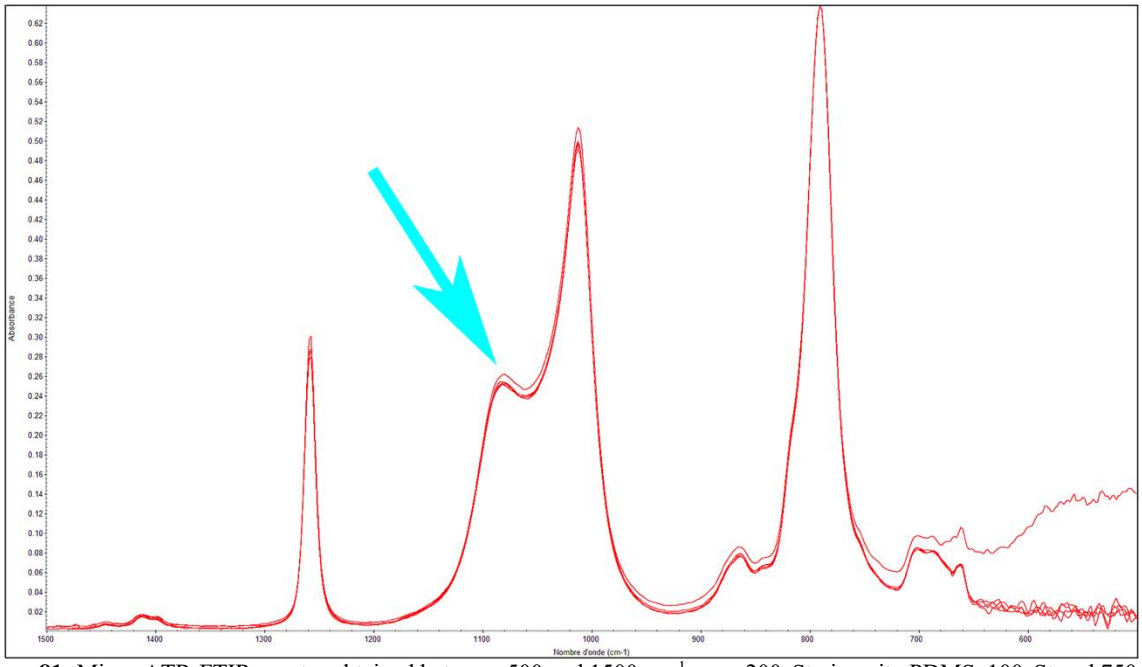


Figure 81: Micro-ATR-FTIR spectra obtained between 500 and 1500 cm^{-1}, on a 200cSt viscosity PDMS. 100cSt and 750 cSt viscosity PDMS are shown in Appendix V. Reproduced from *Burnier C Van Bronswijk W., Massonnet G. (2020) Comparison of spectroscopic methods in detection of silicone-based condom lubricants evidence, Analytical Methods*

This shouldering phenomena can be explained by an increase in the width at half height of the peak, causing the overlap of the peaks and a reduced resolution. The identification of the peak positions showed that there was no significant shift in the peak position between the transmission analysis and the ATR analysis. As the same observation was made for both germanium and diamond crystals, a hypothesis was raised that there was an interaction between PDMS and the crystal, generating changes in relative intensity of the antisymmetric vibration at 1090 cm^{-1}. This interaction might be an issue in evaluating the profile and interpreting evidence in the event both peaks would not be clearly defined. The 1090 cm^{-1} peak is less visible, thus leaving the symmetrical vibration of the Si-O bond and the vibration of the Si-C bond as the only reliable indicators of the presence of PDMS. Regarding repeatability, minimal visual variations were observed, despite the fact that the amount of sample spiked on the analysis cell was not always the same between the replicates. This is due to the ATR mode, as depth of penetration is only a few microns and not the entire thickness of the sample, as with transmission. Preparation and analysis time were short for both techniques.

When applied to liquid PDMS samples, both techniques were found to be unsuitable, as none of the acquired spectra presented PDMS diagnostic peaks. As previously mentioned with transmission methods, polymeric aggregates are formed while solvent evaporates on the crystal. Concerning ATR, aggregates would be randomly found on the crystal and thus very difficult to target for analysis. Additionally, the use of hexane on ATR crystal should be carefully

considered as it may dissolve the glue binding the crystal in position. However, ATR is a very valuable tool when working on bulk samples, or non-diluted samples, as it is a very rapid technique with high accuracy.

DRIFTS

While this technique is generally less frequently used than other modes of infrared spectroscopy, it is widely used to analyse liquids samples adsorbed or dispersed solids in KBr powder matrix [140]. Spectra of pure standards could not be acquired due to spectra saturation. Tests on diluted samples were conducted to assess the possibility of applying this technique to real samples. Diluted solutions of 5 mg/mL and 10 mg/mL were too concentrated and generated saturated spectra. Therefore, five dilutions of concentrations between 0.1 and 2.0 mg ml^{-1} were then prepared and analysed. Spectra exhibited chemical profiles similar to the ones acquired in transmission, with 4 peaks, at the given wavelength for PDMS (Figure 82). These peaks were always present, regardless of concentration. Slight signs of saturation are visible at the doublet of the Si-O bond on the 2.0 mg mL^{-1} spectrum (in red on Figure 82).

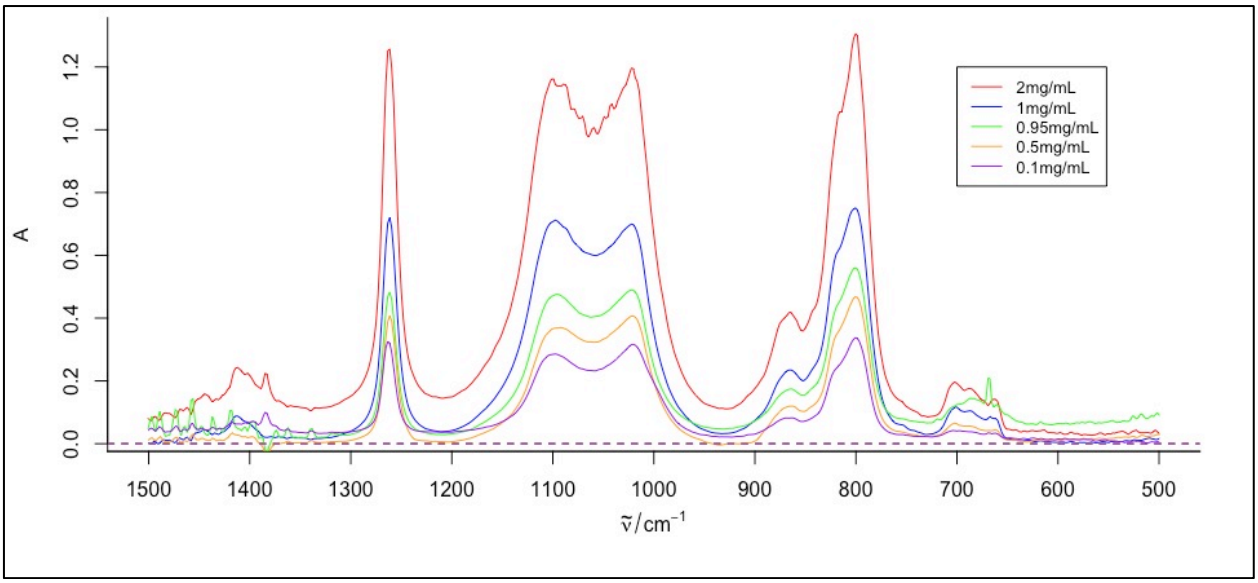


Figure 82 : Illustration of PDMS200 dilution spectra of concentration 0.1 to 2.0 mg/ml after analysis in DRIFTS between 500 and 1500 cm^{-1}. Reproduced from *Burnier C Van Bronswijk W., Massonnet G. (2020) Comparison of spectroscopic methods in detection of silicone-based condom lubricants evidence, Analytical Methods*

The reproducibility was evaluated using three different concentrations. The intensity of the Si-O doublet was found to vary from ~0.3 AU, for 0.1 mg/mL solution, whilst abundance was ~0.4 for the 0.5mg/mL solution. The same observations were made with the 1.0 and 2.0 mg mL^{-1} samples, which exhibited peak intensities of 0.8 and 1.2 AU, respectively. This variation comes from the preparation of the sample for analysis as the KBr pellets are prepared manually

and variations in particle size and distribution can be expected. This generates variable coating of the KBr while depositing the liquid sample on the cell. This variability can be reduced by controlling the particle size when grinding KBr. The limit of detection with this technique was found to be approximately 0.025 mg/mL. Results obtained from the present study suggest that DRIFTS is the most suitable technique for condom traces analysis in case work.

Raman

Reference PDMS Raman spectra illustrated on Figure 83 all exhibit bands resulting from the symmetric and antisymmetric elongations of the groups CH_2 and CH_3 between 3000 cm^{-1} and 2800 cm^{-1} and bands resulting from the vibrations of the Si-C and Si-O bonds, between 800 cm^{-1} and 400 cm^{-1}.

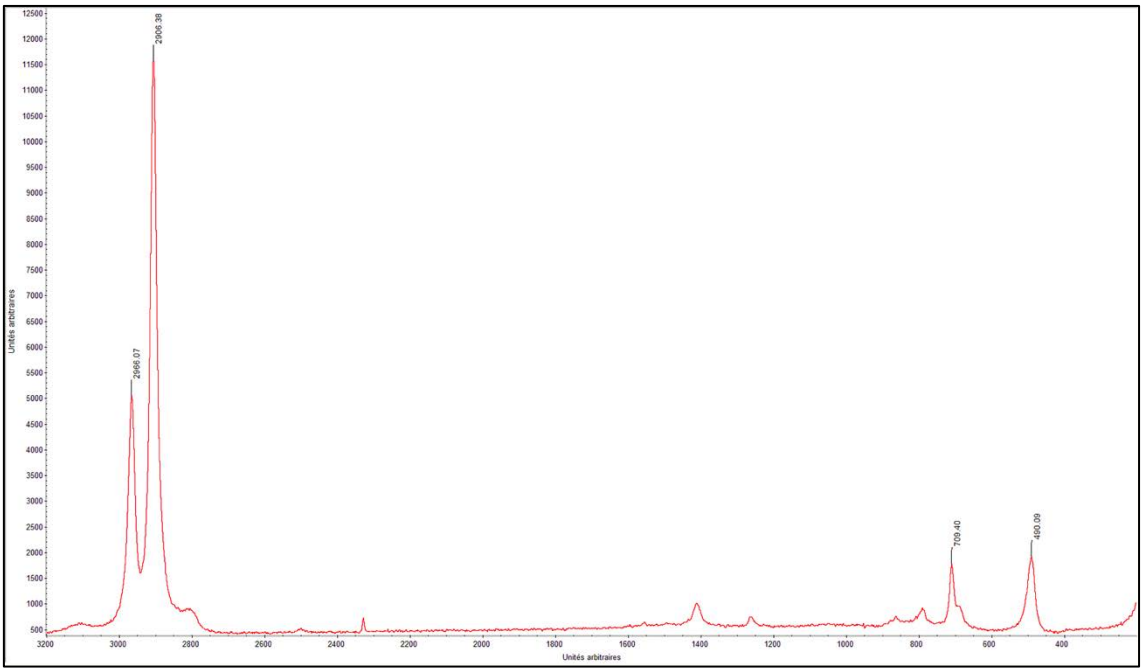


Figure 83 : Illustration of the chemical profile of PDMS, obtained using 514 nm excitation laser. Reproduced from *Burnier C Van Bronswijk W., Massonnet G. (2020) Comparison of spectroscopic methods in detection of silicone-based condom lubricants evidence, Analytical Methods*

Each laser highlighted different scattering ratios between the band groups. Spectra obtained with 488 nm and 514 nm lasers were mainly dominated by CH_2 and CH_3 vibration bands (cf. Figure 84 A and B). However, these peaks are less diagnostic than the peaks originating from the Si-C and Si-O bond vibrations, as they are the most common molecular bonds. Despite good repeatability, a better balance of the relative proportions of the peaks between them would be desirable, as well as improved visibility and definition of the bands coming from the Si-O and Si-C bonds.

Spectra acquired with the 633 nm laser show a better relative balance between the two groups of peaks, with less distant relative intensities and the peaks of the Si-O and Si-C bonds being

better defined (cf. Figure 84 C) but using the 785 nm laser resulted in the best definition of the Si-O and Si-C bands (cf. Figure 84 D); however, greater variability between replicates can be observed as the laser approaches infrared.

Additionally, on spectra acquired with visible lasers (488 and 514nm) two peaks are visible between 1400 cm^{-1} and 1200 cm^{-1}, which are obscured by spectral noise when the laser wavelength increases. These two peaks can be attributed to the harmonics of the C-H bond. This hypothesis is corroborated by the decrease in peak intensity and their disappearance in a ratio similar to that observed for the symmetrical and asymmetrical vibration bands of the C-H bond described above. Although there are bonds suggesting Si-O deformation, the major diagnostic doublet was not found to be Raman active. Therefore, the instrumentation was not found to be suitable for condom lubricant detection, compared to infrared spectroscopy.

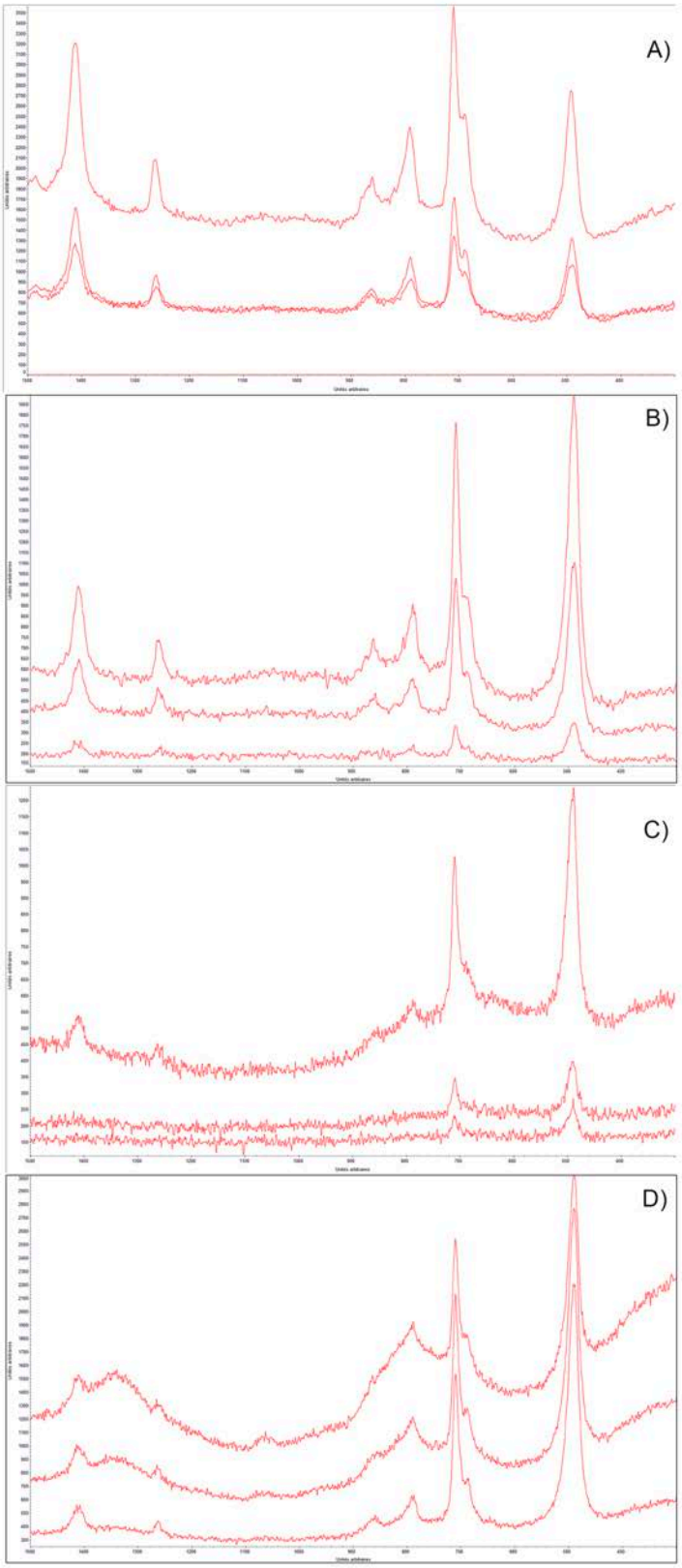


Figure 84 : Raman spectra obtained between 500 and 1500 cm^{-1}, on a 200cSt viscosity PDMS, with A) 488nm laser, B) 514nm laser, C) 633nm laser and D) 785 nm laser. Reproduced from *Burnier C Van Bronswijk W., Massonnet G. (2020) Comparison of spectroscopic methods in detection of silicone-based condom lubricants evidence, Analytical Methods*

Mapping was also investigated given results obtained by other researchers [71], as a potential way to easily detect aggregates after solvent evaporation. However, no conclusive results were

obtained, even though a 100x magnification objective was used. The best spectrum gathered from these analyses is illustrated in Figure 85.

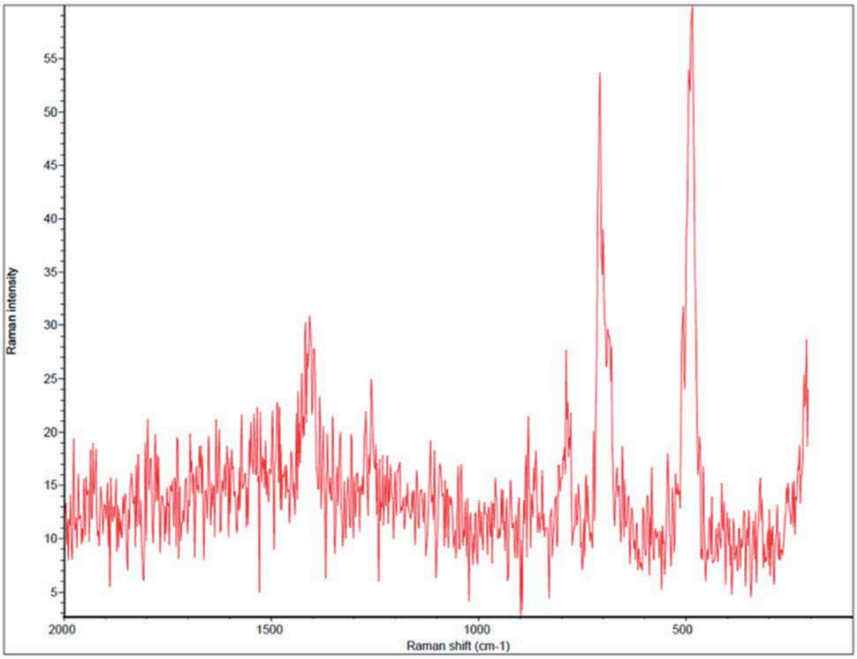


Figure 85: Raman spectrum of identified PDMS aggregates, with a 100x magnification objective. Reproduced from *Burnier C Van Bronswijk W., Massonnet G. (2020) Comparison of spectroscopic methods in detection of silicone-based condom lubricants evidence, Analytical Methods*

8.1.3 Chemical profiles – Comparison

Comparison of the results obtained from pure and diluted PDMS analysed with the 6 aforementioned techniques demonstrated clear differences between the performances of each technique. These differences are partly due to the physical and chemical properties of the polymer in solutions but also in the spectroscopic phenomena, which differ fundamentally according to the technique. Table 29 summarizes the observations of the 4 parameters considered important for the analysis techniques presented as well as the potential application and relevance to case work.

Although all the IR techniques are fundamentally different, the observed response was consistent, under the form of four vibrational peaks, at around 1263, 1090, 1020 and 800 cm^{-1}, the peaks at 1090 and 1020 being the most important ones as they are linked to the O-Si-O bonds, backbone of the target molecule. However, this doublet was not always well resolved in ATR spectra, whereas transmission and DRIFTS techniques offered a fully resolved doublet in their spectra. The micro-transmission spectra showed some saturation on raw material as the detector response below 3% transmission and at lower frequencies was unsatisfactory. No

evidence of O-Si-O doublet was found in any of the Raman spectra, thus indicating that it was not an adequate technique for this type of analysis.

Table 29 : Observation for all the spectroscopic methods used in this study. Reproducibility is here defined as the ability of the instrumentation to obtain the same results when data are acquired within the same conditions. Reproduced from *Burnier C Van Bronswijk W., Massonnet G. (2020) Comparison of spectroscopic methods in detection of silicone-based condom lubricants evidence, Analytical Methods*

Method	Spectral quality (bulk material)	Reproducibility (n=5)	Analysis time [min]	Preparation	Case work application
Transmission	Good O-Si-O doublet well resolved	Medium	~1	Easy	impossible on diluted samples
Micro-Transmission	Spectral saturation O-Si-O doublet well resolved	Good	~2-3	Easy	impossible on diluted samples
ATR	O-Si-O doublet not well resolved	Excellent	~2-3	Easy	impossible on diluted samples
Micro-ATR	O-Si-O doublet not well resolved	Good	~1	Easy	impossible on diluted samples
DRIFTS	Good O-Si-O doublet well resolved Not applicable to bulk material	Medium	~2-3	Needs narrow size range KBr	Good spectra obtained on diluted samples
Raman	O-Si-O doublet not present	Medium	~1	Easy	Possible but difficult

All the studied methods presented a short analysis time and easy preparation, except for DRIFTS, whose preparation time was found to be longer as there is the need to prepare the KBr powder. However, this was found to be an acceptable balance between time and results when considering the quality of the spectra and the possibilities of application to real samples. It was also found that it was not too time consuming to prepare a batch of KBr filled sample cups and to store them at 100°C prior to analysis, sample preparation time being that required for spiking samples on the KBr.

The choice of the most adequate technique will be dictated by the spectral quality obtained mainly from diluted or extracted samples, as this would be more representative of case work samples. In the present study, techniques using microscopy were not found to allow good detection of the liquid samples, due to solvent evaporation. The same issues were encountered with transmission and ATR techniques, where it has not been possible to target PDMS adequately after deposition on the devices.

DRIFTS analysis was found to be clearly able to detect PDMS in solution, with a very low limit of detection, around 0.025 mg/mL. This supports its previous use for condom evidence analysis in case work in the 1990s, although there was no real comparison with any other infrared techniques [11]. Although other vibrational spectroscopy instruments are more commonly used

in forensic sciences, DRIFTS remain the most efficient method to use as a screening method for the detection of condom residues in vaginal swabs collected from real case work.
DRIFTS was found to be qualitatively relevant for the analysis of trace evidence (**Chapter 4**). However, optimization of signal/noise ratios (SNR) is necessary, and a measurement of the distance or of correlation between the spectra using hierarchical cluster analysis (HCA) were realised to ensure good repeatability of the results. In addition, in order to ensure quantitative control in the context of transfer and persistence, a study of the quantitative and/or semi-quantitative potential of the method will be explored. Quantitative analysis aims to be used to modelise the persistence mechanism as a function of a quantity (or a relative quantity), so as to be a more objective.

8.2 Analytical parameters optimization

The data obtained were opened and displayed in transmittance. The display limits (and therefore the integration of the SNR using Omnic® software) were limited to the area between 2200 and 2000 cm^{-1}, then noise analysis was performed. RMS (root mean square), the parameter most often used to characterize the baseline, was determined in the spectrum area between 2200 and 2000 cm^{-1} because it is the most variable area in which the spectrometer provides the most power. SNR was then calculated using the ratio between the expected transmission percentage (ie 100%) and the RMS.

8.2.1 Methodology

8.2.1.1 Identification of factors influencing the Signal-to-Noise Ratio (SNR)

Several experimental designs were conducted in this study, as an iterative process in order to obtain the highest and less variable SNR. All the designs were realized using a single standard solution of bulk PDMS diluted in hexane (1mg/ml) and spiked on a KBr pellet. 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 30A. The chosen FFD plan used four replicates of each point including the central point. This resulted in a total of 20 randomized program experiments. The central point was defined at 64 scans and 4 cm^{-1} because it is the closest point to regular practice in forensic laboratories.

Table 30A : Factors and levels used for the identification of the surface response, using a FFD design

Factor	Level -1	Level 0	Level 1
Scan number	16	64	256
Resolution [cm^{-1}]	8	4	1

The second experimental cycle was led to estimate the effects of each factor. A new FFD was designed with new scan number, chosen within a factor 2 from the central point, i.e. 32 and 128 scans. The resolution variable was modified, to correspond to a variation of a factor 2 around the value of the central point (Table 30B).

Table 30B: Factor and levels used for the calculation of the effects, using a FFD design

Factor	Level -1	Level 0	Level 1
Scan number	32	64	128
Resolution [cm^{-1}]	8	4	2

Finally, a third experimental cycle was an extension of the FFD into a Central Composite Design (CCD), more specifically here, a face centered composite design (FCCD). The remaining points were added to the initial FFD to capture the true relation between the factors and the SNR. For each cycle, effect significance, lack of fit, regression significance and curvature were evaluated. A total of 80 experiments were led for these designs.

Data analysis for experimental designs were performed in Unscrambler X v.10.1 (Camo Software, Oslo, Norway) 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 [101–103], and the curvature of the plan according to a Student's test [101–103]. Several regression models of different complexity (from linear to quadratic) were fitted on the data. The model describing the relation between the factors was then selected based on the highest lack-of-fit p-value and the lowest regression significance p-value.

8.2.1.2 Repeatability evaluation and parameters selection

Repeatability of the instrument is important, especially when it comes to trace evidence analysis. To evaluate the repeatability, two different statistical methods are usually used: principal component analysis and hierarchical cluster analysis, with distance or correlation

measurements. All these techniques will be used to investigate which number of scans provides, for a given resolution, the highest repeatability, with spectra clustered the closest to each other. This will allow the identification of the number of scans which provides the highest repeatability of the data.

8.2.2 Response surface screening

Analyses carried out on the Full Factorial Design were first visually analysed to evaluate the variability among all the replicates, by looking at the variability around the baseline, and the noise variation. The results showed that both number of scan and resolution affect the SNR, as well as their interaction. The quadratic effects were found to be non-significant. The non-significance of the lack of fit (p-value = 0.805) allowed to assume that all main effects are linear in this model. If the number of scans is increased, SNR increases as well, but so does the analysis time. In the same manner, the lower the resolution, the higher the SNR, but the lower the quality of the spectral information will be. Choosing the adequate analytical parameters is a compromise between the amount of spectral information and the SNR.

The visual comparison of the noise observed in the spectra obtained at 16 scans for the resolution at respectively 1 and 8 cm^{-1} present a good repeatability in terms of intensity fluctuation. The noise is very important when the resolution decreases to 1 cm^{-1}. Similarly, at 256 scans, a very good repeatability is observed, with a noise higher if the resolution is higher. Repeatability is globally higher at 256 scans than at 16 scans for a same resolution (cf. Figure 86A and B). The scans at 64 scans and 4 cm-1 have a rather good repeatability (Figure 86C). The impact of the resolution is clearly visible compared to the other sets of analysis.

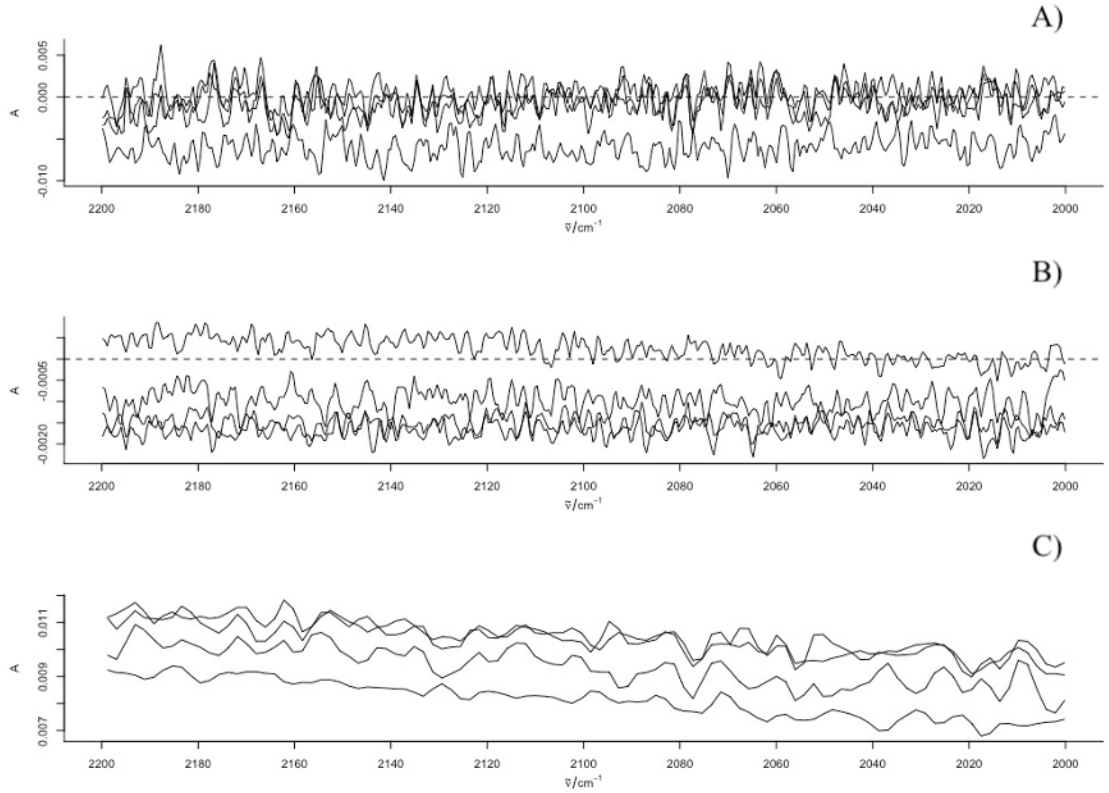


Figure 86: Illustration of the noise of the spectra acquired under different number of scans and resolution, four replicates per design point are presented, A) 16 scans/1cm^{-1}, B) 256 scans/1cm^{-1}, C) 64 scans/4cm^{-1}.

To investigate the variation of the SNR as a function of the number of scans and of the resolution, the noise (RMS) was integrated on the 2200-2000 cm^{-1} region as it is the range where the beam intensity is the greatest. SNR was calculated as follows: $SNR = \frac{Baseline\ \%\ Transmittance\ Value}{RMS}$. The baseline transmittance value was usually 100% but was sometimes slightly lower (i.e around 99.5%) and therefore was adapted as a function of the value obtained for each spectrum.

Table 31 highlighted the lower variability of the central point (64 scans, 4 cm^{-1}). As illustrated in Table 31, the SNR is lower when a high resolution set up is used than when a lower resolution is used. To this effect, a high resolution at 1cm^{-1} is not recommended if planning to work on real cases: when dealing with trace evidence, there's a need to have the highest SNR. Similarly, for the number of scans, a too low number of scans provides a lower SNR and therefore is not recommended.

The variability of the SNR was also investigated as it was assumed that the variability detected on the SNR would be observed as well on the data collected from real samples. Variability was found to increase as the number of scans increases (cf. Table 4). Thus, it seems inappropriate

to expose the sample to too many scans, since not only the analysis time increases, but so does the variability. The coefficients of variation obtained for the low number of scans are less than 10%, which is rather good considering the mode of analysis and the problems related to diffusion that can be observed. These observations confirm that both number of scan and resolutions are important parameter influencing the reproducibility of the data.

Table 31 : Observed coefficient of variation (%) as function of the number scans and of the resolution

Scans	Resolution	SNR	CV %
16	1	283 ± 15	5.60
16	8	883 ±65	7.43
64	4	1424 ±91	6.42
256	1	1440 ±204	14.16
256	8	3662 ± 443	12.09

Scans	Resolution	SNR	CV %
16	1	283 ± 15	5.60
16	8	883 ±65	7.43
64	4	1424 ±91	6.42
256	1	1440 ±204	14.16
256	8	3662 ± 443	12.09

Higher number of scans induce higher variability of the SNR, and the reproducibility is affected as well. Therefore, analyses at 256 scans were discontinued for further investigations. The surface screening showed more repeatable results for a number of scans closer to 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.

8.2.3 Calculation of the main effects

The knowledge acquired in the first cycle of experiment allowed to reduce the factors closer to the centre points. A new two-level factorial design of experiment was run to estimate the effects of each of the factors. Each point was analysed four times to get replicates. Calculation of the main effect of each parameter were realized as described in [142] and respective effects of ~ 3196 (p-value = 0.0001) for the number of scans and~ -2096 for the resolution (p-value = 0.0008) were obtained. These results show that both effects almost equally influence the response of the SNR. Both factors have positive effects. This means that decreasing the resolution (i.e. 8 cm^{-1}) and increasing the number of scans cause an increase in the SNR. The goal is to increase this ratio, since the signal must be maximized with respect to noise. To achieve the goals set, the number of scans must be maximised, and the resolution minimized. The effect of the interaction has also been calculated and is ~ 1496 (p-value = 0.0016). The effect here is half as important as the one of the numbers of scan, and almost as important as

the one of the resolution, while positively affecting the SNR. This interaction is therefore important for the model because its effect is as important as any of the main effects.

This design was still not sufficient to have a complete coverage and understanding of all the interactions. Thus, an extension to a FCCD design which allows computing more complex interactions and create a final response surface modeling with the best understanding of the impact of each parameter was achieved.

8.2.4 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 number of scans and the resolution, then considering their interaction and finally considering second order terms. First order effect were found to be statistically significant, with p-values < 0.001. Within this model, the AB interaction was found to be non-significant, with a p-value of 0.47. Quadratic effects were also investigated but were not found to be significant, with p-values respectively 0.646 for the Scan x Scan (AA) parameter, and 0.388 for the resolution x resolution (BB) parameter. The curvature was not found to be significant, indicating that only a linear model would fit on the data. In addition, the lack of fit was non-significant with a p-value of 0.8051 which indicates the model is not adequate for such a model. The different models were all compared using the adjusted R^2 with a partial Fisher-test. None of the models were found to fit properly. Lack-of-fit values were found to be 0.085 for the first-degree model with interaction, 0.805 for the second-degree model with all the factors and 0.8725 for the second order models without the squared resolution. This indicate that none of these models are likely to fit the data. Surface response obtained for the quadratic interactions and the linear modelling are gathered in Figure 87.

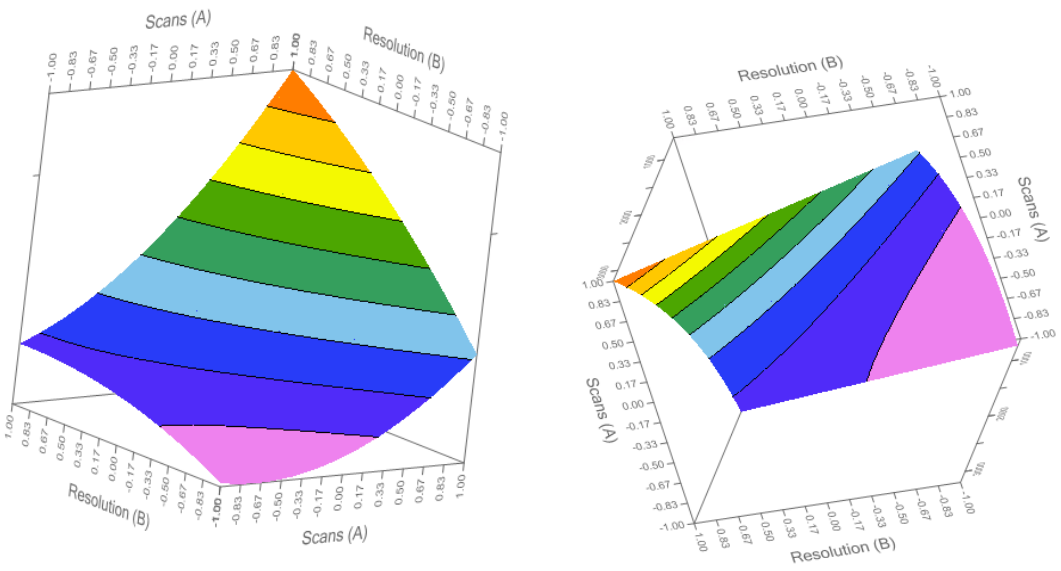


Figure 87: Surface response obtained for the quadratic model with interaction (left) and for the optimization using only the main effects (right). Factors levels are the ones described in table 30B

Multiple regression models were tested and showed out that only the number of scans and the resolution were significant for the surface response. In addition, when removing the quadratic interactions, the obtained surface response was completely linear, suggesting an increase of the SNR with the increase of the number of scans and the decrease of the resolution. To be able to select adequate analysis parameters, focus was set up on literature reporting that the SNR increases according to a power fuction curve of the type $y = ax^b$, the exponential of which is close to a theoretical value of 0.5 [143]. Therefore, optimal analysis parameters will be selected based 1) on the exponential curve for the optimal resolution number, and 2) on the shorter distance or correlation between spectra acquired for a same resolution and different number of scans.

It also needs to be considered that these second and third design cover a very limited area of the global S/N curves. Therefore, it might appear that, on this small area, the global trends are not always respected. However, one of the important parameters to consider is the variability on the sample, and to this extent, there is a need to evaluate the repeatability of the analysis in order to select the most relevant parameters for the forensic analysis of condom evidence.

8.2.5 Repeatability evaluation and parameters selection

8.2.5.1 Resolution

As illustrated in [143], optimal resolution can be selected when plotting the resolution as a function of the number of scans, fitting a power function of the type $y = ax^b$, the exponent of which is close to a theoretical value of 0.5. Relation between resolution and number of scans will be plotted and the parameters of the curves were calculated, as well as the regression coefficients of the latest. The relation between resolution and number of scans is illustrated on Figure 88. However, it has to be considered that a lower resolution won't allow the optimized separation of the infrared signal. In the practice, a resolution of 4 cm^{-1} usually offers the best compromise between the S/N value and spectral separation.

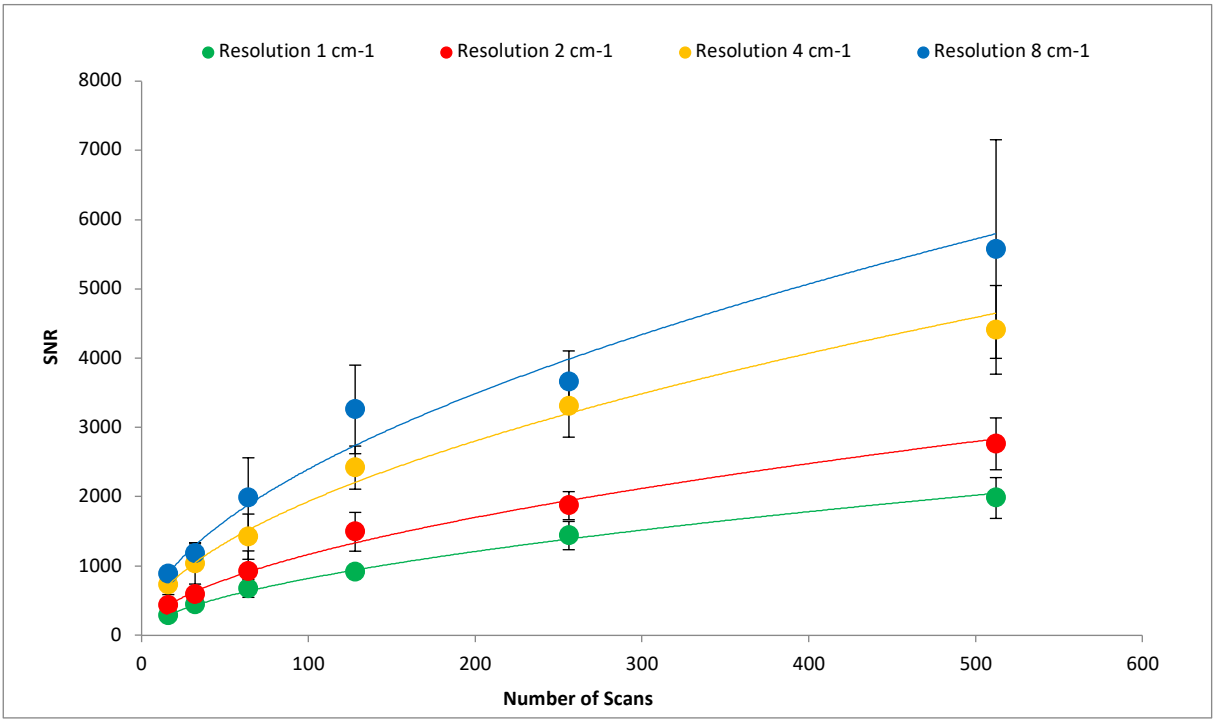


Figure 88 : Illustration of the relationship between scan number and resolution. The observed variability is mainly due to a high within-sample variability.

Parameters of the curves were calculated, as well as the regression coefficients of the latest. (Table 32). Although 8 cm^{-1} resolution is the one which offers the highest SNR, both the regression coefficient and the power function parameter *b* are more fitting to the power function when using a resolution of 4 cm^{-1}. As it is also a very common parameter in most forensic laboratories, the resolution of 4 was selected as a final parameter for further analyses.

Table 32: Resolution impact

Resolution	a	b	R^2
1	88.951	0.4692	0.9334
2	141.65	0.4503	0.899
4	246.51	0.4897	0.9958
8	319.4	0.4253	0.8194

8.2.5.2 Number of scans

To select the adequate number of scans, the repeatability of the analysis should be assessed. Therefore, the data were first plotted into a principal component analysis to evaluate the variability of the different measurements. In addition, hierarchical cluster analyses were used with Ward's Linkage, Euclidean Distance measurement and Pearson's correlation measurements, and different linkage were tested, to see whether the results were consistent.

As illustrated on the PCA results on Figure 89, the spectral data are rather spread out and are not really clustered together. The only ones clustered together are the ones acquired at 64 scans, as represented with a black circle on Figure 89.

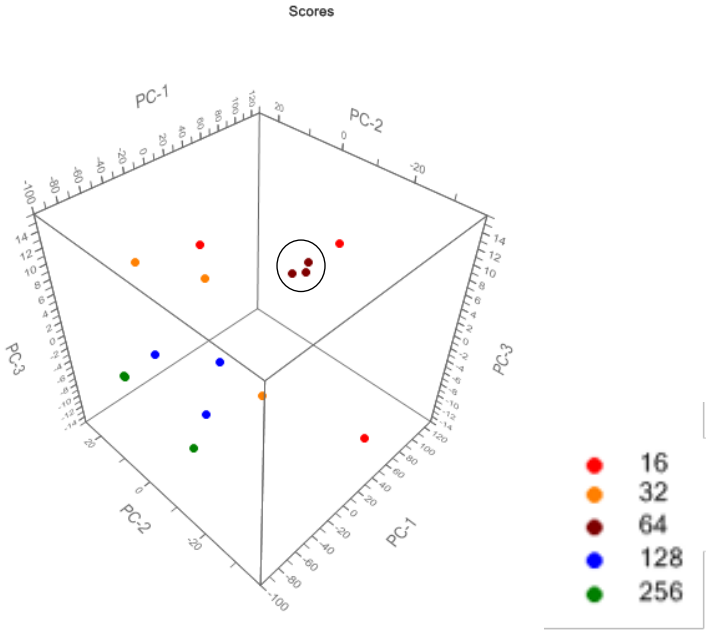


Figure 89: 3-dimensional scores plot of the data acquired on different number of scans but a same resolution. Samples are coloured as a function of the number of scans.

All the hierarchical clusters provided the same results, with the smallest distance or correlation between the spectra being observed at 64 scans independently of the measurement methods or linkage method, thus suggesting a more appropriate repeatability of the data. An example of the results obtained with a Pearson's correlation and a complete linkage analysis is presented on Figure 90. The black circle highlights the measurements obtained for 64 scans. The grey circle outline measurements obtained for 256 scans: interestingly, 2 replicates

acquired at 256 scans are very close to each other, as close as 64 scans ones together, but the third replicate systematically is more distant to the rest of the samples. 256 scans would be a very interesting option of analysis as some authors have used a larger number of scans (256 or 512) to deconvolute the spectra and classify samples of different types. Increasing the number of scans to 512 would not significantly improve the signal-to-noise ratio but increase analysis time by a factor of 10. The analysis time would also significantly increase, with around 4 minutes needed for 64 scans and up to 15 minutes for 256 scans. Hence, this is not a cost-effective compromise based on spectral variability, but it might be requested for specific aims (i.e. spectral deconvolution).

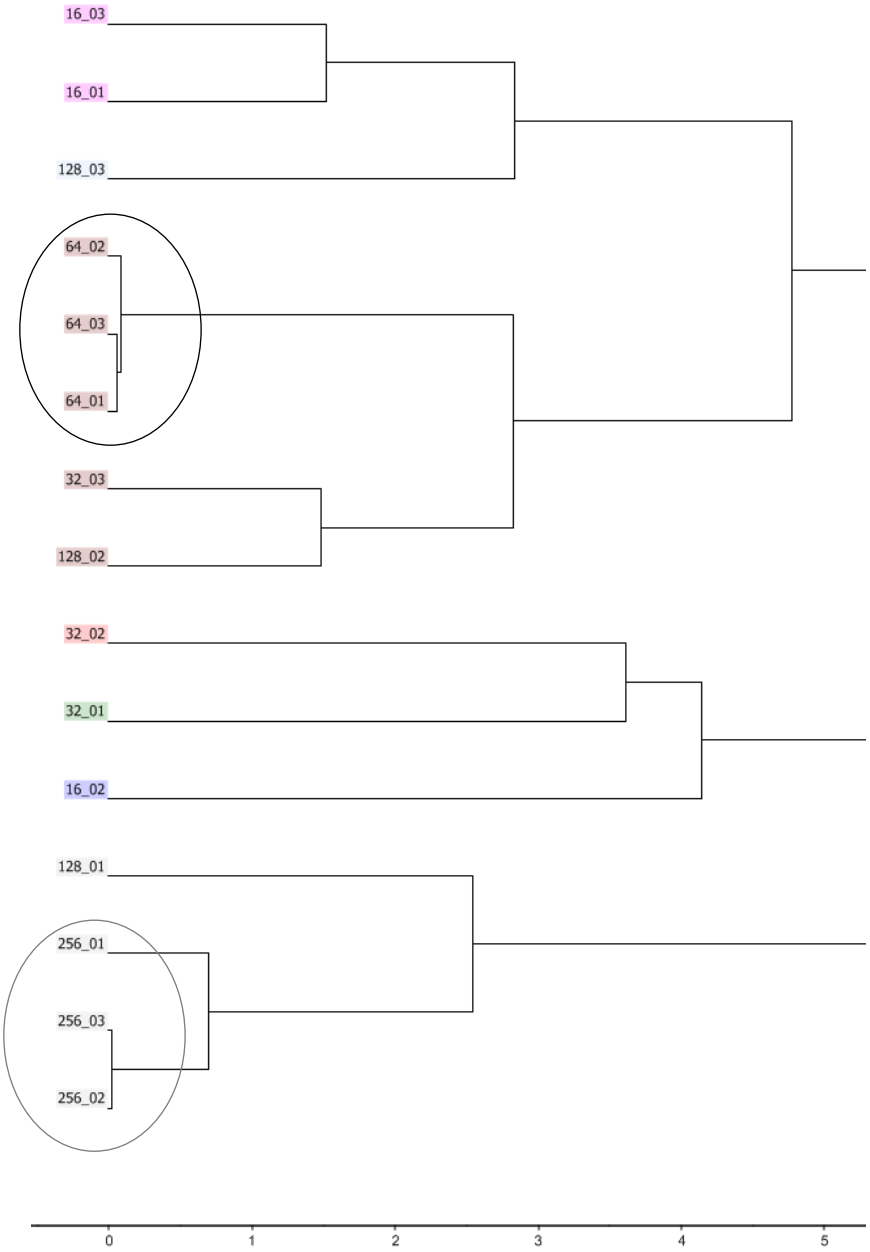


Figure 90: Hierarchical Cluster Analysis obtained with a Pearson's correlation measurement, and a complete linkage clustering

The results presented above show that the best number of scans and the resolution is **64** and **4cm^{-1}**, respectively. These are the parameters that will be used for further analysis.

8.3 Pattern recognition

The market study (**Chapter 4**) was performed on a large sample set sourced from several locations of purchase. Samples were analysed using ATR and chemometrics was applied to the data to observe any potential differentiation of the products. DRIFTS-FTIR was found to be the most relevant FTIR method for liquid analysis and presented very good sensitivity for the detection of silicone-based products extracted from real samples. It has currently been used in most of cases and has been reported as the most adequate technique for this type of analysis. However, most of the pre-existing databases and market studies were performed with either non infrared spectroscopic methods or other types of infrared techniques, mainly ATR-FTIR. One of the current questions to be investigated in this paper was to know if it is possible to combine both DRIFTS and ATR techniques, so that the models created with ATR could be used for predicting samples acquired with DRIFTS analysis and could be filled with additional samples from real cases. In the event that such an approach would be unsuccessful, it would be of great interest to examine DRIFTS data dispersion and if the same type of clustering can be obtained between DRIFTS and ATR data, to find out if a correspondence in terms of patterns obtained with the two methods can be observed.

8.3.1 Material and Methods

8.3.1.1 Sample and preparation

In order to evaluate the applicability of the optimized method to real samples, 16 condoms and 2 lubricants from major distributors and manufacturers on the Swiss market were purchased from Swiss supermarkets and pharmacies (Table 33). All the samples were previously categorized as containing a silicone-based lubricant (**Chapter 4**) except for Fair Squared Sensitive Dry (P11), which is a non-lubricated condom.

Table 33: List of the samples used for this study

N0	Brand	Model	Type	Lubricant	Other Component
P0	Durex	Performa	Latex	silicone	no
P1	Durex	Invisible	Latex	silicone	no
P2	Durex	Natural Feeling	Polyisoprene	silicone	no
P3	Migros	M-Budget	Latex	silicone	no
P4	Migros	Cosano Regular	Latex	silicone	no
P5	RFSU	Profil	Latex	silicone	no
P6	Manix	Contact	Latex	silicone	no
P7	Manix	Skyn Original	Polyisoprene	Silicone	no
P8	Ceylor	Blauband	Latex	Silicone	no
P9	Ceylor	Non-Latex	Polyurethane	Silicone	no
P10	Coop	PrixGarantie	Latex	Silicone	no
P11	Fair Squared	Sensitive Dry	Latex	Non lubricated	Solid Particles
P12	Vitalis	Natural	Latex	Silicone	no
P13	Amor	Nature	Latex	Silicone	no
P14	ESP	Skin	Latex	Silicone	no
L1	Durex	Play play eternal - Perfect glide	Personal lubricant	Silicone	no
L2	Ceylor	Silk sensation	Personal lubricant	silicone	no

Samples used for DRIFTS analysis were prepared to be as realistic as possible. Therefore, cotton swabs were rubbed on unrolled condoms and deposited in a clean glass vial. 1 mL of hexane was then added to each the vial. All the vials were closed and placed in an ultrasonic bath for 15 minutes.

8.3.1.2 Sample analysis

For the **ATR** analysis, the procedure selected is the one described in **Chapter 4**: condoms were rubbed directly on the ATR crystal and analysed with no further preparation. The sampling window was thoroughly cleaned using ethanol and lint-free tissue before each sample, and a background scan of the clean crystal obtained between each replicate acquisitions. For each sample, 5 replicates were acquired. ATR analyses were performed using a Nicolet iS50 FTIR spectrometer equipped with single-bounce diamond crystal ATR accessory. Data collection were carried out using the OMNIC software. Spectral range was from 4000 to 400 cm^{-1}, resolution was of 4 cm^{-1} and 32 co-added scans. ATR correction was performed on all spectra to account for variations in penetration depth based upon wavelength.

For the **DRIFTS** analysis the procedure is described in **section 8.1.1**: KBr was finely ground for about 15 minutes using an electric manual grinder. The finely ground KBr was divided into sample cups and dried for 15 minutes at 100 °C. Each cup was first individually analysed before being spiked with condom extracts using an eVol® syringe. After spiking, samples were put in the oven for 15 minutes at 100 ° C to ensure solvent evaporation. Samples were left to cool

before analysis. For each sample, a series of analyses made up of 5 replicates analysed the same day was carried out. DRIFTS analyses were realised on a Digilab FT 3000 Excalibur Series spectrometer, equipped with a Spectra-Tech 0030-05 Collector II Diffuse Reflectance Accessory (Spectra Tech). Data collection were carried out using Resolution Pro software from Agilent. Spectral range was from 4000 to 400 cm^{-1}, resolution was of 4 cm^{-1} and 64 co-added scans.

8.3.1.3 Data pattern recognition – ATR vs DRIFTS analysis

The results of the 16 samples acquired with the 2 different IR methods were analysed using Principal Component Analysis to identify the potential clustering and classification in the data. The hypothesis to corroborate is the following: samples are not clustered according to their analysis type but according to their sample category. This hypothesis has, to the author's knowledge, never been reported before, these two techniques usually being tested one against the other, and not as the potential of complementary techniques. This investigation aims to verify if samples obtained with DRIFTS analysis can be projected in the market survey discrimination and classification models built with ATR results (**Chapter 4**), and if not, to evaluate if the discrimination patterns obtained on the two techniques are similar. This would allow to transpose the discrimination pattern observed on the entire dataset from one technique to the other, although variability could affect the pattern.

8.3.1.4 Trace vs Reference classification

To investigate the classification of real samples, 2 volunteers had sexual intercourse using 10 different condoms, coming from the list presented in Table 1 as well as from other condom types presented in [144]. 3 blank swabs were collected prior to intercourse, and 3 samples swabs were collected right after intercourse. To avoid any cross-contamination, the volunteers were asked to wait one week between each protected intercourse. Each sample was analysed 3 times, as described for the condom samples, resulting in 9 replicates. A total of 132 analysis were run for this purpose, given that not all the volunteers used the 10 condoms (one volunteer did 9 condoms, the other did 6), and 3 replicates were removed due to instrumental issues. Sample collection was led in accordance with the Swiss Federal Act on Research involving Human Beings (Ordinance on Human Research with the Exception of Clinical Trials) and approved by Swissethics (2018-00690). Informed consent was obtained from all donors, and collected data was entirely anonymized. Based on previous researches [9,11,41], cotton swabs collected from the volunteers were cut from the wooden sticks and individually put in a

glass vial and extracted with 1mL of hexane. The vials were vortexed for 1 min and sonicated for 15 min. The resulting samples were analysed in triplicate.

8.3.2 Comparison of DRIFTS and ATR discrimination models

Most of the previous classification and discrimination models build for condom evidence analysis were constructed using ATR-FTIR. However, DRIFTS has been reported as the most adequate analytical method for the analysis of condom residues when it comes to casework, as described by **section 8.1.2**. This may be an issue for a forensic scientist facing condom evidence in case work, especially if the main concern is the proper identification of a condom, its brand or a specific model, as previous classification and discrimination models were constructed using ATR-FTIR, which is the reason why a model dedicated to DRIFTS analysis should be considered.

It is therefore of great interest to observe if the clustering identified in ATR data is also highlighted in DRIFTS data. Considering the chemical profiles obtained on a batch of different samples using DRIFTS, the main difference between ATR and DRIFTS is based on the silicone-based samples and only on the 1100-1000cm^{-1} region of the spectra. Indeed, it is where the silicon-oxygen double bond presents its symmetric and asymmetric stretching vibrations, which are better resolved and show higher intensity in DRIFTS than in ATR. There seem to be an opportunity here to possibly differentiate using DRIFTS samples that could not have been distinguished with ATR-FTIR.

At first, PCA was computed on the acquired data sets separately. For the DRIFTS analysis data, the variance explained by PC1 to PC3 was respectively 64%, 15%, and 7%. The first three principal components explain 86% of the variance whereas for the ATR data, the variance explained along PC1 to PC3 were respectively 84%, 8%, and 6%, leading to 98% of variance explained. Figure 91 shows the scores of the first three principal components (PC1-PC3) plotted against each other for all data. In this figure, replicates of condom of same *model* are represented by the same color. Figure 91 illustrates the comparison of the PCA scores plot obtained for the ATR (left) and DRIFTS (right) data. Results presented in **Chapter 4** highlighted specific data patterns using ATR-FTIR analysis. A similar structure in the data can be observed, with samples L2 and sample P11 being separated from the rest of the sample set. Similarly to ATR-FTIR results (**Chapter 4**), the rest of the silicone containing samples cannot be differentiated of each other, and it is not possible to differentiate samples coming from different brands or models. Further PC did not help enhancing the discrimination of the

samples. This suggest that although DRIFTS offer better visualisation of the siloxane chemical patterns, it does not enable any improved discrimination of samples.

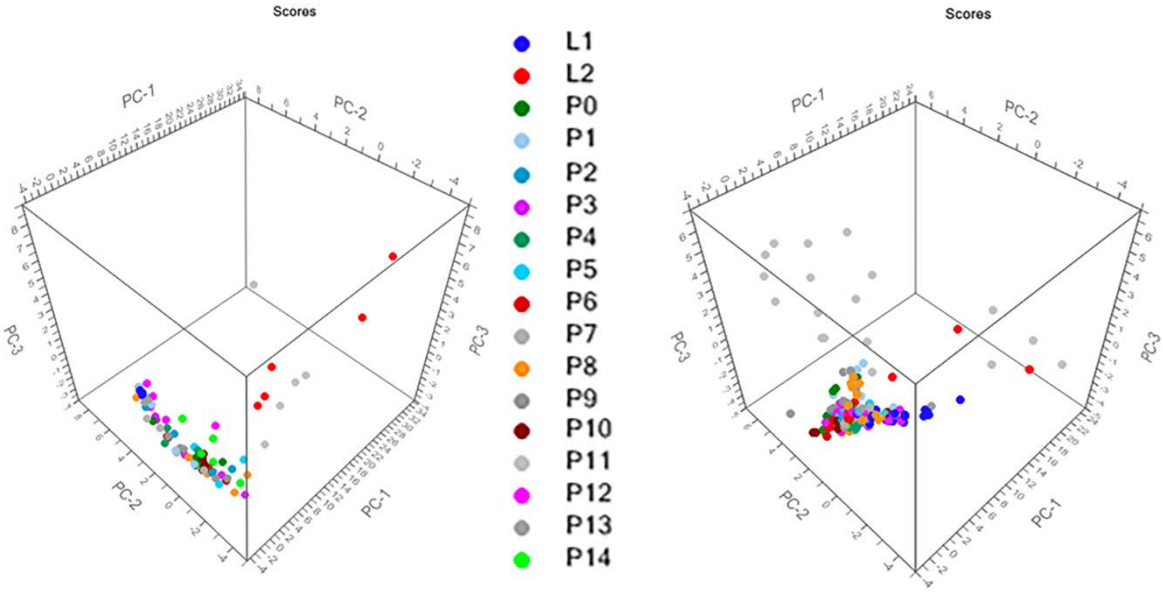


Figure 91: 3-dimensional PCA scores plots showing the distribution of the samples acquired with ATR-FTIR (on the left) and DRIFTS-FTIR (on the right) for comparison. Legend numbers refers to Table 33.

A very important difference that can be noted when comparing the two models presented on Figure 91 is the dispersion of the data: DRIFTS model presents a far more important dispersion of the data than ATR model. This can easily be explained by two main reasons: the sample preparation and the extraction procedure. Regarding the sample preparation, DRIFTS analysis required the manual preparation of KBr pellets followed by spiking the liquid sample. Not only the KBr grinding might not always reproducible between all the pellets, but an important modification of the KBr surface is generated at the moment of spiking, which is a source of variation.

Secondly, the samples analysed using DRIFTS were samples extracted from the condom whereas the ATR data were acquired by rubbing the sample on the crystal. Not only will there be variation resulting from the transfer, but also the extraction efficiency has not been evaluated, as it is known from [41] only that the extraction allows adequate extraction of the target compounds. ATR was not found to offer significant results for the analysis of condom evidence on swabs. *In situ* analysis was shown to be applicable on reference material (i.e. when condom was rubbed on a cotton before squeezing it on the ATR crystal) but produced no results from real samples, when an external matrix (i.e. vaginal matrix) was present. *In situ* analyses present considerable risk of contamination if DNA extraction has not been previously processed. Previous researchers reported that DNA extraction did not affect silicone-based

residues analysis using hexane extraction on the same cotton swab [15]. The use of extracted samples is not adequate for ATR of analysis, as at the moment of the deposition, the solvent evaporates, generating randomly deposited PDMS aggregates on the ATR crystal, and thus causing reproducibility issues. On the other hand, DRIFTS was reported as the most adequate technique for the analysis of real samples in case works [11] and a very sensitive technique. In this case, an extraction step is mandatory. However, data collected with DRIFTS analysis cannot be projected in the classification model as chemometrics algorithm will separate samples according to the instrumental analysis first, before handling different brand/models. This may be an issue for a forensic scientist facing condom evidence in case work, especially if the main concern is the proper identification of a condom, its brand or a specific model, as previous classification and discrimination models were constructed using ATR-FTIR, which is the reason why a model dedicated to DRIFTS analysis should be considered.

Although the number of samples is limited in this study, the analysis of such a big dataset as presented in **Chapter 4** would unlikely provide more accurate results, considering that condom production is regulated by international ISO norms. The conclusions of a discrimination and clustering investigation of a bigger dataset would be unlikely to provide different results on the discrimination of condoms than the ones observed in Chapter 4. Should further discrimination be required, other analytical instrumentations such as DART-TOF-MS would be recommended given their discrimination ability [10,21].

8.3.3 Trace vs Reference classification

Another recurrent and important question is to know whether real traces classify the same way pure lubricant samples do [144]. To this aim, samples collected from real samples were analysed. The visual analysis did not allow to differentiate samples coming from traces from the reference, except for the ones coming from sample P11, which is not a silicone-based condom. The real samples were then processed and added to the PCA plots to investigate their possible discrimination pattern within the reference material (Figure 92).

As illustrated in Figure 92A, trace samples are clustered within the same pattern as the one obtained with reference material. Traces present a slightly higher variability than the reference material when it comes to the silicone content (Figure 92B). Replicates of the same samples were clustered together. Most of the traces were clustered very close to the reference material, thus suggesting that the model would be appropriate to classify traces at the moment of their transfer and that the chemical profiles are not affected by matrix residues which is not surprising considering the type of extraction processed on the samples. Indeed, non-lubricated

condom traces were found to be clustered in the same zone (in terms of PC eigenvalues) than the non-lubricated reference P11. In a similar manner, silicone containing traces were found to cluster within the silicone-containing reference cluster. These observations are very interesting, as they suggest that the chemical profile gathered from condom lubricants after intercourse is not affected at T0 by the receiver or the contact to generate a different pattern or a new specific cluster in the PCA plots.

In addition to Principal Component Analysis, Hierarchical Cluster Analysis was also performed, as an unsupervised method to see the similarity between the data, without taking into account any other information. All the traces and the references were used, and 16 cluster were asked. All of the different distance measurements and linkage measurement revealed the same pattern: P11, L2 and the traces coming from these references were clustered together and presented a higher distance to the rest of the samples. Considering the silicone samples, the cluster analysis failed to correctly group the traces with their corresponding condom, independently of the type of linkage or distance/correlation measurements. These results also illustrates that whilst traces can be clustered with the reference material, it is not possible to link a trace to a specific material. This informs that when a trace is recovered, inferring on its exact source might not be possible, although in most casework it might not even be relevant, as the questions usually target the presence or the absence of traces. Whether it is a condom or another type of sample can also be answered, but the exact source of the trace cannot be inferred, as the chemical profiles are all undistinguishable.

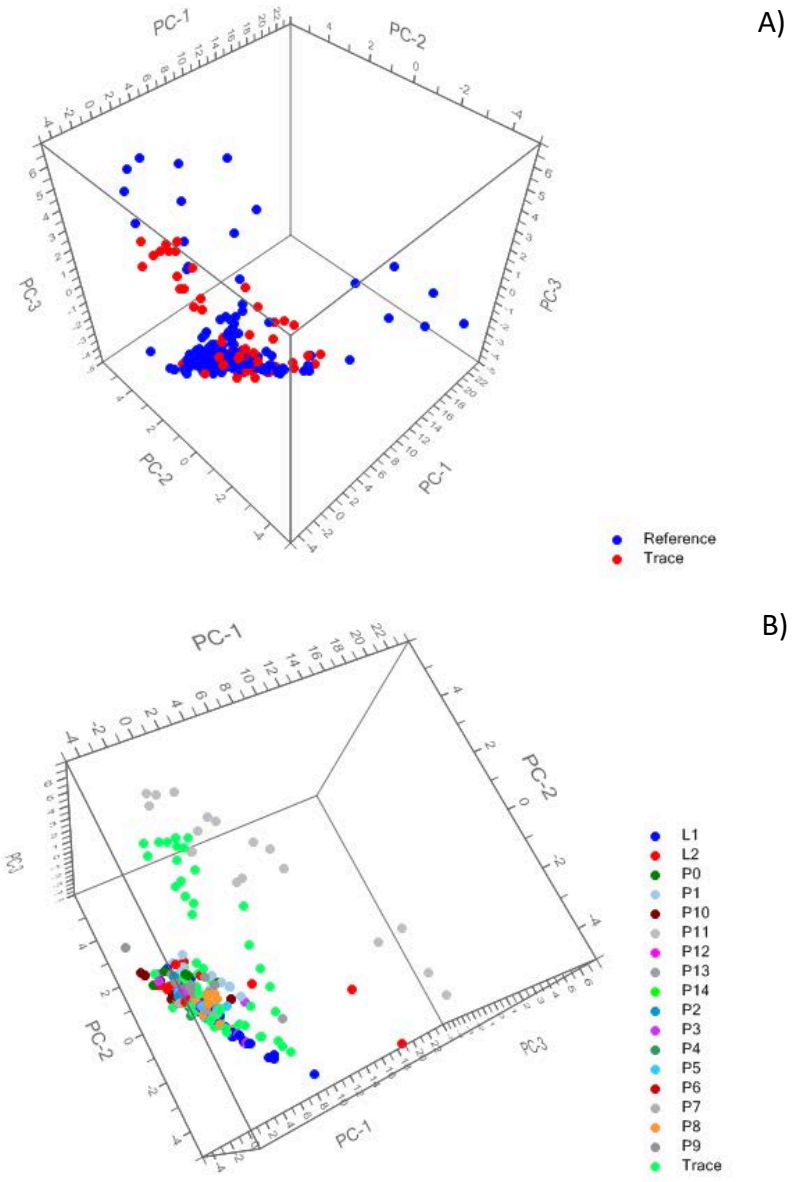


Figure 92 : 3D-scores plot of the PCA on 16 reference samples (n= 5) and 15 samples collected from human volunteer (n=9) analysed with DRIFTS, A) distinguishing traces and references, B) distinguishing all the different types of condom used as reference material

This suggest that although DRIFTS offer better visualisation of the siloxane chemical patterns, it does not enable any improved discrimination of samples. There is a definite need of another technique to allow proper discrimination of samples, and one such technique to investigate is py-GC/MS. Although previous research has investigated mass spectrometry methods for sample discrimination, and obtained interesting results, condom discrimination was not achieved [10] or papers did not focus on discriminating a condom population [16,17,20,44].

8.4 On the quantification of polymers using optimized DRIFTS-FTIR

The objective of quantitative analysis within condom evidence, and more specifically silicone-based lubricants, is to allow to modelise persistence as a function of the quantity detected at different post-coïtal time. Quantitative analysis is not common in infrared spectroscopy, but some publications present this type of analysis on results obtained in ATR [109,110,145–149] and DRIFTS [150–152]. None of these research reported the use of an internal standard, although it is usually needed for quantitative purposes. However, using an internal standard in spectroscopy would need the compound to give only one band in a given zone of the spectrum, and in our case not to be detected in further py-GC/MS analysis. As this could not be set up, no internal standard was used. An external calibration was therefore used. The number of points to use for the external calibration was set up at 5, located at similar interval from each other, with the linearity domain of the instrumentation. The spectra acquired in the various studies are all processed before performing the calibration. Common preprocessing according to [109,110,145–149] are the correction of the baseline, derivatives (first or second) as well as corrections of scattering effects (MSC-Multiplicative Signal Correction) or normalization (SNV-Standard Normal Variate). Unit vector normalisation (UVN) and range normalization (RN) are also commonly recommended, with UVN being the more common. Several combinations of pretreatments are presented in the literature and provide good quantitative results [109,110,145–152]. However, it has been stated that the best way to obtain quantitative data using DRIFTS is to perform a Kubelka-Munk transformation. This is a function frequently applied to diffuse reflection spectra to derive quantitative information [153–155]. Therefore, various combinations of preprocessing, presented in Table 34 were tested, based on the results presented in the literature. Then, a linear PLS (Partial Least Square) regression was performed, using the NIPALS algorithm. The regression models obtained were studied to evaluate the possibility of using them for quantitative purposes. PCR (Principal Component Regression) was also tested but did not provide better results than PLS regression.

Table 34 : Pretreatments tested for the calibration, regression and prediction coefficient

Pretreatment	Regression	Prediction
1st derivative	0.859	0.854
2nd derivative	0.957	0.894
SNV	0.909	0.835
MSC	0.884	0.804
1st derivative + SNV	0.862	0.853
2nd derivative + SNV	0.550	0.085
1st derivative + MSC	0.574	0.503
2nd derivative + MSC	0.908	0.759
Baseline Correction (BL)	0.965	0.732
BL+ 1st derivative	0.987	0.894
BL+ 2nd derivative	0.952	0.873
BL + SNV	0.971	0.897
BL + MSC	0.980	0.861
BL + KM	0.982	0.852
BL + 1st derivative + SNV	0.982	0.797
BL + 2nd derivative + SNV	0.968	0.819
BL + 1st derivative + MSC	0.59	0.282
BL + 2nd derivative + MSC	0.834	0.820

Although the present approach produces interesting results, the Kubelka-Munk approach, described in [101,155], was used to evaluate if regression and validation parameters could be enhanced. Spectra were baseline corrected, adjusted to 0% reflectance, to limit the effects of dispersion, and then normalized to reduce the variability caused by the texture of the surface. The Kubelka-Munk (K-M) conversion was then applied according to the equation

$$F(R) = \frac{(1-R)^2}{2R} \quad \text{(Eq. 1)}$$, R = reflectance

Before conversion, spectra were adjusted to a maximum reflectance of 1 before conversion because the K-M function does not handle well values close to 0 (function tending to infinity), like those obtained using standardization [153]. Here again, a PLS-type linear regression was performed, using the NIPALS algorithm. However, with a calibration quality of 0.823 and regression quality of 0.837, Kubelka-Munk algorithm did not enhance results compared to most of the pretreatments described in Table 9.

The best result obtained in terms of calibration and prediction quality among the pre-treatments was the combination Baseline Correction + 1st derivative. The quality of the calibration regression was 0.987, while the prediction quality was about 0.894. When applied to known concentration samples for validation using external samples, the model failed to adequately quantify samples and provided negative concentrations, or errors larger than the estimated concentration. The other preprocessing approaches from Table 9 presented the exact same

issue. On the contrary, Kubelka-Munk processing was found to be the more adequate processing to achieve quantification.

Linearity domain of the analytical method was investigated, to evaluate the relevant domain to lead a quantification study. According to the Beer-Lambert law, infrared absorbance is proportional to the concentration of the sample. This generally applies to transmission analyses, but several investigations have also applied this rule to reflection analyses, with good results. DRIFTS analysis has, so far, not been listed as a linear quantitative method. The few studies that mention it essentially report the process of data processing, once acquired. The saturation limit was first tested. Solutions of various concentrations of standard were analysed and saturation of the detector was obtained for concentrations over 1.5 mg/mL of PDMS in hexane, which was therefore considered as the maximum value of the linearity range. The calibration curve was then measured at concentrations of 0.05, 0.1, 0.25, 0.5, 0.75, 1.00, 1.25 and 1.50 mg/mL. Figure 93 compiles all the calibration data obtained at 1-week intervals after preprocessing, for a total of 63 values. Results presented in Figure 93 indicate that the linearity range is below 1 mg/mL, this concentration being the maximum before the point of inflection giving rise to the plateau area of the quantitative analysis. Similarly, the minimum concentration used for the calibration was 0.1 mg/mL. Indeed, the 0.05 mg/mL concentration is very low and does not enable good quality spectra to be acquired. The errors on the concentration are only greater. The linearity domain studied for the calibration is thus between 0.1 and 1 mg / ml (Figure 94).

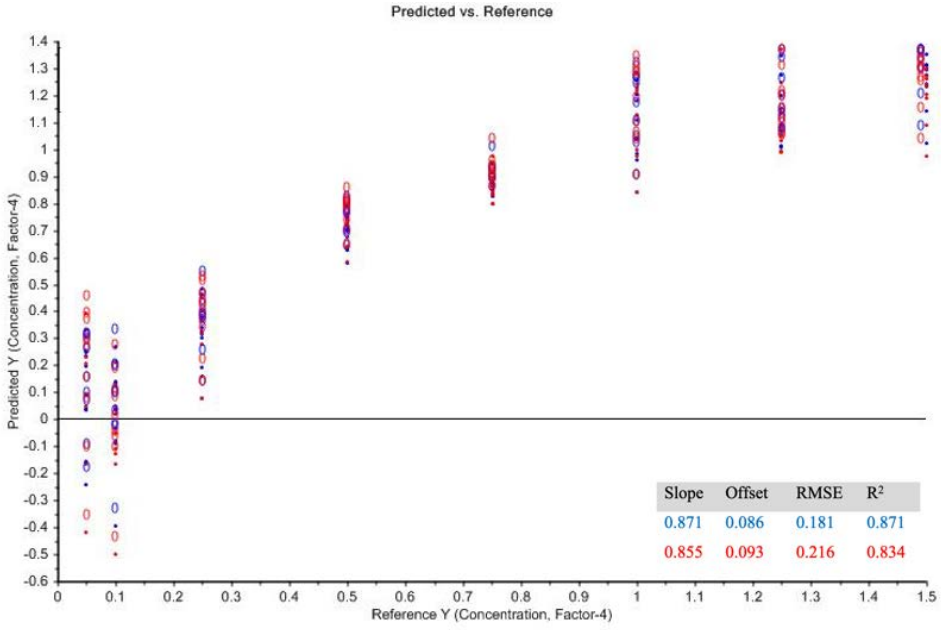


Figure 93 : Linear regression between 0.05 and 1.5 mg/mL

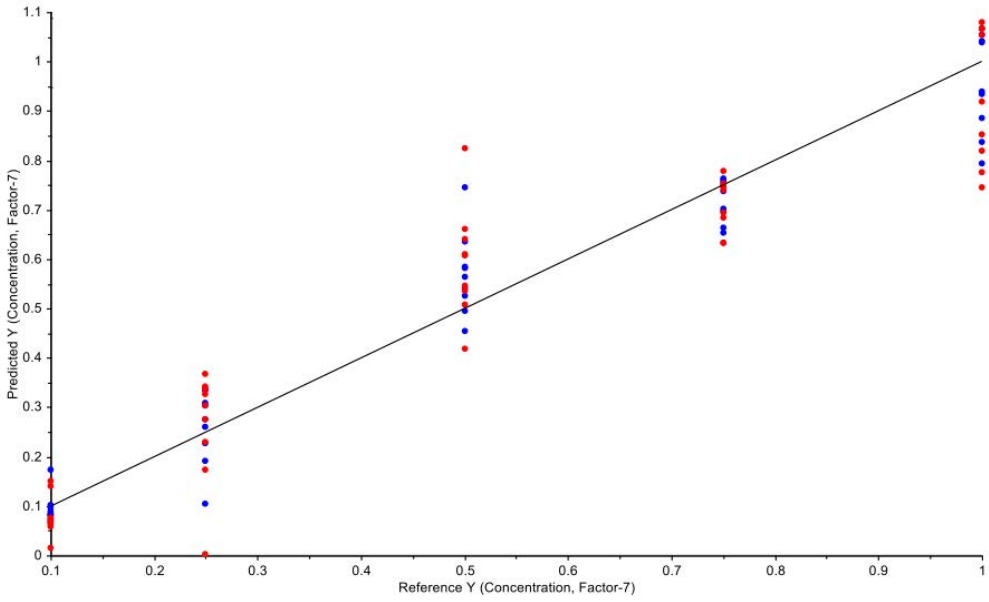


Figure 94 : Linear regression after Kubelka-Munk processing, 45 spectral acquisition, wavelength domain 700-1300 cm^{-1}. Black line is the trend line for X=Y

The regression coefficient for the calibration on the entire dataset was found to be around 0.94, which was considered satisfactory. However, the prediction coefficient is lower than 0.9, which was not sufficient for the desired experiments in this work, especially when considering case work applicability. A strong variability was also observed for the 0.5 mg/mL concentration point, whose prediction on the concentration was oscillating between 0.4 and 0.85 mg/mL. Considering this observation, R^2 might not be the best parameters to discuss the quality of the regression.

As the samples were acquired one week apart, the preparation of the KBR pellets was not the same. Variation in the preparation of the analytical support may explain a variation in the estimated concentrations, as particle size and diameter are not consistent between acquisitions. The series of calibrations were therefore separated according to the date of acquisition, and a PLS regression was performed on each of these series. Table 35 presents the regression and prediction results obtained for each calibration series.

Table 35 : Regression and prediction coefficient for each calibration curve

	Regression		Prediction	
	R^2	**RMSE**	**R^2**	**RMSE**
01	0.988	0.035	0.863	0.129
02	0.965	0.0604	0.940	0.0855
03	0.994	0.0235	0.956	0.0732

A good correlation was observed between the expected and measured concentrations, with correlation coefficients of over 0.95 for all the regressions. The resulting models should have good potential for predicting the concentration of unknown samples. The correlation coefficients are not all above 0.9, and the errors associated with a low correlation coefficient are important (> 0.09). These observations suggest that a quantitative analysis is possible with the DRIFTS analysis, as long as the subsequent processing of the data is adequate.

A calibration series should be run before experiments on the same batch of samples, in order to be applicable for quantification purposes. Although it is difficult to obtain a good general calibration, individual calibrations seem to offer good quality calibration and would be recommended for each batch of analyses. Significant variations were observed in quantitative analysis between days. As the same operator was running the samples, this suggests that the variation related to sample preparation is an issue.

The detection limit was estimated by progressive dilutions of a PDMS solution that were individually analyzed until the 4 characteristic target peaks were no longer visible in the spectra. Samples containing an initial concentration of 0.01 mg/mL were detectable and clearly identifiable. The smallest concentration still detectable was **0.005 mg/mL**. The limit of detection was therefore estimated at 0.005 mg/ml. 8 replicates of the 0.005 mg/mL solution were analyzed at this minimum concentration and all had a consistent profile.

An attempt to validate the quantification method was performed using external calibration before each analysis, using, respectively, a standard batch consisting of a series of 5 calibration

levels. The parameters of the linear regression were obtained for each calibration and are presented in Table 36.

Table 36: Linear regression (y = ax + b) parameters for PDMS quantification

Calibration	Intercept	Slope	R^2
1	0.006	1.011	0.9884
2	0.017	0.965	0.9402
3	0.0027	0.994	0.9561

The concentration range for the validation procedure was between 0.3 and 1.0 mg/mL at 0.1 mg/mL intervals. The lower limit solutions (0.1 and 0.2 mg / mL) were excluded from the validation because the coefficients of variation were very high, respectively 97% and 148%. The solutions of lower (0.3 mg/mL) and higher concentration (1.0 mg/mL) of the linearity curve were injected 7 times to observe the repeatability of the extremums, and the respective coefficients of variation were 41.22% and 19.98%. These values are fairly high and extremely surprising because they do not reflect at all the qualitative observations of the spectra obtained, as illustrated in Figure 95.

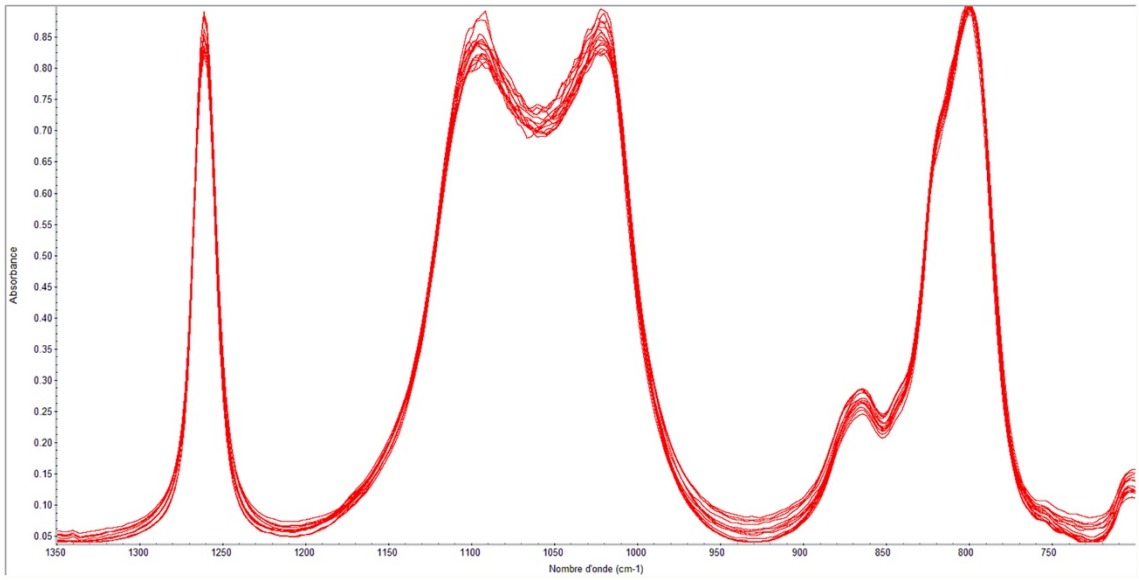


Figure 95: Repeatability for 7 replicas of the 0.3 mg/mL solution, after data preprocessing

To estimate the accuracy of the method, standard solutions were analysed three times in triplicate at different days. The data presented in Table 37 were obtained using One-Way ANOVA [156,157]. These data were used to obtain the precision on all the samples (i.e., global standard deviation, SD in Table 37), which takes into account both intra and intergroup analysis variances, expressed as Standard Deviation Within Sample (SDWS) and Standard Deviation Between Sample (SDBS) in Table 37. In addition, the ratio between the mean measured

concentration (MMSC) and the analysed theoretical concentration (ASC-Analysed Standard Concentration) was calculated to obtain the mean recovery percentage (MR-Mean Recovery).

Table 37: Validation parameters using One-way ANOVA

ASC (mg/mL)	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
MMSC (mg/mL)	0.16	0.38	0.47	0.54	0.28	0.45	0.46	0.81
MR (%)	54.71	96.21	95.01	90.00	40.85	56.62	51.72	81.65
SDWS (mg/mL)	0.064	0.094	0.138	0.188	0.143	0.203	0.172	0.150
SDBS (mg/mL)	0.088	0.111	0.147	0.176	0.154	0.178	0.191	0.142
SD (mg/mL)	0.018	0.00023	0.00062	0.00035	0.171	0.120	0.188	0.033
CV (%)	11.24	0.05	0.13	0.66	59.95	26.57	40.55	4.12

Thus, as shown by the results of Table 37, the precision of the analytical method varies between 59.95% and 0.05% (expressed in terms of coefficients of variation). The average recovery rate is 70.85 (± 22.16) %, which does not cover an acceptable area of analysis. Indeed, the average recovery rates are generally of the order of 100 ± 20%. Only one area of the curve corresponds to these criteria and is between 0.3 and 0.5 mg/mL.

To better visualize the variations and problems related to the validation of this technique, an accuracy profile has been drawn (Figure 96). The calculated concentrations (MMSC) were related to the analysed concentrations (ASC). The accuracy has been compared to the true answer (i.e. X = Y). Generally indicative of the bias of a method, the difference between the true response and the concentrations obtained by calculations indicate that the method does not allow adequate quantification of samples, despite a calibration performed during the day. In Figure 96, the acceptance limit values are also drawn, within ± 20% of the true value (broken lines). Concentrations from 0.4 to 0.6 mg/mL have values close to the theoretical value, and errors that fall within the acceptance limits. The rest of the samples do not belong to the acceptance domain of the method.

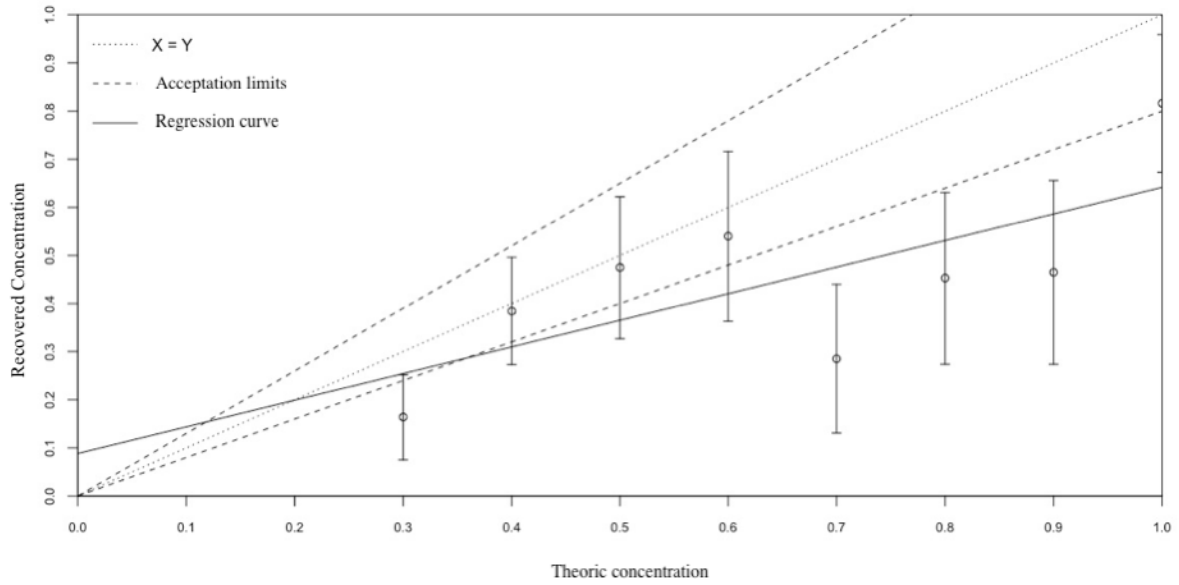


Figure 96: Accuracy profile, using MMSC as function of ASC

The obtained quantification results seem random and show little consistency between them. Several hypotheses can explain these observations. First, the calibrations each have a different error rate which can significantly affect quantification. However, this is unlikely because the prediction errors of the calibrations are very small and do not explain this quantification variability. In addition, the samples are analysed the same day as a series of calibration analysis. The second hypothesis comes from the assumption that the spectra expressed in Kubelka-Munk units follow the Beer-Lambert law, according to which the absorbance is proportional to the concentration. In our case, the following equation should be true:

$$F(R) = Ax + B \quad \text{(Eq. 3)}$$

with x the analyte concentration, A the relation $\frac{k_{analyts}}{s_{KBR}C_{KBR}}$ and B the relation $\frac{k_{KBR}}{s_{KBR}}$, k the molar absorption coefficient, s the molar diffusion coefficient and C the concentration. This equation implies that specular reflection is negligible, and that the diffusion coefficient is constant at any point in the sample. The third hypothesis of validity of this equation is that the concentration of analyte is not too important, to enter the linear range of the detector, which has already been previously determined and therefore verified. All the different points likely to generate such variability were presented in [158], and were carefully studied. All of them could be excluded as a source of variation except one, which is linked to sample preparation, and is the particle size after grinding. Following [158], "*The intensity of the bands decreases sharply when the average grain size of the analyte increases, but it varies little according to that of the diffuser*". If this statement is valid when a solid is mixed directly with the KBr powder, it is also relevant when dealing with polymer in solutions. The polymers are adsorbed on the KBr particles and

form a coating (Figure 97). If grain size varies, the distribution of PDMS around KBr grains will differ and so will resultantly coating. The contact area is maximized when the KBr particles are small, which increases the infrared signal. Moreover, it is impossible to control how the particles percolate through the KBr, and whether they remain on the surface or not. If the KBr particles are too small, adsorption may not occur, whereas if they are too large, the results will not be repeatable.

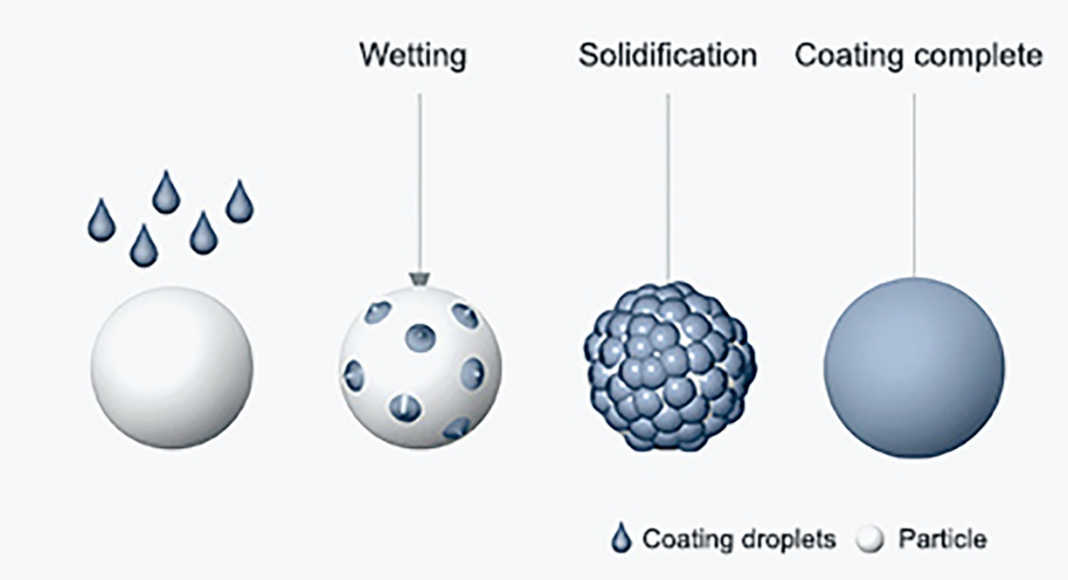


Figure 97: Coating illustration. Source: https://www.myschlick.com/en/pharma-foodwelt/502/coating/drum-coating/ (last view: May 22nd 2020).

Based on the above observations and reflections, it can be concluded that sample preparation, and more specifically the use of a liquid sample, is the factor that most impacts the analytical result and affects quantification. Although the instrument's stability can be questioned, the modification of the surface of the KBr pellet might be the key point of the variation. Indeed, at the time of the deposition of the sample on the KBr, a deformation of the surface of the pellet was observed and does not occur reproducibly. As the DRIFTS technique analyses the surface, the light reflection and scattering processes are not repeatable. This hypothesis is also confirmed as literature reports the ability to do quantitative analysis using DRIFTS-FTIR on solids exclusively [171,172,173,175]. No references dealing with liquid samples and quantitative analysis were found.

Proper quantification in accordance with analytical reference standards [109,110,149,151,152,156,157,159,160] as well as a reliable and accurate quantification models are not possible, despite data processing adapted to the type of analysis and recommended in several references [11,101,153,154]. On the other hand, when studying the limit of detection, the different concentrations seemed to be very distinct in terms of quality.

Semi-quantification, or at least an indication of the estimated quantity of the sample, should be considered. This aims to assist in further modelisation of persistence as a function of a concentration, or with semi-quantitative data as a function of a relative concentration.

8.5 Investigation of Semi-Quantification

The previous data were processed using PCA to evaluate if the algorithm is able to detect differences within samples that are initially coming from different concentrations. Clusters linked to concentrations would be expected. The scores plots obtained were observed in three dimensions so that the various factors could be studied, and the following statement confirmed or invalidated: it is possible to estimate the concentration present on a sample.

A total of 75 samples were used to study potential to estimate sample concentration using PCA. Each concentration used during the calibration (0.1-1.0 mg/mL) was analysed 6 times and 15 replicates separated into 5 sets of 3 analyses for different known concentrations related to an extraction procedure. The scores plot generated from the raw data is presented in Figure 98. Some samples seem to stand out, but no other principal components enabled better separation. Samples were not distinguishable based on their concentration content, as different concentrations were not clustered separately. Therefore, pre-treatments are necessary to succeed in obtaining a potential separation.

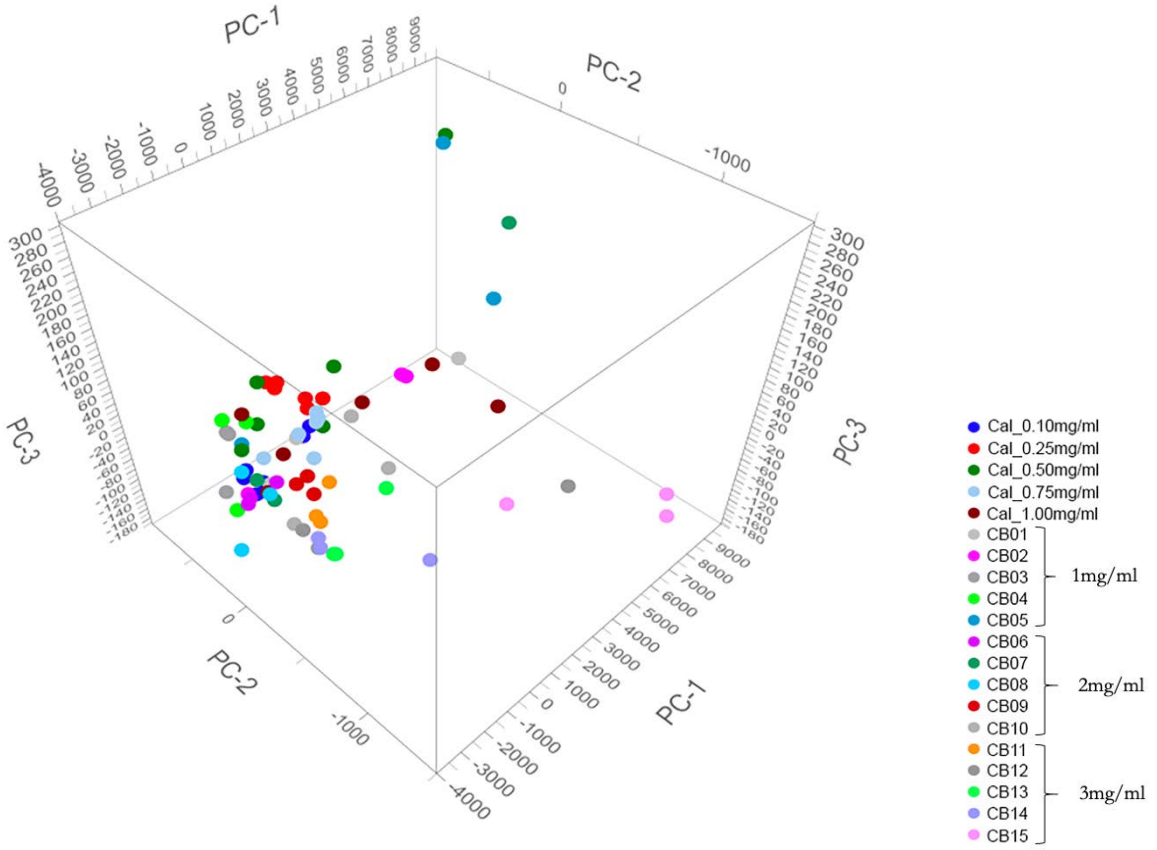


Figure 98: 3D Scores plot of the PCA realized on the raw data, along the three first principal component. Further principal components did not allow to enhance separation and/or visualization,

12 different pre-processing methods were tested as previously for quantification (Table 38).

Table 38 : Preprocessing tested for separation of samples following their concentration level

Preprocessing tested for separation	
Baseline correction (BL)	BL + 1st der
BL + Unit Vector Normalisation (UVN)	BL + 1st der + SNV
BL + UVN + Offset 1	BL + 1st der +MSC
BL + UVN + Offset 1 + Kubelka Munk	BL + 2nd der
BL + UVN + SNV	BL + 2nd der + SNV
BL + UVN + MSC	BL + 2nd der + MSC

Scattering corrections were tested as these can help reduce the displacement of the spectra along the vertical axis by multiplicative effect due to particle size. Applying these corrections was expected to improve the separation of samples of different concentrations.

Baseline correction did not enhance the separation of the samples but generated a slight spreading of the data (Figure 99A), suggesting that the algorithm might actually detect differences, but these might not be linked to concentration variations. Unit vector normalization and offset put at 1 [101,155] results in samples to be entirely clustered together (Figure 99B),

without any significant relevant groupings. Finally, the Kubelka-Munk transformation spread out the data, but no clusters were visible (Figure 99C). These observations suggest that these processing approaches do not distinguish concentration variations within the dataset. However, this is known to be working, for other types of evidence as shown in [101,155].

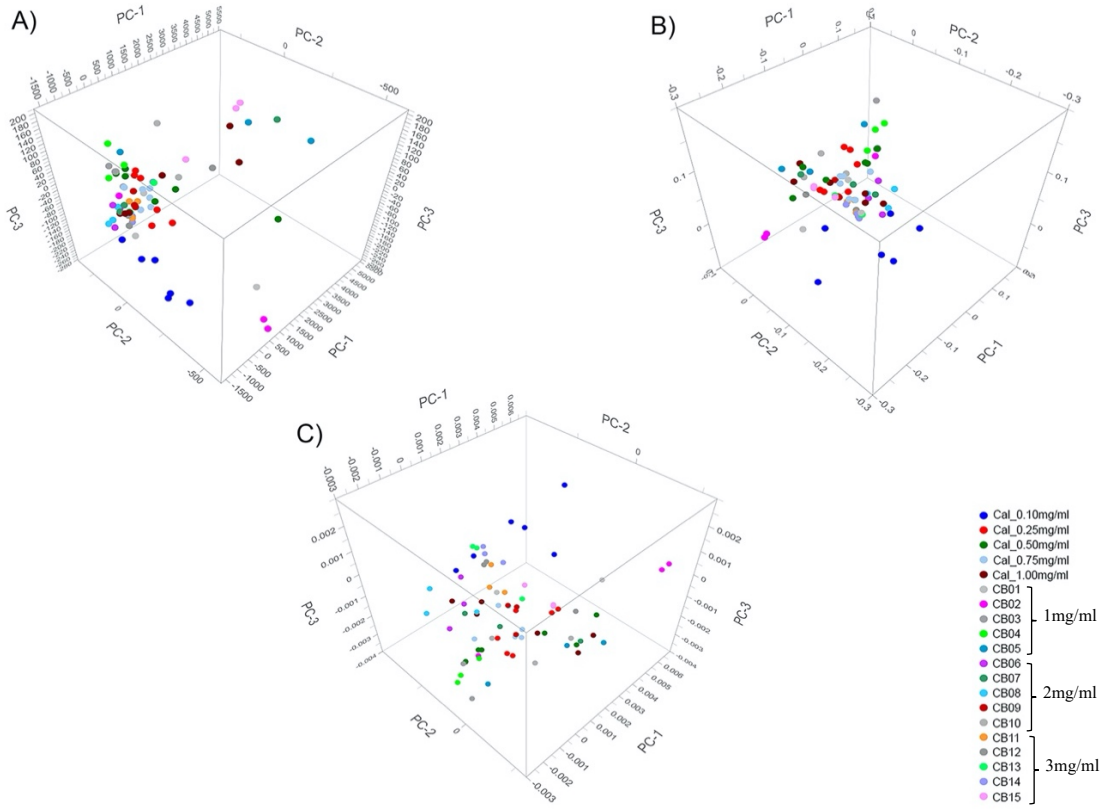


Figure 99: 3D Scores plot of the PCA realized A) after baseline correction, B) after baseline correction + vector normalization + offset, C) Kubelka-Munk transformation according to [101,155] . The three first principal component are presented, no other principal component allowing to enhance separation and/or visualization.

Derivation algorithms are known to improve the spectral quality and were tested to enhance the separation. The first derivative makes it possible to isolate a few replicas, in particular samples 01, CB02 and CB15 (Figure 100A). The clusters that could be formed do not correspond at all to the clusters that are expected, knowing what was spiked for the analysis. The second derivative in Figure 100B also illustrates possible separation of some replicates, but again clusters were not consistent with the real content of the samples.

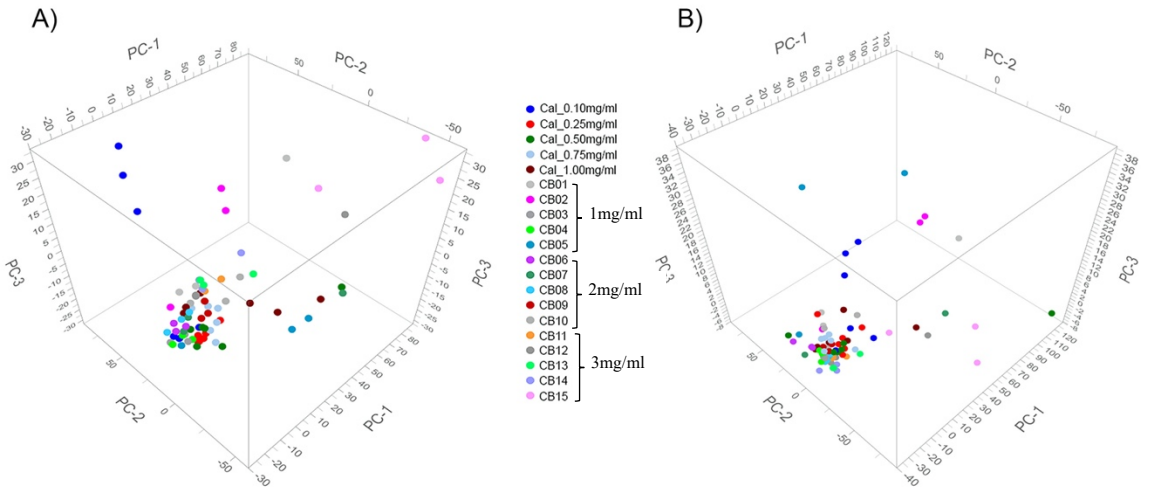


Figure 100: 3D Scores plot of the PCA realized A) after baseline correction + 1st derivative, B) after baseline correction + 2nd derivative. The three first principal component are presented, no other principal component allowing to enhance separation and/or visualization.

As highlighted by [161], scattering corrections do not improve the quality of the spectrum if the sample is in a solid state, which led to no better results on the separation of the samples than with the raw data (Figure 101).

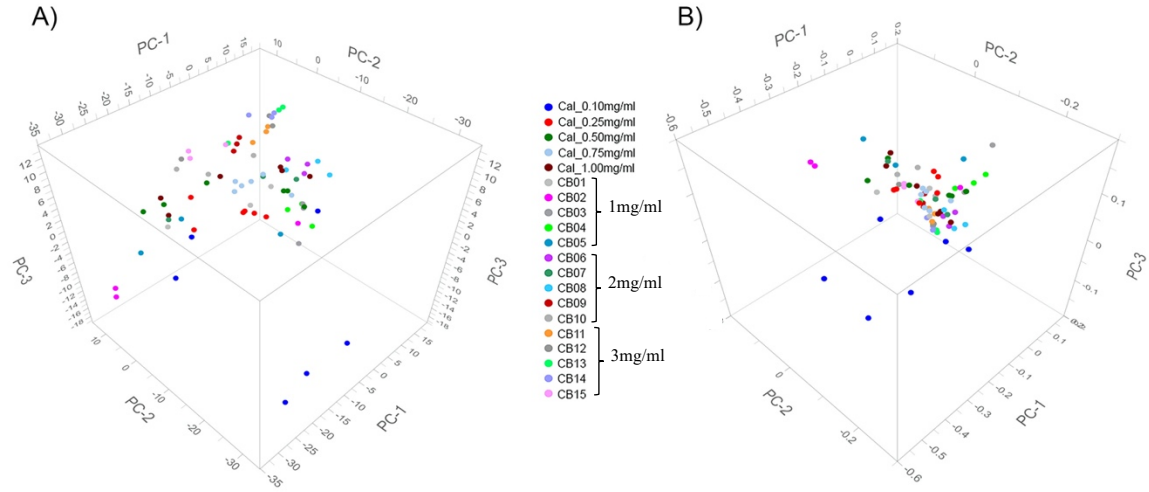


Figure 101 : 3D Scores plot of the PCA realized A) after SNV B) after MSC corrections. The three first principal component are presented, no other principal component allowing to enhance separation and/or visualization.

The use of SNV or MSC treatments following a derivative process did not show any clustering corresponding to the expected groups. SNV pre-treatment after a second derivative caused samples to be projected more disparately, so as to avoid obtaining a mass of samples at the same location, but the samples were not classified as expected. None of these pre-treatments are therefore satisfactory for achieving groupings by level of concentration.

Of the 12 different pre-processing tested, none provided better separation of the samples compared to the raw data. No classes were observed that corresponded to concentration variations, as the samples were all clustered together. This highlights that while DRIFTS-FTIR is a very sensitive method and a powerful screening method for condom lubricants, a confirmatory method such as py-GC/MS may be better suited for discriminating samples of different concentrations. However, applied to real samples, quantification seems obsolete because the amount of evidence present on the cotton swab is likely to vary according to the way the sampling is done, especially when considering self-sampling methods. The smear performed will not necessarily be representative of the contents of the vaginal matrix or the exact concentration and depends on several uncontrolled parameters.

Highlights

This chapter investigated FTIR analysis for condom residues. The main results are the following:

- FTIR and Raman spectroscopy were investigated for PDMS analysis in a forensic context: DRIFTS-FTIR was found to be the most relevant technique for traces, more specifically liquid extracted residues, considering sensitivity, analysis time, sample preparation and application to real samples;
- Optimal analytical conditions for DRIFTS analysis are 64 scans, 4 cm^{-1};
- ATR-FTIR model was not found to be directly usable with DRIFTS-FTIR data. However similar discrimination pattern was observed;
- Condom discrimination was not enhanced when using DRIFTS-FTIR data;
- Quantification and semi-quantification were not realisable within concluant results: different concentration samples could not be distinguished, due to a strong variation in the sample preparation, as well as instrumental variability.

Chapter 9: An investigation of parameters assisting forensic interpretation of the evidence: Background

This chapter is based on the following articles (Appendix I) :

Article 9 : Hermelin A., Fabien L., Fischer J., Saric N., Massonnet G., Burnier C., (2021) *Analysis of Condom Evidence in forensic science: Background survey of the human vaginal matrix using DRIFTS-FTIR and pyrolysis-GC/MS*, Forensic Science International

The analysis of the chemical composition of condom evidence has seen increasing focus in forensic research. This in an effort to be able to successfully analyze condom lubricants, more specifically silicone-based lubricants, and overcome analytical issues facing condom evidence. These lubricants were found to be preferentially extracted with hexane and detected by FTIR, followed by py-GC/MS. It is recognized that there is a need to identify not only the analytical parameters that would allow evidence to be detected, but also the factors that would influence their detection in casework. Issues regarding the lack of knowledge on the interaction between the target compounds and the vaginal matrix have already been highlighted in **Chapter 2**, and were outlined again in **Chapter 7**, when considering case work analysis. Although research has been led to answer questions concerning the transfer and persistence of condom residues, the question of the background is still pending. This chapters aims to be a comprehensive study of the qualitative composition of the human vaginal matrix and to report the prevalence of silicone-based residues amongst a given population.

The vaginal matrix is made up of a stratified squamous epithelium, characteristic of the mucous membranes that are found in moist areas of the human body, such as the esophagus or the mouth (Figure 102).

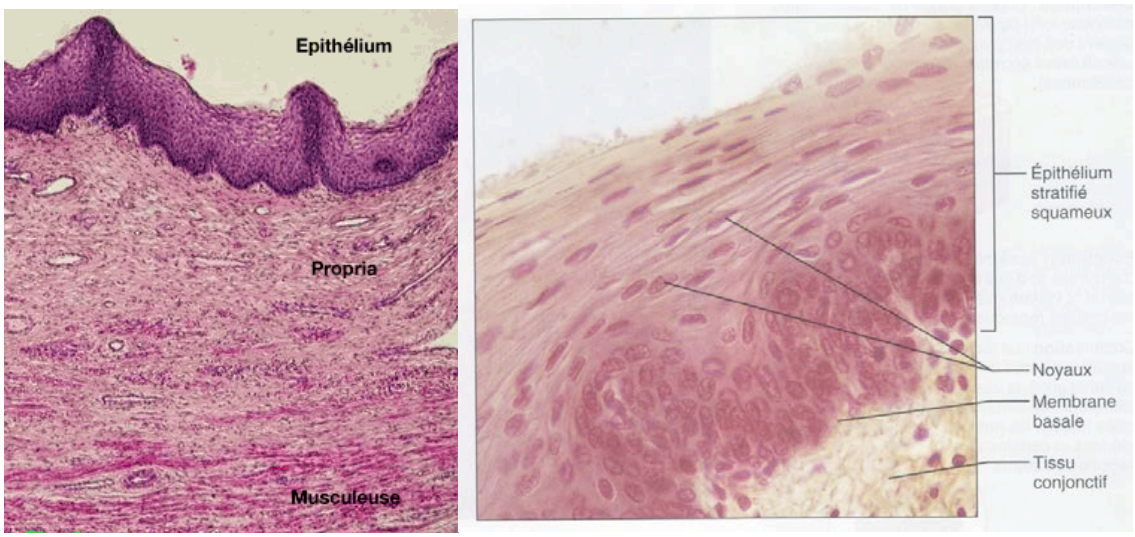


Figure 102 : Histologic structure of the vaginal matrix (left) and stratified squamous epithelium (right) *[45]*.

The epithelial tissues have a polarity: the apical surface is exposed outside the organism, and the basal surface is attached to the underlying connective and muscle tissue. The cells of the apical surface carry for the most part microvilli, increasing the surface area and thus to improve the absorption of substances. The basal surface lies on top of the basal lamina, a support sheet composed of glycoproteins and collagen fibers. The basal lamina acts as a scaffolding allowing the migration of epithelial cells for the growth or repair of organs. The epithelia are innervated but are not vascularized. The cells are then nourished by substances which diffuse from the underlying connective tissue. The further the cells are from the basement membrane, the less they are nourished by the connective tissue, and the less viable they are [45]. The cells of the apical surface are constantly abraded and replaced by the process of mitosis of the cells of the basement membrane. The cells of the surface layer are rich in glycogen, to be able to survive. The glycogen produced by the epithelial cells is used to protect the vagina. The lactobacilli present naturally in the vagina anaerobically metabolize glycogen to lactic acid, giving the vagina an acidic pH of 4.0 - 5.0. This acidity is toxic to sperm, but it helps protect the vagina against infections [45,96,165]. At the same time, the vaginal mucosa is permanently moistened despite the absence of glands, thanks to three types of secretions: cervical mucus, mucus and transudate. Cervical mucus is secreted by glands located at the level of the cervix and flows along the vaginal wall, thus implying a constant humidification of the mucous membrane. The secretions are however subject to physiological variations due in particular to the period of the ovulatory cycle, the taking of hormones (e.g. HRT, contraceptives) or sexual arousal [57,58,96,97]. Mucus and transudate are produced during sexual arousal. Mucus comes from the vestibular glands located on either side of the vaginal opening. Transudate is produced by the venous system and which percolates through the mucosa by osmosis. It then flows from the

walls of the vagina, ensuring good lubrication [45,46]. The exact composition of the vaginal matrix is not completely known. However, some articles have listed and reported compounds that can be easily detected, and they are reported in Table 39 here under.

Table 39 : Composition of the vaginal matrix

Compound Class	Type of compounds [45,46,57,166–168]
Trace elements	Calcium, iron, magnesium, zinc
Electrolytes	Potassium, chlorine, sodium
Proteins	Albumin, immunoglobulins, lactoferrin, glycoproteins (mucins)
Organic Acids	Lactic, citric, acetic, propionic, butyric acid
Amino Acids	Histidine and other amino acids
Lipids	Triglycerides, cholesterol, phospholipids
Degradation products	Urea, amines
Carbohydrates	Glucose, fructose, maltose, glycogen
Enzymes	Vaginal peptidases, lysozyme, oxidases, peroxidases, alkaline phosphatases, lactate dehydrogenase
Cells	Macrophages, flaky epithelial cells, granulocytes
Other	Water, squalene, pyridine, immunoglobulins

There are many reported medical studies investigating changes in the vaginal matrix under certain circumstances, such as infections, diseases, pregnancy or surgery. However, none of these studies report observations of spectral or chromatographic data that would provide information regarding possible siloxane content. In 2015, Orphanou [169] analysed various human secretions using ATR-FTIR spectroscopy and broadly reported the variations that could be observed, which are very relevant for forensic purposes. Since then, most research regarding condom evidence has focused on the development of analytical techniques to enable the detection of condom residues, but interactions with the vaginal matrix has only been recently reported [76]. However, to assist the interpretation of evidence, there is a need to conduct a prevalence study as well as report possible interactions, or specific problems linked to interactions, that may be observed with condom target compounds.

9.1 Material and Methods

9.1.1 Demographics

42 volunteers each provided 3 swabs, which were analyzed in triplicate, which resulted in a total 378 analyses for the FTIR technique. The data from 8 volunteers who presented results that could be misinterpreted and suggest the presence of silicone in their FTIR spectra were analysed using py-GC/MS instrument. The questionnaires the volunteers were asked to complete are presented in Appendix IV. The volunteers were aged between 18 and 35 years old. They were asked to self-sample 3 cotton swabs from their vaginal matrix, at any time of

the day, independently of any specific activity. Volunteers were asked not to collect samples during menstruation. Information was collected regarding the date of their last menstruation, the sampling date and the hour and if they had had intercourse in the week before sampling. If they had intercourse the week before the sampling, they were asked to report when the intercourse was protected, and when it last happened.

9.1.2 Sample preparation and analysis

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

For DRIFTS analyses, KBr was finely ground for about 5 minutes using an electric mechanical grinder. The finely mixed KBr was placed in sample cups and dried for 15 minutes at 100 ° C. When the bulk material was deposited on the KBr it was observed that it could not be absorbed on to it. Hence solution samples of each PDMS were used and individually deposited on the dry KBr filled cups using an eVol® syringe. Four cups filled with just KBr were kept for blanks. After spiking with 10µl of solution, samples were put in an oven for 15 minutes at 100 ° C to ensure solvent evaporation. Samples were left to cool down before analysis. A blank was taken every 3 analyses. For each solution, a series of analysis made up of 5 replicates analysed the same day was carried out. Reference material from PDMS and glycogen were also run the same way. Analysis parameters for DRIFTS were taken from **Chapter 8**.

For the py-GC/MS analysis, 10 µl of the solution was spiked into the quartz tube[††] on glass wool and left to evaporate before analysis. Three replicate samples were prepared from each donor to take into account homogeneity in the sample composition as well as the variation due to the instrumentation and sample preparation. Py-GC/MS analytical conditions are the ones presented in **Chapter 5**.

9.1.3 Preliminary considerations

The method selected for samples collection asked the volunteers to self-sample. Although this is a minimally invasive method, causing minimal restriction for the volunteers, large variations can be generated during sampling: a difference in pressure may cause a

[††] The analysis were run on CDS Analytical instrument, using quartz tubes, which is different from the Frontier Laboratory instrument used in Chapter 5.

variation in the amount of material collected. In addition, several factors are likely to influence the matrix content as previously highlighted in **Chapter 2**. As the vaginal matrix undergoes cyclic evolution, parameters such as the time of the menstrual cycle, hormonal intake, specific medication, pathologies, use of toiletries or genetics will influence the composition of the samples. The questions asked to the volunteers were deliberately very general and were related to the time of the menstrual cycle and previous protected intercourse, which could lead to detect silicone-based lubricants. No personal questions regarding medications, pathologies, personal hygiene or hormonal intake were asked.

As reported in **Chapter 2** and **Chapter 4**, several research showed that silicone-based lubricants were easily distinguished from the vaginal matrix and could be readily detected using the analytical framework presented in this study. As the present study focuses on silicone-based compounds, apolar solvent, i.e. hexane, was used as an extraction solvent. Therefore, a limited number of non-polar matrix constituent are expected to be detected. Water-based molecules are not likely to exhibit strong infrared signal and will not be detected using py-GC/MS, due to the degradation process, generating H_2O and CO_2, which are not detected by the mass spectrum. These compounds may instead be easily analysed using GC/MS. Therefore, the focus of this work was constrained only to the non-polar fraction.

The hypothesis set to justify the analysis of only 8 volunteers using py-GC/MS is the following: if samples who presented possible traces of PDMS using FTIR were negative after the py-GC/MS analysis, the ones whose FTIR spectra did not present any PDMS traces are unlikely to present a positive py-GC/MS result. This is based on the limits of detections of both instrumentations, which were found to be very close: 0.025 mg/mL for FTIR, 0.01 mg/mL for py-GC/MS.

Finally, regarding data processing, several normalization and preprocessing were investigated (similar to the ones presented in **Chapter 8**), including normalization to major peaks of the IR spectra (i.e. cholesterol peaks) or of the pyrograms (i.e. cholesta-3,5-diene), in order to maximize the between sample variability and minimize within sample variability. The use of an internal standard, although being one of the more common way to standardize data, is not recommended when using pyrolysis-GC/MS, as recombination would happen, and chromatographic patterns might not be reproducible. The various combination of processing did not allow to enhance the separation of between and within sample variability. Therefore,

these observations suggest that the extracts coming from different women are rather homogenous and there is no major difference between the different volunteers given their different lifestyles. This is due to the hexane extraction which only extracts apolar compounds. A polar extraction with methanol might show other variations. Methanol extraction was not tested in here as this chapter aims to focus on silicone-based residues detection, as a the most likely compound to be detected when it comes to condom evidence. Therefore, it is possible to focus on knowing if silicone traces can be distinguished from the background.

9.2 Qualitative analysis of the vaginal matrix

9.2.1 FTIR Analysis

All 378 spectra acquired were found to have very good reproducibility between sample replicates. Typical DRIFTS spectra obtained from three different samples are illustrated in Figure 103A. Blank swabs were analysed to ensure the cotton swabs were not contaminated with silicone and were not offering a chemical pattern within the area of interest (Figure 103B) In this study, the main vibrational bands identified in all background vaginal matrices were found to be comparable to those identified in earlier research into vaginal secretions from a qualitative point of view [169]. However, many differences were observed with the results from [169]. Indeed, Orphanou used ATR-FTIR and deposited the samples directly on the ATR crystal [169] whereas the present study used DRIFTS analysis, based on its relevance in the forensic analysis of condom residues (**Chapter 8**). As an extraction procedure was required in this study, some compounds might not be visible in the extracts, due to their polarity. Except for the bands coming from CH_2/CH_3 vibrations between 2850 and 2920 cm^{-1}, bonds coming from the cotton swabs were not dominant in the vaginal matrix swabs, thus suggesting that the vibrations observed in the collected spectra could be attributed to the vaginal matrix components. As illustrated on Figure 103B, bonds coming from the cotton swabs are not observed in the blank extracts, nor in the vaginal matrix swabs (Figure 103A). Therefore, the vibrations observed in the collected spectra (Figure 103A) can be attributed to the vaginal matrix components.

Given the chemical structure of cellular membranes and the use of hexane as the extraction solvent, one could reasonably expect IR bands characteristic of glycoproteins, glycolipids, peripheric proteins or cholesterol to be present in the spectra. The presence of glycogen, nucleic acid and amino acids was also expected.

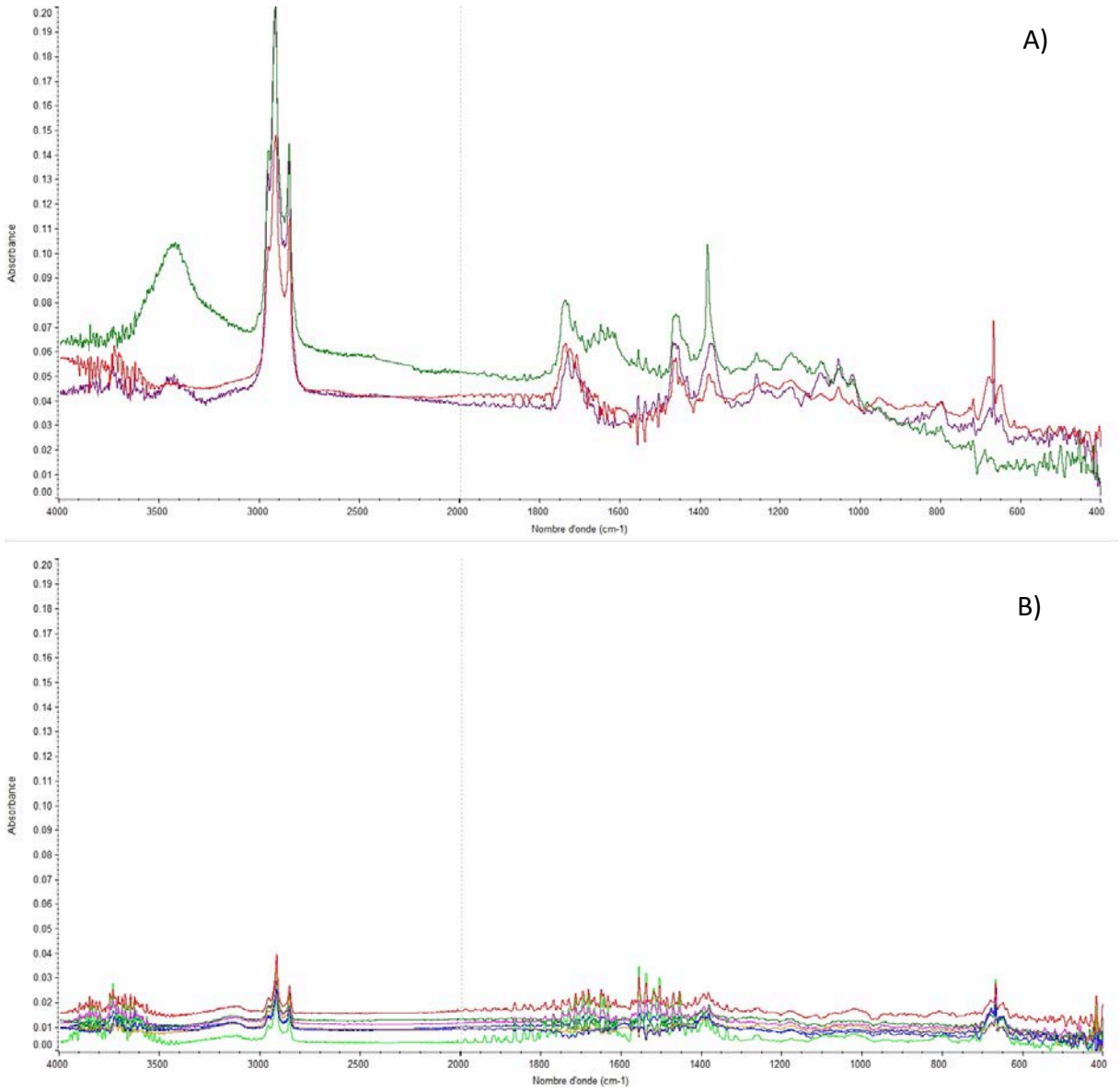


Figure 103 : Spectra acquired A) from the extracts of the vaginal matrix of different volunteers, B) from the extracts of the blank cotton swabs.

Proteins were observed through C=O stretching at 1649cm^{-1} and C-N stretching at 1542 cm^{-1} which are characteristics of Amide I and Amide II bonds. Additional peaks coming from the methyl bending of proteins were observed at 1467 and 1383 cm^{-1}. However, these peaks were found to be significantly more important than the ones reported by Orphanou [169]. This suggests either a high concentration of proteins or the presence of other molecules that present significant C=C vibrations as well as bending and stretching of C-O-H and OH bonds. However, if the protein concentration was higher, it is likely that Amide I and Amide II bands would also be significantly larger, which was not observed. In addition, vibrations at 2850, 2920 and 2960 cm^{-1} were found to be significantly more important than the ones presented in

[169]. This observation is more likely to be due to the extraction step, as apolar solvent was used: therefore, non-polar bonds appear to be dominating the spectra.

Lipidic material was indicated by the free hydroxyl (OH) bond around 3432 cm^{-1}, asymmetric vibrations from the C-H bond present at 2850, 2920 and 2960 cm^{-1} , 1467 and 1383 cm^{-1} ,as well as C-O vibrations at 1055 cm^{-1} and C-C backbone vibration at 938 cm^{-1}. Comparisons with reference spectra found in the literature [167,170–172] indicated that these peaks were associated with cholesterol. Traces of cholesteryl stearate were also observed in the spectra. Steroids were also found to produce several various vibration bands in the domain between 600 and 1400 cm^{-1}. As well as cholesterol, steroid hormones (e.g. progesterone and estrogens) secreted along the menstrual cycle may also be detected in infrared spectra. Given the strong apolar composition of these molecules, it is not surprising to see them being extracted from hexane [173]. The concentrations of these hormones vary naturally during the menstrual cycle [45] and could possibly affect sample discrimination (see section 8.3).

Carbohydrates were detected in the form of glycogen, with the main bands observed in the region 1034-1126 cm^{-1} [169]. Although these bands were present in the background spectrum and in the area where Si-O doublet vibration would appear (1020/1090 cm^{-1}), it was previously observed that the peaks from silicone-based lubricants are well resolved from vaginal secretion spectra as shown in Figure 104. This means they would be unambiguously indicative of PDMS in vaginal matrices, which is very promising, when considering that one of the main questions is also to know if silicone residues can be distinguished from the background.

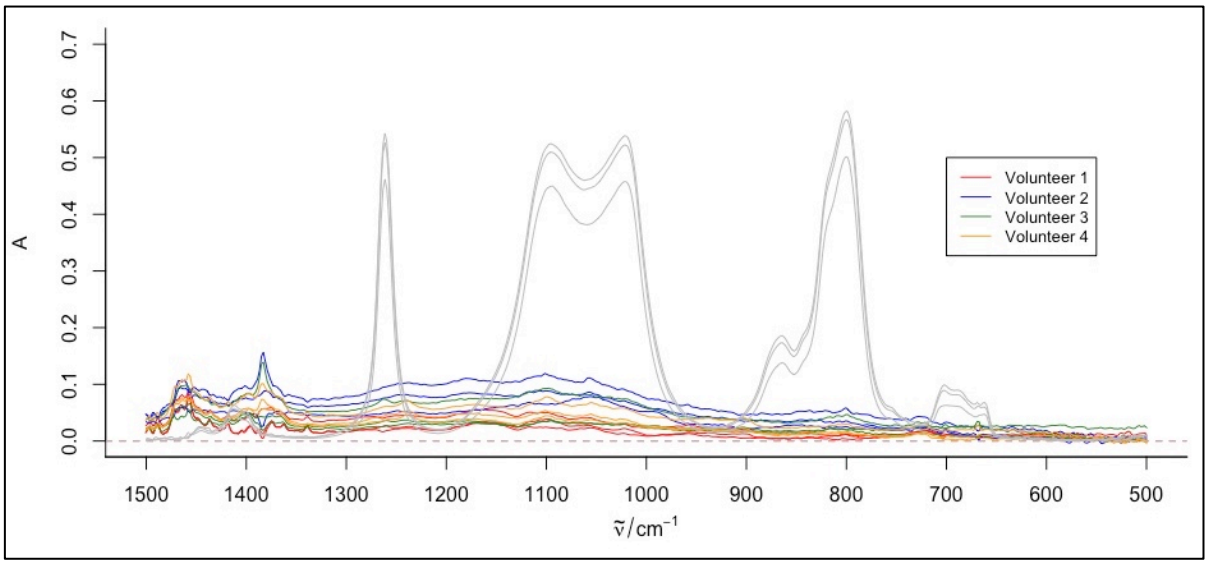


Figure 104 : Infrared spectra of vaginal secretions from 4 different volunteers and an overlay of PDMS reference spectra (in grey), between 500 and 1500cm^{-1}. For each volunteer, the 3 replicates acquired are presented. Reproduced from *Burnier C Van Bronswijk W., Massonnet G. (2020) Comparison of spectroscopic methods in detection of silicone-based condom lubricants evidence, Analytical Methods*

As there could be some misattribution of peaks to either glycogen or PDMS in the eventuality of the presence of traces, reference material of glycogen and PDMS were analysed and compared, as illustrated in Figure 105. If the Si-O doublet appears to be in a similar region than peaks from glycogen, Figure 105 also highlights that the bonds at 1263 cm^{-1} and 800 cm^{-1}, which are known to be diagnostic peaks for PDMS (**Chapter 8**), are very important and are not overlaid with any other components coming from glycogen. Therefore, the presence in the spectra of the four diagnostic peaks of PDMS, i.e. 1263, 1090/1020 and 800 cm^{-1}, is necessary to infer on the presence of PDMS in the sample.

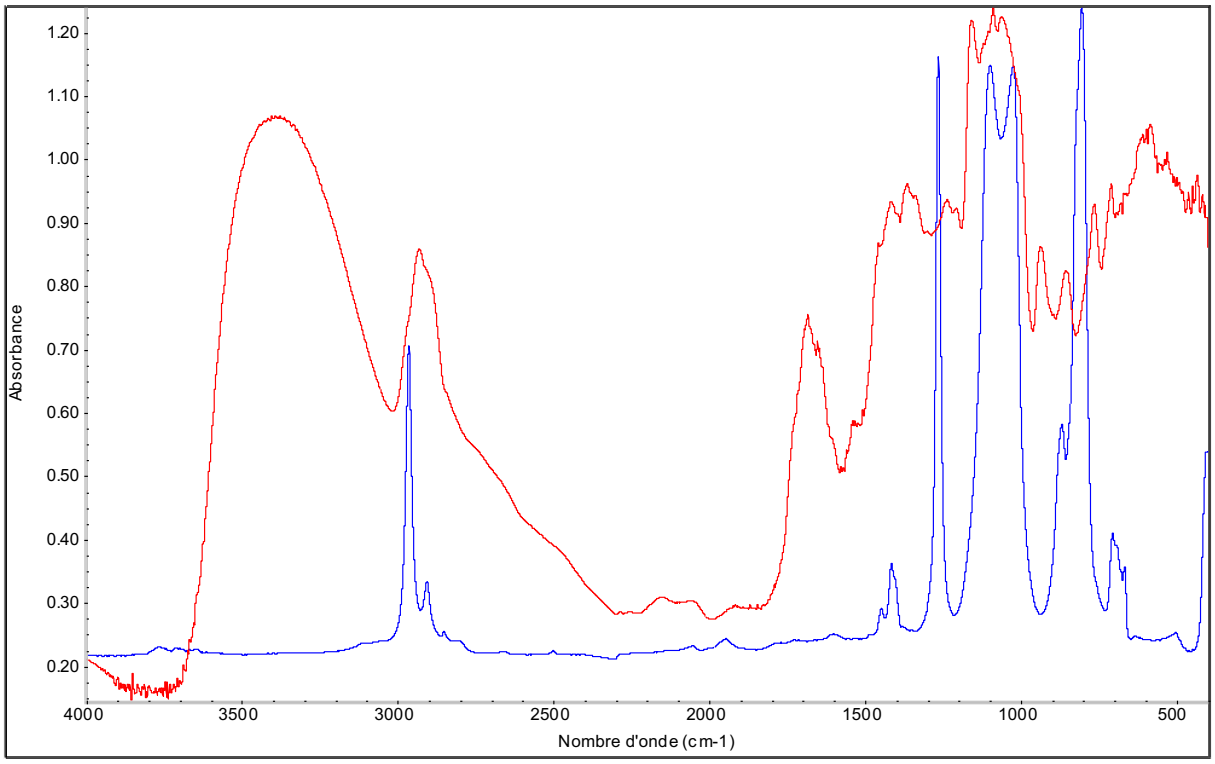


Figure 105: Overlay of reference spectra of glycogen (in red) and of PDMS (in blue).

Based on these results, it was possible to identify the most informative regions of the IR spectra for exploring dataset variation through chemometric analysis (Figure 106 and Table 40).

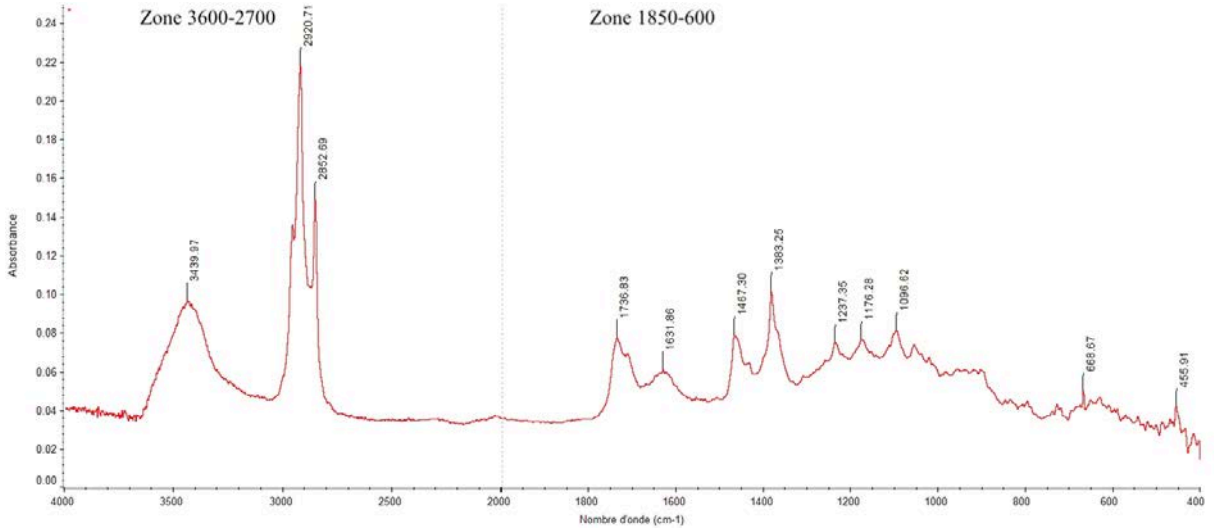


Figure 106: Example of a vaginal matrix spectrum obtained after hexane extraction. The shaded zones were selected for further chemometric analysis.

Variations of the vaginal matrix were studied visually, with no indication of strong variation between the different volunteers from a qualitative point of view. The spectra obtained from the 42 volunteers were largely similar, with no significant distinguishing peaks. Variations in the abundance of the peaks were observed in the region between 1850 and 600 cm^{-1}, which may be explained by natural variations as well as by the impact of the different contraception devices used. Sampling should also be considered as a source of variation.

Table 40 : Major vibrational bands corresponding to proteins, lipids and various material observed in the IR spectra of vaginal matrix extracts [169,170,174,175]

Peak Position [cm^{-1}]	Vibrations	Compounds	Category
3150-3600	Large OH-bond		
2960	C-H_2 stretch	Esters	Proteins, Lipids
2920	C-H stretch (1st carbon)	Aliphatic Chains	Proteins, Lipids
2850	C-H stretch (2st carbon)	Aliphatic Chains	Proteins, Lipids
1730	C=O stretch	Fatty acids	Lipids
1713	C=O stretch	Carboxylic Acid	Organic and Amino acids
1630	C=O stretch	Amide I	Proteins
1542	N-H and C-N	Amide II	Proteins
1465	C-H_3 asymmetric bend	Aliphatic Chains	Lipids
	C-H_2 symmetric bend		
1380	C-H_3 symmetric bend	Aliphatic Chains	Lipids
1200-1250	C-N stretch	Amide I/II	Proteins
1175	C-C-O stretch	Saturated esters	Lipids
1100	C-O stretch	Secondary alcohol	Lipids (cholesteryl stearate)

From the information obtained from the volunteers, 66% (28/42) did not have any sexual intercourse in the week before sampling. Of those that reported sexual intercourse, 3 volunteers (21.4%) reported the use of a condom. Intervals were 2 days before sampling, 7 days before sampling and unknown interval. These spectra were first targeted to evaluate whether silicone traces were present (Figure 107).

In the spectra of two out of the three volunteers, traces of silicone material were suspected to be present (Figure 107A and B), with very low concentration band. Given that not all the bands were present, and they are not well defined, it is difficult to conclude that silicone is present in the sample. These samples were first targeted using py-GC/MS to confirm or infirm this observation. No other spectra were found to present any silicone material. In the spectra of two out of the three volunteers, traces of silicone material were detected, with very low concentration bands. These two samples were first targeted using py-GC/MS to investigate this observation. Regarding the third volunteer (Figure 107C), presence of silicone is excluded, as none of the diagnostic peaks from PDMS are visible. No other spectra were found to present any silicone material.

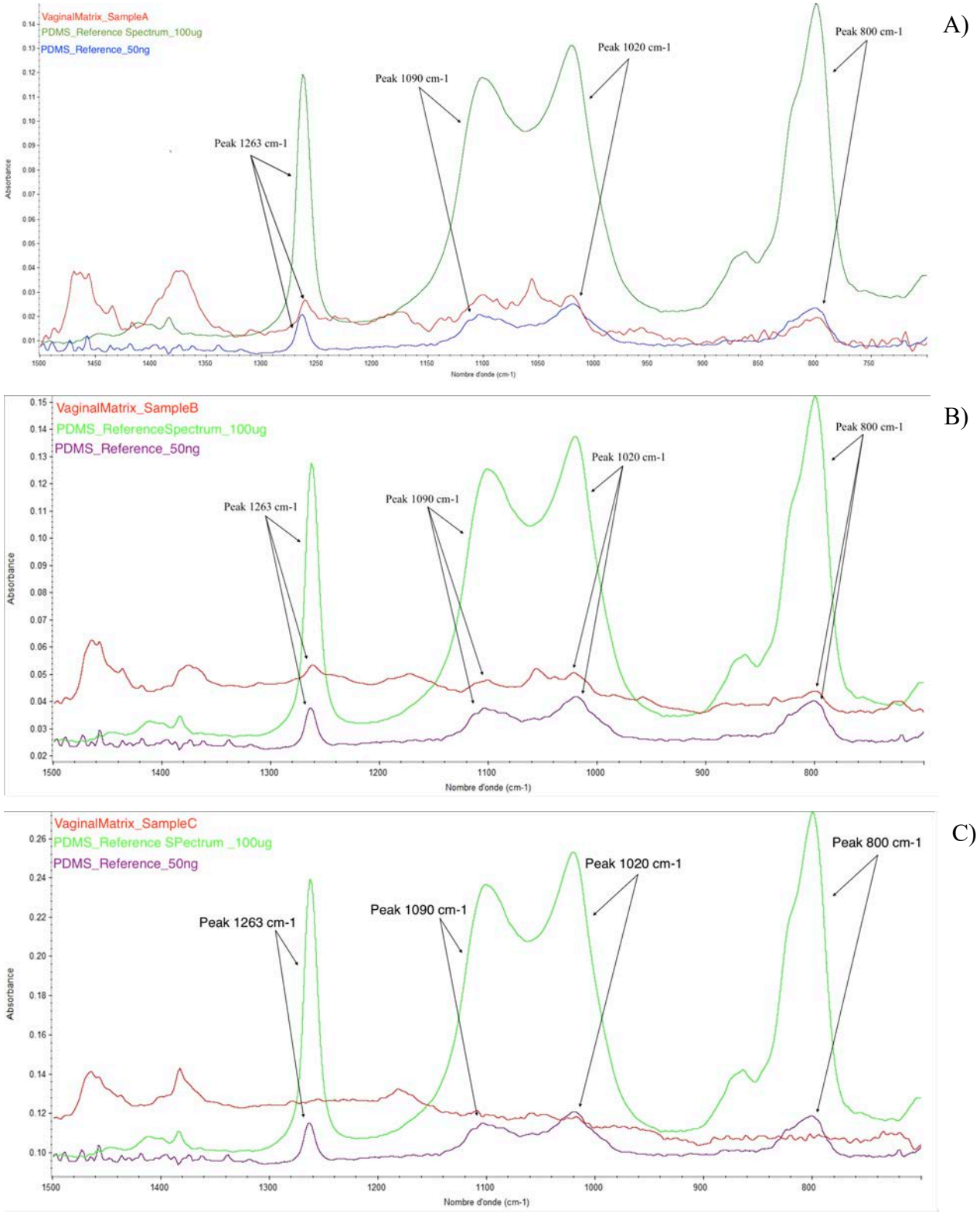


Figure 107: Illustration of the FTIR spectrum obtained from the volunteers who reported sexual intercourse in the week before sampling. A) 2 days before sampling, B) 7 days before sampling, C) unknown time interval. Spectra are displayed between 700 and 1500cm^{-1} to enhance readability. Overlay of a 100µg PDMS reference spectrum (green) and 50ng PDMS reference spectrum (red) are used for comparison.

9.2.2 Py-GC/MS Analysis

Before processing any qualitative analysis, blank swabs were run and evaluated in order to avoid considering compounds coming from the cotton as coming from the vaginal matrix. 12 compounds were detected in all extracts coming from the cotton swabs. They were respectively toluene, ethylbenzene, styrene, 1,2,4-trimethyl-benzene, alpha-methylstyrene, 3-phenyl-1-propyne, naphthalene, 1,2-dihydro-1-phenyl-naphtalene, undecane, nonadecane, and indene. Most of these compounds were present in very low abundance and were not detected in the vaginal matrix pyrograms. These products are likely to be coming from the glue used during the production of the cotton swabs. It was also found that although these compounds could originate from the swabs, the relative abundance of some of these peaks was higher in the vaginal matrix pyrograms, suggesting that pyrolysis of vaginal matrix residues also produces this type of compounds. Althouth this is a very unlikely hypothesis, the relative amount of these compounds was therefore subtracted to the one obtained from the vaginal matrix swabs.

All the pyrograms acquired were found to have very good reproducibility between sample replicates. Some differences were noticed between the different volunteers, with certain peaks being detected only in certain chromatograms. Typical spectra obtained from three different volunteers are illustrated in Figure 108A. Major differences were observed when compared to case work samples presented in Chapter 7, as the present background study shows chemical profile very different from the negative results obtained in three out of four of the case work. Multiple hypothesis can be drawn to explain that difference, such as the different instrument or sampling (doctors sampling in **Chapter 7**, self-sampling in this chapter). Sampling effect cannot be neglected, as in one case doctors sampled the evidence, and in the second, volunteers self-sampled. Variations in the pressure put to collect the traces is likely to affect the recovery and therefore the chemical profile obtained. However, the main difference stands in the type of cotton swabs used: we have used cotton swabs mounted on a wooden stick (COPAN 150C swabs) whereas cotton swabs used by SARC are mounted on a plastic stick (COPAN Interpath Services H043N). Given that the blank cotton swab from **Chapter 7** contained styrene and its degradation products, one can understand that the plastic stick has desorbed its content in the extraction solvent. The COPAN 150C swabs also contains these aromatics compound, which makes it more likely that these compound come from the glue used during the manufacturing process. Blank pyrogram from COPAN150C cotton swab are shown in Figure 108B for comparison.

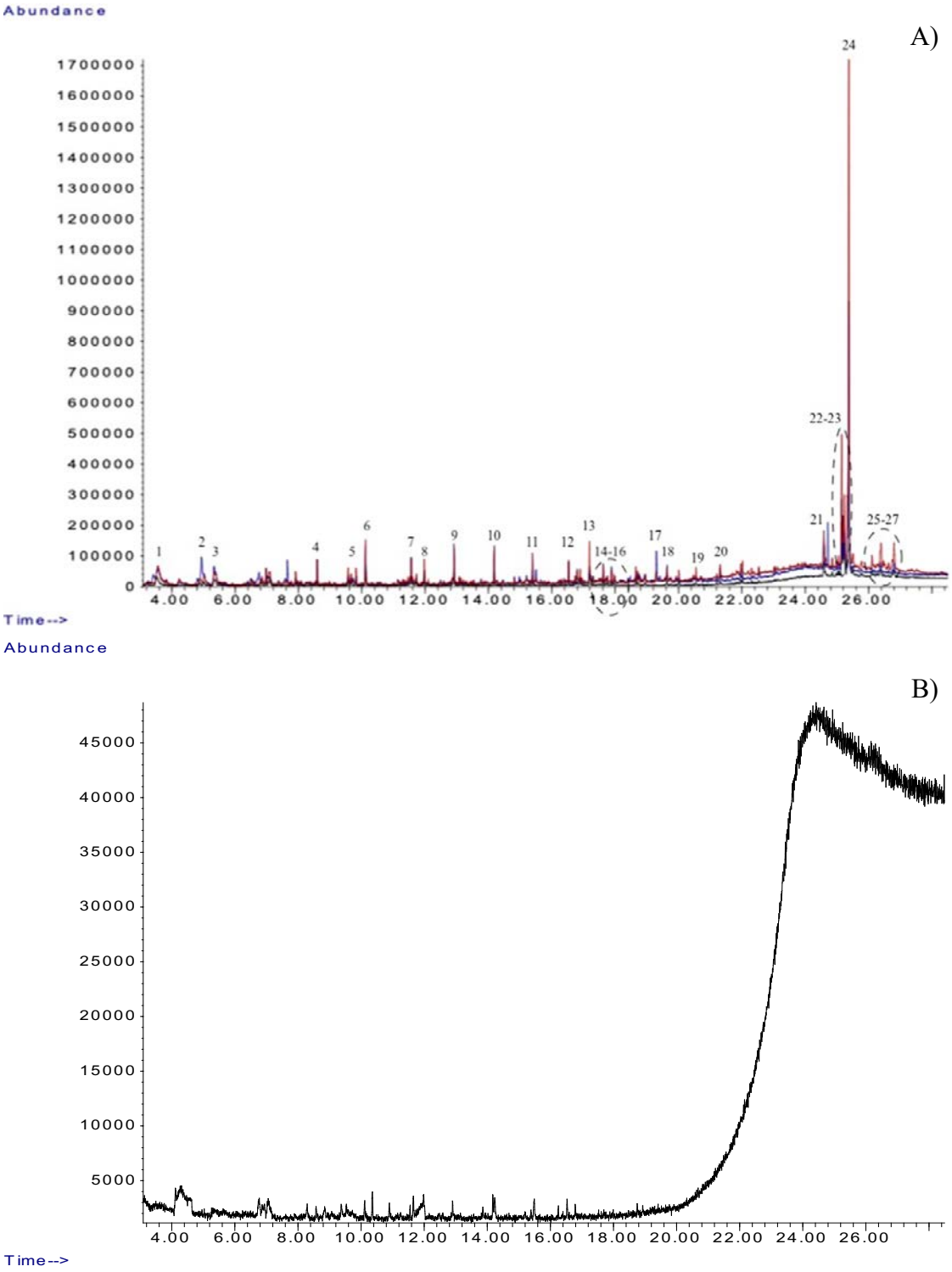


Figure 108: A) Typical pyrogram obtained for three different volunteers. Annotated peaks are the ones that were found to be coming from the vaginal matrix, and not from the cotton swabs. B) Blank for comparison

The major peaks seen towards the end of the pyrogram, i.e. peaks 21-27 in Figure 108A, are found systematically in all the pyrograms. These peaks were attributed, using their mass spectra, literature [92,176–179] and databases, to cholesterol and stigmasterol derivatives (Table 41), which are two compounds classified as steroids. These molecules are found in cell membranes but are also the basic structure of sex hormones such as estradiol or progesterone.

The presence of steroid hormones in the extracts had already been noted during the infrared analysis of the same samples.

The majority of the compounds detected in the pyrograms had a naphthalene or indene base or were small molecules such as xylene and styrene. Compounds such as phenanthrene and anthracene, as well as their derivatives were also detected. Literature [92,179] confirmed that these compounds did indeed come from the degradation of cholesterol and steroid-based compounds present in the samples. Compounds which had an abundance higher than three times the noise (threshold set at 30,000 AU) and which were sufficiently distinct from the background noise for integration are reported in Table 41.

The smallest peaks present in the pyrograms, with abundances approaching the limit of 30 000 AU, presented mass spectra characteristic of products derived from cholesterol and analogous steroids. These compounds could have been attributed to naphthalene derivatives (for example 2-ethenyl-, 1-ethyl-, 1,5-dimethyl-, 2,3-dimethyl-) or to phenanthrene derivatives. These were not detected in the blank analysis from the cotton swabs. Certain compounds, such as dienol or compounds containing hydroxy groups, may be produced from the breakdown of estradiol and estriol, which are the two most important female sex hormones produced by the human body. Their chemical structure presented below (Figure 109), includes phenolic groups as well as one or more alcohol groups. Considering recombination happening during the pyrolysis process, the presence of these molecules might explain the hydroxy or phenolic content detected in the minor peaks in the pyrograms.

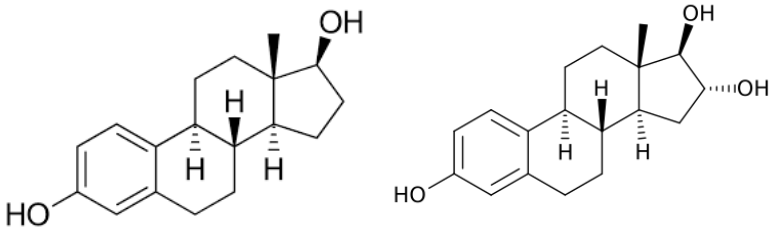


Figure 109: Chemical structure of estradiol (left) and estriol (right)

Table 41: Identification of the compounds found in all the pyrograms obtained from the vaginal matrix. Peak numbers are related to Figure 108A. Database used was NIST18.

Peak	Compound	RT [min]	Target ion [m/z]	Qualifiers [m/z]
1	Toluene	3.530	91	79
2	p-xylene	4.929	91	106, 77
3	Styrene	5.354	104	78, 91
4	1-Undecene	8.589	154	117, 97, 83
5	Trimer containing:		104	
	1-Methylindene	9.562	130	115, 102, 77
	2-Methylindene	9.667	130	115, 102, 77
	Naphtalene, 1,2-dihydro	9.813	130	115, 91
6	1-Dodecene	10.116	128	97, 83, 70
7	1-Tridecene	11.550	83	97, 111, 196
8	Naphtalene, 2-methyl	11.719	142	115, 129, 158
8b	Naphatalene, 1-methyl-	11.964	142	115, 126, 89
9	1-Tetradecene	12.908	83	196, 125, 111
8c	Naphtalene, 1,3 dimethyl	13.124	141	156, 115, 127
10	1-Pentadecene	14.179	83	210, 182, 111
11	1-Hexadecene	15.385	97	224, 125, 111
12	1-Heptadecene	16.522	97	238, 125, 111
13	Naphtalene,1-(1,1-dimethylethyl)-7-methoxy	17.186	214	199, 143, 91
14	1-Octadecene	17.612	83	252, 125, 111
15a	Phenanthrene	17.699	178	152, 89, 76
15b	Anthracene	17.752	178	152, 89, 76
16	Tetradecanal	17.880	82	194, 96, 138
15c	Phenanthrene,2-methyl-	18.952	192	165, 176, 95
17	n-hexadecanoic acid	19.296	73	129, 213, 256
18	5-Eicosene, (E)-	19.634	97	111, 125, 280
19	1-nonadecene	20.549	97	111, 125, 266
20	1-docosene	21.307	97	111, 125, 308
21	Cholesta-2,4-diene	25.148	368	255, 353, 106
22	Unknown_25.18	25.185	197	352, 144, 117
23	Cholesta-4,6-dien-3-ol, (3 beta)-	25.236	366	247, 143, 91
24	Cholesta-3,5-diene	25.370	368	353, 147, 247
25	Cholest-7-en-3one-4,4-dimethyl	26.098	207	193, 348, 181
26	Cholesta-7,9(11) dien-3-ol-4,4-dimethyl	26.390	207	398, 344, 281
27	Stigmasta-3,5-diene	26.804	207	147, 396, 81

The pyrograms were further examined for other steroid hormones derivatives and pyrolysis products, based on the report by [180]. No residues characteristic of progesterone or androsterone, nor any of their derivatives or pyrolysis products were detected in the pyrograms. The remainder of the compounds listed in Table 41 are 1-alkenes. According to [181], 1-alkenes are generated from the pyrolysis of triglycerides. It was previously suggested that the compounds observed in the FTIR spectra and pyrograms originate from cell membranes. Cell

membranes contain, amongst other compounds, glycoproteins, cholesterol and glycolipids. The previously described cholesterol residues do not explain the presence of 1-alkenes. Triglycerides are very common fatty acids, which are found in both phosphoglycerides (e.g. phosphatidylcholine) and glycolipids who present e.g. stearyl, oleyl, linoleyl acyl groups [92,93] which can totally explain the presence of 1-alkenes in the pyrograms.
Although most of the peaks detected in the pyrograms were sourced from cholesterol derivatives and glycolipids, indoles were also detected in small amounts, though not abundant enough to integrate for chemometric purposes. However, indole is a typical marker from protein compounds [182]. 1,2-Cyclopentanedione, 3-methyl was also found as a marker from carbohydrates [182].

From a qualitative point of view, the pyrograms showed no signs of the presence of silicone traces. Regarding samples that were found to present possible evidence of silicone in the infrared spectroscopy, the sample presented in Figure 107C was confirmed to be negative to silicone residues. The overlay of the samples with the blank associated illustrated that it was likely that the vaginal sample A (Figure 107A) would contain some silicone, but an extraction of the ions would be necessary. Regarding sample B (Figure 107B), the overlay did not allow to visually distinguish any peaks from the background
The procedure previously led in **Chapter 7** for case work was again led and extracted ion chromatograms of the silicones patterns for the vaginal matrix sample A and B, showed traces of the main D3 to D5 degradation, but in very low abundance. Given that the abundance was not over 3x the noise at the same retention time for any of the two samples, the presence of the compounds in the matrix cannot be fully assessed. These observations support the hypothesis that silicone compounds are not naturally found in the vaginal matrix.

9.3 Variation of the vaginal matrix

9.3.1 FTIR Analysis

The aim here is to assess whether statistical differences can be observed within a population of women, based on variations in absorbances. Based on previous studies on the classification of human samples according to population characteristics [174], spectra were pretreated using the Savitsky-Golay second derivative, which corrects for baseline variations and allows for the separation of overlapping peaks.

PCA of the overall dataset (378 infrared spectra) revealed that 75% of the variance within the dataset was accounted for by the first seven PCs. The scores plot constructed from the first 3 PCs (Figure 110) revealed no significant differences in composition were observed between the 42 volunteers. PCA of data processed with Savitsky-Golay second derivative was compared against PCA following baseline correction and range normalization, where 95% of the variance was explained by the first seven PCs. While the cumulative variance of the range normalized data was higher than that of the data processed with derivation, it was found that baseline correcting and normalizing the data generated a broader spread of the samples within the scores plot. However, no trends were visible in the dataset, and it was not possible to see influence from certain region of the spectra.

As the vaginal matrix undergoes cyclic changes, the relative amounts of molecules linked to sexual hormones (such as estriol, estradiol or progesterone) that could be detected in the spectra are reflective of compositional changes along the cycle. Investigation into discrimination of the samples based on the time in the menstrual cycle found that no commonalities at the different phases of the menstrual cycle, which is likely due to inter-donor compositional differences. Increased numbers of replicates were projected randomly from each other, indicating disparities between replicates collected at various times of day.

The disparate projection of samples from the 42 volunteers is caused by inter and intra-donor variation, as well as variations due to sample collection and analytical impact, which creates difficulties in interpreting the dataset in its entirety. PCA indicates that there are no sufficiently significant changes in the infrared spectra to differentiate between women nor between different stages of the menstrual cycle.

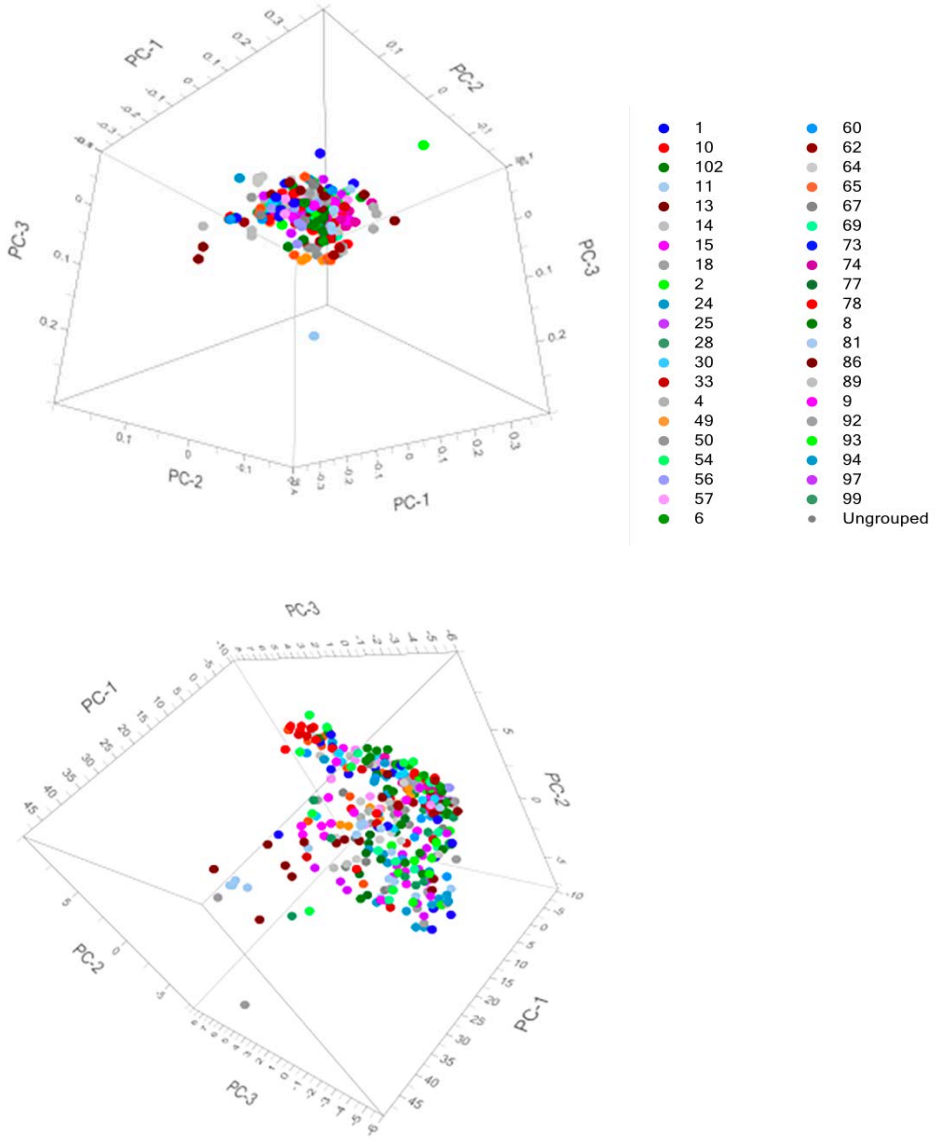


Figure 110: 3-dimensional scores plot generated from the first 3 PCs, demonstrating the distribution of vaginal matrix composition collected from 42 volunteers, after second derivative (top) and range normalization (bottom). Samples are colored by individual volunteer.

9.3.2 Py-GC/MS Analysis

PCA of the overall dataset (24 pyrograms, from 8 different donors) showed that 90% of the variance within the dataset was accounted for by the first seven PCs. The scores plot constructed from the first 3 PCs (Figure 111) exhibited that no significant differences in composition between the 8 volunteers. Although strong variability was observed, the same pattern was observed as with the infrared data. No trends were visible in the dataset. The loadings plots demonstrated that PC1 was influenced by styrene and xylene, and PC2 by cholesta-3,5-diene and cholesta-2,4-diene. These compounds were also found to generate separation along PC3.

The rest of the compounds were not found to contribute significantly to sample separation. The rest of the variables were found to be too close to 0 to be relevant for discrimination purposes.

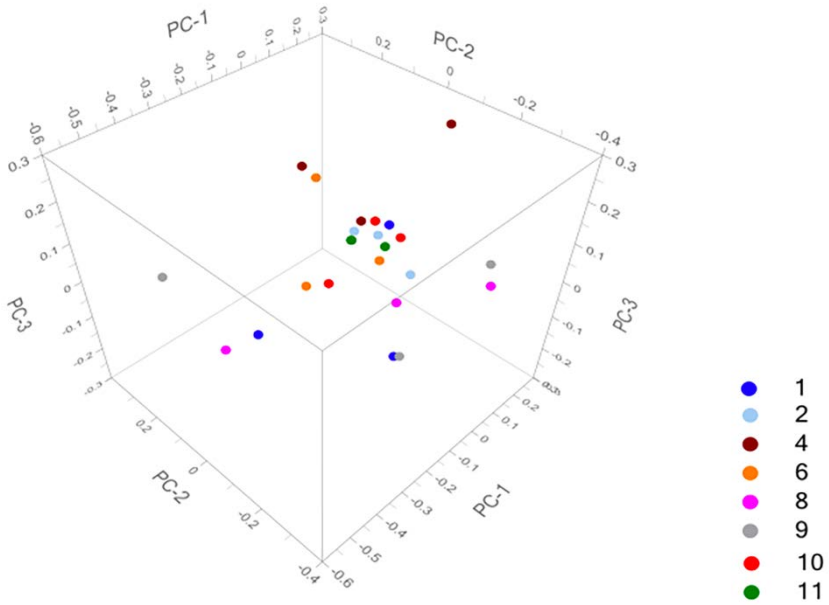


Figure 111*:* 3-dimensional scores plot generated from the first 3 PCs, demonstrating the distribution of vaginal matrix composition collected from 8 volunteers, after areasum normalization and double square root processing.

The disparate projection of samples from the 8 volunteers is caused by inter and intra-donor variation, as well as variation in sample collection and analytical impact. PCA of the dataset indicated that there are no statistically significant differences in the pyrograms to observe any differences between women nor between different times of the menstrual cycle.

9.4 Discussion

The observations described above indicate that the endogenous prevalence of silicone compounds in the vaginal matrix is zero. This is true for the pilot study presented here, studying a small population of women within a restricted age group (18-35 years). If certain spectra suggested the presence of a silicone compound, the extraction and py-GC/MS analysis did not reveal any trace of these compounds, even in samples from women who had protected intercourse, between 2 and 7 days prior to sampling. No additional precisions were obtained on the intercourse and its nature due to the formulation of the questionary (see Appendix IV). No instances occurred where a volunteer had protected intercourse in the 24 hours preceding sampling, in which case silicone traces could have been detected.

The data obtained made it possible to understand the structure of the vaginal matrix, in order to identify relevant peaks for the analysis of real samples. The application of data pretreatments

(for example normalization or derivation) or multivariate statistical analyses (for example PCA) did not enable any classification linked to the volunteers or any stages of the menstrual cycle. Intravariability was found to be as important as intervariability, preventing differentiation between samples from different sources. It is therefore necessary to standardize the acquisition and analytical methodology, by validation according to reliable and repeatable statistical models, so as to be able to subsequently reduce the intravariability, while retaining a sufficient intervariability to discriminate samples from different sources. Although the utility of it might be questionable, the presence of different populations based on the vaginal matrix content, and variations due to the menstrual cycle or the intake of certain type of contraceptive might affect the chemical profile of the matrix. Therefore, being able to distinguish these differences would be of interest so as to be able to see these when encountered in casework.

In addition, the results obtained in this study raise more fundamental questions about the value of acquiring more data or of modeling and trying to understand the impact of parameters that seem to be individual as a whole. The acquisition of a larger data set, particularly in terms of pyrolysis-GC/MS analysis, is necessary so as to obtain a model which can be considered statistically reliable. In view of the data now obtained and considerations regarding the observed variability, the acquisition of more data will inevitably result in the creation of an unreadable grouping at the center of the 3D scores plot, in a similar manner to the FTIR data. The grouping of the data obtained between them, despite different sources, is explained on one hand by the effects of the extraction and on the other hand by human cellular composition. As the extractions were carried out using an apolar solvent, the compounds extracted were apolar vaginal matrix constituents. However, these apolar compounds are mainly those which compose the cell membrane, which shows little variation between individuals. The expected variations in terms of membrane thickness or hormonal effects are not large enough, given the additional variability of the extraction, operator and instrument, to be detected. Variations due to the pyrolysis itself, as it is a process that is difficult to monitor, should also be considered. Finally, the modeling of interpretive parameters is generally carried out so as to derive from the data a model which is applicable to the entire considered population. Knowing that each woman has different vaginal microbiome, which undergoes regular cyclical variations, it the influence menstrual cycle on the data warrants investigation. However, such information would be specific to each woman, and the model might never be applicable in general. These results are however promising for future practical use, since the results show that once the traces are extracted, there is *a priori* no vaginal matrix residues detected in the py-GC/MS pyrograms. Thus, only lubricant residues are likely to be detected, making it more

relevant for case work investigation. This doesn't inform on matrix effect, which will happen during the elapsed time between the intercourse and the sampling.
Subsequent extraction for polar compounds using methanol followed by a GC/MS analysis would inform on the polar composition of the vaginal matrix and highlight questions related to water-based lubricant analysis and interactions with the vaginal matrix.

Highlights

This chapter investigated FTIR and py-GC/MS analysis of vaginal matrix residues. The main results are the following:

- The apolar fraction of the human vaginal matrix exhibits a high degree of complexity.
- Full characterization and discrimination of different matrix is challenging.
- The most informative FTIR spectral region was found to be between 700-1850 cm^{-1} and 2700-3600 cm^{-1}.
- 27 compounds were detected using hexane extraction followed py-GC/MS.
- FTIR showed possible traces of silicone in 3 out of 42 samples, but py-GC/MS did not support this hypothesis.
- Several uncontrolled factors could affect the sample and were not initially monitored.
- To obtain more representative results, further investigation on larger population are needed and more personal parameters should be monitored.

Chapter 10: A preliminary investigation of parameters assisting forensic interpretation of the evidence – I: Transfer study

This chapter is based on the following article (Appendix I):

Article 3: Burnier C., Van Bronswijk W., Massonnet G. (2020) *Comparison of spectroscopic methods in detection of silicone-based condom lubricants evidence,* Analytical Methods, Vol. 12.

Article 12: Saric N., Fabien L., Hermelin A., Fischer J., Massonnet G., Burnier C., (2021) *A preliminary investigation of transfer of condom lubricants in the vaginal matrix*, Forensic Science International

Multiple researches have focused on different, but complementary, approaches to be able to exploit the condom evidence. Most studies focused on the composition of the condoms and the discrimination of samples present on various markets (as **Chapter 4**). More rarely, chemical markers in the vaginal matrix were investigated to identify which compounds were the most easily detected and what could be found in traces [7-9, 11]. Another important aspect seems to have been neglected by researchers: the question of interpretative parameters. This approach generally takes into account parameters such as occurrence, background, transfer and persistence parameters. Despite the recurrence of such questions, there's is no existing model to assist the forensic scientist to understand what happens during the transfer as well as after a sexual intercourse, and thus to allow adequate interpretation of the evidence. This is likely due to barriers related to this type of study. Indeed, the variations observed in a profile are influenced by various factors which are difficult or even impossible to control (**Chapter 2**) and whose effects are for the most part unknown (e.g. the effects of the contact itself (duration, intensity...), or the influence of the vaginal matrix) as highlighted in **Chapter 2**. Added to this are ethical considerations, which make these studies long and tedious but demonstrate the need to increase research on the subject.

As described by Burnier and Massonnet [14], several factors are likely to influence the trace and its recovery by influencing the transfer and persistence of the evidence. Both the initial composition and recovered composition of the evidence are significantly affected by different influence factors, classified in five groups: the donor, the receiver, the contact, the elapsed time between intercourse and sample and the activity of the receiver between the elapsed time [14]. This chapter details a preliminary study into the parameters of transfer, by exploiting data acquired with the optimized infrared technique (**Chapter 8**). These parameters have already

been reported, although not exhaustively, by New Zealand researchers using py-GC/MS, and in view of the importance of the inter and intra-donor variation observed in volunteers of the background study (**Chapter 9**), it was considered more judicious to present the preliminary results of the infrared analysis, without going further in the analytical sequence. This chapter is a preliminary investigation of the transfer of silicone lubricants, which is studied in a fundamentally qualitative and semi-quantitative perspective, i.e. question the influence of the phenomen of tranfer on the FTIR profile obtained. This chapter will not discuss quantitative dimensions, such as the amount of silicone transferred from the condom to the vaginal matrix.

10.1 Material and methods

10.1.1 Chemicals and solutions

Hexane of analytical grade was from Sigma Aldrich (USA) and was used as received. PDMS 200cSt obtained from Sigma Aldrich (USA) was diluted in hexane at concentrations of 0.1mg/ml and 1 mg/ml. A 5µl syringe eVol XR ® from SGE Analytical Science was used to deposit the samples on the KBr pellets. Cotton swabs used for sample collection were COPAN150C ones.

10.1.2 Human sample collection

Investigation of transfer and persistence needed the participation of human volunteers. The sample collection was done following the protocol accepted by the Ethics Committee (see Appendix IV).

To investigate the donor (i.e., condom) effect, 2 volunteers had sexual intercourse using 10 different condoms, listed in Table 42. 3 blank swabs were collected prior to intercourse, and 3 samples were collected right after intercourse. To avoid any cross-contamination, the volunteers were asked to wait one week between each protected intercourse (see Appendix IV for the detailed procedure, after acceptation by the ethics committee). Each sample was analysed 3 times, resulting in 9 replicates for a same donor. A total of 132 analysis were run for this purpose.

Table 42 : List of the donors used for the donor effect investigation and their features, with the number attributed and which receiver used which samples. Composition is known based on observations from Chapter 4, Chapter 5 and Chapter 6.

No	Donor	Type	Lubricant	Other Components	Receiver 1	Receiver 2
D1	Ceylor Blue	latex	silicone	no	X	X
D2	Ceylor Gold	latex	water-based	Glycerin, PEG, nonoxynol-9	X	X
D3	Ceylor Ultrathin	non latex	silicone	no	X	X
D4	Ceylor Green	latex	none	no	X	
D5	Manix Contact	latex	silicone	no	X	X
D6	Durex Natural	latex	silicone	no	X	
D7	Durex Gefuhlsecht	latex	silicone	no	X	
D8	Manix Orgazmax Plus	latex	silicone	Propylene Glycol	X	
D9	Manix Skyn	non latex	silicone	no	X	
D10	Prix Garantie	latex	silicone	no	X	
D11	Manix Fraise	latex	silicone	no		X

To investigate the receiver (i.e. volunteer) and therefore matrix effect, the donor was fixed as the Ceylor Blue condom (latex silicone lubricated condom). In the eventuality the volunteer asked for a latex free condom, the selected condom was Manix Skyn. These choices were made as they are common condoms found on the market, and they are not statistically distinguishable based on their chemical composition. 9 volunteers had sexual intercourse using the provided condom, and samples were taken right after the intercourse. Here again, blanks were collected prior to intercourse to ensure the absence of contamination.

10.1.3 Sample extraction, preparation and analysis

As presented in Chapter 9, cotton swabs collected from the volunteers were cut from the wooden sticks and individually put in a glass vial and extracted with 1mL of hexane. The vials were vortexed for 1 min and sonicated for 15 min. The resulting samples were analysed in triplicate.

For DRIFTS analyses, KBr was finely ground for about 5 minutes using an electric mechanical grinder. Mixed KBr was placed in sample cups and dried for 15 minutes at 100 ° C. Cups filled only with KBr were kept for blanks. A standard sample was run within each analysis batch to ensure analytical instrument quality. After spiking with 10µl of extract solution, samples were put in an oven for 15 minutes at 100 ° C to ensure solvent evaporation. Samples were left to cool down before analysis. A blank was taken every 3 analyses. Analysis parameter for DRIFTS were taken from **Chapter 8**.

10.2 Results

10.2.1 Matrix effect investigation

To evaluate the matrix effect and see if the chemical observed is affected by interference with either the cotton swab or the matrix, three different types of samples were used:

- Standard reference PDMS diluted in hexane, at various concentrations between 0.1 and 1.0 mg/mL (substrate: none)
- Cotton swabs spiked with various concentrations of PDMS between 1.0 and 3.0 mg/mL (substrate: cotton swab)
- Cotton swabs with residues self-sampled from the 9 volunteers (substrate: cotton swab + vaginal matrix)

This allows evaluation of whether there are any specific patterns linked to dilution, to cotton swabs and/or to the human matrix. No visual differences were observed as illustrated in Figure 112.

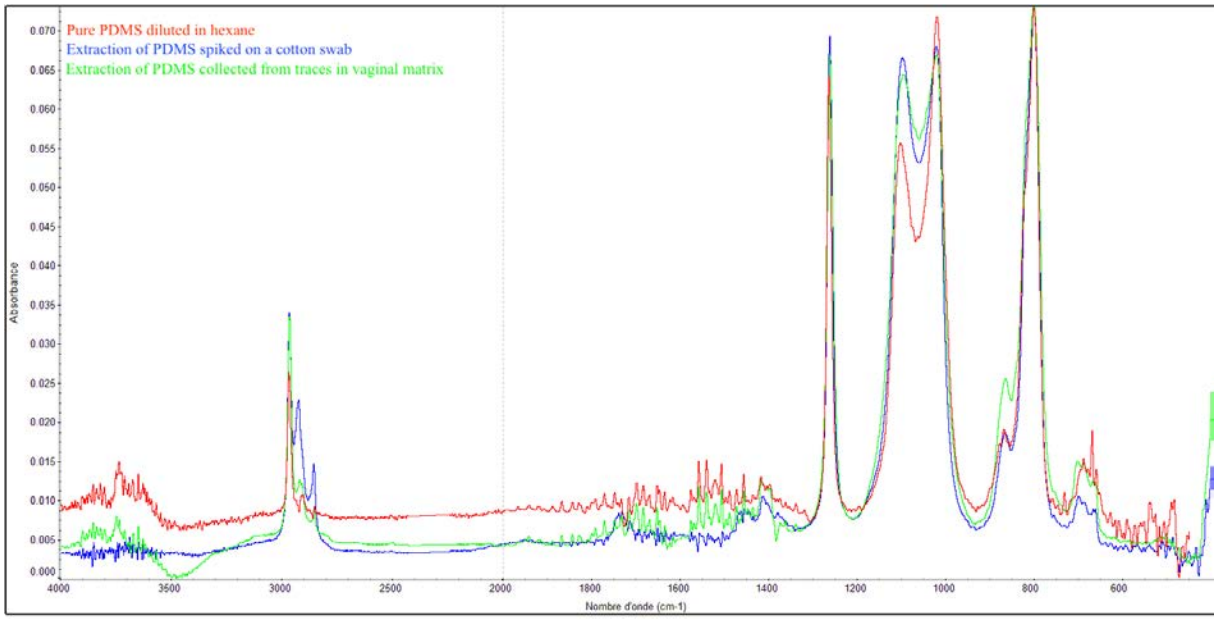


Figure 112: Illustration of the chemical profile obtained from pure PDMS diluted in hexane (red spectrum), extract of PDMS spiked on a cotton swab (blue spectrum) and extract of PDMS collected as trace evidence in a vaginal matrix after intercourse (green spectrum).

Principal component analysis was performed to see if the samples were statistically distinguishable. Results for the matrix effect investigation are presented on Figure 113.

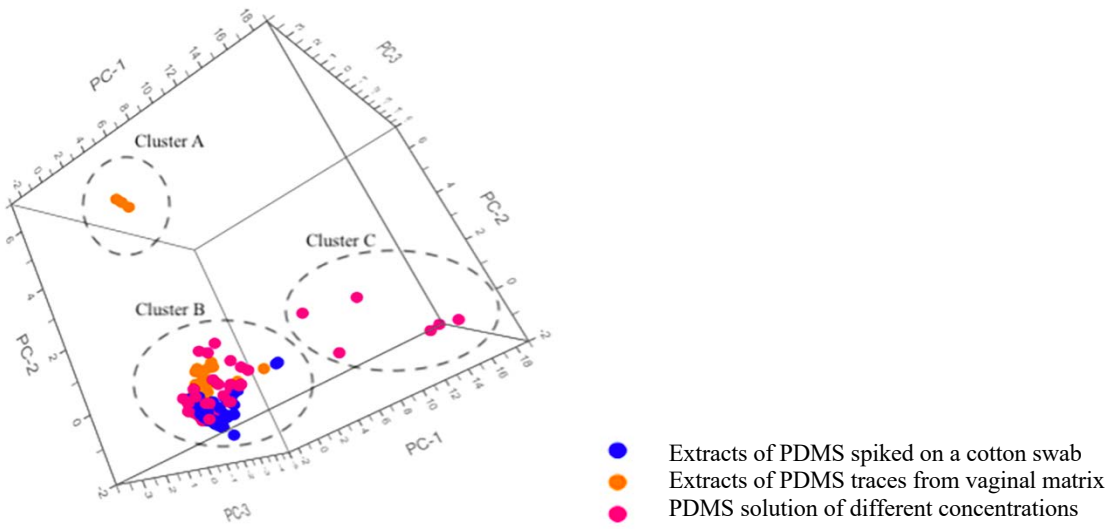


Figure 113 : 3D-Scores plot obtained from the PCA on the data used to investigate matrix effect. Several clusters are outlined, but more importantly is outlined the variability of the samples. PC 1 to 3 are presented. Separation is made according to the matrix samples were extracted from.

Three clusters can be observed from Figure 113:

- Cluster A contains 3 replicates from extracts of PDMS traces in the vaginal matrix, separated from the rest of the dataset
- Cluster C contains 7 replicates from the PDMS standards
- Cluster B contains the rest of the sample analysed.

These data highlight the significant variability that can be encountered in case work. Indeed, samples from Cluster A were found to originate from a single volunteer, whereas all the rest of the receivers were found to be clustered together (Cluster B). Therefore, the separation of Cluster A from the rest of these replicates might be due to the receiver rather than to the matrix effect. Investigation of the loading's plots indicated that the separation of this cluster from the rest of the data was due to the CH_2/CH_3 content, which was previously assigned to cell membrane components and sex hormones. Variation in the concentrations of these compounds in this sample type may contribute to this clustering.

Samples constituting Cluster C were found to all come from the reference PDMS samples. Loadings plots highlighted that this cluster was separated from the rest of the samples due to its silicone content. Variations in concentration would explain such clustering. However, as previously outlined in regarding DRIFTS analysis, significant variability can be observed for this type of analysis due to the sample preparation. The particle size of the ground KBr used for the analysis might generated variable adsorption of the silicone molecules and various coating phenomena. These phenomena were reported to significantly affect the variability and thus are more likely to be the reason of the separate cluster.

Regarding Cluster B, it was not found to be possible to differentiate the samples prepared from different substrates. These observations show that hexane extraction might not be affected to the same extent as methanol extraction in terms of matrix effect, as most of the matrix is expected to be aqueous/hydrophilic, and thus not soluble in hexane. This might also reinforce that the chemical model presented in **Chapters 4, 5**, and **6** would be sufficient as an interpretation tool and that classification can be applied to trace evidence without any major concerns. The preprocessing method previously used to discriminate samples found to be unable to account for variability due to matrix effect, as samples were not projected according to their matrix in PCA scores plots. Investigation of further principal components (up to 7) did not enable further separation of the samples.

10.2.2 Donor effect – Condom

To investigate the effect of the donor, 11 donors from the initial market dataset were used. Two volunteers (receivers) were selected to have protected intercourse with at least 5 of the different condoms (donors). The volunteers self-sampled immediately after transfer (T= T0).

Given that the receiver might be affecting the results, PCA was performed separately on the data acquired for receiver 1 and receiver 2. Results are presented in Figure 114 for Receiver 1, and in Figure 115 for Receiver 2. As this section aims to observe the difference between the donors, and its consistency, data acquired for both volunteers will not be computed together. This will be presented in section 10.2.3.

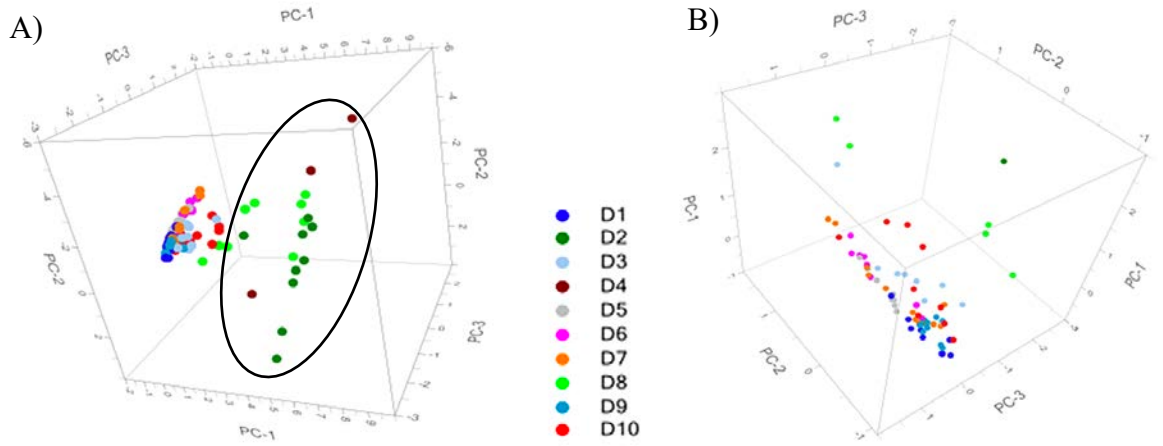


Figure 114 : 3D-Scores plot obtained from the PCA on the data used to investigate donor effect with **receiver 1**. PC 1 to 3 are presented. Samples are colourised according to donor (i.e. condom). For each donor, replicates are n=9. A) considering the whole dataset B) afer removing samples circled on Figure 111A

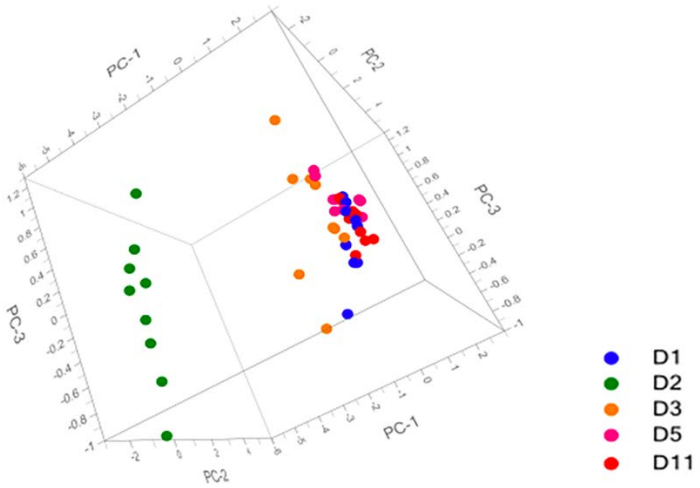


Figure 115 *:* 3D-Scores plot obtained from the Principal component analysis on the data used to investigate donor effect on **receiver 2**. PC 1-3 are presented. Separation is made according to the donor. For each donor, replicates are n=9.

Figure 114A shows the PCA plots obtained when discriminating donors according to their chemical profiles. Donors D2, D4 and D8 were found to be statistically differentiated from other donors. Visually, sample D2 and D8 were found to present a different chemical profile than the other samples, as illustrated on Figure 116.

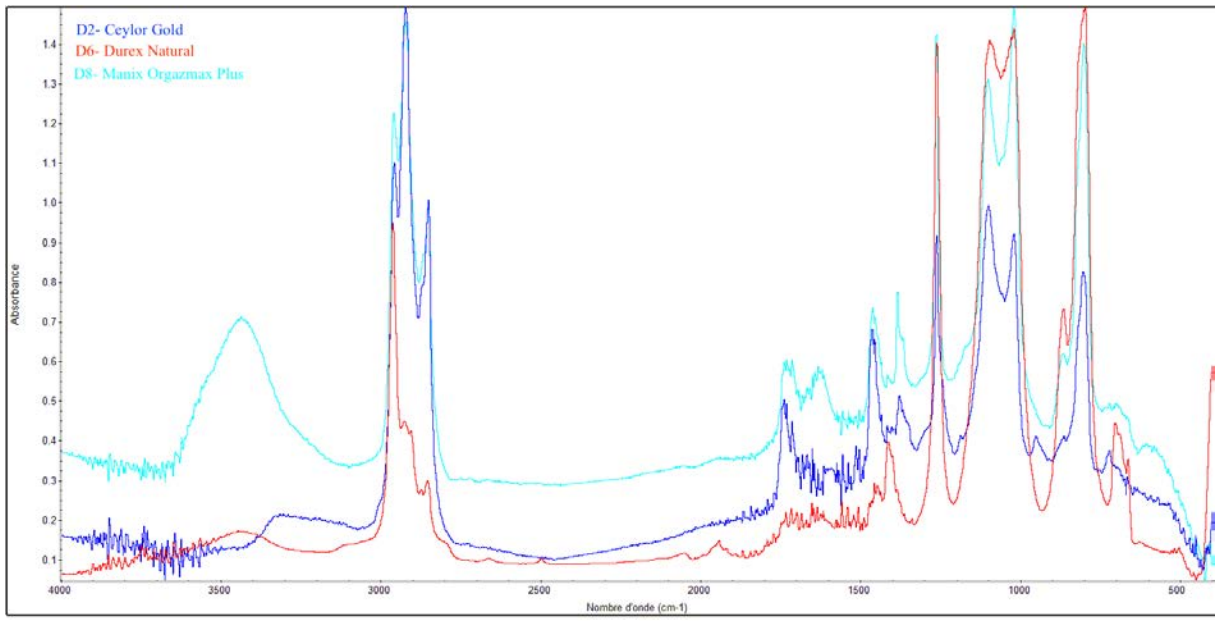


Figure 116*:* Illustration of the different DRIFTS-FTIR chemical profile obtained for Durex Natural condom (in red), Ceylor Gold condom (in dark blue) and Manix Orgazmax Plus condom (in light blue). These chemical profiles perfectly match the ones previously observed when leading the market study using ATR-FTIR.

These observations are consistent with the market survey (**Chapter 4**), as donors D2 and D8 were already found cluster separately from the rest of the condom samples. Regarding donor D4, such a classification is not surprising as it is a dry condom type, which means it is not lubricated. It looks like D4 is clustered with samples D2 and D8 and such an observation was

previously observed with FairSquared Sensitive Dry sample, which was clustered with non-silicone-based samples (see **Chapter 4**).

Further observations on Figure 114A and 115 also indicate that the composition of the body of the condom does not affect the chemical profile of the trace. This observation was already noted during the market study but is now also confirmed to be the case for trace evidence.

As donors D2, D8 and D4 significantly impacted discrimination, these samples were removed from the dataset and PCA was rerun on the remaining samples. Figure 114B illustrates the scores plot generated from the reduced dataset. Samples from donors D10 and D3 were found to present significant variability. However, this variability can be considered normal and more likely to be linked to the analytical instrumentation or the self-sampling procedure than to other factors. The variability observed in the data is in accordance with the variability observed in the ATR dataset from the market study.

In both cases, further principal components up to PC7 were used to project the data, but none of them enabled improved separation of the samples in the dataset.

The same procedure was applied to samples collected from receiver 2. As illustrated on Figure 115, donor D2 was again found to be clearly separated from the rest of the dataset, which confirms previous observations in the market study and receiver 1. The pattern observed on Figure 115 strongly resembles the pattern observed on Figures 11 and 13 in **Chapter 4** which illustrated the various types of chemical profiles observed within the condom population. Donor D2 is known to be a Category 4 sample on Figure 11 (**Chapter 4**). All the other condoms are known to be Category 1 samples (silicone-based lubricants). Therefore, the clustering of donor D2 in Figure 115 is due to the variability within the condom samples.

Discrimination patterns are in agreement with previous observations on the discrimination of condom profiles conducted with another instrument (i.e. ATR) and applied on raw samples (i.e. non extracted condom rubbed on ATR crystal). Further investigation of principal components did result in further separation of the samples. As it has previously been highlighted in **Chapter 4**, it is interesting to investigate whether the classification of real traces is applicable to a pure lubricant sample mode. The projection of the traces samples within the model containing the overall data is presented in Figure 92.

10.2.3 Receiver effect – Woman

To investigate the effect of the receiver, the donor was fixed (here, silicone-lubricated condom), and 9 volunteers (here after receiver) were asked to self-sample post-coïtal residues.

Typical spectra obtained from real samples are presented in Figure 117. No interaction with the matrix was noted, and spectra presented clear and well-defined Si-O-Si and Si-C stretching bands, thus confirming the efficacy of DRIFTS analysis for case work. PDMS was detected in all the swabs that were collected right after coitus. From a qualitative point of view, abundances were found to vary between different volunteers but also within the same volunteer (Figure 117). Such variation is likely to happen due to self-sampling or parameters linked to the contact itself and cannot be reasonably monitored.

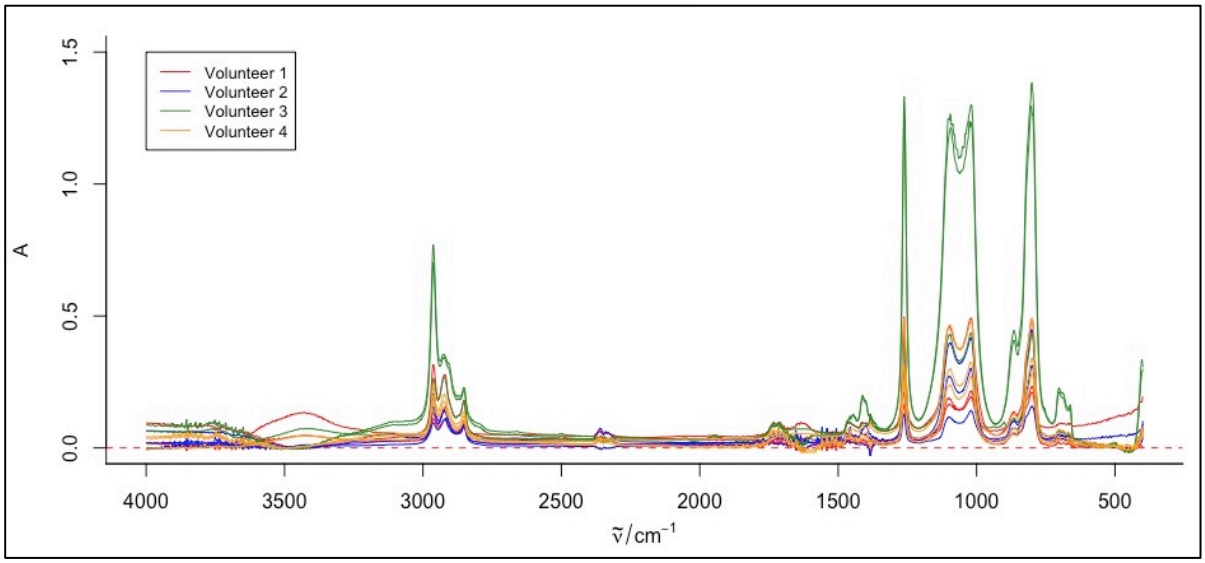


Figure 117 : Infrared spectra of post-coïtal transferred residues from 4 different volunteers. For each volunteer, the 3 replicates acquired are presented. Reproduced from *Burnier C Van Bronswijk W., Massonnet G. (2020) Comparison of spectroscopic methods in detection of silicone-based condom lubricants evidence, Analytical Methods*

The PCA scores plot shown in Figure 118 contains two main clusters, one consisting of replicates from receiver R7, the second containing the rest of the samples. Investigation of PCs 4 to 7 did not produce any further enhancement of sample clustering.

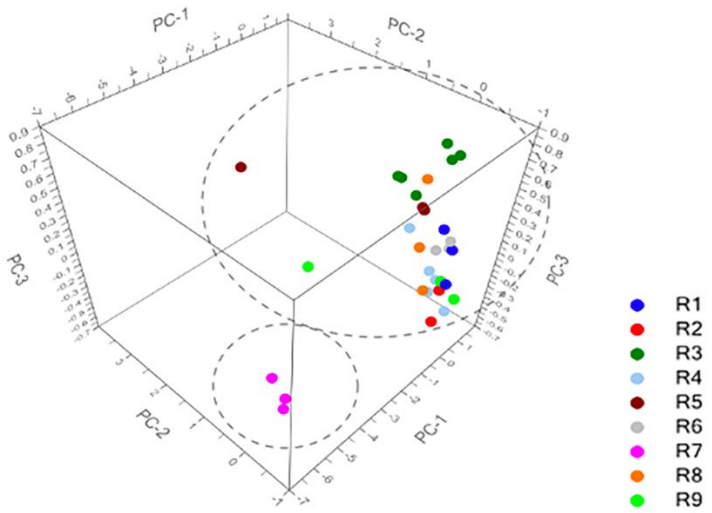


Figure 118: 3D-Scores plot obtained from the Principal component analysis on the data used to investigate receiver effect. PC 1-3 are presented. Separation is made as a function of the receiver. Transfer was generated with silicone-lubricated condom (Ceylor Blue).

Such a strong separation of the samples can originate from variation in self-sampling procedure or from the analytical instrumentation. Samples coming from receivers R7, R8 and R9 were run on the same analysis day, which means it is unlikely that the variability of the sample preparation would be the main reason for R7 to be separated from the rest of the samples. Observation of the spectra obtained from R7 compared to the rest of the dataset coupled to the analysis of the loading plots obtained from the PCA computation allowed to note that the difference of abundance between the two bands coming from the Si-O doublet (Figure 119) was the source of the variation.

Although Figure 118 shows no significant differences between the volunteers when it comes to the relative abundance of the Si-O doublet band, similar difference of abundance was observed on Figure 111, with pure PDMS samples analysed after dilution in hexane. Variation of concentration might be the most likely hypothesis to explain the variation. A difference in sample preparation or sampling cannot be left aside but given that the rest of the samples are clustered together, it is highly unlikely that these would be the major reason of the separation.

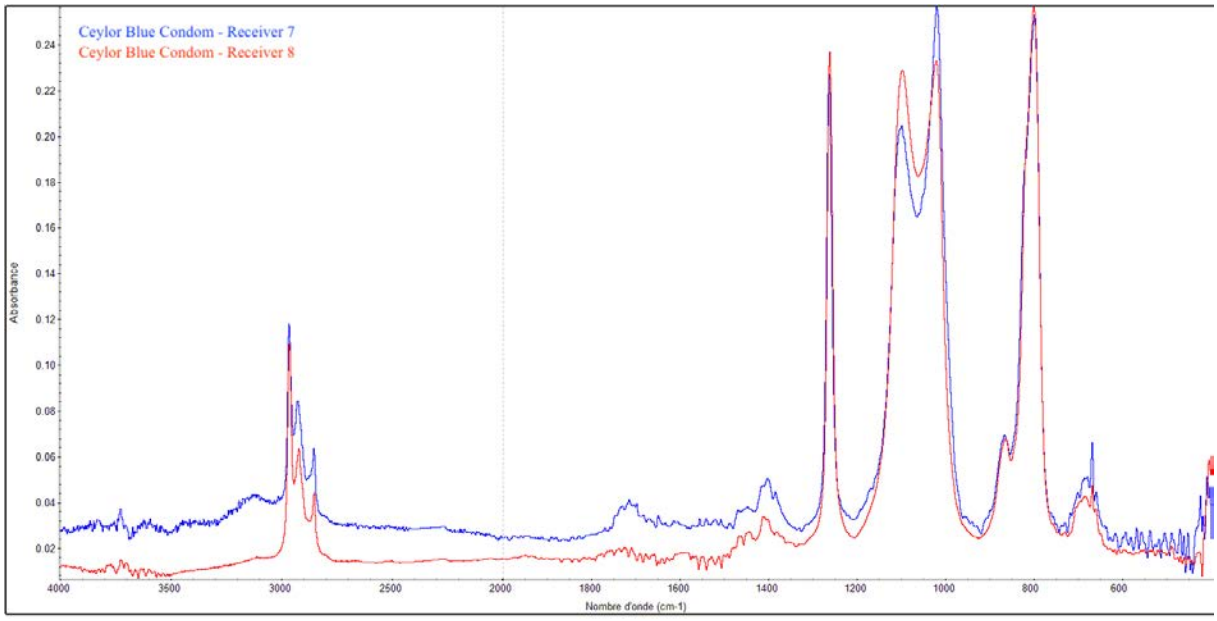


Figure 119: Illustration of the difference between Receiver 7 and 8, samples collected at T = T0

As receiver R7 was responsible for the major clustering, the corresponding samples were removed from the dataset and PCA was rerun on the remaining samples. Figure 120 illustrates the results of the new PCA. Receiver 3 was found to be clustered separately from the rest of the samples. Other samples were found to cluster together. Spectra were found to be visually similar, with no distinguishable features that could be used for sample separation according to the receiver. As the algorithm is able to detect a significative difference between spectra coming from Receiver 3 and the rest of the receivers, multiple hypothesis can be drawn, the main ones being:

1. Self-sampling was stronger or lower than for the other volunteers, which would lead to a difference of concentration, that would not be visually detectable, but the algorithm would be able to detect.
2. Interactions between the matrix and the lubricants have generated modifications. Although these interactions have not really been reported and investigated and knowing that PDMS is highly nonpolar and not supposed to be reactive to most of the microbiota, this hypothesis is not very likely.
3. The matrix has overcome some important changes at the time of the experiments, which explains the separation. Given the information obtained from the volunteer, there is no way to confirm that statement.

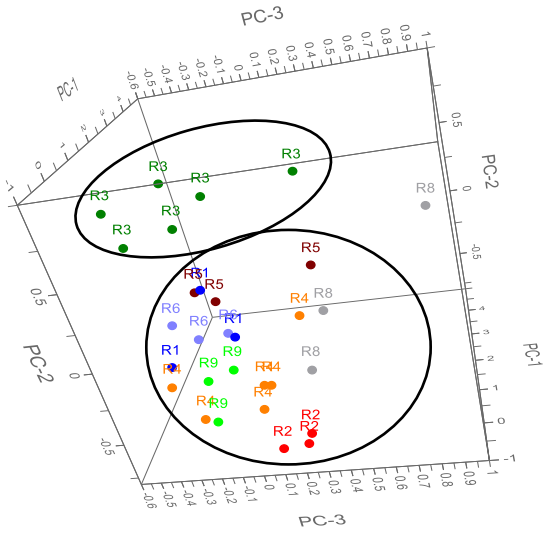


Figure 120: 3D-Scores plot obtained from the Principal component analysis on the data used to investigate receiver effect. Receiver 7 removed from dataset. PC 1-3 are presented. Transfer was generated with silicone-lubricated condom (Ceylor Blue and Manix Skyn).

The variation in the dataset is consistent with variability within the donors. However, given previous observations on a larger sample set, it is more likely that the present observations would be better explained by a hypothesis other than inter-donor variation. The self-sampling procedure introduces significant variability within the data. Therefore, although most of the data were found to cluster together independently of the receiver, the strong variation observed is more likely to be due to uncontrolled factors.

10.3 Discussion

The transfer of traces of condoms analyzed by DRIFTS-FTIR were evaluated using different visual and statistical tools. However, the data collected within the framework of this study were significantly influenced by uncontrolled factors, such as the sampling, carried out in this work in the form of self-sampling. Similarly, to the previous attempt to realize a quantification procedure using DRIFTS analysis (**Chapter 8**), the data acquired presented a strong variability, which makes it difficult to know if the variation is due to human effect or if it comes from instrumental effects. DRIFTS-FTIR was also previously found to be a very good screening technique but was not found to be accurate nor reliable for quantitative purposes, which is why a purely qualitative and semi-quantitative approach was adopted in this chapter.

The experiments described illustrate the potential to distinguish condoms in a similar way to what was observed during the construction of the infrared profile database. The effect of the matrix after extraction of the traces of the cotton support proved to be practically nonexistent,

the diluted silicone standards, and spiked cotton having statistically indistinguishable profiles from the samples from a living matrix.

The results obtained are no less interesting, but it is clear that additional studies must be carried out in order to assess the impact of the various influence factors and whether it is possible to construct a more relevant statistical model. The estimation of the persistence of known traces (by blind analysis) must also be carried out in order to test the practical applicability and the reliability of such models. This is to select the most suitable type of model. Finally, validation procedures, both instrumental and statistical, are vital prior to practical application.

Highlights

This chapter investigated transfer of condom evidence using DRIFTS-FTIR. The main results are the following:

- Discrimination pattern from the condoms are not affected by matrix residues and is not distinguishable from the ones obtained on reference material;
- The donor effect is more important than the receiver effect;
- Matrix residues are not detected in transferred extracts;
- Vaginal matrix do affect the discrimination of chemical profile collected;
- Self-sampling significantly affects the variability of the sample;
- The use of a physical model before application to simulated real samples would be advisable, so as to be able to control more factors.

Chapter 11: A preliminary investigation of parameters assisting forensic interpretation of the evidence – II: Persistence study

This chapter is based on the following article (Appendix I):

Article 3: Burnier C., Van Bronswijk W., Massonnet G. (2020) *Comparison of spectroscopic methods in detection of silicone-based condom lubricants evidence,* Analytical Methods, Vol. 12.

Article 13: Fischer J., Saric N., Fabien L., Hermelin A., Massonnet G., Burnier C., (2021) *Persistence of condom silicone-lubricants in the vaginal matrix in sexual intercourses,* manuscript submitted to Forensic Science International

This chapter details a preliminary study into the parameters of persistence, by exploiting data acquired with the optimized infrared technique (**Chapter 8**). The importance of persistence has been highlighted in many casework studies (**Chapter 7**), where the interpretation of the evidence was complex, due to a very long elapsed time between the aggression and the collection of the evidence. Concerning the interpretation of the results, more specifically negative results, it has been shown that trace detection can be affected by persistence and that information about time and activity of the victim is often missing when samples are collected by doctors before the forensic process. Elapsed time is one of the key parameter influencing the detection of a trace. It is important that the forensic scientist is aware of that information, in order to be able to assess the persistence of the traces. These parameters have already been reported, although not exhaustively, by New Zealand researchers using py-GC/MS, and in view of the importance of the inter and intra-donor variation observed in volunteers of the background study (**Chapter 9**) and of the transfer study (**Chapter 10**), it was found necessary to investigate the different factors affecting the persistence, as illustrated in **Chapter 2**.

11.1 Material and methods

11.1.1 Chemicals

Hexane of analytical grade and PDMS 200cSt were purchased from Sigma-Aldrich. PDMS was diluted at a concentration of 1mg/mL and was used as a control of the instrumentation along the entire analysis. KBr powder was purchased from Acros Organics.

Regarding human sample collection, a self-sampling procedure was selected, as it is less invasive. COPAN 150C cotton swabs (Copan Inc., USA) were distributed in the kits, as well

as Ceylor Blue or Manix Skyn condoms. A 5µl syringe eVol XR ® from SGE Analytical Science (Australia) was used for all the dilutions and spiking realized in this study.

11.1.2 Sample Collection

To investigate the persistence, 9 volunteers aged between 18 and 35 years old, each provided 4 swabs, which were analyzed in triplicates. Condom used for the purpose of this study were Ceylor Blue condom (classic condom), and Manix Skyn as latex free condom for the volunteers who asked. Volunteers were asked to self-sample 2 cotton swabs from their vaginal matrix before protected sexual intercourse, and 2 cotton swabs after the intercourse, at different post-coïtal time (up to 36 hours). Not all the volunteers completed the entire procedure. Table 43 resumes the available material for the study. The swabs were not collected sequentially from one event, but from 5 different events, so that swabbing a first time wouldn't influence the next one. One volunteer did more experiments and collected swabs at T6h, T12h, T18h, T24h and T36h. These were obtained from one event as collecting at such times is relatively constraining for the participants. To avoid wasting material and being able to confirm analysis if needed, only half of the swabs were extracted and analysed.

Table 43 : Available material for the study

	T0	T0.5h	T1h	T2h	T4h
Returned kits	9	8	5	7	4
Number of swabs (blank and post-intercourse)	36	32	20	28	16
Swabs used (blank and post-intercourse)	18	16	5	7	4
Number of analysis (post-intercourse)	27	24	15	21	12

Volunteers were asked not to collect samples during menstruation. Information was collected regarding the date of their last menstruation, the sampling date and the hour and if they had had intercourse in the week before sampling. If they had intercourse the week before the sampling, they were asked to report when the intercourse was protected, and when it last happened. To avoid any cross-contamination, the volunteers were asked to wait one week between each protected intercourse.

Sample collection was led in accordance with the Swiss Federal Act on Research involving Human Beings (Ordinance on Human Research with the Exception of Clinical Trials) and approved by Swissethics (2018-00690). All the volunteers gave their informed consent, and collected data were fully anonymized.

11.1.3 Extraction and Sample Analysis

The sample extraction and analysis procedure used in this study follows the method developed by Burnier et al. [76]. Cotton swabs were separated from the wooden sticks, deposited in a glass vial and soaked into 1mL hexane. Vials were then vortexed for 1 min and sonicated for 15 min.

KBr was manually grinded before being deposited in sample cups. Cups were dried at 100° C for 15 minutes. 10µl of extract solutions were spiked on the pellets and put back in the oven for 15 minutes at 100° C. A control pellet was spiked with 10µl PDMS solution, a solvent blank pellet was spiked with 10µl pure hexane and the other unused pellets were kept as instrumental blanks. Analysis parameters are the ones presented in **Chapter 8**.

11.2 Results

11.2.1 Short term persistence – Time interval: 0-4h post coïtus

Following the transfer study, the persistence of the traces of 8 donors was investigated over an initial post-coital interval ranging from 0 to 4 hours. This interval was first selected to see how silicone-based lubricants were behaving within the vaginal matrix. The time was then extended to 36 hours, as discussed in section 11.3.2.

In order to ensure that the trace observed came from a single transfer, blanks of the vaginal matrix were collected before the transfer. None of the swabs contained any trace of silicone, thus attesting that the silicone residues, if detected, would indeed come from contact generated with the donor condom. Persistence modeling was undertaken using different statistical tools (PCA and PLSR) in order to assess the possibility of developing a method that allows the "dating" of the trace as a function of the abundance of peaks observed. The procedure was inspired from [174,183–185] who illustrated that PCA and PLSR were valuable tool to separate samples from various concentration.

Persistence was first investigated by visual inspection of the spectra. A variation in abundance was observed between the different volunteers, similar to the one observed within the transfer study (Figure 117), which confirms an impact of the sampling method, given the use of self-sampling technique.

Silicone lubricants were detected in all the analysed samples between T0 and T4h after the protected intercourse. All the blanks were found to be blanks, thus making the results of the persistence relevant. Visual inspection of the spectra obtained from a same donor for the whole interval revealed a decrease of the abundance on this time interval (Figure 121). As observed

in other type of evidence, a massive decrease of the concentration is observed within the first hours. In this case, a significant loss in the intensity was observed: at T0 the Si-O-Si doublet presented an absorbance of ~ 0.28, which drops down to an absorbance of ~0.1 after 1 hour, and ~0.05 after 2 hours (Figure 121). Note that intensity varies at T0 as a function of the contact, the volunteer and the sampling itself, which generates variations in the absorbance as illustrated in Figure 117.

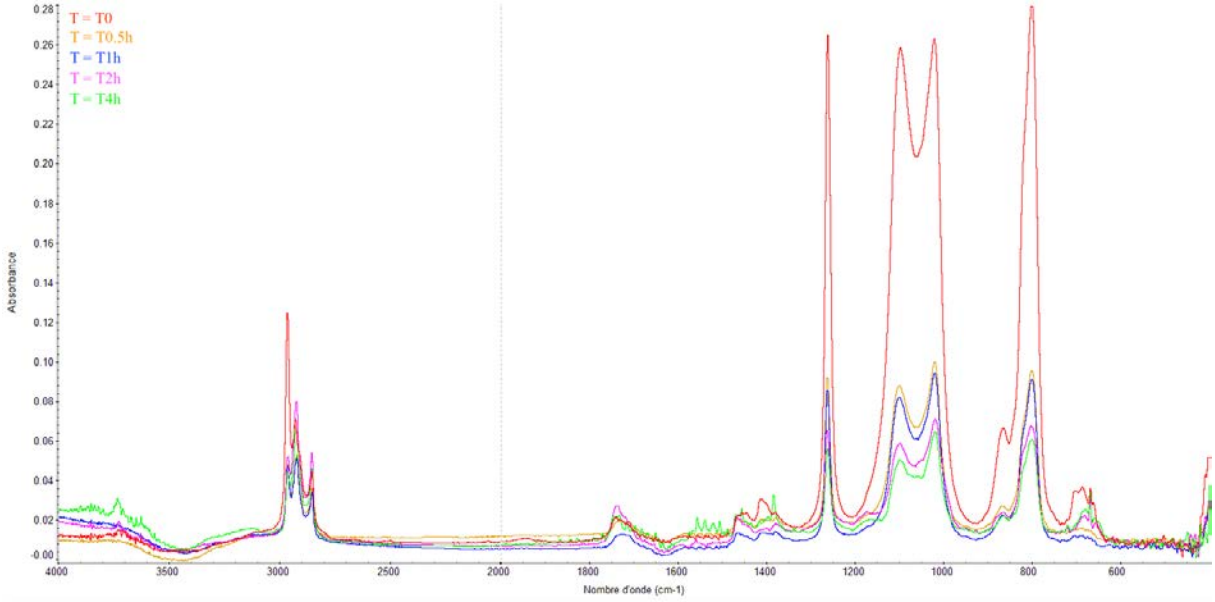


Figure 121: Illustration of the persistence of silicone-lubricant for one volunteer, between T0 and T4h after intercourse. Only one replicate is represented for matter of readability.

Then, principal component analyses were performed on the spectral areas selected in the background study. It was thus noted that the samples obtained despite contact with the vaginal matrices were all projected closely and could not be differentiated according to post-coital time (Figure 122). Although visual observation of the spectra made it possible to note differences, the statistical analysis shows that it is not possible to determine the time interval within the limits selected here. At this stage, it can be concluded that silicone compounds are indeed present up to 4 hours after protected intercourse, in variable concentrations, and that it is not possible to infer on the recency of the intercourse.

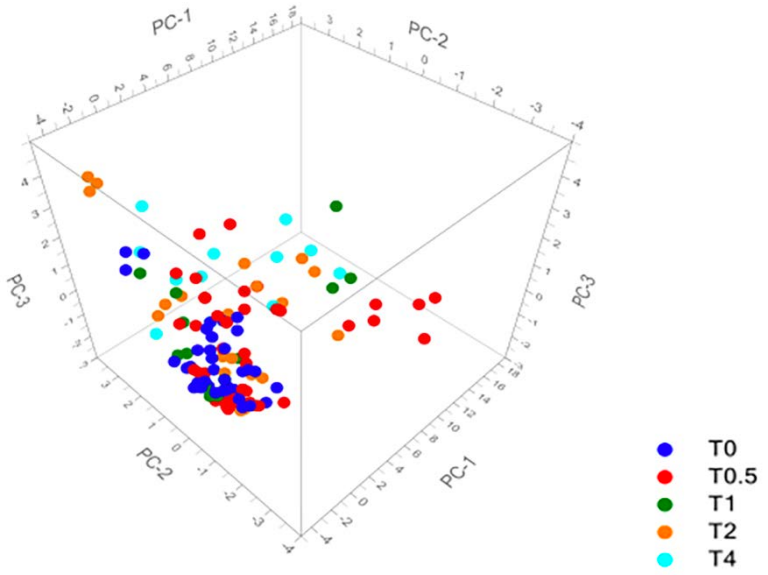


Figure 122: 3-dimensional scores plots of the FTIR spectra obtained from traces collected at various time after sexual intercourse, along PC 1, 2 and 3. Samples are classified according the post-coital time, independently from the volunteer.

Significant variation was observed, however, knowing that the samples were observed independently of the volunteer who was the source of the samples. Such variability, notably at T0, was observed before in this chapter and was attributed to variation from the volunteer. The samples were therefore represented according to the volunteer in Figure 123.

The persistence related PCA revealed the same information as that previously obtained in the transfer study, namely that, most samples are grouped independently of the parameters inherent to the different volunteers, and that no cluster is visible. While no post coital interval group was clearly distinguished from the others, the variations observed are also not explained by the volunteer. Observation of the loadings plot highlights two areas of interest in the discrimination of the samples obtained: the area related to the Si-O doublet from the silicones, and the area relating to the presence of CH_2-CH_3 groups in the molecule, area which was shown to be representative in particular of the presence of lipids and proteins in the vaginal matrix. Considering the self-sampling procedure, it is more than likely that the variation observed amongst the collected data are due to sampling variations, which mask the variation from the matrix activity. Indeed, literature reports that the vaginal matrix, following contact with external material, tends to get excrete it using an “autowash” system [45].

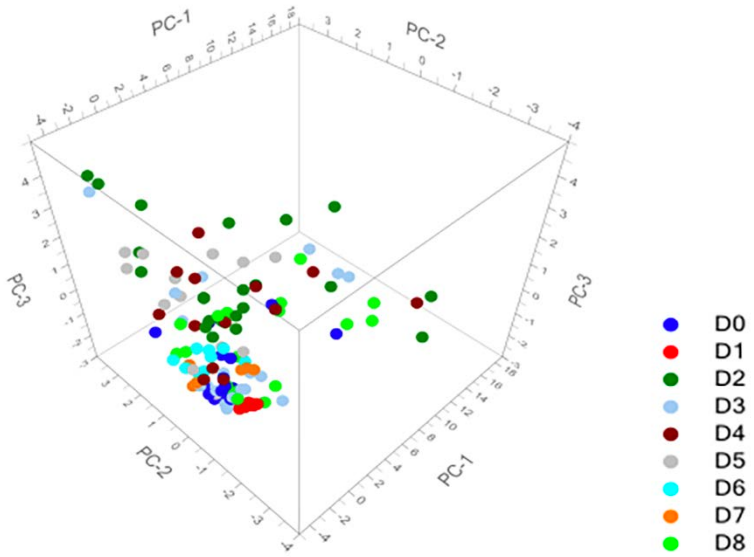


Figure 123: 3-dimensional scores plots of the FTIR spectra obtained from traces collected at various time after sexual intercourse, along PC 1, 2 and 3. Samples are classified according the volunteer, independently from the post-coital time.

In addition, persistence was also studied using PLS regressions on the 0-4h post-coital domain. As mentioned earlier, evaluating the persistence of a trace is important for successfully interpreting the observed trace, which can be modeled based on a set of comparison traces obtained under controlled conditions. However, the models created must be particularly robust regarding a certain number of unknown factors such as, for example, the parameters linked to the contact itself, or to the sampling technique. This is why, following this reflection, a PLSR model was first built using the dataset (Figure 124). The observations highlight significant variability and poor-quality regression. Given the variability, the model as presented is not likely to be able differentiate older traces from fresh traces.

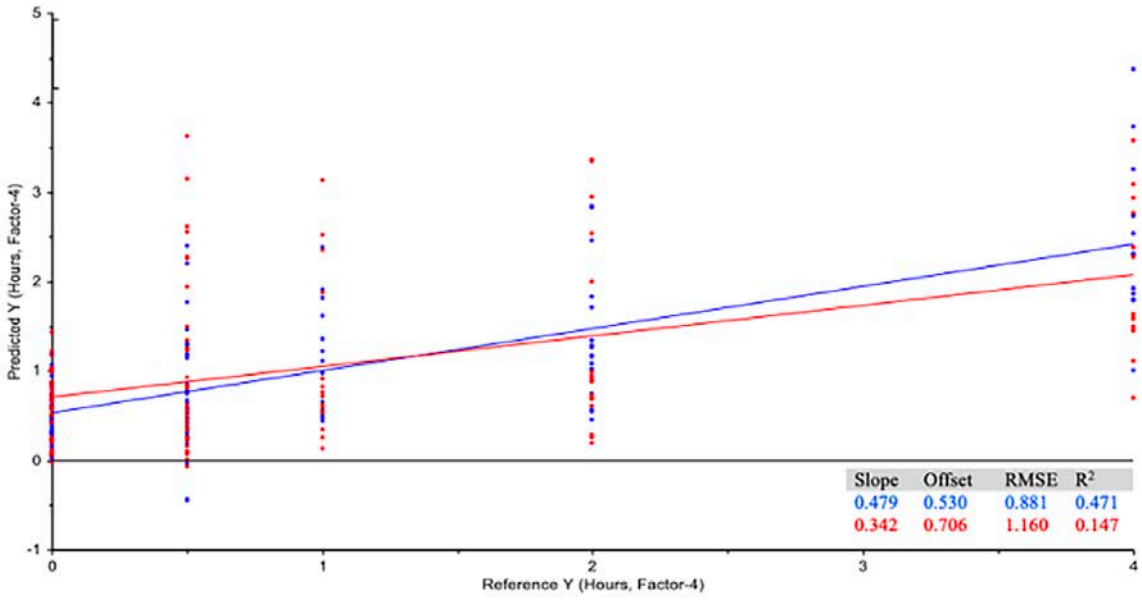


Figure 124: PLS-Regression obtained based on the overall spectra obtained from 0 to 4 hours after intercourse. Calibration (blue) and cross-validation (red) curves were calculated.

The variation observed in Figure 123 was expected as observed when overlaying all the spectra, such as in Figure 117 previously for the transfer T0, and in Figure 125 here under after 4 hours post-coïtus.

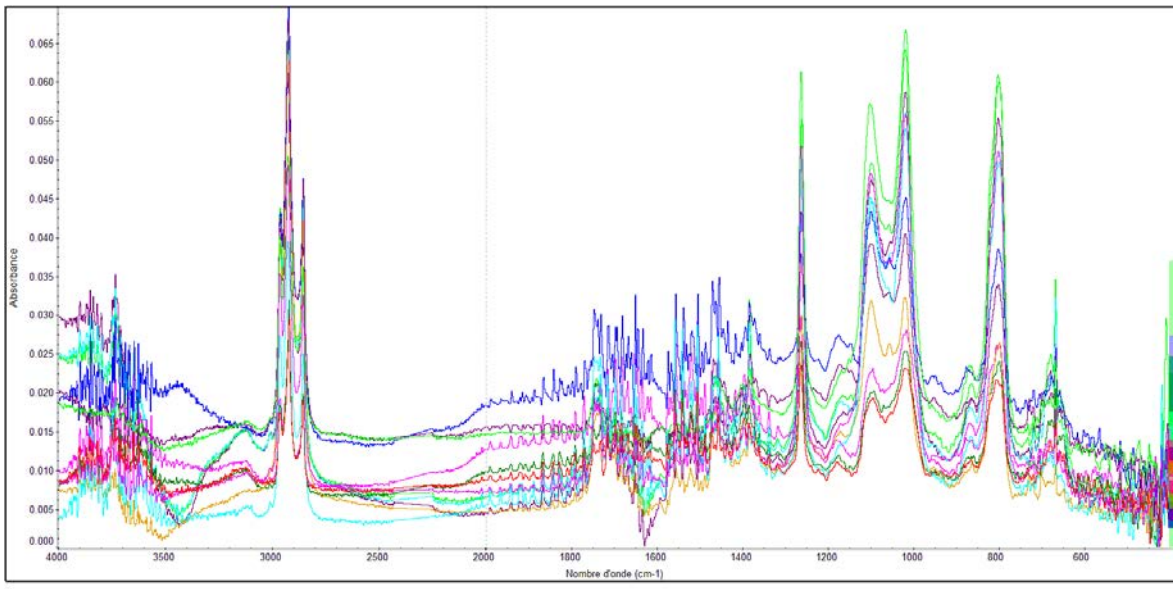


Figure 125 : Illustration of the variability of the transferred residues from the 4 different volunteers after 4 hours post-coïtus. For each volunteer, the 3 replicates acquired are presented.

PLS regression is affected by uncontrolled factors, which may be related to a particular volunteer. Given the observations previously made on all the data at the transfer level and previous conclusion regarding self-sampling issues on the observation of true variations, PLS regressions were individually performed on the data obtained from three different volunteers

(Figure 126), including all the replicas obtained for the five post-coital persistence points. For the traces obtained for each volunteer, the models obtained are rather of good quality since the quality of the regression exceeds 90% in the three cases, which is not surprising given the small number of points. The precision is variable given the great variability obtained on the data. Analytical effects can explain this variation, as shown above (see **Chapter 8**, Section 4.2.2 and 4.2.3).

Persistence was studied here by PLS regressions over a limited time range from 0 to 4 hours. In practical cases where an activity-level assessment would be necessary, the time between the assault and the sample can be greater than 4 hours, as illustrated by the scenarios obtained in real cases (See **Chapter 7**, section 7.2 and 7.3). Therefore, time ranged was extended up to 36 hours and results are presented in the next section.

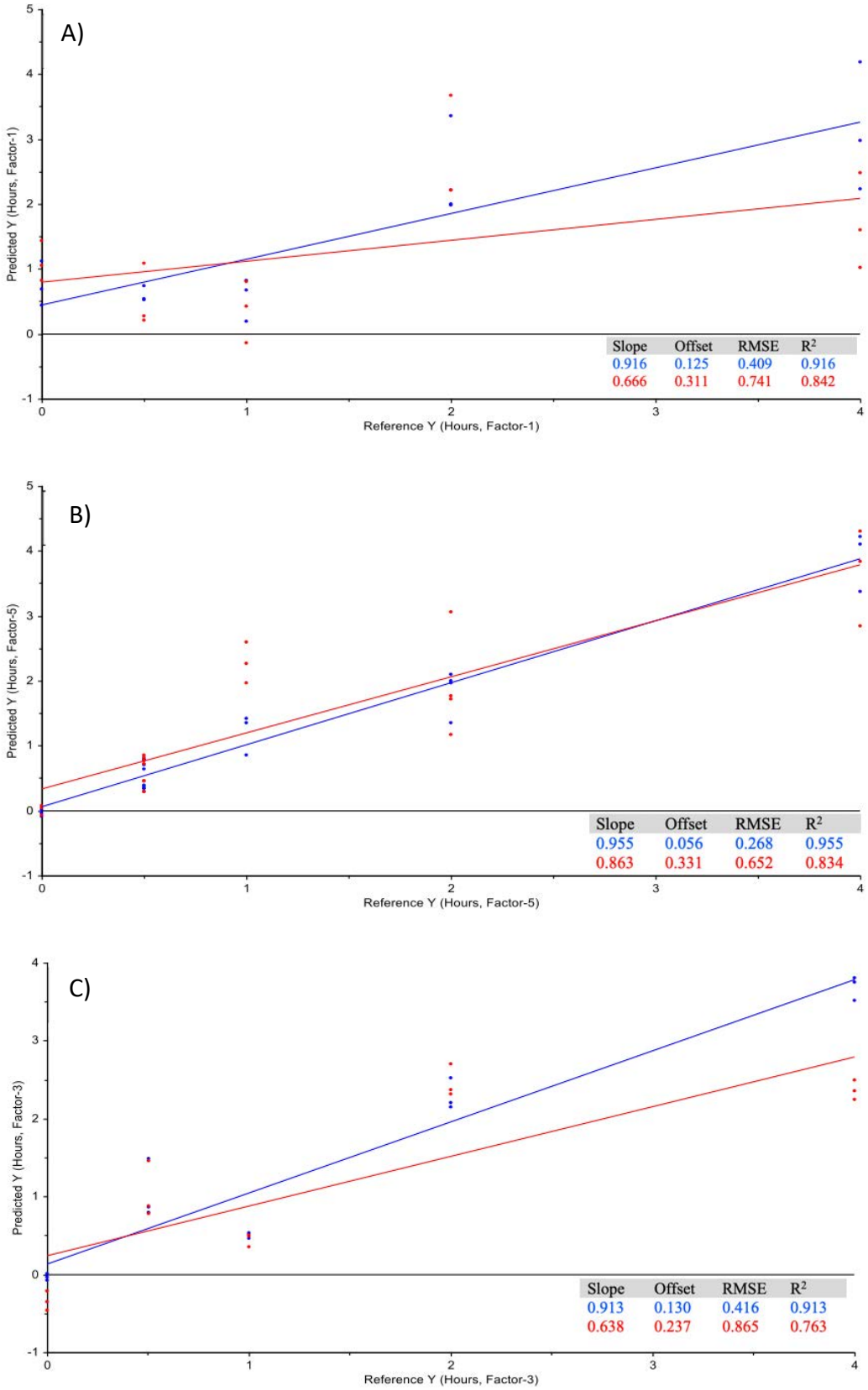


Figure 126: PLS Regression obtained based on spectra acquired between 0- and 4-hours post coïtus. A, B and C come from three different volunteers. Calibration (blue) and cross-validation (red) curves were calculated.

11.2.2 Long term persistence – Time interval: 0 to 36 hours post-coïtus

While the models have been shown to detect traces on a similar substrate (known factor), their performance is not particularly robust against unknown factors, such as the sample. It was found interesting to see at which moment the limit of detection was triggered when it comes to real samples. Subsequently, traces collected from a single volunteer between 0 to 36 hours after a protected intercourse were analysed and data are presented here after.

Visual observation of the spectra collected at different postcoital time intervals between 6 and 24 hours, are presented on their own scale on Figure 127 to highlight the difference with the matrix residues, and on the same scale on Figure 128 to illustrate the loss over time. The matrix was not found to significantly affected at 6 hours. However, with the significant decrease with time (12 and 18 hours), the matrix effect could be observed, with peaks from the matrix being almost as intense as the ones coming from PDMS. After 24 hours, PDMS peaks were not consistently detected in the spectra, with sometimes as a small trace of the 800 cm^{-1} peak visible, and a spectrum dominated by the matrix profile.

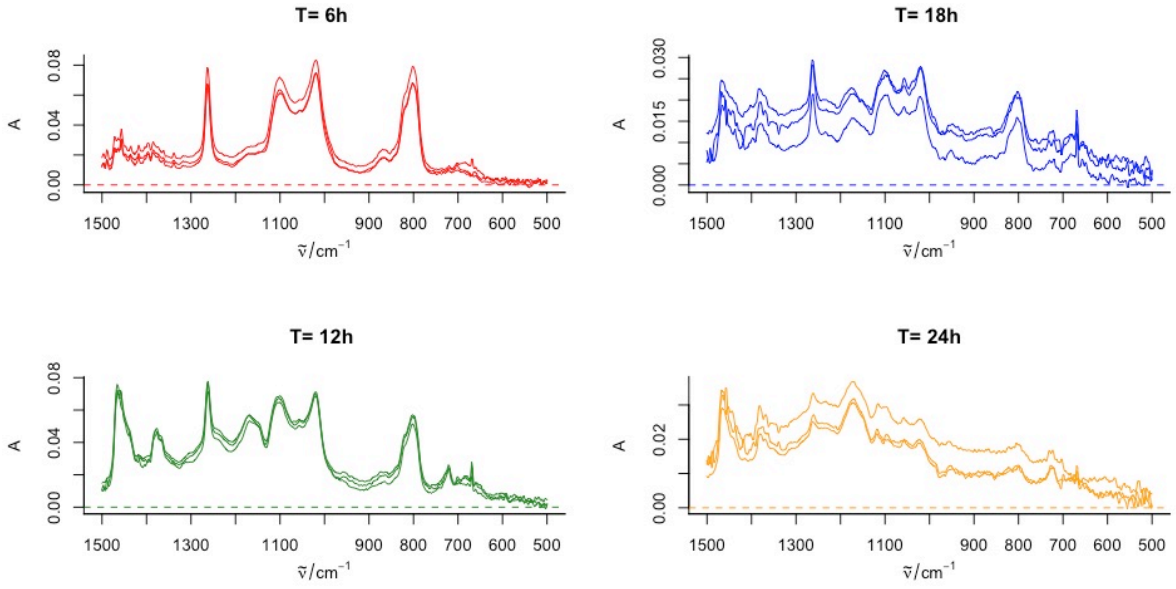


Figure 127: Infrared spectra of post-coïtal transferred residues after 6, 12, 18 and 24 hours, between 500 and 1500cm^{-1}. Spectra are represented on their own scale. Reproduced from *Burnier C Van Bronswijk W., Massonnet G. (2020) Comparison of spectroscopic methods in detection of silicone-based condom lubricants evidence, Analytical Methods*

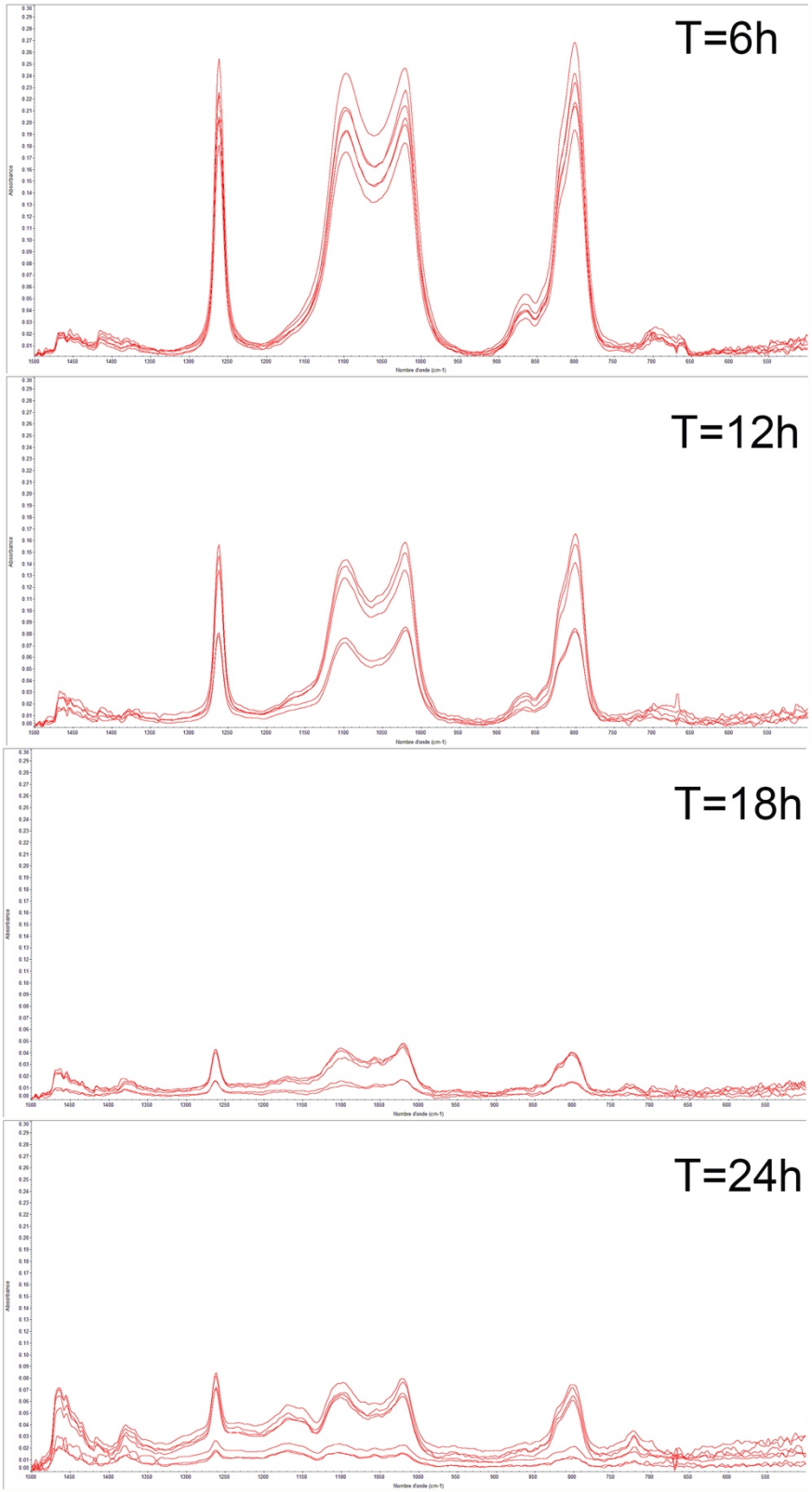


Figure 128 *:* Infrared spectra of post-coïtal transferred residues after 6, 12, 18 and 24 hours, between 500 and 1500cm^{-1}. Results are presented on the same scale. Reproduced from *Burnier C Van Bronswijk W., Massonnet G. (2020) Comparison of spectroscopic methods in detection of silicone-based condom lubricants evidence, Analytical Methods*

When reaching 36h post-coïtal time, spectra were found to be similar to the ones obtained for the background (Figure 129), with no apparent peaks coming from the silicone lubricant. Therefore, the results show that after 18 hours, silicone lubricants can be detected without any

major issues. Within a 24 hours' time interval, repeatability is significantly affected, with half of the spectra not presenting any of the characteristic bands of the silicone lubricants. Over that time, the present results did not allow the detection of silicone lubricants.

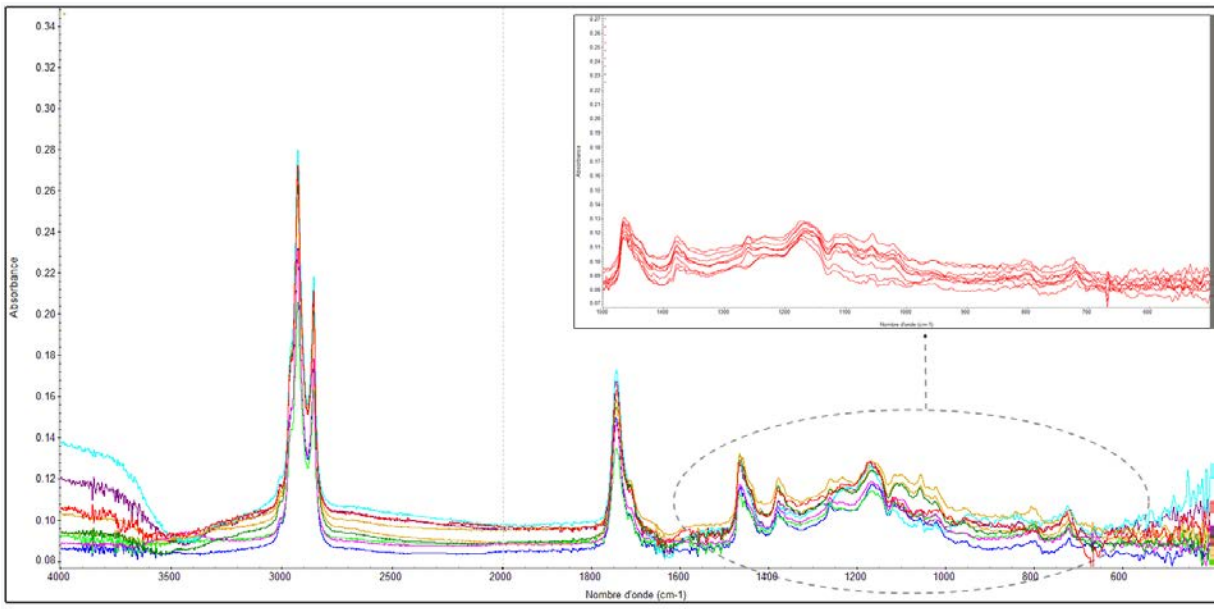


Figure 129: Infrared spectra of post-coïtal transferred residues at **36 hours** post coïtus, with a focus in the zone between 500

Principal component analyses were performed on the spectral areas selected in the background study. Data obtained are presented in Figure 130, plotted as a function of the time.

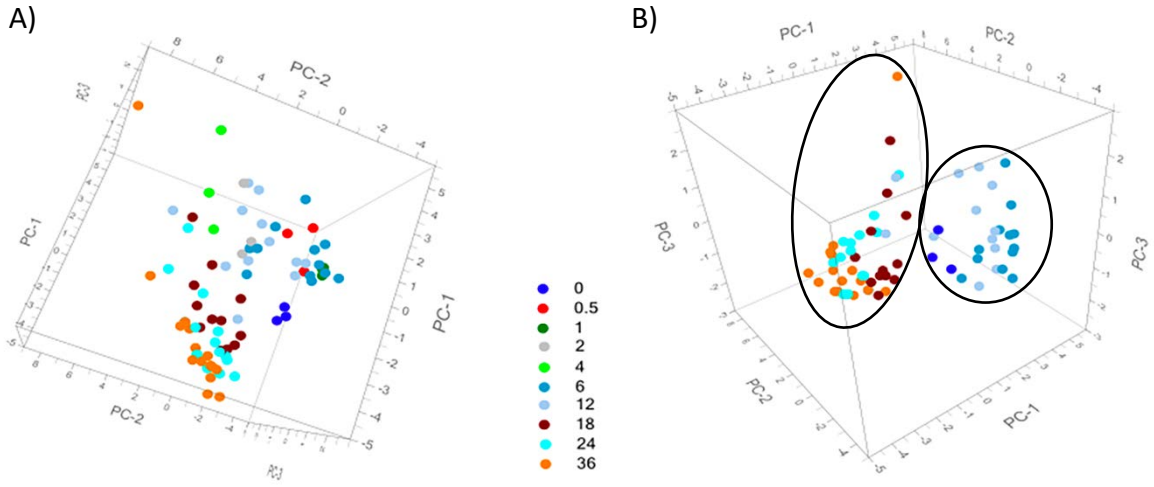


Figure 130: 3-dimensional scores plot obtained on the data collected by the same volunteer, between 0 and 36 hours after intercourse. Colors are linked to different post-coïtal time. A) all the data, B) after removing data between 0 and 6 hours

The corresponding loadings plots demonstrate that the silicone concentration is the most important separation factor according to the first two main components. Two main clusters can be observed on Figure 130B (highlighted with the black circles), one containing sample collected from 0 to 12 hours, and the second one containing samples collected after 12 hours.

The cluster obtained from data collected after 12 hours seems to be rather uniform, with a significant overlap of data acquired between 24 and 36 hours. A strong variability is also observed. As this cluster is clearly separated from the one made of samples collected up to 12 hours post-coïtus, it is likely that the algorithm is able to detect variation in concentrations, and thus in the elapsed time between intercourse and sampling time. However, it does not seem that the algorithm is powerful enough to distinguish samples that could contain small concentration variations.

The activity level of the volunteer was also considered. The volunteer reported taking a shower before collecting the T=6h samples. Given that samples were still found to cluster closely to T=0h samples, this activity, in this specific case, was not found to affect the traces. The volunteer reported no activity (i.e. lie down or sit) after T=6h, up to T=18h, except going to the toilets, which might be the source of the variation observed for samples collected at T=12h. Collection of evidence at T=18h was done early morning. Considering almost no activity from the volunteer, and that the activity that would be likely to significantly affect the evidence (i.e. showering) was not found to have any impact on traces, the observed variations cannot be linked to a given activity, and it makes it more likely that the pattern observed would be due to a matric effect.

Regarding PC3, the bands linked to the vaginal matrix appeared to be those which generate discrimination. A matrix effect is therefore not negligible and may explain why the samples at 36 hours are well separated from the fresh samples or obtained at 12 hours post-coitus. Although previous observations in **Chapter 9** helped understanding that vaginal matrix were not affecting separation of samples, this was only observed on immediate post-coïtal sampling. A longer post-coïtal time might generate uncontrolled interactions which could be the source of such variations. However, this is not known and has never been referenced. The variability observed on the data is very important and reminds of the calibration trials led in **Chapter 7** regarding DRIFTS-FTIR analysis, which highlighted the lack of precision of the analytical instrumentation.

Following the same procedure described previously, a PLSR model was built from the dataset using the replicates (Figure 131). The obtained model was not satisfactory since the quality of the regression does not exceed 90%. Significant deviation was observed for each of the data point, and precision of the model varies between 0 and 36 hours, especially for samples collected after 12 hours and 36 hours. These models appear able to distinguish between the oldest (24-36h) and the newest (0-4h) traces. The precision is reduced when considering traces between 12 and 24 hours. PLS Regression does not seem to be the most relevant technique to

modelise the persistence of condom evidence when exposed to various unknown influence factors, as resulting models are not robust. Given the absence of standardization of the sample collection (i.e. self-sampling variations) and the limited sample size, small deviations from the intended protocols have large negative impacts on the ability to achieve consistent inferences. These results outline that the variability observed on condom residues collected in controlled conditions is not easy to understand. Unknown influence factors linked to the contact itself would affect the modelisation of the persistence, as well as known but non controllable influence factors, such as self-sampling. Indeed, self-sampling influence the amount of traces collected and that can be thus detected. Analytical instrumentation and its variability as well as the extraction procedure and sample preparation are likely to influence the observed results, and more specifically PCA, by creating a significant variability, which does interact with the intrinsic variability of the traces, leading to a lack of robustness for PLS models.

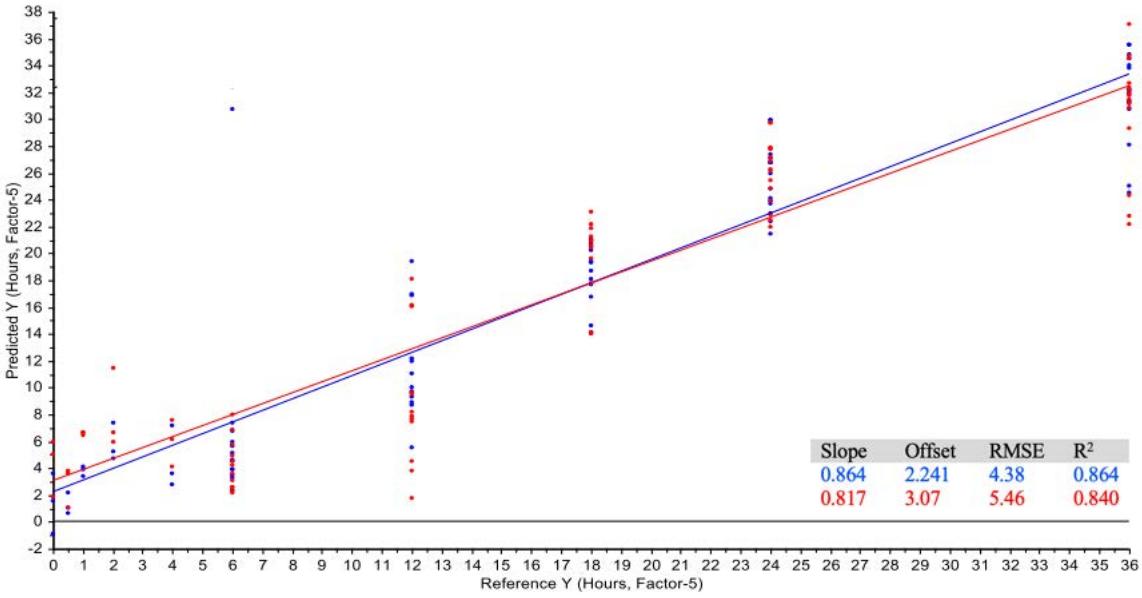


Figure 131: PLS Regression obtained based on spectra acquired between 0- and 36-hours post-coïtus. from one volunteer. Calibration (blue) and cross-validation (red) curves were calculated.

Considering that persistence is usually represented under the form of an exponential-linear regression, fitting such a regression on the data was investigated. Abundance of the peak at 1263 cm^{-1} was extracted in each spectrum at from T0 to T36. Mean and standard deviation were calculated, plotted into a graph (Figure 132) and a regression curve was fit on the data. The regression drawn presented good recovery regression, with a R^2 of 0.990, thus confirming that the persistence modelisation is following an exponential decay. Coefficient of variations calculated on the data illustrated that the bigger the elapsed time, the higher the error, with over 80% variation at 24 and 36 hours. This is also confirmed by the fact that multiple replicates and

confirmation by another experimenter were needed to assess the absence of a chemical profile in the collected spectra.

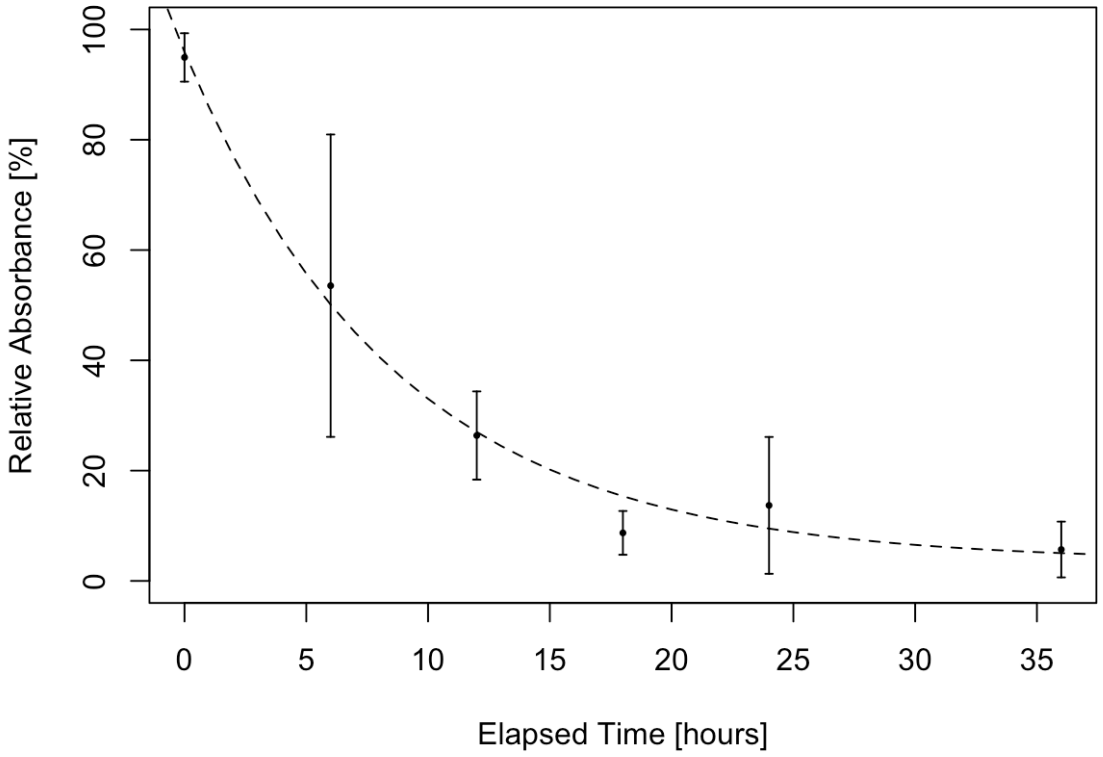


Figure 132:Exponential Linear Regression fitted on the data collected between 0 and 36 hours after intercourse. Parameters of the fitted curve are $y = 92.39e^{-0.1142x}$, with a R^2 of 0.990.

These results illustrate that even though there might be multiple unknown factors, exponential-linear regressions allow a better modelisation of the persistence than PLS regression. The regularity of such a modelisation cannot be assessed as only one volunteer participated to this experiment. However, this is encouraging for further studies.

On the basis of the data analyzed in this study, it can be inferred that the idea of a single model applicable in a homogeneous manner is perfectly inadequate and illusory. If the procedure were to be limited to the use of known factors, the use of comparison material from the identified victim would be required, but this practice would be ethically questionable. These ethical questions were already highlighted in fingermark ageing works, where authors showed out that a model would need to be built individually and replicating an environment similar to the one questioned

11.3 Computing transfer, persistence and background data

The entire dataset obtained for transfer, persistence and background study, constituted of 570 spectra, was computed together to investigate if the discrimination algorithm was able to

distinguish all the data. Results are presented on Figure 133. Statistical analysis of the data revealed two main clusters. The first one is mainly constituted of background samples as well as some transfer and persistence samples, which seem to be T36h samples. The second cluster circled in black on Figure 133A is exclusively constituted of transfer and persistence data. In order to better observe the separation of the samples and their distribution within the background clusters, the second cluster was removed and PCA analysis was rerun on the remaining data (Figure 133B). As illustrated the remaining transfer and persistence samples are clearly mixed with the background noise samples, and it is not possible to distinguish them.

The first observation coming out of Figure 133A is that samples collected at T0h were clearly separated from samples collected from the background study. This finding is confirmed by the loading of the PCA analysis (Figure 134A) which indicate that the vaginal matrix variation is responsible for the separation along PC1 and PDMS content for the separation along PC3.

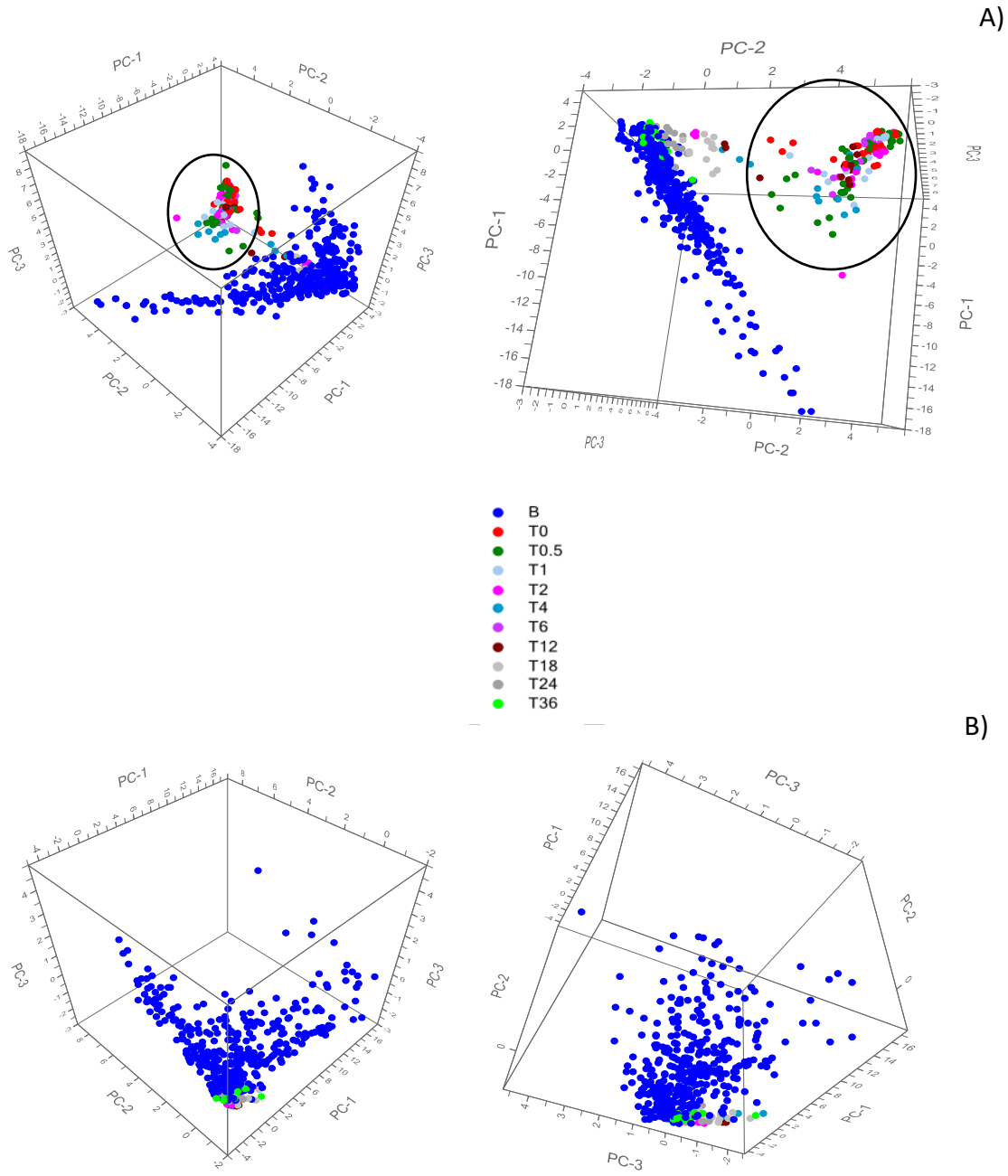


Figure 133: 3-dimensional scores plot obtained for the discrimination of 570 spectra coming from background (blue), transfer (red) and persistence; a) entire dataset plot, b) PCA plot after removing data separately clustering (black circles on figure 117a).

After studying the transfer parameter, which was found to be clearly distinguishable from the background, comes the question of the persistence data. It is interesting to note that the analysis results do not form a cluster which gradually decreases towards the background noise. Instead, three clusters are observed: one clustered with the transfer data, one clustered with the background data and one in the middle. This seems to indicate that persistence does not follow a continuous decreasing trend, but rather a spontaneous loss of PDMS after a certain period of

time, more precisely after 12 or 18 hours. By observing Figure 133A and 133B, it is possible to estimate after how many hours it is likely to find PDMS in the vaginal matrix. According to the data in graph 130A, it is still possible to make a distinction (from a statistical point of view) between the data collected between 0 and 4h post coital and the background noise. The loadings (Figure 134) also seem confirm this finding. Indeed, the separation of the clusters seems to be correlated with the presence of PDMS. As mentioned above, Figure 133B was obtained by removing the clusters distinguished from the background noise, to observe if the remaining persistence data could be separated from the background cluster. The remaining persistence data were not found to be distinguished from the background data. However, the persistence data remain relatively grouped together, which is correlated with the results of the loading (see Figure 134B), which nevertheless has detected an inverse correlation to the presence of PDMS.

Taking these observations into account, the maximum persistence time would be 18 hours, with the sensitivity of this specific instrument. It is also interesting to note that for very short persistence times (≤T4h), it is possible to observe that some of the samples analyzed are confused with the background noise, for example for samples collected at T0.5h and T4h. These observations, considering previous considerations using PLSR regression, highlight that the volunteer is a major source of variation, especially when considering self-sampling techniques. There are also many uncontrolled parameters which are likely to influence the presented results, one of the most important one being the contact itself. In conclusion, the results indicate that after intercourse with a condom, PDMS is found in the vaginal matrix immediately after intercourse. In addition, it is possible to distinguish these samples from background noise. With regard to persistence, it would seem that the traces of PDMS do not gradually decrease, but rather sudden after 12 or 18 hours. The estimated persistence time on the data collected is corroborated in other studies

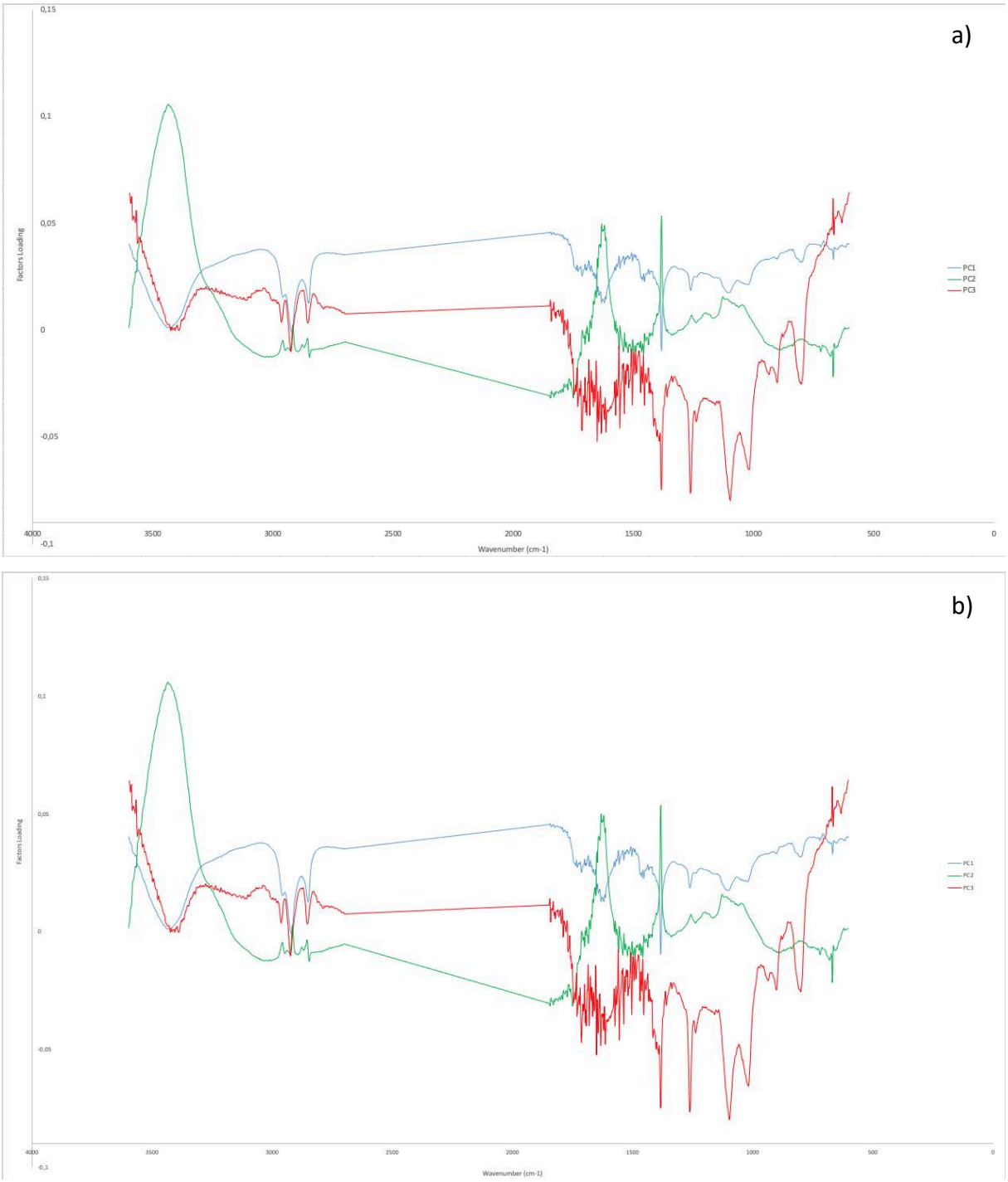


Figure 134: Loading plots obtained a) for Figure 130A and b) for Figure 130B

11.4 Discussion

The persistence of traces of condoms analyzed by DRIFTS-FTIR can be modeled by different statistical tools (PCA, PLSR). However, the data collected within the framework of this study were significantly influenced by uncontrolled factors, such as the sampling, carried out in this work in the form of self-sampling. Similarly, to the previous attempt to realize a quantification procedure using DRIFTS analysis (**Chapter 8**), the data acquired presented a strong variability,

which makes it difficult to know if the variation is due to human effect or if it comes from instrumental effects. DRIFTS-FTIR was also previously found to be a very good screening technique but was not found to be accurate nor reliable for quantitative purposes.

The experiments highlighted the current difficulties in reducing the variability of the chosen persistence parameters due to different volunteers. On the basis of the data analyzed in this study, it can be inferred that the idea of a single model applicable in a homogeneous manner is perfectly inadequate and illusory. If the procedure were to be limited to the use of known factors, the use of comparison material from the identified victim would be required, but this practice would be ethically questionable. These ethical questions were already highlighted in fingermark ageing works, where authors showed out that a model would need to be built using the suspect (if you can find them) and replicating the exact environment of the evidence [174,183–185].

PCA-based persistence models and PLS regressions provided information on the variability of the samples obtained. It was noted that the newer traces (0-4 hours), could be differentiated from older traces (24-36 hours). In the scenarios of the real cases described in **Chapter 7**, the traces were at least 12 hours old and collected up to 20 hours after the alleged assault. If the traces have persisted for up to 24 hours, the presented technique would be able to detect it. However, non-detection of the trace cannot be attributed to the absence of a protected intercourse. Several other factors, unknown or not controlled, can be the source of the non-detection of the trace. As previously mentioned, the specific post-coital activity of the victim or the use of an unlubricated condom can explain why traces may not be detected after an interval of 12-24 hours, although according to [15] , “PDMS is expected to be detected if the swabs are taken within approximately 24h (..)”. These same authors concluded their persistence study by stating that “There are many sources of variation for this type of trail, including the length of intercourse, the efficiency of self-swabbing by the volunteers, variable hygiene practices and differences in the body's natural ability to excrete and transferred lubricant”.

The results obtained are no less interesting, but it is clear that additional studies must be carried out in order to assess the impact of the various influence factors and whether it is possible to construct a more relevant statistical model. The estimation of the persistence of known traces (by blind analysis) must also be carried out in order to test the practical applicability and the reliability of such models. This is to select the most suitable type of model. Finally, validation procedures, both instrumental and statistical, are vital prior to practical application.

Highlights

This chapter investigated persistence using DRIFTS-FTIR. The main results are the following:

- Persistence parameters can be modelled using multivariate models
- Spectra did not completely correlate with post-coital interval, although a trend was observed
- The model is more influenced by uncontrolled factors such as the sampling, and the analytical variations, which masks the intrinsic variation of the sample
- Up to 18 hours post intercourse, samples can be distinguished from the background and PDMS traces can be detected.
- After 24 hours post intercourse, the reproducibility of the results are questionable and it makes it more difficult to assess whether a trace is present or not. However, PDMS traces were still detected after 24 hours in some samples.
- The use of a physical model before application to simulated real samples would be advisable, so as to be able to control more factors.

Chapter 12: General discussion

Part of this chapter is based on the following article (Appendix I) :

Article 15: Burnier C., Grisoni C., Kelly M., DeTata D, Pitts K, Hicks T., (2020) *Considerations on the interpretation of condom evidence in forensic casework,* Manuscript in redaction

12.1 Key points for further research development

In reviewing the main elements obtained during this research, as well as pre-existing publications, several key elements were identified as necessary to consider in the development of an analytical methodology for the analysis of traces of condoms and its applicability to the forensic field. These are:

- target compounds and analytical techniques
- the composition of the market
- the trace support considered – vaginal matrix - as well as its characteristics
- parameters and models related to transfer and persistence

These elements must be studied in the order given above. The first step is to choose the target compounds that are relevant from a forensic point of view as well as amenable to reproducible, reliable and statistically validated analytical techniques, before being able to study the composition of the market. The study of the market must be representative, covering not only the samples which constitute the heart of the subject but also all those which can potentially generate false positives or interact with or modify traces. At this stage, it is necessary to understand the prevalence of each component in the population, and the more frequent combination of components. Such a study thus makes it possible to determine the frequency and the constancy of a given characteristic in the population, thus providing a possibility of interpreting the evidence observed at the level of the source. Once these parameters are known, it is possible to study various parameters which are necessary for an interpretation at the activity level, but also to decide on the absence of trace, and thus to create models of transfer and persistence leading to the construction models of interpretations.

This doctoral research has made it possible to carry out a first part of this research process. Results were obtained for these key elements by targeting the study of silicone-based lubricants by two analytical techniques. The sections below discuss the results in order to draw realistic prospects for the development of further research in this area.

12.1.1 Target compounds and analytical techniques

As previously demonstrated, there are a large number of interesting compounds that make up condom residues. Within the framework of the development of an analytical and interpretative procedure and application in the forensic field, the ideal target compounds should be:

- present on the majority of condoms and differentiable from common contaminants or from other sources
- as stable as possible in a living matrix (good persistence, not affected by the matrix itself), but not too much so as not to be "permanent"
- measurable with analytical techniques usable in a practical context, which implies an additional series of criteria:
    - availability to forensic units, at reduced cost
    - differentiation between target compounds and potential contaminants
    - ability to analyze traces from various supports
    - be compatible with DNA analysis
    - simple and quick to use

These parameters were used to orient this thesis towards the study of silicone-based lubricants by FTIR (DRIFTS and ATR) and py-GC/MS. As part of a market study, GC/MS was also selected to investigate the composition of water-based lubricants. Microscopic examination was not investigated for solid particles, but considering they are on most of the condoms, this might be of great interest for further research.

Most of the research carried out in the field of condom traces conducts a two-stage analytical sequence, with an initial technique, and a second technique which confirms the observations, and allows a more in-depth interpretation of the results. Such flowcharts suggesting the use of several techniques in sequence, for the purpose of screening and then confirmation, are recommended by forensic organizations such as SWGMAT or ENFSI. These choices make it possible to quickly prioritize the samples and limit the destruction of the sample, in particular thanks to the FTIR analysis. If the FTIR analysis is negative, it can be tempting to conclude that there is no trace, and a subsequent analysis is not necessary. However, techniques such as GC/MS and py-GC/MS undoubtedly have lower detection limits than infrared spectroscopy and can detect older traces than the latter technique.

A preliminary comparison of the different modes of analysis by spectroscopy was carried out to select the most accurate technique. Both DRIFTS-FTIR and py-GC/MS were developed and optimized on reference samples, then verified on real samples, in order to minimise intra-sample variability. The initial hypothesis was that silicones with different viscosities could

exhibit different chemical profiles, and that condoms of different brands and models could be differentiated, due to the addition of other components during the manufacturing process. 200 cSt viscosity silicone was chosen, as a reference because it was the most common on the market. Standards of different viscosities (100cSt, 200cSt, 350cSt and 750cSt) were also analysed, but there were no observable differences. Confirmation and validation of the optimized analytical parameters were then carried out using two condoms of completely different brands and models.

Optimization of the analytical conditions was carried out using experimental designs, in order to understand how the various parameters influenced the analytical variability. These parameters could then be chosen so as to optimize the robustness. Considerations relating to the quantification or semi-quantification of the compounds present in the samples were also investigated. Although time-consuming, this part of the PhD research proved to be crucial, since it made it possible on the one hand to ensure that the results obtained were repeatable (and therefore exploitable), and on the other hand to understand how to interpret the data obtained.

Compared to the published literature, particularly in the field of condom trace analysis, this optimization step has proved to be innovative, in particular with the aim of obtaining the lowest variability achievable in the data collected from sample replicates. This is a crucial step in the further aim of discriminating between samples from different sources. The results of this research have been published.

12.1.2 Market survey

12.1.2.1 General observations

Infrared spectroscopy carried out on reference samples identified the region 700-1500cm^{-1} as a region of interest which contains most of the information related to silicone compounds, and thus being the most discriminant zone of the spectra. Analysis of the samples found on the market revealed several qualitative variations. The 166 samples making up the analyzed database could be classified both visually and statistically into four main categories, based on their chemical composition. 80% (132 samples out of 166) of the dataset was found to contain silicone-based compounds.

Exploratory analysis of the market structure revealed that condoms did not contain exclusively silicone-based lubricants, and that some had a profile characteristic of water-based lubricants. It was not possible to group samples according to their brand, model, lot number or the place they were purchased. No impact of the latex, polyurethane or modified latex condom body was observed. The discrimination of the samples was based mainly on their content of silicone-

based or aqueous compounds, which required developing a two-step discriminatory approach to be able to inform on the initial class samples were coming from.

Concerning the analyses carried out using py-GC/MS, the study of the initial composition of the samples highlighted patterns specific to condoms, with cyclic oligomers characteristic of the degradation of PDMS which dominated the chromatograms. Analysis were led on 70 out of 132 silicone-based samples analysed with ATR-FTIR, due to removing replicate samples (same brand, same model but different lot number). The variation of the 70 silicone-based samples was studied on 50 compounds characteristic of PDMS degradation, 10 of them being cyclic oligomers D3-D12, the other 40 being minor compounds derived from the degradation of silicone. These compounds allowed the classification of the 70 samples into four main groups, based on their composition and the variation in the abundance of the peaks observed. Chromatographic patterns specific to silicone-based condoms were also identified. These are clearly distinguishable from the chromatographic patterns of lubricants and intimate hygiene products as well as those of condoms also containing other, aqueous compounds. As the degradation of silicone is a function of the length of the siloxane backbone, examination of the different chromatographic patterns and their statistical separation led to the conclusion that the two types of PDMS (methylterminated and hydroxyterminated) are used on condoms and appeared to be specific to these products. PDMS used in lubricants or other products did not present the same chromatographic pattern.

GC/MS analysis confirmed previous hypotheses based on FTIR and py-GC/MS analysis results. 94% of the condoms contained only silicone-based lubricants, 2% contained silicone lubricants with benzocaine and polyethylene glycol traces, 1.5% contained silicone and propylene glycol lubricants and 1.5% of the condoms on the market study were non silicone-lubricated. A strong variation in the analytical composition was observed amongst samples that were not condoms. 26 chemical compounds were used to discriminate the samples, leading to the separation of the 166 samples into 12 major groups.

It should be noted that the aromas which seemed to be present, lower than 5% concentration, in certain samples were not detected by any of the analytical techniques mentioned above. Two possibilities have been considered, namely that they are volatile organic compounds (VOC) requiring a different analytical technique for its analysis (for example SPME), or that they are present in concentrations too low to be able to be detected. In all cases, it is clear that these compounds do not meet the criteria for presence in most samples or for analysis by techniques readily available to forensic laboratories. Regarding spermicides, nonoxynol-9 was detected only in one sample (*Sample 124 - Ceylor Gold condom*) out of 166. While it does not meet the

criteria for presence in most samples, knowing that its use in condoms will soon be banned in because of its carcinogenic properties, as well its association with increased HIV transmission rates, it is still an interesting indicator if detected in a sample.

12.1.2.1 Analytical comparison

This research saw the development of three analytical techniques, dedicated to being used in sequence for the analysis of silicone and water-based lubricants. However, one might reasonably question the relevance to use these methods consecutively, and what the benefits might really be. Therefore, an analytical comparison is interesting to consider on two points: the analytical side (qualitative analysis) and the statistical side (discrimination and classification). Parameters such as sensibility, selectivity and specificity are also to be discussed. The relevance of such a comparison should be handled with great care, as FTIR analysis were run directly on the samples themselves, whereas an extraction with polar (methanol) solvents for water-based compounds and with apolar (hexane) solvents for silicone-based compounds were run before any GC/MS or py-GC/MS analysis. This might affect the recovered chemical profiles.

Water-based lubricants

The two methods used for water-based lubricants are ATR-FTIR and GC/MS.

From the qualitative point of view, spectra acquired with ATR-FTIR presented relatively similar patterns, with a strong OH-bond and additional peaks in the 800-1600 cm^{-1} region, which allowed to group the samples in 5 major categories, up to 7 when considering subcategories (see **Chapter 4**). Glycerin, oils, PEG and water were found to be the major components detectable in the IR profiles. Other minor components indicated on the product label that could have been relevant for discrimination purposes were not assessable. This is due to the presence of a mixture of components, presenting similar chemical configuration, with the main bonds being vibrating in similar region of the IR spectrum and either being vibrating at the same wavenumber or not being well resolved from a more abundant peak.

On the other hand, GC/MS allowed to detect 26 different compounds in the analysed dataset. Each lubricant was presenting a slightly different chemical pattern, which allowed to visually distinguish all the products as their compositions were different. From the 7 groups observed in the ATR-FTIR qualitative groupings, up to 12 groups were created with the help of GC/MS analysis (see **Chapter 6**). The visual discrimination is therefore enhanced by GC/MS analysis

as it showed out more peaks, whereas ATR-FTIR only shows the bonds coming from the major components of the mixture.
The statistical comparison is slightly more complex as the ATR-FTIR classification models were built with the aim to discriminate silicone and water-based lubricants and then focus more specifically on silicone lubricants, whereas GC/MS classification model was specifically built for the separation of water-based lubricants. Therefore, a statistical comparison of the two methods is unrealistic as data were not acquired and processed for the same purposes. Limits of detection were not investigated on any of these instruments and thus will not be discussed here.

The relevance of GC/MS after ATR-FTIR analysis ultimately depends on the question faced by the forensic scientist. When the question is whether a silicone or water-based product was used, ATR-FTIR is adequate, as a rapid screening method. When it comes to a possible comparison question where the overall chemical profile needs to be assessed, GC/MS would be more relevant, as it allows to separate all the components of a mixture and even inform on the concentration of each of the detected compound.
Application to casework in **Chapter 7** showed the possible sequence between polar and apolar extraction procedures so as to allow both py-GC/MS and GC/MS analysis to be performed. As illustrated in **Chapter 9**, DRIFTS-FTIR was successfully used for the detection of silicone-based lubricant and **Chapter 10 and 11** illustrated the possibility to distinguish traces from the vaginal matrix. This has not been evaluated for water-based lubricants. It is therefore not possible to draw any conclusion on the relevance of GC/MS analysis after DRIFTS-FTIR analysis for water-based lubricants analysis.

Silicone-based lubricants

The two methods considered for silicone-based lubricants are ATR-FTIR and py-GC/MS. DRIFTS-FTIR analysis will also be discussed in terms of trace evidence.
From of general point of view, the qualitative analysis of the ATR-FTIR spectra acquired on silicone containing products illustrated similar vibration bands for all the products. Few products presented less resolved IR peaks which allowed to distinguish some of the samples. With the silicone-containing products, three groups were created based on the difference of the chemical profile.
Py-GC/MS highlighted that some samples that were initially clustered as water-based samples in the ATR-FTIR model were also containing silicones, as illustrated with Ceylor Gold (Sample

124) sample which was found to contain hydroxy-terminated silicone lubricant in addition to PEG and nonoxynol-9. These observations confirmed ATR-FTIR observations that condom presented at least 2 different types of chemical profile, although the groups obtained with py-GC/MS were exclusively based on the different silicone types, whereas ATR-FTIR groupings was also taking into account the water-based composition. Silicones that were not initially distinguishable using ATR-FTIR or DRIFTS-FTIR were found to present significantly different chromatographic patterns that allowed to enhance the discrimination of the samples from a visual point of view. The visual discrimination is therefore enhanced by py-GC/MS analysis.

The statistical comparison here again seems to be more complex as there has not been a specific FTIR model built exclusively for the discrimination of silicone lubricants within the silicone dataset. Therefore, a statistical comparison of the two methods is unrealistic as data were not acquired and processed for the same purposes. Both techniques were found to encounter an issue with Sample 181 (Durex Perfect Play Glide) which was a lubricant whose profile was qualitatively not distinguishable from the condom ones. In both cases, the classification algorithms were successful on the prediction of this sample. When it comes to attributing a source to one silicone-based profile, ATR-FTIR was found to offer 13.3% false positive and 6% false negative, whereas py-GC/MS model presented 5% false positive and 2% false negative, which is a significant enhancement on the prediction.

In terms of selectivity, py-GC/MS was found to be more selective as it was possible to detect silicones in samples that were initially clustered with water-based lubricants samples in ATR-FTIR. Both techniques are specific, as they inform on the molecular signature of the analysed target. However, specific is again enhanced with the use of py-GC/MS for silicone lubricants, as chemical profiles were visually distinguishable between condom and other silicone lubricants. Limits of detection were investigated with DRIFTS-FTIR as it was found to be the most relevant method for trace evidence analysis. Detection was possible up to 0.025 mg/mL with DRIFTS-FTIR, whereas py-GC/MS allowed a detection up to 0.01 mg/mL, which is a slight enhancement, but would not justify the use of py-GC/MS solely for silicone-based lubricants analysis.

Conclusion

The previous subsections have discussed the relevance of the use of 3 analytical techniques in a row for condom lubricants analysis. Advanced chromatographic techniques such as GC/MS

and py-GC/MS were found to enhance the quality of the discrimination of the samples. It is not possible to compare the applicability to trace evidence, as DRIFTS-FTIR was used on transfer and persistence data, and the chromatographic methods were used on casework, thus limiting the comparison potential.

Considering the overall picture, a framework with a screening and a confirmation method are quite frequently encountered in the forensic practice. It allows laboratories that are not equipped with some of the used published techniques to still be able to provide with some information. In the condom evidence context, the use of FTIR or chromatographic techniques depends on the initial question. FTIR can provide relevant information on the profile, and help orientating the investigation. Py-GC/MS and GC/MS would be more relevant when it comes to discrimination questions or links between material collected from the suspect (i.e. condom, lubricant bottles seized) and the questioned evidence, although this has not fully been investigated in this research. Finally, DRIFTS-FTIR was found to be a relevant method for transfer and persistence modelisation, but the use of py-GC/MS would probably enhance the quality of the modelisation.

12.1.3 Trace support – Vaginal Matrix

Several trace supports are likely to be investigated when looking for traces of condoms. While the samples received by the forensic laboratories are mostly collected from the victim (vaginal, anal, endocervical or vulvar swabs), other trace media may be encountered, such as the victim's underwear, such as illustrated in the Malkinson case [22]. This doctoral research focused on the vaginal matrix, by analogy with DNA, on the assumption that these samples would be more often encountered in forensic practice.

The spectral areas 700-1850 cm^{-1} and 2700-3600 cm^{-1} as were identified as being the most interesting based on FTIR analyses carried out on several volunteers. These regions contain the majority of the available and relevant spectral information, particularly in relation to the components of the vaginal matrix (i.e. lipids, proteins).

A py-GC/MS study of the composition of the vaginal matrix after collection on a cotton swab and extraction using hexane, was carried out and characterized 27 compounds present in vaginal matrix samples which were not detected in the products analyzed during market research, including cholesterol and its degradation products. It was not possible to classify volunteers into different groups, regardless of the technique used, based on these compounds.

12.1.4 Parameters and model related to transfer and persistence

Several samples were collected at various post-coital intervals and were analyzed using DRIFTS-FTIR in order to define relatively stable aging parameters (depending on the various influence factors), and construct of a stable and reproducible model base. A study by Tottey *et al.* [15] documents the persistence in pyrolysis-GC and the effect of various influencing factors. However, this study does not provide statistical modeling of the persistence parameters, nor does it establish a model taking into account the various influencing factors.

The selected spectral regions were transformed into absorbance units and then pretreated in order to be able to study the persistence parameters. Several pretreatments were applied, and baseline correction followed by a range normalization proved to be the most suitable pretreatment. The other pretreatments reduced the variability of the sample composition too much, and also impacted the variation related to persistence, so that no relevant model could be obtained. It is also important to note that from the start of this study, the parameters are not quantitative, and that their modeling was approached on a semi-quantitative level in view of the instruments used.

Persistence parameters were used in the construction of multivariate models, namely PCA and PLSR. The robustness of the models was assessed by testing several influencing factors, namely the donor and the recipient. Spectra did not completely correlate with post-coital interval, although a trend was observed. The regressions are interesting, but it appears that the model is more greatly influenced by uncontrolled factors such as the sampling technique, and the analytical variation due to manual sample preparation, which masks the variation intrinsic to the sample.

Persistence analysis allowed to successfully detect silicone lubricants up to 18 hours post intercourse. After 24 hours, the chemical patterns are getting overlaid by the matrix chemical information and peaks are not constantly observed, which makes the assertion of the presence of a silicone lubricant less reproducible.

12.2 Operationalization of the procedure and recommendations

12.2.1 Sampling and medical examination

Operationalization and implementation of condom evidence analysis in case work practice is nonetheless a complex procedure, which requires various aspects to be taken into account, from the initial investigation and evidence collection to the interpretation of the data. Indeed, condom evidence should ideally be integrated in an existing forensic procedure, in such a way to minimize the impact on routine protocols.

The aim of this research was to establish a procedure that can easily be integrated into an existing protocol, such as that used by practitioners in the field of DNA analysis. Several discussions between the various partners involved in the investigation of such traces in the state of Western Australia revealed that these traces are only collected by doctors if the testimony of the victim suggests the use of a condom. The subsequent analysis of these swabs was not systematic, and the samples were not sent to a qualified forensic laboratory. Medical examiners also reported that they were not confident with correct practices regarding condom evidence collection. However, the implementation of an analytical procedure admits that the samples have been collected and preserved. The first point to consider with a view to operationalizing this procedure is to **inform the doctors** in charge of caring for victims of sexual assault so that forensic evidence can be collected.

Medical examination recommendations edicted by Blackledge in 2007 are still valid:

- **use of non-lubricated latex or plastic examination gloves,**
- **sample condom evidence prior to the rest of the medical examination,**
- **avoid the use of any lubricants for this type of sampling**

There are currently no other existing rules on sampling for condom evidence. An important point that should be mentioned is the **choice of the swabs** used. The swabs selected for use in research (started in 2016) are made of cotton and mounted on a wooden stick. This was the type of swab used at that time by the CURML forensic genetics unit, which prepares and distributes collection kits for the Swiss-French cantons. Interviews with SARC doctors revealed that this type of swab was also used in all of their collection kits (COPAN Interpath ServicesH043N). During 2018, the UGF decided to change the type of swab used to FLOQSwabs® nylon swabs, as these significantly improve the quantity of samples collected and allow better desorption of DNA. Tests were carried out with these new swabs to evaluate their impact on condoms residues [25]. Significant interference was observed in infrared spectroscopic analysis, and the desorption of silicone compounds was found to be less than with cotton swabs, with the chosen extraction procedure. In addition, these swabs are made of a plastic stem which is likely to interfere during the extraction process, as illustrated in **Chapter 7**, As a result, this research was only carried out on cotton swabs. The conclusion of the present study on real samples are therefore only applicable to this swab type. Therefore, it would be recommended to **add at least one cotton swab dedicated to condom evidence collection in the sexual assault evidence collection kits** distributed to hospitals. **Blank swabs should also be sent** and labelled as such,

to ensure the absence of contamination and avoid similar issues as the ones encountered in the Malkinson case.

As medical examiners are usually the first investigators to be involved in sexual crimes, it is important that they gather not only the medical evidence but also additional information that are important for further interpretation of the forensic evidence by the forensic scientist. Sampling is the most important part of the forensic process, and in our case medical examiners are crucial and determinant in this process.

The Sexual Assault Resource Center (Perth, Western Australia) updated their recommendations and guidelines regarding condom evidence in sexual assaults after several discussions between forensic practitioners and researchers regarding condom evidence analysis. As illustrated in Figure 135, the initial sheet completed by the forensic nurse or the medical examiner was very vague. Therefore, interpretation of the evidence from a Bayesian approach considering background, transfer and persistence parameters was hardly possible due to the lack of collected data.

Other specimens – Non biological eg: leaves, dirt, etc		
Swab for lubricant/condom residue		
Other:		
Other:		
Other:		
Other:		

Figure 135: Initial sheet for evidence collection – SARC

The updated SARC sheet for condom evidence collection is presented in Figure 136. This new sheet asks more questions that are relevant for the scientist such as:

- the use of products that could generate false positive during the forensic analysis
- the background question, so as to make sure that the observed evidence, if any, is related to the event itself, rather than to another legitimate activity
- the activity level, so as to understand what could explain the possible absence of evidence

These questions might look like being completely irrelevant for a medical examiner, but they are more than important for the interpretation of the evidence, as this will be discussed in section 12.3. Although it might be surprising not to see a clear information of the time elapsed between the alleged assault and the examination, one can easily calculate this time from the date and time information provided at the top of Figure 136.

Examination	Date	Time
Allegation / assault	Date	Time

Emergency Case details						
Nature of allegations						
Condom used	Yes	No	Unknown			
Showered / Bathed / Douched afterwards	Yes	No				
Products used	(soap, gel, pessary, personal lubricant, shampoo, conditioner)					
Other information						
Sex before assault	Yes	No	**Condom used**	Yes	No	Unknown
Sex after assault	Yes	No	**Condom used**	Yes	No	Unknown

Other specimens – Condom residue / Non biological eg: leaves, dirt, etc	Collected
Swab for lubricant/condom residue Vagina ☐ Anal ☐	
Batch blank reference swab	
Other:	
Other:	
Other:	

Figure 136: Updated sheet for condom evidence sampling – SARC

In a recent presentation from Dr Maire Kelly and Dr Kari Pitts at the FAMSACA conference November 6th 2020, some additional considerations for medical examiners and recommendations on sampling were presented and shown in Figure 137 here under.

What are we swabbing for and where?

- *What*: Looking for transferred chemicals/lubricant from the outside of the condom
- *Where*: This will most likely be at the entrance to and lower vagina and along the vaginal walls
- *Not* in the pool of fluid at the top of the vagina (fornices) or within the cervix (not sperm, no tails)
- Therefore no need to collect HVS or ECS swabs

Best method to sample the vaginal walls

- Collect LVS swabs (for Forensic Biology) as per usual
- Then collect a *SPIRAL VAGINAL* swab
- Insert a swab (moistened if necessary) and spiral up the length of the vagina gently rotating slightly and then back down
- Then insert speculum and look for injuries and collect HVS, ECS swabs (Forensic Biology) as per usual

What else to ask

Sex

- Sex before and after
- Was a condom used
- Was any sexual lubricant used
- Is so, what type

Bathing and lubricants

- Have they bathed or showered
- What products did they use including shampoo and conditioner
- Have they douched or used any feminine hygiene products

What else to do

- Collect within 48 hours for both vaginal and/or anal penetration condom residue
- Document collection site – vagina, anal
- Complete appropriate documentation
- Explain reason for collection eg. Vaginal penetration with a condom
- Mention if shampoo, condition, lubricant etc has been used by the patient and when
- Include a batch blank unopened swab (all states and territories) - important

Figure 137: Recommendations presented by Dr Maire Kelly at FAMSACA conference, November 6th 2020

12.2.2 Forensic analysis of the evidence

The implementation of the procedure described within the framework of this doctoral thesis was oriented on a practical approach, based on the needs of forensic practitioners. On the one hand, certain forensic laboratories have thus expressed the need for an optimized analytical method as well as databases of chemical profiles, in order to be able to answer the questions posed by the investigations (namely, whether a condom was used or not). This is why it was necessary not only to obtain reliable and reproducible experimental conditions, but also an understanding of the market structure and the possible distinctions between different populations. On the other hand, discussions with forensic experts on cases dealing with condom traces raised several questions regarding the discrimination of chemical profiles of condoms as well as alternative sources. These discussions also revealed significant shortcomings in the classification of the samples, and the error rates associated with these classifications. It is with a view to answering these practical questions that this research is oriented.

Considering the operational needs of forensic practitioners as well as observations within this research, a few recommendations can be drawn so as to establish a consistent framework for condom analysis evidence.

Extraction and Sample preparation

Various extraction and analytical techniques could be applied. The one selected for this research purpose was simply taken from the literature and tested for applicability. Because of this, the extraction procedure is likely to significantly negatively affect the sensitivity and reproducibility of the analytical procedure. Some solutions to tackle these issues can be suggested: further research should be performed in order to evaluate extraction efficiency and investigate possible means for improvement like extract concentration. Other extraction techniques could be considered, although the simplicity and applicability to small cotton swabs should always be kept in mind.

Given that both polar and non-polar compounds can be of forensic interest, the following extraction procedure would be recommended:

The cotton head should be individually removed from the wooden stick with a disposable scalpel. The cotton swab is then placed into a disposable glass vial, soaked in 1mL hexane and then placed in an ultrasonic bath for 15 minutes. The recovered cotton swabs should then be dried and re-extracted using methanol with 0.1% diphenylmethane as an internal standard (IS). Batch blank cotton swab should be submitted to the same procedure to obtain the matrix chemical profile, so that non-pertinent peaks can be determined.
Extracts should be stored at less than 4 °C until analysis.

Numerous limitations were also encountered, especially when confronted with real cases. Concentration of the samples is always required, so as to ensure that trace amounts can still be detected. Therefore, **a major addition to the present procedure is to fully evaporate the extraction solvent (i.e., hexane) and to reconstitute the extract in 100 µL of solvent for analysis**. This procedure is modified from [15,18]. While the question of the source of a sample can be resolved as long as traces are detected, questions regarding the absence of evidence are still pending.

Analytical instrumentation and sequence of methods

As previously described (**Chapter 2**), numerous analytical approaches could potentially be applied to analyse condom evidence. However, many of them have never been investigated on real samples. Every technique is different and has its advantages and disadvantages which should be investigated for efficient application to case work samples. In this research, DRIFTS-FTIR, py-GC/MS and GC/MS were selected, as they have previously been **successfully used in case work** and chromatographic instrumentations have demonstrated good sensitivity and reproducibility.

These methods are supposed to be used in sequence, with the extracts to be analysed by DRIFTS-FTIR as a first screening method, allowing to decide whether it is necessary to use both py-GC/MS and GC/MS or only one of these methods for confirmation purposes. From an operational point of view, the procedures presented in this work, especially py-GC/MS and GC/MS, are currently used by ChemCentre, the forensic laboratory of Western Australia, DRIFTS-FTIR being a not so commonly used method in forensic sciences. Analytical conditions are the ones described in **Chapter 5** and **Chapter 6** and the limitations of each

technique are to be considered. Although ATR-FTIR was used in **Chapter 4**, it is not the most relevant method for casework analysis.

As a matter of fact, all the discrimination models were built on raw or standardized material, and not on trace evidence. Knowing that py-GC/MS is sensitive to any changes within the amount of material inserted for the analysis, it is possible that trace samples might not be projected with the real group they originate from. That does not mean that the model does not work for trace evidence, but rather highlights the importance to create a discrimination model based on traces and investigate the possibility to attribute a source to a trace chemical profile, similarly to previous work done by Gueissaz in 2013 for tire traces [91].

To assess the presence of a silicone trace in casework samples, following recommendations are made:

- DRIFTS-FTIR analysis: the four bands characteristic from PDMS, i.e. at 800, 1020, 1090 and 1263 cm^{-1} should be present in the spectra, as they were found to be correlated to each other.
- Py-GC/MS analysis: the smallest oligomer, aka D3 oligomer, should be present. If D4 and D5 are present, it would be relevant to consider them as well. Given that D3 peak was the most abundant, this compound would be more likely to be detected in low concentrated samples.

Finally, for an optimal use of the present framework, the analytical techniques should be validated according to standards (i.e. ISO17020, ISO 17025) so as to respect the principles of accreditation (ChemCentre being a NATA-accredited laboratory). This includes to report sensitivity, selectivity, specificity and sensibility of each technique so as to be able to use them in the forensic practice.

In a recent presentation from Dr Maire Kelly and Dr Kari Pitts at the FAMSACA conference November 6th 2020, some additional considerations for forensic scientists and recommendations on analysis were presented and shown in Figure 138 here under.

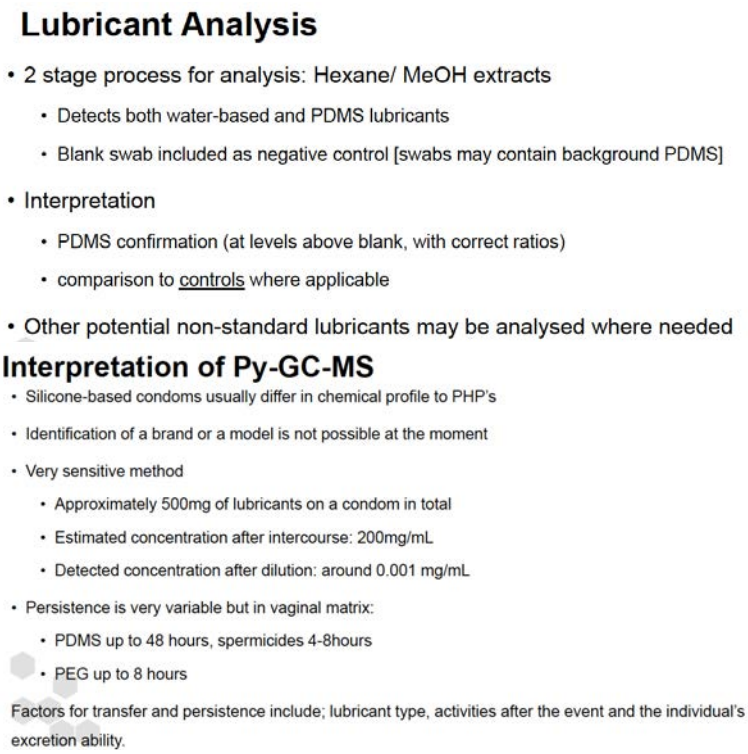


Figure 138: Recommendations presented by Dr Kari Pitts at FAMSACA conference, November 6th 2020

12.3 Interpretation considerations

In the context of condom evidence, several compounds can be targeted and analyzed using different techniques. Silicone-based lubricants, for example, will be preferentially extracted with hexane and detected by DRIFTS-FTIR and py-GC/MS. The water-based lubricants and polar additives will instead be extracted with ethanol or methanol and analyzed in DRIFTS-FTIR with GC/MS analysis as a confirmation method. If solid particles are to be analysed, crossed polar microscopy analysis is recommended. Based on the results presented in this research, it is possible to determine that the analytical methods DRIFTS-FTIR and py-GC/MS enable the detection of silicone lubricants extracted with hexane, which have been brought into contact with a living matrix (i.e., the vaginal matrix). The lowest concentration that could be detected by py-GC/MS for condom lubricants was 0.01 mg/mL. The impact on other components when confronted with the vaginal matrix was not treated in this work. Knowing that the analytical framework developed in this research allows silicone-based condom evidence to be detected, the main question how to interpret the analytical results. What would a positive result mean? How about a negative one? In respect with the hierarchy of the propositions presented in section 2.5, considerations regarding interpretation of the evidence to answer these questions will be separated in a first section regarding the source level, and a second section regarding the activity level.

12.3.1 Source level

The source level questions the origin of the evidence, whose nature is known. Data used to interpret source level evidence include the number of times a characteristic (in this study a chemical profile) is observed in a given population, the probability that the pattern cannot be observed, that patterns can be observed when they should not be, or that it would be an artifact. These data are usually published. Considerations regarding the interpretation of positive and negative results at the source level will be discussed.

Two possibilities can explain a positive result: a false positive or a true positive.
A false positive indicates a detection, but not of the intended target. A well-known example of false positives in the forensic community involves immunochromatographic tests, which do not distinguish human blood and animal blood.
A true positive indicates detection of the correct target, in our case being PDMS. The techniques used are not quantitative, which implies that the detection limit of the instrument has to be reached to obtain a profile. Provided that target compound is detected, the question arises as to whether a profile can be extracted. The two instrumental techniques used have shown that it is possible to extract a chemical profile if a trace is detected. If the evidence comes from the action of interest, several questions arise, such as the possibility to assess a source, at which level (unicity, individualization), or if two profiles can be compared. Three source level scenarios are presented below, with false positive and true positive interpretation discussed.

Scenario 1: Determine if a condom was used. The alternate hypothesis is no condom was used.
In this scenario, the false positive would come from a substance that would react like PDMS but is not. In the case of py-GC/MS analysis, the chromatographic pattern of PDMS is specific, and characteristic of this molecule (although it is affected by the amount of recovered sample). The probability of having another molecule with similar chemical profile is therefore very low. Regarding DRIFTS, error rate is slightly higher, given that we are only looking for 4 specific bands in the spectra. Indeed, one should always keep in mind that siloxanes are a chemical family, whose chemical structure is based on the presence of Si-O backbone. Many crossed polymers siloxanes can be found, with different conformations, such as copolymers (e.g. epoxy-siloxanes), or organo-silicon molecules (e.g. silsesquioxanes) [186,187]. All these molecules are known to present the same 4 major bonds.

The true positive is a characteristic profile of the PDMS, which can be extracted and projected into the discrimination model presented in Chapter 5 of this study. The source can be obtained by classifying traces in the classification model created, and the error rates are known.

Scenario 2: Determine if a condom was used. The alternate hypothesis is a lubricant was used.
In this scenario, the false positive would come from a silicone lubricant that would react like the one from the condom. The true positive would come from the silicone lubricant. Results from the ATR-FTIR market study (**Chapter 4**) highlighted that 5 lubricants were found to present a silicone chemical profile which was not visually distinguishable from the condom ones. However, with the help of chemometrics, only one lubricant was still indistinguishable from the condom chemical profiles. FTIR analysis classification reported a 6% false negative and 13 % false positive error rate when all the samples were put together.
Results from **Chapter 5** (py-GC/MS) have shown that only one lubricant out of the 7 silicone-based lubricants found on the market presented a chemical profile that was visually and statistically clustering with condom lubricants. All the other lubricants (6 out of 7) were found to be visually distinguishable from the condom lubricants pattern. The classification model presented reported a 2% false negative error rate and 5% false positive error rate. Compared to FTIR, Py-GC/MS is therefore more adequate when one wants to know whether the chemical profile originates from a condom or a lubricant, as long as it is a silicone containing product.

Scenario 3: Determine if a condom from a given brand or model was used. The alternate hypothesis is a condom from another brand, or another model was used.
The methodology developed in this research does not allow this question to be answered. Condoms of different brands and models could not be discriminated by the statistical model. The analytical instrumentation used in this study were not sufficient to answer this question. Other analytical instrumentations might answer this question.

These three scenarios involve the detection of a trace. However, one of the questions that preoccupies forensic experts today is how to interpret the absence of a trace. Two possibilities can explain a negative result: a false negative or a true negative.
A false negative can have several sources. First, the trace may have characteristics that do not allow it to be detected with the methods proposed. For example, a water-based lubricated condom will not give any response in pyrolysis-GC- MS, since the compounds will decay into CO_2 and H_2O, which will not be detected by the mass spectrometer. Similar scenarios will occur

with non-lubricated condoms or lubricated with solid particles. If the condom used did contain a silicone-based lubricant, then the trace may be present, but in a too small quantity to be detected. Here we must consider the sensitivity of the analytical technique and the sampling. However, analytical sensitivity is easier to monitor compared to sampling, given that sampling implies a human being and therefore multiple differences, even though a common protocol is accepted amongst practitioners. Compounds inhibiting the detection of the trace may also be present, for example if the samples are collected during menstruation. Although this has never been investigated, it is known that menstruation will affect the evidence; for this reason, in the background, transfer and persistence studies, volunteers were asked to avoid sampling during the menstruation period.

The true negative can be explained either by the absence of trace (assuming that evidence would transfer) or by the absence of transfer. This raises considerations regarding transfer and persistence, which are linked to the activity level, and are discussed in the following section.

12.3.2 Activity level

The level of activity addresses the question of whether a trace is present, how it got there or if not, how the absence of a trace be can explained. This level takes into account three additional parameters compared to the source level: background, transfer and persistence.

When a trace is detected and its source has been determined, it is important to know how to explain its presence. Three scenarios can be envisaged:

1. The trace is present naturally and continuously in the vaginal matrix and can be referred to as ‘natural background’. For example, a woman using an intimate hygiene product containing silicone is likely to have traces of silicone in the vaginal matrix unrelated to protected intercourse. Traces of silicones will be detected systematically regardless of when the sample is taken.
2. The trace originates from a legitimate action. It is the concept of background as generally considered in the forensic field and which considers the prevalence of a chemical profile in a population for legitimate reasons. This is the use of condoms or lubricants in the context of consensual relationships.
3. The trace originates from sexual assault.

The distinction between scenarii 2 and 3 implies that it is possible to precise how the traces would differ from the *production of the trace* point of view, which questions the genesis of the

trace. This has not been investigated, but some omics approach on the vaginal matrix itself might help solving these questions.

Scenarii 1 and 2 considering the background are extremely difficult to differentiate during a prevalence study. The study detailed in **Chapter 9** also did not differentiate the two populations and considered the background as a whole, as there were no specific restrictions for the volunteers to be part of the study. The results showed that the prevalence of traces of PDMS in the vaginal matrix in the population was very low. Consequently, the probability that the observed trace does indeed come from a transfer and not from the background noise is extremely high. However, some reservations must be considered with regards to this study:

- The number of volunteers obtained cannot be considered as representative. Prevalence studies such as those presented in the medical area [189–193] are generally carried out on significantly larger populations, usually of more than 100 volunteers, and often containing 1000 or 2000 volunteers. This then provides much more reliable and relevant statistical information.
- Two populations should be considered to study the background reliably.
    - The first population should consider women who do not use condoms with their partners. Thus, if traces of silicone compounds are detected, they will not come from a legitimate activity or sexual assault involving a condom, but rather from a natural background. This determines the prevalence of the compounds present naturally in the population.
    - The second population should consider
        - Women who have protected intercourse on a regular basis when having sex with their partners.
        - Women who use lubricants on a regular basis when having sex with their partners.

      This will assess the prevalence of silicone compounds in a population that habitually uses condoms or lubricants.

    Considering these two populations will allow the background to be assessed more reliably than the exploratory study presented in this work.

In addition, the population used in this research was very specific: aged between 18-35 years old, non-pregnant women, within an academic context. Working with such specific populations is mandatory so as to be able to control most of the parameters, but a loss of realism is expected. In addition, as the volunteers were aware of the type of study, they might have avoided a daily use of certain products.

- These remarks also imply that specific questions must be asked of the victim of sexual assault, such as whether they regularly use condoms or lubricants for sexual intercourse and, in the case of a lubricant, obtain a sample in order to compare the chemical profile of the trace with that of the lubricant. Although it is not possible to distinguish the condom population, reference material should be collected, as PDMS and water-soluble compounds might be present. It might also appear that other techniques would be able to be more successful at discriminating reference and clustering the traces within the adequate cluster. These questions seem invasive but are extremely important for the interpretation of the presence of a trace.

When no trace is detected it is important to understand why. Here again, three scenarios can be envisaged at the activity level:

- There is no evidence. Although absence of evidence is not evidence of absence, the non-detection of a trace can be due to the absence of contact.
- The evidence did not transfer. Multiple explanations can explain that lubricants did not transfer: the contact time was not long enough; the contact surface was not sufficient or the intensity of the activity (as described by Locard (1920) [138]) is too low. It is possible that after a given time, the surface compounds have been completely transferred into the matrix and that a state of equilibrium is reached. If the intercourse persists after this time, losses by material removal should be considered. Other scenarios such as forced oral sex with a condom preceding vaginal penetration, or sexual assault by multiple aggressors can explain the traces not to transfer.
- The evidence did not persist. Similarly to other types of traces, several factors will influence persistence of condom traces. As described in **Chapter 2**, the characteristics of the donor (i.e., the type of lubricant used), the characteristics of the receiver (i.e. the vaginal matrix which changes cyclically), the contact time, the time elapsed between the assault and the collection of the trace, and the activity of the victim are likely to affect persistence. From the point of view of the forensic analyst, most of these parameters are not controllable, and the issues raised are very similar to those encountered in fingerprint dating [174] or fingerprint classification [184,185,194].

The preliminary study in **Chapter 10 and 11** investigating transfer and persistence was carried out on a small number of samples, which does not allow definitive conclusions to be drawn. However, several trends were highlighted, and three main factors were identified as contributing to the observed variations, namely, voluntariness, sampling technique and

analytical variation. While analytical variability can be controlled and reduced through standardization and validation of the procedure, the other two parameters cannot be controlled under any circumstances. As demonstrated by [15], the persistence of traces differs among women, which was confirmed in **Chapter 11** when two different techniques were used. Sampling was also found to affect the quality of the traces but is not directly affecting the persistence. The creation of a human model therefore seems very complex if it is to be achieved in this way.

In a similar manner to medical studies seeking to bring new drugs to market, one option that could be considered would be to create a physical model, such as the one described in [195], that allows the various influencing factors to be controlled and understood, either in a univariate or a multivariate model. Only then should human modelling be considered, to evaluate how these parameters change when confronted with a human matrix. This type of research requires collaboration with researchers from outside the forensic field, including physicians, biologists, and toxicologists.

12.4 Development and future work

Establishing an analytical procedure for the analysis and interpretation of traces of condoms described in this research gives a pragmatic vision of the question, by defining a tangible and concrete procedure. The analytical parameters proposed are objective since they have been optimized using experimental designs. In addition, the potential for discrimination and classification of samples according to each of the analytical techniques is associated with error rates obtained on a statistical basis. These results are however valid only within the framework of this study, under the limitations which are imposed by the analytical choices. The main limitations are:

- The infrared DRIFTS procedure has extremely high qualitative and quantitative variability. It is neither standardized nor validated and could not be developed quantitatively.
- The extraction technique is not optimized.
- The procedure only applies to samples containing either a silicone-based or aqueous lubricant. More specifically with regards to interpretation, only silicone-based samples were studied.
- The individual (i.e., the person) at the source of the trace must be known and available to collect comparison traces.

- The traces in question must have been collected in the vaginal matrix. Other types of substrates were not investigated.
- Transfer and persistence were only investigated on DRIFTS-FTIR analyzes. The results obtained suggest that certain questions must be resolved before moving on to a model in py-GC/MS.
- Py-GC/MS being sensitive to sample quantity, traces analyses are likely to suffer from variations that need to be monitored and reported.

To reduce these limitations, several lines of research can be proposed. The first relates to the analysis of the sample, the second to the question of the composition of the samples and the last to the interpretation of the results.

The analytical procedure implemented as part of this work has not been standardized. Some parameters have been optimized, but no validation has been performed. The failures encountered when attempting to quantify samples using DRIFTS-FTIR, knowing that other researcher had succeeded in doing so, suggests that significant variations are generated by the operator, within the sample preparation step. An instrumental variability cannot be excluded. Research focusing on standardization of the procedure, with minimization of the operator's influence and an understanding of clear analytical variability should be considered. Likewise, the extraction technique was based on information from the literature [41] and confirmed within a master's thesis led in 2017 [25]. Although this is a working method, other solvents could be compared, and tests of extraction efficiency and yield should be carried out. This would help to understand if a trace is not detected because it is not extracted, not concentrated enough or simply not present.
With regard to the sample composition, only the lubricants were studied. Samples which are not lubricated, or which contain a large concentration of solid particles have yet to be studied. Other analytical techniques should be selected, such as microscopy, to answer these questions. It is also a question of whether the investigation of these traces is relevant, given the proportion of non-lubricated condoms in the population available on the market. Other questions relating to the question of lubricants of different viscosities and the capacities of various techniques to differentiate them, further research is necessary, although Gareth Campbell and Manolita Monzo in their respective research works [25,119] have not successfully reproduced Blackledge's results [9]. The issue of other sources of silicone lubricants is still pending, as this study focused on products that could be found in the vaginal matrix.

With regards to the interpretation of the results, several lines of research can be proposed. Other issues must first be resolved, in particular those related to the extraction of the sample and to the analytical validation.

Population prevalence studies should be repeated in a larger population, so that the conclusions drawn are more reliable than those presented in this study. Two populations of women should be considered, so as to separate the natural impact of the matrix from the impact of regular use of lubricants or preservatives.

A physical model should be developed for transfer and persistence. If the option of a multivariate analysis to identify the factors which influence the transfer of the trace (and potentially its persistence) is strategically recommended, it will also be a question of understanding how the contact affects the transfer, and which parameter contact is the most important. This is necessary before moving onto a human model, which involves a living and evolving matrix. The issue of the self-sampling technique should be addressed given the variations it generates. The collection of traces by a qualified doctor could make it possible to reduce the variability linked to sampling. Other trace media could be investigated, such as the victim's underwear, with considerations to alternative sources of silicone, such as detergents. If that is done for silicone-based lubricants, it should be done again with a similar procedure for water-based lubricants and the polar fraction of the vaginal matrix. Finally, ‘omic’ approaches which are currently very popular in the forensic field could also be the subject of interesting future research, to investigate, for example, the reaction of the matrix to the use of specific lubricants.

Finally, it is necessary to emphasize that, whatever the developments or modifications made, complete validation steps are necessary. Validation must go far beyond the analytical method, as steps such as sample preparation, building models, harvesting or even analyzing the material necessary to build the model must be included.

Chapter 13: Conclusion

Detecting and interpreting traces left behind in the course of criminal activity is the daily task of the forensic scientist. They seek to know if the traces can be revealed, if they are present for a legitimate reason or if they have withstood the ravages of time. The same is true for traces of condoms, the analysis and interpretation of which are a relatively recent problem since they have only been studied for around forty years. However, due to the complexity of the composition of the samples and their interactions with a living trace support, such as the vaginal matrix, no reliable analysis methodology has been developed and validated to date. There are only a few databases clearly referencing the potential for the discrimination of samples or their classification, and studies on traces in contact with a living matrix are rare. It is therefore particularly worrying that forensic experts may decide on the source of a trace, without the parameters used in the determination being precisely documented. This point illustrates perfectly the need for further research on the subject.

For this reason, the present work has focused on the evaluation of the development an analysis sequence for condom traces which is applicable in real cases, and which clearly documents the specificity of the compounds thus detected, as well as the classification error rates for each method used. This line of research has indeed been identified as necessary in order to answer the fundamental questions of the source of a trace, on the basis of the state of the art. This research was more particularly articulated around the study of six main parameters: namely the target compounds, the analytical techniques, the market composition, the specificity of the compounds, the trace support and its characteristics as well as the parameters and models for transfer and persistence.

Thus, an analytical framework adapted to condom evidence was developed and optimized. The compounds present in the various condoms available on the international market as well as intimate hygiene products, likely to be found in the general population were analyzed by FTIR, py-GC/MS and GC/MS. For all the analyses, various preprocessing methods were used in order to reduce within sample variability and to maximize between sample invariability, so as to be able to differentiate samples from different sources. The statistical models created have shown that condoms are predominantly only silicone-lubricated, but that a small percentage of them also contain water-based compounds or are solely water-based lubricated, which can cause problems detection and persistence issues in real cases. At the same

time, personal hygiene products and personal lubricants generally presented different profiles than those of condoms. Certain lubricants presented a profile indistinguishable from condoms in FTIR, but could be differentiated in py-GC/MS. A single sample of silicone lubricant (*Durex Perfect Play Glide*) could not be distinguished from the condom population.

The analytical framework was validated using proficiency trials and applied to case work samples. Correct classification of the samples was possible, and traces were successfully detected in case work. Application to real cases has highlighted the need to understand interpretive parameters other than the frequency of appearance of a characteristic. Indeed, the main question of interpretation that arose is how to interpret the absence of traces. For this, parameters such as transfer, and persistence must be investigated. A background study made it possible to determine the composition of the vaginal matrix and to observe variations within a given population. The prevalence of silicone-based compounds in the given population was found to be zero. Finally, a pilot study on transfer and persistence was carried out. However, modeling of the persistence parameters was more affected by the analytical variability and the variability due to the self-sampling than by variations in the vaginal matrix. Further investigations are still needed, including py-GC/MS modelisation of transfer and persistence.

This research made it possible to complete gaps noted in the literature such as the absence of a database, the absence of study on the peculiarities of spectra or pyrograms, the effects of latex or the absence of latex on the chemical profile obtained or even the possibilities offered by the different instrumental techniques in terms of sample discrimination. No previously reported study using the chosen analytical techniques combined the results with a chemometric analysis. And yet, the latter, as well as all the parameters mentioned above, has proved to be crucial in the understanding in particular of the frequency of appearance of the characteristics in the population, a basic parameter which allows the forensic evaluation at the level of the source. One of the largest publicly available databases has been produced, using three different techniques, and coupling of the information obtained within the different analytical instrumentation has made it possible to achieve the objective of understanding the market and studying the specificity of the compounds of interest. In addition, this database was used during the analysis of real cases, and it was found that if the detection of the trace did not pose problems for investigative purposes, the non-detection of the trace, on the other hand, is more problematic. This methodology has built the foundation for further study of the very important interpretative parameters of transfer and persistence, through the study, by DRIFTS-FTIR, of the effects of the various known influence factors (i.e., donor and receiver) and the

limitations due to variability and unknown influencing factors (i.e., contact, elapsed time, sampling).

In conclusion, this work is only the beginning of a structured and transparent research on the establishment of a methodology for the analysis of condoms traces. Indeed, the six elements studied here form the basis of research on the subject and must be considered iteratively. Many areas for further development are still open and must be studied in order to assess whether, ultimately, validation of a method according to the interpretative model is possible. Validation of analytical techniques, according to established criteria, should be carried out before carrying out a transfer and persistence study. Persistence modeling should take into account both univariate (regressions) and multivariate (PCA and PLSR) models, and their robustness should be assessed, before proceeding with a Bayesian assessment approach. However, due to the variability of the samples and the evolving nature of human matrices, it is important to keep in mind that the implementation of such a methodology is complex, especially if one wishes to decide on the level of activity. Many questions remain unresolved, for example regarding the analysis of non-lubricated samples, and it is more sensible to first resolve these questions before claiming to have built a reliable model for trace interpreting.

Chapter 14: References

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Chapter 15: Appendix

Appendix I: List of the publications

Given the length of the manuscript and the publications, the latest will be appended in another appended PDF document.

Appendix II: Experimental design – Detailed planification for py-GC/MS Optimization

1st experimental cycle: surface response screening

A first FFD (Full Factorial Design) experiment plan was generated using Unscrambler X (Camo Software, Norway) to observe the response surface. The parameters used are described in the Table .

Factor	level -1	level 0	level 1
Temperature	420	620	920
Temps	10	20	30

Table A: Factors and level used for the identification of the surface response, using FFD design

The chosen FFD plan used two replicas of each point and three replicas for the central point. This resulted in a total of 11 randomized program experiments. The central point was defined at 620 ° C and 20 seconds because it is the pyrolysis conditions used during the evaluation phase of the chromatographic conditions. Good abundance of the various peaks and a profile consistent with those presented in the literature had been obtained. This plan made it possible to make a wide screening of the response surface and Table B summarizes the first series of experiments conducted.

Analysis	Temperature	Time
Plan_01	620	20
Plan_02	420	30
Plan_03	920	10
Plan_04	920	10
Plan_05	620	20
Plan_06	420	30
Plan_07	920	30
Plan_08	420	10
Plan_09	420	10
Plan_10	620	20
Plan_11	920	30

Table B: Experimental conditions for the first cycle of experiments

The parameters of the transfer line and the interface were not changed during the experiments and were set at 30 ° C and 275 ° C respectively. Indeed, as previously shown by Gueissaz (2013), a temperature too low of these two parts of the instrument leads to a recondensation of the particles and the entirety of the pyrolyzed compounds is not analysed in the GC.

In a second step, eight additional analyzes were added to the plan. The latter come from the desire to study the variability of the extreme points, namely the couples 420°C /10s, 920°C /30s and 620 °C/20s. The experiences added for this plan are shown in Table C.

Analysis	Temperature	Temps
Plan_12	620	20
Plan_13	420	10
Plan_14	920	30
Plan_15	920	30
Plan_16	620	20
Plan_17	420	10
Plan_18	920	30
Plan_19	420	10

Table C: Additional experiments

2nd experimental cycle: effect calculation and surface response modelisation

The knowledge obtained during the first cycle of experiment made it possible to reduce the zone of experimentation of the measurement of the temperature. The new temperature levels were chosen within ± 100 ° C of the central point. The time variables have not been modified but correspond to a variation of ± 10 seconds around the value of the central point. The new levels are shown in Table

Factor	level -1	level 0	level 1
Temperature	520	620	720
Time	10	20	30

Table D: Choice and codification of the new levels

A new FFD-type plan was therefore drawn with 7 experiments which are presented in Table E. The purpose of these experiments is to estimate the effects of the factors. The first experimental planning required several point analyses of the experimental design as well as the integration of several compounds. Since this was very time-consuming, a new response factor was chosen. Compound D3 was chosen as a new response factor since the increase in the presence of this compound was related to a decrease in the coefficients of variation. The remaining 5 oligomers, D4 to D8, were retained for integration. The abundance of D3 was then subjected to the data processing procedure in Section 2.5. On the basis of the results obtained, a first model of regression will be sketched.

Analysis	Temperature	Time
FFD_01	720	20
FFD_02	520	30
FFD_03	720	20
FFD_04	920	30
FFD_05	720	20
FFD_06	520	10
FFD_07	920	10

Table E: Second experimental planification

3rd experimental cycle: optimising the surface response

The previously presented FFD plan has been supplemented by a CCD (Central Composite Design) plan, which makes it possible to add axial points and thus to better investigate the interactions between the pyrolysis parameters. This type of plan makes it possible to measure the quadratic and cubic effects of the variables. The planning in Table F has previously required taking into account the results of the previous planning (see Table E). Here again only the pyrolysis temperature has been modified.

Analysis	Temperature	Time
CCD_01	720	30
CCD _02	520	10
CCD _03	720	10
CCD _04	520	30
CCD _05	920	10
CCD _06	920	30
CCD _07	720	20
CCD _08	520	20
CCD _09	720	20
CCD _10	720	20
CCD _11	920	20

Table F: CCD planification

On the basis of the results obtained, regression models will be sketched, and the most suitable model can be used for an application to real samples.

Data preprocessing

As indicated by Gueissaz (2013), pre-treatment by normalization at the sum of the areas is a pre-treatment suitable for GC/MS pyrolysis analyzes because the sample quantity can be variable between the depositions. This pre-treatment allows the quantitative comparison of the

various analyzes. The area of each i-th compound is divided by the sum of the areas of the n compounds considered. The calculation is as follows:

$$A_{i\,norm} = \frac{A_i}{\sum_{j=1}^{n} A_j}$$

with A the integrated area for the compound, i and j the indices of the integrated compounds. Once the areas are normalized, other pre-treatments can be applied. This point being important for the subsequent treatments, a complete section is dedicated to him.

The calculation of means, standard deviations and coefficients of variation.

Finally, the mean and standard deviation of each compound were calculated on the basis of standardized and pre-processed data. The coefficients of variation are also calculated according to the following formula:

$$CV\ (\%)\ \frac{SD_i}{\bar{x}_i}\ x\ 100$$

3rd experimental cycle: adjusting the experimental design

New analyzes were carried out to recreate an FFD plan by considering the analyzes already carried out. The new centre point was set at 720 ° C and 20 seconds of pyrolysis. It turns out that the total variance observed on the replicas made for this time / temperature pair is smaller than the variance of the 620 ° C point and 20 seconds. Thus this seems to indicate that the most suitable temperature to obtain a minimum variability is greater than the point of degradation obtained by DTG curves. A new FFD plan was therefore developed (Table G) and the first two previous models were re-evaluated in the light of new experiences.

Analysis	Temperature	X1	Time	X2
FFD2_01	920	1	10	-1
FFD2_02	920	1	30	1
FFD2_03	720	0	20	0
FFD2_04	520	-1	10	0
FFD2_05	720	0	20	0
FFD2_06	720	0	20	0
FFD2_07	520	-1	30	1

Table G : Experimental conditions for the adjustment of FFD planification

4th Experimental cycle: Method optimisation

New analyzes were carried out for the purpose of optimization, completing the new FFD plan. The temperatures and pyrolysis times used for this new experimental design are presented in Table H. The chosen optimization plan will not be a star plan but an FCC (Faced Central Composite) plan that will act as an extension. of the FFD plan already used (Figure A).

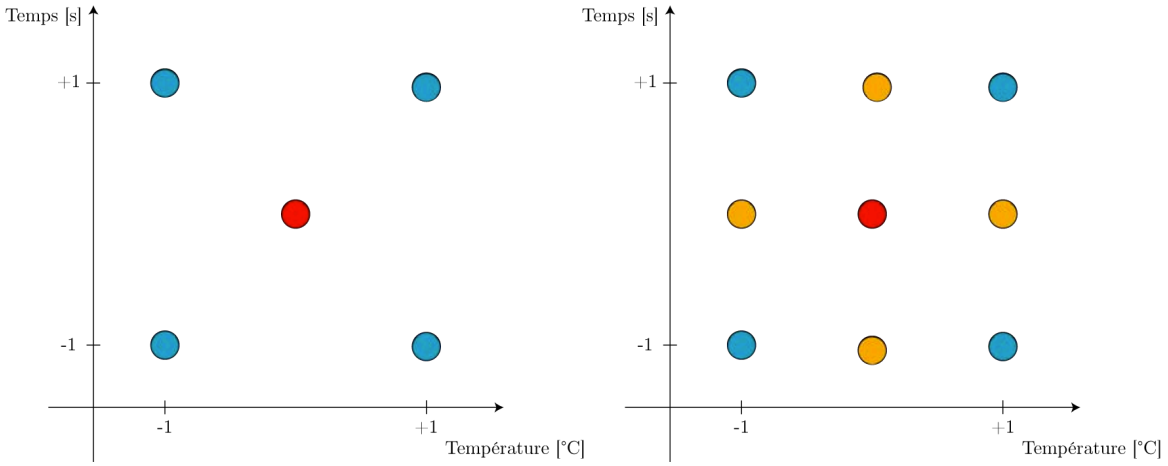

Figure A: Illustration of the FFD planification (left) and FCCD planification (right)

Analysis	Temperature	X1	Time	X2	Relative Abundance
CCD_01	520	-1	20	0	0.953
CCD_02	720	0	10	-1	0.939
CCD_03	720	0	30	1	0.937
CCD_04	920	2	20	0	0.931

Table H : Experimental conditions for the additional CCD analysis

Appendix III : Sample listing

Sample Nº	Brand	Model	Lot	Place	Sample Type	FTIR Chemical Profile	py-GC/MS Chemical Profile	GC/MS Chemical Profile	Other observations
1	Durex	Extra Safe	1000026318	NZ	Condom	Silicone	Silicone degradation; D3-D13 detected	-	Analysed NZ
2	Durex	Extra Safe	1000010957	NZ	Condom	Silicone	*Duplica Sample 1*	-	Analysed NZ
3	Durex	Extra Safe	1000106077	NZ	Condom	Silicone	*Duplica Sample 1*	-	Analysed NZ
4	Durex	Extra Safe	1000049207	NZ	Condom	Silicone	*Duplica Sample 1*	-	Analysed NZ
5	Durex	Extra Safe	1000046754	NZ	Condom	Silicone	*Duplica Sample 1*	-	Analysed NZ
6	Durex	Extra Safe	1000041595	NZ	Condom	Silicone	*Duplica Sample 1*	-	Analysed NZ
7	Durex	Extra Safe	1000049636	NZ	Condom	Silicone	*Duplica Sample 1*	-	Analysed NZ
8	Durex	Extra Safe	1000041595	NZ	Condom	Silicone	*Duplica Sample 1*	-	Analysed NZ
9	Durex	Extra Safe	21306074	NZ	Condom	Silicone	*Duplica Sample 1*	-	Analysed NZ
10	Durex	Classic	1000063396	NZ	Condom	Silicone	Silicone degradation; D3-D11 detected	-	Analysed NZ
11	Durex	Unknown	1000010958	NZ	Condom	Silicone	Silicone degradation; D3-D12 detected	-	Analysed NZ
12	Durex	Pleasure Me	10939364	NZ	Condom	Silicone	Silicone degradation; D3-D9 detected	-	Analysed NZ
13	Shield	XL	PN29803	NZ	Condom	Silicone	Silicone degradation; D3-D13 detected	-	Analysed NZ
14	Durex	Mutual Climax	1000026346	NZ	Condom	Silicone	Silicone degradation; D3-D13 detected	Benzocaine, PEG (traces)	Analysed NZ
15	Durex	Unknown	1000015117	NZ	Condom	Silicone	*Duplica Sample 11*	-	Analysed NZ
16	Durex	Unknown	1000011869	NZ	Condom	Silicone	*Duplica Sample 11*	-	Analysed NZ
17	Durex	Unknown	1000012759	NZ	Condom	Silicone	*Duplica Sample 11*	-	Analysed NZ
18	Durex	Classic	10121993	NZ	Condom	Silicone	*Duplica Sample 10*	-	Analysed NZ
19	Durex	Mutual Climax	1000039272	NZ	Condom	Silicone	*Duplica Sample 14*	Benzocaine, PEG (traces)	Analysed NZ
20	Durex	Intimate Feel	1000044392	NZ	Condom	Silicone	Silicone degradation; D3-D11 detected	-	Analysed NZ

21	Durex	Intimate Feel	10837252	NZ	Condom	Silicone	*Duplica Sample 20*	-	Analysed NZ
22	Durex	Mutual Climax	1000039272	NZ	Condom	Silicone	*Duplica Sample 14*	Benzocaine, PEG (traces)	Analysed NZ
23	Durex	Confidence	1000059628	NZ	Condom	Silicone	Silicone degradation; D3-D11 detected	-	Analysed NZ
24	Durex	Banana	14F2456B	NZ	Condom	Silicone	Silicone degradation; D3-D11 detected	-	Analysed NZ
25	Durex	Pleasure Me	1000039189	NZ	Condom	Silicone	*Duplica Sample 12*	-	Analysed NZ
26	Durex	Pleasure Me	1000042518	NZ	Condom	Silicone	*Duplica Sample 12*	-	Analysed NZ
27	Durex	Confidence	1000045624	NZ	Condom	Silicone	*Duplica sample 23*	-	Analysed NZ
28	Durex	Unknown	22B09482	NZ	Condom	Silicone	*Duplica Sample 11*	-	Analysed NZ
29	Durex	Unknown	1000049211	NZ	Condom	Silicone	*Duplica Sample 11*	-	Analysed NZ
30	Durex	Unknown	1000064796	NZ	Condom	Silicone	*Duplica Sample 11*	-	Analysed NZ
31	Durex	Classic	10135039	NZ	Condom	Silicone	*Duplica Sample 10*	-	Analysed NZ
32	Durex	Strawberry	14F1293S	NZ	Condom	Silicone	*Duplica Sample 130*	-	Analysed NZ
33	Durex	Apple	14F1293A	NZ	Condom	Silicone	Silicone degradation; D3-D11 detected	-	Analysed NZ
34	Ansell	Contempo-Rough Rider	1011081616	NZ	Condom	Silicone	Silicone degradation; D3-D11 detected	-	Analysed NZ
35	Durex	Strawberry	14F2456S	NZ	Condom	Silicone	*Duplica Sample 130*	-	Analysed NZ
36	Durex	Orange	14F2456O	NZ	Condom	Silicone	Silicone degradation; D3-D9 detected	-	Analysed NZ
37	Durex	Classic	21306193	NZ	Condom	Silicone	*Duplica Sample 10*	-	Analysed NZ
38	Durex	Thin Feel	10847195	NZ	Condom	Silicone	Silicone degradation; D3-D9 detected	-	Analysed NZ
39	Durex	Orange	14F1293O	NZ	Condom	Silicone	*Duplica Sample 36*	-	Analysed NZ
40	Durex	Confidence	1000179693	NZ	Condom	Silicone	Silicone degradation; D3-D9 detected	-	Analysed NZ
41	Gold Knight	Chocolate	PC29801	NZ	Condom	Silicone	Silicone degradation; D3-D9 detected	-	Analysed NZ
42	Marquis	Flavoured	PG1202	NZ	Condom	Silicone	Silicone degradation; D3-D11 detected	-	Analysed NZ
43	Durex	Extra Safe	1000136970	NZ	Condom	Silicone	*Duplica Sample 1*	-	Analysed NZ

44	Marquis	Regular	1005096	NZ	Condom	Silicone	Silicone degradation; D3-D10 detected	-	Analysed NZ
45	Gold Knight	Strawberry	PS2980	NZ	Condom	Silicone	Silicone degradation; D3-D11 detected	-	Analysed NZ
46	Durex	Extra Safe	1000174930	NZ	Condom	Silicone	*Duplica Sample 1*	-	Analysed NZ
47	Ansell	SKYN-Original	1705403516	NZ	Condom	Silicone	Silicone degradation; D3-D10 detected	-	Analysed NZ
48	Ansell	Lifestyles-Ultra Thin	1504111016	NZ	Condom	Silicone	Silicone degradation; D3-D13 detected	-	Analysed NZ
49	Ansell	Lifestyles-Ultra Thin	1611060216	NZ	Condom	Silicone	*Duplica Sample 48*	-	Analysed NZ
50	Ansell	Lifestyles-Regular	1705591616	NZ	Condom	Silicone	Silicone degradation; D3-D11 detected	-	Analysed NZ
51	Ansell	SKYN Original	1705743116	NZ	Condom	Silicone	*Duplica Sample 47*	-	Analysed NZ
52	Ansell	Lifestyles-Regular	1701081516	NZ	Condom	Silicone	*Duplica Sample 50*	-	Analysed NZ
53	Ansell	Lifestyles-Zero	AK017A04	NZ	Condom	Silicone	Silicone degradation; D3-D13 detected	-	Analysed NZ
54	Ansell	Lifestyles-Party Variety-Snake Skin Textured	1512032416	NZ	Condom	Silicone	Silicone degradation; D3-D9 detected	-	Analysed NZ
55	Ansell	Lifestyles – Party Variety - O'Max	1603780316	NZ	Condom	Silicone	Silicone degradation; D3-D11 detected	-	Analysed NZ
56	Ansell	Lifestyles – Party Variety -Tutti Frutti	1607192816	NZ	Condom	Silicone	Silicone degradation; D3-D10 detected	-	Analysed NZ
57	Ansell	Lifestyles – Party Variety -Warm/Cool	1605512216	NZ	Condom	Water based	Non analysed (no silicones)	PPG, Glycerin	Analysed NZ
58	Ansell	Lifestyles – Party Variety – Glow in the Dark	AGP610A	NZ	Condom	Silicone	Silicone degradation; D3-D10 detected	-	Analysed NZ
59	Ansell	SKYN-Elite	1702093216	NZ	Condom	Silicone	Silicone degradation; D3-D10 detected	-	Analysed NZ

60	Ansell	Lifestyles – Assorted – Banana Bump Studded	1701142016	NZ	Condom	Silicone	Silicone degradation; D3-D10 detected	-	Analysed NZ
61	Ansell	Lifestyles – Assorted – Sonic Strawberry Ribbed	1606011716	NZ	Condom	Silicone	Silicone degradation; D3-D10 detected	-	Analysed NZ
62	Ansell	Lifestyles – Assorted – Berry Blast Smooth	1601151216	NZ	Condom	Silicone	Silicone degradation; D3-D10 detected	-	Analysed NZ
63	Ansell	Lifestyles – Assorted – Vanilla Thriller Smooth	1512101016	NZ	Condom	Silicone	Silicone degradation; D3-D10 detected	-	Analysed NZ
64	Ansell	Lifestyles – Assorted – Choc Ripple Ribbed	1610022416	NZ	Condom	Silicone	Silicone degradation; D3-D10 detected	-	Analysed NZ
65	Ansell	Lifestyles – Assorted – Mintensity Studded	1512050316	NZ	Condom	Silicone	Silicone degradation; D3-D10 detected	-	Analysed NZ
66	Ansell	Lifestyles – Assorted – Sonic Berry Ribbed	1702111116	NZ	Condom	Silicone	Silicone degradation; D3-D10 detected	-	Analysed NZ
67	Ansell	Lifestyles – Assorted – Banana Bump Studded	1612032416	NZ	Condom	Silicone	*Duplica Sample 60*	-	Analysed NZ
68	Ansell	Lifestyles – Party Mix – Choc Ripple Ribbed	1611602316	NZ	Condom	Silicone	Silicone degradation; D3-D10 detected	-	Analysed NZ

69	Ansell	Lifestyles – Party Mix-Dynamint Studded	1703090316	NZ	Condom	Silicone	Silicone degradation; D3-D10 detected	-	Analysed NZ
70	Ansell	Lifestyles – Party Mix – Warm Smooth	1612122916	NZ	Condom	Water based	Non analysed (no silicones)	PPG, Glycerin	Analysed NZ
71	Ansell	Lifestyles – Party Mix – Tutti Frutti Smooth	1608452116	NZ	Condom	Silicone	Non analysed	-	Analysed NZ
72	Ansell	SKYN-Intense Feel	1612813316	NZ	Condom	Silicone	Non analysed	-	Analysed NZ
73	Ansell	SKYN-Extra Lubricated	14707PI16	NZ	Condom	Silicone	Non analysed	-	Analysed NZ
74	Hero	Natural	7365	NZ	Condom	Silicone	Non analysed	-	Analysed NZ
75	Hero	Ultra Thin	7367	NZ	Condom	Silicone	Non analysed	-	Analysed NZ
76	GLYDE	Maxi	PN32503	NZ	Condom	Silicone	Non analysed	-	Analysed NZ
77	GLYDE	Slimfit	PN31564	NZ	Condom	Silicone	Non analysed	-	Analysed NZ
78	Durex	Intense Stimulating	1000214762	NZ	Condom	Silicone	Non analysed	-	Analysed NZ
79	Durex	Performa	1000170528	NZ	Condom	Silicone	Silicone degradation; D3-D9 detected	-	Analysed NZ
80	Durex	Together	1000174855	NZ	Condom	Silicone	Non analysed	-	Analysed NZ
81	Durex	Love	1000033421	NZ	Condom	Silicone	Non analysed	-	Analysed NZ
82	Durex	Close Fit	1000065555	NZ	Condom	Silicone	Non analysed	-	Analysed NZ
83	Durex	Real Feel	1000243647	NZ	Condom	Silicone	Non analysed	-	Analysed NZ
84	Four Seasons	Naked – Pink Strawberry	X27150902	NZ	Condom	Silicone	Non analysed	-	Analysed NZ
85	Four Seasons	Naked Bubble gum	X31150901	NZ	Condom	Silicone	Non analysed	-	Analysed NZ
86	Four Seasons	Naked-Chocolate	X26150903	NZ	Condom	Silicone	Non analysed	-	Analysed NZ

87	Four Seasons	Naked – Banana Yellow	X33150902	NZ	Condom	Silicone	Non analysed	-	Analysed NZ
88	LELO HEX	Original	X35160502	NZ	Condom	Silicone	Non analysed	-	Analysed NZ
89	Four Seasons	Extra Strength	X31141101	NZ	Condom	Silicone	Non analysed	-	Analysed NZ
90	Four Seasons	Glow	16N3253	NZ	Condom	Silicone	Non analysed	-	Analysed NZ
91	Sir Richard's	Ultra-Thin	13N3983	NZ	Condom	Silicone	Non analysed	-	Analysed NZ
92	GLYDE	Vanilla	PV27711	NZ	Condom	Silicone	Non analysed	-	Analysed NZ
93	GLYDE	Strawberry	PS21131	NZ	Condom	Silicone	Non analysed	-	Analysed NZ
94	GLYDE	Wild Berry	PW25701	NZ	Condom	Silicone	Non analysed	-	Analysed NZ
95	GLYDE	SuperMAX	PN26161	NZ	Condom	Silicone	Non analysed	-	Analysed NZ
96	GLYDE	Blueberry	BB23801	NZ	Condom	Silicone	Non analysed	-	Analysed NZ
97	GLYDE	Cola	BL28311	NZ	Condom	Silicone	Non analysed	-	Analysed NZ
98	GLYDE	Ultra	PN34381	NZ	Condom	Silicone	Non analysed	-	Analysed NZ
99	GLYDE	Maxi	PN32503	NZ	Condom	Silicone	Non analysed	-	Analysed NZ
100	GLYDE	SlimFit	PN31564	NZ	Condom	Silicone	Non analysed	-	Analysed NZ
108	Wet Stuff	Gold Water based personal lubricant	9D729	AUS	Lubricant	Water based	-	PPG, Glycerin	
109	Ansell	LifeStyles Luxe Silicone-based lubricant	19051507DR	AUS	Lubricant	Silicone	Silicone degradation; D3-D6 detected; absence of minor peaks	Non analysed (non soluble in methanol)	
110	Ansell	Manix Contact	1606380216	CH	Condom	Silicone	Silicone degradation; D3-D13 detected	-	
111	Ansell	Manix Natural	1601012016	CH	Condom	Silicone	Silicone degradation; D3-D13 detected	-	
112	Ansell	Manix Orgazmax Plus	1507200316	CH	Condom	Water based + silicone traces	Silicone degradation; D3-D8 detected	PPG	

113	Ansell	Manix Endurance	1409151416	CH	Condom	Water based + silicone traces	Silicone degradation; D3-D9 detected	PPG
114	Ansell	Manix Fraise Gourmande	1408211216	CH	Condom	Silicone	Silicone degradation; D3-D10 detected	-
115	Ansell	Manix Xtra Pleasure	1411082316	CH	Condom	Silicone	Silicone degradation; D3-D9 detected	-
116	FairSquared	Sensitive dry	0953BA14228	CH	Condom	Dry condom (no profile) + silicone traces	No Chemical Profile detected	-
117	FairSquared	Original	0796IF14180	CH	Condom	Silicone	Silicone degradation; D3-D10 detected	-
118	FairSquared	Max Perform	1491PF13371	CH	Condom	Silicone	Silicone degradation; D3-D8 detected	-
119	Migros	M-Budget	IUR-010	CH	Condom	Silicone	Silicone degradation; D3-D13 detected	-
120	Migros	Cosano Regular	OUR-097	CH	Condom	Silicone	Silicone degradation; D3-D11 detected	-
121	Migros	Cosano Sensual	OUR-176	CH	Condom	Silicone	Silicone degradation; D3-D12 detected	-
122	Migros	Cosano Feeling 0.05mm	OUR-149	CH	Condom	Silicone	Silicone degradation; D3-D12 detected	-
123	Coop	Prix Garantie	1594ZF16451	CH	Condom	Silicone	Silicone degradation; D3-D9 detected	-
124	Ceylor	Gold	160831P	CH	Condom	Water based, silicones traces	D3- D4 detected.	Glycerin, PEG (traces), nonoxynol-9
125	Ceylor	Thin Sensation	1626B2S	CH	Condom	Silicone	Silicone degradation; D3-D12 detected	-
126	Ceylor	Non-Latex UltraThin	660801U	CH	Condom	Silicone	Silicone degradation; D3-D10 detected	-
127	Ceylor	Strawberry	153332P	CH	Condom	Silicone	Silicone degradation; D3-D12 detected	-
129	Durex	Performa	1000176796	CH	Condom	Silicone	*Duplica Sample 79*	-
130	Durex	Strawberry	1000271385	CH	Condom	Silicone	Silicone degradation; D3-D13 detected	-
131	GLYDE	Silver Lubricated	PN34261	AUS	Condom	Silicone	Non analysed	-

132	GLYDE	Condom 53mm Premier Water Based Lubricant	(B) 51711	AUS	Lubricant	Water based	-	No sample left over
133	Ansell	SKYN Maximum Performance Lubricant	19051103JP	AUS	Lubricant	Silicone	Silicone degradation; D3-D7 detected, absence of minor peaks	Non analysed (non soluble in methanol)
134	Durex	Play Massage 2 in 1	447X1	AUS	Lubricant	Water based	-	PPG
135	Four Seasons	Lush Lubricant with Aloe Vera	9B722	AUS	Lubricant	Water based	-	PPG, Glycerin
136	Four Seasons	Nature Lubricant Vegan Friendly	9A716	AUS	Lubricant	Water based	-	PPG
137	Durex	KY Jelly	488X2	AUS	Lubricant	Water based	-	Glycerin
138	Four Seasons	Massage Oil	BG668	AUS	Massage oil	Oil based	-	Pentadecanoic acid 14-m,ethyl methylester; 8,11-octadecadienoic acid methyl wster
139	Durex	Play Feel, Pleasure Gel	213X4	AUS	Lubricant	Water based	-	
140	Ansell	LifeStyles Silky Smooth Water Based Lubricant	19042006	AUS	Lubricant	Water based	-	Glycerin, 2-Phenoxyethanol
141	Durex	Naturals Intimate Gel	919Y3	AUS	Lubricant	Water based	-	PPG, Glycerin
142	Astroglide	Naturally Derived Liquide	A011874	AUS	Lubricant	Water based	-	2-phenoxyethanol, Xylitol
154	Multi-Gyn	Active Gel	1033164	AUS	Intimate products	Water based	-	Glycerin, Octanediol

155	Summer's eve	Feminine Wash Sensitive Skin	0217H0161	AUS	Intimate products	Water based	-	Alcane patterns, PEG (traces), others
156	Femfresh	Feminine deodorant spray	(B)1079351	AUS	Intimate products	Silicone	No clear pattern. May be due to the gaz form.	Non analysed (impossible to solubilise)
157	Femfresh	Daily Intimate Wash	101702163	AUS	Intimate products	Water based	-	Triethylcitrate, alcane patterns
158	Vagisil	Soothing Oatmeal Cream	Z19D126	AUS	Creams	Oil based + silicone traces	Traces of D4	Phenoxyethanol, Octanediol, Cetene, Heptadecanal,
159	Canesten	Intimate Discomfort Cool Cream Gel	GPO1KPL	AUS	Creams	Water based + oil based	-	Pentanediol, Hexanediol, Glycerin, Oxalic Acid, Alcane patterns
160	Vagisil	Intimate Wash Fresh Plus	B91653	AUS	Intimate products	Water based	-	Glycerin, Benzoic Acid, Alcane patterns, Others
161	Dermeze	Moisturising Cream	B15866	AUS	Creams	Water based + oil traces	-	Glycerin, Phenoxyethanol, Octadecanoic acid ocyl ester, Tetradecene, Hexadecene, Cyclododecane
162	Essentials	Barrier Cream	1081235	AUS	Creams	Water based + oil traces	-	Glycerin, Phenoxyethanol, Hexadecene, Cyclohexadecane, Glycerol-1-palmitate
163	Rosken	Intensive Moisture Hand Cream	B14519	AUS	Creams	Water based + oil traces	-	Glycerin, Cetene, Octadecene
164	Love My Ink	Tattoo Cream	30789	AUS	Creams	Water based + oil traces	-	Glycerin, Phenoxyethanol, Octadecanoicacidocylester, Tetradecene, Cyclotetradecane, Cyclododecane, Octadecene
165	Sasmar	Classic Personal Lubricant	160271C	AUS	Lubricant	Water based	-	PPG, Glycerin
166	Sensuous	Smooth & Warming Lubricant	8433821	AUS	Lubricant	Water based	-	2-phenoxyethanol, oxybisoctane

167	Sensuous	Frenzy Extreme Pleasure Gel for Women	870	AUS	Lubricant	Water based	-	2-phenoxyethanol, oxybisoctane
168	Ansell	Skyn Intimate Moments	19031301DR	AUS	Lubricant	Water based + Silicone traces	Silicone degradation; D3-D10 detected	PPG, Octanediol
169	Ansell	Skyn Intense Feel Non-Latex Condoms	1905013316	AUS	Condom	Silicone	Silicone degradation; D3-D8 detected	-
170	Pjur Med	Vegan Glide Intimate Personal Lubricant	32965-01	AUS	Lubricant	Water based	-	PPG, 2- oxybispropanol,
171	Astroglide	Diamond Silicone Gel Personal Lubricant	A011819	AUS	Lubricant	Silicone	Silicone degradation; D3-D6 detected; 8 major peaks between 14 and 28 minutes	Non analysed (non soluble in methanol)
172	Astroglide	Gel Personal Lubricant	A011395	AUS	Lubricant	Water based + Silicone traces	Slight detection of siloxanes patterns, only D3-D7, no minor compounds	Glycerin
173	Astroglide	Personal Lubricant and Massage Oil	A010316	AUS	Lubricant	Oil based	-	methyltetradecanoate, pentadecanoic acid, methylstearate, glycerol-1-palmitate, octadecenol, octadecenoic acid, hexadecanoic acid
174	Astroglide	Waterproof Silicone Liquid	A011415	AUS	Lubricant	Silicone	Silicone degradation; D3-D6 detected; absence of minor peaks	Non analysed (non soluble in methanol)
175	Astroglide	Warming Liquid Personal Lubricant	A011326	AUS	Lubricant	Water based	-	PPG, Glycerin
176	Astroglide	Water based Personal lubricant	A011494	AUS	Lubricant	Water based	-	PPG, Glycerin
177	Astroglide	Strawberry Liquid Personal Lubricant	A011452	AUS	Lubricant	Water based + Silicone traces	Slight detection of siloxanes patterns, only D3-D7, no minor compounds	PPG, Glycerin

178	Four Seasons	Naked Black Condom	Z07180301	AUS	Condom	Silicone	Silicone degradation; D3-D10 detected	-	
179	Four Seasons	Stubbed & ribbed Stimulating condoms	18N0363	AUS	Condom	Silicone	Silicone degradation; D3-D11 detected	-	
180	Checkmate	ExtraSensitive Lubricated Condoms	1809122816	AUS	Condom	Silicone	Silicone degradation; D3-D10 detected	-	
181	Durex	Play Perfect Glide	541X1	AUS	Lubricant	Silicone	Silicone degradation; D3-D10 detected	Non analysed (non soluble in methanol)	Indistinguishable from condom patterns
182	Durex	Climax Stimulating Gel	2231W7	AUS	Lubricant	Water based	-	PPG, Glycerin	
183	Durex	Comfort XL	1000417937	AUS	Condom	Silicone	Non analysed	-	
184	Ansell	Lifestyles ribbed condoms	18009812116	AUS	Condom	Silicone	Silicone degradation; D3-D10 detected	-	

Appendix IV: Ethic protocol

Change history

Version Nr	Version date	Modified without version change	Description, comments	Control
1.1	2018-02-26		Initial version	CB
1.2	2018-03-03		Modification contexte et justification	CB
1.3	2018-03-05		Modification project design	CB
1.4	2018-03-31		Corrections orthographes + modification hypothèses + remarques diverses (contenu forensique)	GM
1.5	2018-04-04		Corrections des remarques après contact avec GM + compléter le résumé du projet (HRO Annexe 2)	CB
1.6	2018-05-14		Corrections selon retour de la CER-VD	CB
1.7	2018-09-25		Corrections selon indications CER-VD	CB

Analyse des traces de préservatifs dans les cas de pénétration vaginale

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Législation:	Ordonnance relative à la recherche sur l'être humain à l'exception des essais cliniques (HRO) [1].
Type de projet :	Projet de recherche impliquant des sujets humains
Catégorie de risque:	A
Responsable du projet:	**Professeure Geneviève Massonnet** Ecole des Sciences criminelles Quartier UNIL-Sorge Bâtiment Batochime CH-1015 Lausanne Phone : 021 692 46 16 Mail: Genevieve.Massonnet@unil.ch
Condition de santé :	Aucun problème médical. Etude des traces de préservatifs après des rapports protégés chez des participantes.
Durée du projet :	42 mois
Version du plan et date :	Version 1.7, 29.05.2018

FORMULAIRE DE SIGNATURE DU PROTOCOLE

Titre de l'Etude	*Analyse des traces de préservatifs dans les cas de pénétration vaginale*

La responsable du projet a approuvé la version du protocole **1.6 (datée du 14.05.2018)** et confirme par la présente que le projet doit être réalisé conformément au protocole, aux exigences légales suisses [219,220], version actuelle de la déclaration de l'Association médicale mondiale d'Helsinki [221] et les principes de la bonne pratique clinique.

Responsable du projet:

Professeure Geneviève MASSONNET

Lausanne,	Signature

Investigatrice du projet:

MSc. Céline BURNIER

Lausanne,	Signature

Sommaire

GLOSSARY OF ABBREVATIONS

BASEC	*Business Administration System for Ethical Committees*
CRF	*Case report form*
FOPH	*Federal Office of Public Health*
HRA	*Human Research Act*
HRO	*Ordinance on Human*
Py	*Pyrolyse*
FTIR	*Infrarouge à transformée de Fourier*

SYNOPSIS (RESUME)

Project Leader	Prof. Geneviève Massonnet, Ecole des sciences criminelles, Bâtiment Batochime, Université de Lausanne, CH- 1015 Lausanne, Tel. 0041 21 692 4616, genevieve.massonnet@unil.ch
Titre du projet	*Analyse de traces de préservatifs dans les cas de penetration vaginale*
Identifiant du projet	Projet de Doctorat en Sciences Forensiques
Version du projet et date	*Version 1.7, 25.09.2018*
Catégorie de risque	*Categorie A*
Type de recherche:	Ce projet de recherche vise à mettre au point une méthodologie permettant l'analyse de traces de préservatifs. Ensuite, il vise à étudier les paramètres forensiques de bruit de fond, transfert et persistance ainsi que les facteurs qui les affectent. Les écouvillons de prélèvement ainsi que les informations du donneur seront libellés avec un code alphanumérique assigné à chaque participant.
Design d'étude:	Le design de cette étude implique le développement d'une méthodologie analytique (FTIR- py-GC/MS) pour la détection et la caractérisation des traces de préservatifs. Cette méthodologie sera ensuite appliquée pour une analyse quantitative ou semi-quantitative de traces dans une matrice vaginale, obtenues à l'aide de volontaires. Les données résultantes seront analysées à l'aide de statistiques multivariées pour investiguer les variations des échantillons dus aux facteurs affectant les traces. Parallèlement, les résultats seront utilisés afin de modéliser, sur la base d'une statistique bayésienne, les paramètres de bruit de fond, transfert et persistance, afin de permettre l'interprétation de l'indice scientifique au tribunal.

Contexte:	Les traces de préservatifs sont une trace retrouvée de plus en plus dans les cas de viols ou d'agressions sexuelles, mais elles ne sont généralement pas ou peu exploitées. Ces traces servent comme preuve associative et peuvent permettre d'expliquer le déroulement d'une action et ainsi confirmer ou infirmer le témoignage des parties en cause. Les travaux forensiques actuels sur ces traces se sont principalement attelés à mettre en place une méthode analytique pour l'analyse de ces traces, souvent à l'aide de standards de références. Or, la mise en application dans des cas réels implique de considérer une matrice, i.e. la matrice vaginale, ainsi que tous les facteurs de variations qu'elle engendre. Ceci a très peu été étudié. De plus, la détection des traces de préservatifs dépend de nombreux paramètres tels que le transfert, l'activité de la victime ou encore le mode de prélèvement. Ainsi, certaines lacunes restent dans la compréhension actuelle des traces de préservatifs et de leur interprétation. Des données plus fondamentales sont nécessaires du point de vue tant qualitatif que quantitatif sur les traces de préservatifs ainsi que leur transfert, leur persistance ou encore les facteurs influençant leur persistance au cours du temps.
Objectif(s):	L'objectif principal de cette recherche est d'extraire et des détecter, par FTIR et py-GC/MS les traces de préservatifs provenant de prélèvement vaginaux. Ensuite, il s'agira de modéliser les paramètres forensiques tels que le bruit de fond, le transfert et la persistance
But(s):	Le but final de cette recherche est de mieux comprendre les principes de bruit de fond, transfert et persistance et les variations qui les affectent. Ceci permettra la création d'un modèle bayésien pour l'interprétation des résultats lorsque des cas réels dans lesquels des traces de préservatifs seront retrouvées et devront être évaluées en tant que preuve au tribunal.

Critères d'inclusion / exclusion	Les critères d'inclusion et d'exclusion de l'étude sont les suivants: *Critères d'inclusion* • Age : de 18 à 35 ans • Bonne santé habituelle • Pas de maladie gynécologique pouvant entraver les prélèvements *Critères d'exclusion* • Allergie aux préservatifs • Absence de contraception efficace • Grossesse • Incapacité de comprendre/suivre le protocole • Refus de signer le formulaire de consentement • Problèmes linguistiques limitant la compréhension
Procédure du projet:	Des prélèvements vaginaux dans une population donnée ainsi que après un rapport protégé seront collectés. Les composés du préservatif seront extraits et analysés par FTIR et py-GC/MS. Ensuite, des statistiques multivariées seront utilisées pour étudier la variation des échantillons et la reproductibilité des analyses en fonction des quantités relative des composés. Ces résultats seront également utilisés pour modéliser, à l'aide d'une statistique bayésienne, les paramètres de transfert, persistance et bruit de fond. A cet effet, les informations des participantes (comme l'activité, la période du cycle menstruel ou encore leur type de contraception) pourront être utilisées pour aider à l'interprétation des variations ou de groupements observés.
Nombre de participants	*Nous aimerions collecter des échantillons sur 100 participantes pour l'étude de bruit de fond et 50 participantes pour l'étude de transfert et de persistance. Ces nombres permettent de créer une population de participant assez large par rapport à ceux décrit dans la littérature et permettent une investigation préliminaire des facteurs d'influence ainsi qu'une modélisation statistique adéquate. Le nombre est choisi au vu des contraintes de temps et peut être diminué si la représentabilité des résultats est statistiquement atteinte.*
Durée du Projet	Le projet de recherche est planifié sur une durée de 42 mois, à partir du 1er juin 2018
Lieu de réalisation:	Ecole des Sciences Criminelles, Université de Lausanne (UNIL)

Considérations statistiques	*La complexité des résultats obtenus nécessiteront l'utilisation de la chimiométrie pour extraire l'information des spectres et des chromatogrammes obtenus.* Les traitements statistiques seront réalisés sur les spectres infrarouges ainsi que sur les pyrogrammes obtenus en py-GC/MS dans le but d'optimiser les méthodes, sélectionner les variables appropriées puis d'étudier les données. Ensuite, une statistique de type bayésienne ainsi que des traitements statistiques adéquats seront utilisés pour créer le modèle de décision et les modèles de transfert, persistance et bruit de fond.
Autres considérations méthodologiques	*n.a.*
Risques et bénéfices	Les résultats de ce projet devraient fournir une information utile en science forensique et ouvrir de nouvelles aires de recherches en ce qui concerne les traces de préservatifs et ce afin d'aider les enquêteurs et la justice. Au vu de la nature de la procédure d'échantillonnage, il y a un petit risque de blessure au moment du prélèvement. Il est prévu d'expliquer aux participantes comment réaliser les prélèvements afin d'éviter toute blessure. Toutes les précautions nécessaires seront prises pour minimiser un accès non autorisé aux documents complétés par les participantes et qui pourrait mener à leur identification par un tiers.

1 Contexte et justification du projet

Lorsqu'une enquête pour viol ou pour agression sexuelle est ouverte, la première trace recherchée est la trace ADN. En effet, s'il n'y a pas eu utilisation de préservatif, l'ADN de l'agresseur devrait être retrouvé sur la victime, en quantité variable. Le sperme est le liquide biologique le plus souvent recherché dans ce type de cas (agression d'un homme envers une femme) et les supports de traces sont des échantillons gynécologiques ainsi que d'autres supports tels que les vêtements de la victime ou d'autres objets reliés au cas. Une étude indique que sur 335 femmes examinées à la suite d'un viol, 82% d'entre elles ont du sperme dans le vagin [222]. Ainsi, toute la procédure est axée sur les cas où de l'ADN est retrouvé [223].Lorsque du sperme n'est pas détecté, bien que d'autres traces puissent aider à résoudre le cas (par exemple ADN de contact, fibres), la présence de traces de préservatifs sur les écouvillons des prélèvements vaginaux permettrait de prouver la pénétration.

L'absence d'ADN dans ce type de cas peut s'expliquer entre autres par l'utilisation d'un préservatif, bien qu'il ne s'agisse pas d'une majorité des cas. En effet, bien que de récentes études montrent une nette augmentation du nombre de cas où les préservatifs sont utilisés, les statistiques policières obtenues à Los Angeles montrent que sur 20% des cas de viols rapportés, 11 à 15% d'entre eux l'ont été avec des préservatifs. L'utilisation de préservatifs pourrait donc expliquer l'absence d'ADN et une analyse forensique de ces traces devrait être réalisée.

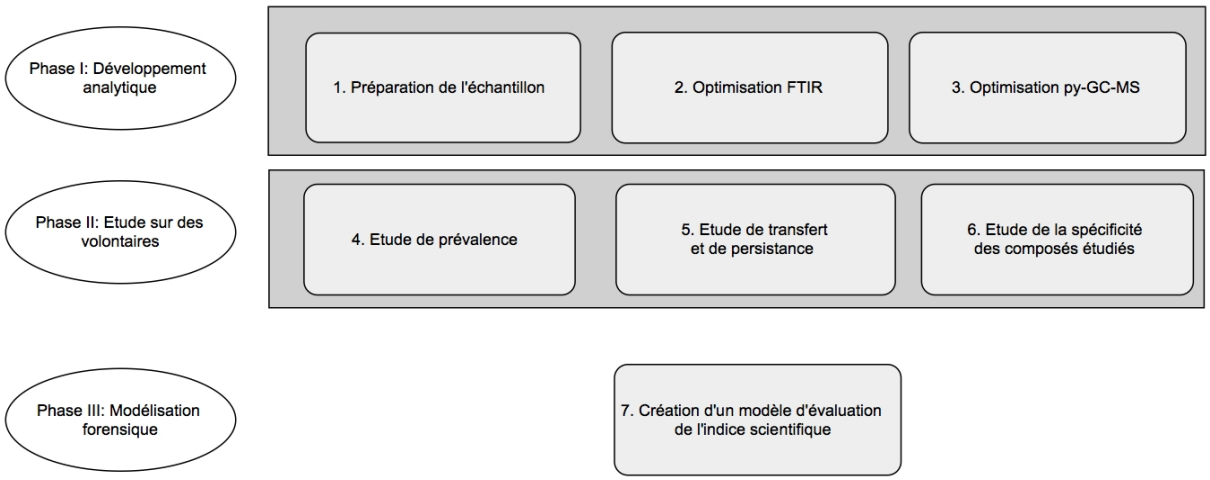
L'exploitation forensique des traces de préservatif est proposée en premier par Berkefeld comme le rapporte Blackledge [224]. Conformément au principe de Locard selon lequel tout contact laisse une trace, le préservatif laissera, dans le cas d'un viol, une trace, plus ou moins abondante, dans le vagin de la victime, mais également sur d'autres zones comme la zone externe des organes génitaux féminins. Ainsi, le préservatif peut être une preuve associative pour établir le *corpus delicti* et vérifier la pénétration [225–227]. La trace de préservatif peut également être utilisée comme une preuve pour déterminer la validation des arguments de chacune des parties : ce n'est pas l'acte sexuel mais son déroulement qui est remis en cause [228].

La littérature et les protocoles analytiques sont multiples et aussi divers que le panel analytique à disposition. La plupart de ces études ont été réalisées aux Etats-Unis ou en Australie. En Europe, quelques recherches ont été conduites en Angleterre ou en Italie. Ce sujet ne semble à ma connaissance pas avoir été l'objet d'études conduites en Suisse. De plus, l'interprétation du résultat est parfois complexe. Si le résultat est positif, cela signifie qu'il y a eu un contact avec un produit contenant ce type de molécule. Il est nécessaire de prendre des précautions quant à l'affirmation selon laquelle un préservatif aurait été utilisé puisque d'autres produits peuvent contenir les molécules ciblées dans le cadre des préservatifs [229]. Des faux positifs ne peuvent par ailleurs être exclus. Il est généralement difficile de tirer des conclusions d'un résultat négatif en science forensique car le résultat négatif peut provenir de l'absence de l'échantillon mais également d'un manque de sensibilité de la méthode ou de prélèvements inadéquats par exemple. Ainsi, si la victime n'a pas eu d'activité pouvant générer la perte des traces de préservatifs et que le PDMS n'est pas détecté par la méthode, cela signifie qu'il n'y a pas eu le port de préservatif. Toutefois, pour Coyle et Anwar (2009), trop de facteurs peuvent influencer le résultats négatifs comme le temps écoulé entre l'acte et le prélèvement, les techniques de prélèvements et les variations inhérentes aux kits de prélèvements [229].
La littérature s'est limitée à une évaluation qualitative du résultat sur peu d'échantillons réels et atteste la présence d'un préservatif si certains pics caractéristiques des préservatifs sont présents dans les divers résultats d'analyses, sans évaluer la pertinence de ces pics ni leur spécificité. Peu d'auteurs ont essayé de vérifier leurs résultats avec des standards analytiques. De plus, l'éventualité de l'usage d'autres produits pouvant contenir les mêmes composés que ceux ciblés dans les diverses études n'a que peu été relevée [228,230–232]. La spécificité des composés cibles ainsi que les possibilités de faux positifs et de faux négatifs n'ont donc pas été entièrement étudiées. De plus, aucune évaluation des résultats analytiques par une approche logique, l'approche du rapport

de vraisemblance (LR) [233], mettant en concurrence au moins deux propositions exclusives, ne semble avoir été étudiée. Il s'agit pourtant d'une approche reconnue en sciences forensiques [234].

La présente étude vise la mise en place d'une méthodologie permettant la détection, l'analyse chimique et l'exploitation des traces de préservatifs dans les cas de pénétration vaginale. Une analyse chimique du prélèvement vaginal pourra être utilisée lorsque les autres traces font défaut (par exemple la trace ADN) mais également comme technique supplémentaire pour aider à confirmer ou infirmer le lien établi (par exemple dans le cas de la rupture du préservatif) entre la victime et son agresseur, ainsi que les témoignages de chacune des parties.

Le but de ce travail est donc de développer une approche forensique qui permette d'identifier une trace de préservatifs sur un support de trace (i.e. les écouvillons de prélèvements de la médecine légale). L'approche développée doit être simple et rapide à mettre en œuvre, être applicable en routine tout en garantissant un minimum de faux positifs et de faux négatifs. Une fois l'analyse réalisée, l'interprétation du résultat analytique devra être exploitable au tribunal. Ainsi une approche bayésienne considérant le bruit de fond, le transfert et la persistance des traces de préservatifs devra être développée. La recherche va se dérouler comme présenté sur l'image ci-dessous.



2 objectifs et conception du projet

2.1 Objectifs

Cette étude est une étude exploratoire car il n'existe à notre connaissance aucune étude de ce type. Il nous est donc difficile de déterminer le pouvoir de l'étude et nous pensons qu'une étude préliminaires est nécessaire pour définir le nombre de cas en vue d'autres études prospectives et cliniques.

L'objectif principal de cette recherche est d'obtenir de plus amples connaissances sur les traces de préservatifs, leur variabilité et la possibilité de les analyser par infrarouge (FTIR) et pyrolyse –GC/MS après un temps donné. Cette recherche se focalise principalement sur la modélisation de paramètres forensiques tels que le bruit de fond, le transfert et la persistance.

Dans un premier temps, la méthodologie analytique sera développée sur la base d'études préexistantes, dans le but d'obtenir une séquence simple et qui permette d'obtenir un résultat analytique de qualité.

Une fois la méthodologie analytique mise en place, quatre objectifs majeurs pourront être étudiés :

- *Objectif 1* : Etudier la prévalence (c'est à dire le bruit de fond) du PDMS dans une population donnée et en fonction de leurs habitudes.

- *Objectif 2* : Etudier les paramètres de transfert (c'est à dire directement après un rapport protégé) et de persistance (c'est à dire à des temps post-coïtaux pouvant aller jusqu'à 72 heures) des traces de préservatifs dans la matrice vaginale.
- *Objectif 3* : Etudier les paramètres influençant le transfert et la persistance des traces de préservatifs dans la matrice vaginale (par exemple la période du cycle menstruel ou l'activité de la personne)
- *Objectif 4* : Etudier la spécificité des composés provenant des préservatifs face à l'utilisation d'autres produits pouvant contenir des composés analogues (par exemple des lubrifiants intimes).

2.2 But premier de la recherche

Le but final de cette recherche est de mieux comprendre les principes de bruit de fond, transfert et persistance et les variations qui les affectent. Ceci permettra la création d'un modèle bayésien pour l'interprétation des résultats lorsque des cas réels dans lesquels des traces de préservatifs seront retrouvées et devront être évaluées en tant que preuve au tribunal.

2.3 Conception du projet

Ce projet est conçu pour le développement d'une méthodologie pour la détection des traces de préservatifs dans une matrice vaginale combinant une analyse infrarouge (FTIR) et une analyse par pyrolyse-GC/MS, ainsi qu'une modélisation semi-quantitative des paramètres de bruit de fond, transfert et persistance des composés ciblés dans la matrice. Le développement des méthodes FTIR et py-GC/MS ainsi que les analyses subséquentes seront réalisées à l'Ecole des Sciences Criminelles. La récolte d'échantillons sera réalisée par des volontaires recrutés au sein de population majoritairement de l'UNIL.

Les échantillons consistent en des prélèvements vaginaux réalisés à l'aide d'écouvillons en coton et prélevés après un rapport protégé chez des volontaires en couple. Les participantes prélèveront elle-même les échantillons chez elles, bien que cela implique une variation dans l'échantillonnage. Il est en effet reconnu que la modélisation forensique représentative de la réalité passe par la réalisation d'analyse à l'aide de volontaires. De plus, l'utilisation de simulants vaginaux ne permettent pas de prendre en considération les variations dues à la population étudiée et ne sont pas représentatifs de la matrice vaginale et de son évolutivité. C'est pourquoi il nous est nécessaire de travailler avec des participants humains vivants.

Les méthodes FTIR et py-GC/MS pour la séparation et la détection des composés ciblés seront d'abord développées à l'ESC, sur des standards de référence. Les traces seront toute collectées sur le même type d'écouvillons que ceux utilisés par les hôpitaux dans les kits d'agressions sexuelles. Une méthode d'extraction simple sera développée pour isoler la fraction apolaire, contenant du PDMS, pour l'analyse en FTIR et py-GC/MS, tout en étant compatible avec les contraintes imposées par les deux méthodes. Au vu des potentiels résidus de cellules dans les extraits, d'autres étapes pourront être envisagées tels que la filtration ou une seconde extraction de type liquide –liquide afin de limiter les contaminants et les interférences liées à la matrice. L'identification des composés cibles sera réalisée par comparaison visuelle avec des standards de référence et les bases de données NIST. Les protocoles seront établis pour monitorer et évaluer la fiabilité de la méthode analytique.

Pour atteindre l'objectif principal de ce projet, nous souhaitons collecter des échantillons provenant d'une population plutôt large, en comparaison de ce qui a été déjà décrit dans la littérature. Les échantillons pour l'étude de prévalence seront collectés dans une population de 100 participantes et pour l'étude de transfert/persistance dans une population de 50 participantes, principalement au sein d'étudiants et de membres de l'Université de Lausanne. Bien que de plus grands nombres de participants soient requis pour obtenir des données représentatives et statistiques, il est également reconnu qu'il est nécessaire de faire un compromis pratique considérant le design expérimental. 3 réplicas seront demandés lors de chaque prélèvement, sur une période d'un mois, ce qui permet

d'étudier les effets d'intravariabilité (reproductibilité des échantillons obtenus par une même participante) et d'intervariabilité (variation des traces obtenues par des participantes différentes). La collecte d'échantillon sera accompagnée d'un questionnaire sur des facteurs tels que l'âge, le dernier jour des règles, le contraceptif utilisé et l'activité entre le rapport et le prélèvement (par exemple douche, toilettes, sport). Comme il y a des risques que l'obtention d'échantillons d'une population assez grande soit compliquée, nous estimons que la collecte d'échantillon et l'analyse se déroule sur toute la durée du projet.

3 population du projet et procedure d'Etude

3.1 Population du projet, critères d'inclusion et d'exclusion

Cent cinquante femmes avec un partenaire stable depuis au moins 4 semaines et prenant la pilule ou un contraceptif autre que le préservatif seront enrôlées soit :

- 100 femmes pour l'étude de prévalence
- 50 femmes pour l'étude de transfert et persistance.

Les critères d'inclusion et d'exclusion de l'étude sont les suivants:

Critères d'inclusion

- Age : de 18 à 35 ans
- Bonne santé habituelle
- Pas de maladie gynécologique pouvant entraver les prélèvements

Critères d'exclusion

- Allergie aux préservatifs
- Absence de contraception efficace
- Grossesse
- Incapacité de comprendre/suivre le protocole
- Refus de signer le formulaire de consentement
- Problèmes linguistiques limitant la compréhension
- Etudiantes de l'Ecole des Sciences Criminelles, ayant un rapport de hiérarchie avec les investigatrices du projet.

3.2 Mode de recrutement et procédure de consentement éclairé

En ce qui concerne le recrutement des volontaires pour l'étude de prévalence, nous prévoyons de contacter les femmes en passant par leur gynécologue lors de leur pratique clinique. Des kits contenant une feuille explicative de l'étude, un formulaire d'activité à remplir par la participante (voir annexe), des écouvillons stériles scellés et une enveloppe de retour (affranchie et adressée) seront distribués. Les femmes sont libres de retourner ou non le kit. Nous admettons un consentement implicite si elles retournent le kit.

En ce qui concerne le recrutement des volontaires pour l'étude de transfert et de persistance, nous prévoyons de contacter les femmes dans une population locale ciblée (étudiants et personnel de l'UNIL) ainsi qu'une procédure de recrutement par affiche sur le site de l'UNIL. Les participantes s'annonceront par email et/ou en personne à la Prof. Massonnet et/ou MSc. Céline Burnier.

Les participantes potentielles seront informées verbalement et par écrit des objectifs de la recherche, ainsi que de leurs droits en matière de confidentialité et de leur droit de retirer leur consentement à n'importe quelle étape du projet. Si elles acceptent de participer à l'étude, toutes les participantes devront signer un formulaire de consentement avant que toute collecte d'échantillon puisse être effectuée. Les participantes auront également l'occasion de poser des questions concernant le projet à ce moment. Les participantes recevront les coordonnées du ou des chercheurs impliqués dans le projet s'ils souhaitent poser des questions ou demander à se retirer du projet et faire détruire leurs échantillons, données et documents associés.

Aucune procédure de screening n'est nécessaire pour ce projet.

3.3 Procédure d'étude

La durée du projet pour chaque participante est une période de 1 mois au maximum, car seulement deux séries de prélèvements seront réalisées par chaque participante.
En ce qui concerne l'étude de prévalence, la période de collection nécessite le temps du prélèvement et le renvoi de l'enveloppe réponse.
En ce qui concerne l'étude de transfert et de persistance, la première période de collecte peut prendre du temps pour obtenir le consentement des volontaires avant la réalisation des prélèvements (p.ex. nécessité d'en parler avec son partenaire). Chaque participante recevra deux kits de prélèvements scellés contenant un préservatif, 6 écouvillons de prélèvement codés (serviront de réplicas pour étudier la variabilité), 1 formulaire d'explication du prélèvement, 1 questionnaire d'activité (cf. Annexe) ainsi qu'une enveloppe retour adressée et affranchie pour renvoyer les échantillons.
Nous demandons aux participantes de faire dans un premier temps 3 prélèvements avant le rapport protégé afin de s'assurer qu'il n'y ait pas de résidus de PDMS dans la matrice. Ensuite, nous demandons que les participantes aient un rapport protégé avec leur partenaire avec le préservatif fourni dans le kit. Ensuite, les participantes devront réaliser trois prélèvements à l'aide d'écouvillons et ce à des temps post-coïtus indiqués sur le kit. Les temps de prélèvements peuvent varier entre 1 heure et 48 heures après le rapport.
Afin de pouvoir étudier le maximum de paramètres, nous demandons aux volontaires de bien vouloir respecter les conditions en fonction du scénario qui leur est attribué et le temps de prélèvement demandé.

- Pas de relation sexuelle non-protégée dans les 3 jours avant le prélèvement
- Pas de relation sexuelle avec préservatif dans les 7 jours avant le prélèvement
- Pas d'utilisation de lubrifiant vaginal dans les 7 jours avant le prélèvement
- Pas de lavage vaginal avec du savon dans les 3 jours avant le prélèvement
- Pas de prélèvement en période de menstruations

Une fois les prélèvements réalisés, nous demandons aux volontaires de mettre les écouvillons fermés ainsi que les formulaires dans l'enveloppe retour adressée et affranchie et de nous renvoyer le tout par poste. Il est également possible pour les volontaires de déposer l'enveloppe dans la boîte aux lettres de l'ESC au Batochime.

Les échantillons collectés et réceptionnés par l'investigateur seront conservés au congélateur (Laboratoire BCH-6119, Ecole des Sciences Criminelles, UNIL) à -4°C avant de suivre la procédure d'extraction. La partie coton de l'écouvillon sera découpée puis déposée dans un vial de 2mL. 1mL d'hexane sera ajouté pour l'extraction puis le vial fermé sera mis au bain à ultrasons pour optimiser l'extraction. Ensuite, l'écouvillon sera retiré du vial et l'extrait restant pourra être analysé. Deux méthodes d'analyses seront utilisées :

1. Une méthode de spectroscopie infrarouge, qui sert de méthode de screening afin d'assurer qu'une trace est bien présente
2. Une méthode de chromatographie couplée à de la spectrométrie de masse, de type pyrolyse-GC/MS, qui sert de méthode de confirmation du résultat infrarouge et sur la base de laquelle des analyses statistiques pourront être réalisées.

3.4 Codage des données et retrait de l'étude

Les écouvillons de prélèvements seront codés à l'aide d'un code alphanumérique et placé dans les kits avant la distribution aux participantes, de sorte à ce que l'analyste ne connaisse pas la personne qui a rendu l'échantillon.

Chaque participante est libre de se retirer de l'étude à tout moment, sans justification.

Une participante ne respectant pas les conditions posées par l'étude peut être retirée de l'étude.

En cas de retrait de l'étude, les données et le matériel biologique (écouvillons de prélèvement) recueillis jusque-là seront tout de même analysés, ceci afin de ne pas compromettre la valeur de l'étude dans son ensemble.

4 statistiques et methodologie

4.1. Plan d'analyse statistique

Détermination de la taille de l'échantillonnage

Au vu de la nature exploratoire de ce projet, la population échantillonnée sera constituée de 150 participantes (100 pour la phase de prévalence, 50 pour la phase de transfert/persistance). Le nombre total d'échantillon constitue un set de données suffisant pour réaliser des analyses de statistiques multivariées et bayésiennes.

Traitement des données

Des prétraitements (p.ex. correction de ligne de base, normalisation, alignement de pics) des spectres infrarouge et des pyrogrammes seront appliqués avant toute analyse statistiques afin de minimiser l'intravariabilité des échantillons. Une semi-quantification relative sera utilisée puisqu'il a été démontré que la pyrolyse d'un échantillon peut produire différentes concentrations de pyrolysats et différents types de pyrolysats selon la quantité d'échantillon introduite pour l'analyse ainsi que selon la température de pyrolyse. De plus, une semi-quantification relative à la somme des aires de pics permet de prendre en considération les effets de l'analyse.

Les traitements statistiques des données infrarouges et des données chromatographiques seront réalisées à l'aide des logiciels disponibles à l'ESC (contenant R, Unscrambler X, RésolutionPro 4 et MatLab, ainsi que les logiciels des machines tels que Omnic® et Chemstation®) dans le but d'optimiser les méthodes, de sélectionner les variables appropriées et de dessiner des courbes de distribution adéquates (fitting curve) par rapport aux données récoltées.

4.2. Gestion des données manquantes

Dans le cas où une participante serait retirée du projet, toutes les données personnelles et codées appartenant à la participante ainsi que tous les échantillons non analysés seront détruits. Une participante de remplacement sera cherchée, si cela est possible et nécessaire.

5 Aspects regulatoires et de securite

5.1 Règles locales/Déclaration de Helsinki

Ce projet de recherche sera mené en accord avec le protocole, la Déclaration d'Helsinki, [3], les principes de Bonnes Pratiques Cliniques, l'amendement de recherche sur l'humain (HRA) et l'ordonnance de recherche sur l'humain (HRO) [1] ainsi que toutes les autres règles locales s'appliquant. La responsable du projet (Prof. Massonnet) et l'investigatrice du Projet (MSc. C. Burnier) reconnaissent leurs responsabilités.

5.2 Notification de sécurité et mesures de protection (HRO Art. 20)

La responsable du projet et l'investigatrice du projet sont avisées dans les plus brefs délais (dans les 24 heures) si des mesures de sécurité et de protection immédiates doivent être prises pendant la réalisation du projet de recherche. La commission d'éthique sera informée par le biais de BASEC de ces mesures et des circonstances qui les nécessitent dans un délai de 7 jours.

5.3 Evénement grave (HRO Art. 21)

Si un événement grave se produit, le projet de recherche sera interrompu et le comité d'éthique sera informé des circonstances par BASEC dans un délai de 7 jours selon HRO Art. 21[7].

[7] A serious event is defined as any adverse event where it cannot be excluded, that the event is attributable to the sampling of biological material or the collection of health-related personal data, and which:
a. requires inpatient treatment not envisaged in the protocol or extends a current hospital stay;
b. results in permanent or significant incapacity or disability; or

5.4 Radiation
Aucune source de radiation ne sera utilisée dans cette recherche.

5.5 Amendements
Des changements substantiels à la mise en place du projet, au protocole et aux documents de projet pertinents seront soumis au Comité d'éthique pour approbation conformément à HRO Art. 18 avant la mise en œuvre. Les exceptions sont des mesures qui doivent être prises immédiatement afin de protéger les participants.

5.6 Fin du projet
La commission d'éthique sera avertie de la fin du projet dans les 90 jours. A la fin des analyses, les échantillons seront détruits et aucun transfert de données et/ou d'échantillons avec d'autres laboratoires ne sera effectué.

5.7 Assurance
n.a.

6 Autres aspects

6.1 Considérations éthiques
Aucune extraction d'ADN en vue d'obtenir un profil à des fins d'identification n'est prévue et aucun processus de comparaison ne pourra être effectué par l'ESC. Nous assurons également que cela ne sera pas effectué par des tiers. De plus, nos méthodes ne permettent pas d'obtenir l'information ADN des volontaires.

6.2 Evaluation des risques et des avantages
Des données plus fondamentales sont nécessaires afin de mettre en place une stratégie d'analyse des traces de préservatifs dans un contexte forensique (e.g. victimes de viols chez lesquelles aucune trace ADN n'aurait été détectée) et sur les mécanismes de transfert, et de bruit de fond (prévalence) de ces traces dans la matrice vaginale ainsi que sur la façon dont la trace évolue avec le temps et selon différents facteurs influents. Une telle connaissance aiderait à atteindre trois objectifs clés rapportés dans la littérature forensique (par ordre d'importance et de faisabilité):
• Développement, amélioration et compréhension éclairés des méthodes d'analyse de ces traces,
• Nouvelles connaissances dans le domaine forensique en terme de bruit de fond, et évaluation des mécanismes transfert et persistance des traces,
• Évaluation de la possibilité d'évaluer la preuve scientifique au tribunal sur la base d'un modèle statistique fiable et valide.

Bien que les résultats du projet n'offrent aucun avantage direct aux participantes, les résultats de ce projet fourniront potentiellement une orientation utile pour la recherche future de ces traces chez des victimes d'agressions sexuelles ou de viol.

Les risques de grossesse en cas de rupture du préservatif sont peu importants puisque nous nous assurerons de l'utilisation d'un autre moyen de contraception chez la femme. Bien que la nature de l'échantillonnage soit invasive, les participantes réaliseront les prélèvements elle-même, avec pour consigne de ne pas se faire mal lors du prélèvement. Le risque physique devrait donc être minimal. Aucune information concernant la santé des participantes ne seront collectées. Les risques liés à l'identification des donneurs par l'accès non autorisé aux données seront minimisés en étiquetant les écouvillons et les questionnaires avec des codes alphanumériques plutôt qu'en identifiant les détails des donneurs et en limitant l'accès aux données non codées (incluant les formulaires de consentement) aux personnes directement impliqué dans le projet de recherche (Prof. Massonnet et MSc. Burnier).

7 Gestion et protection des données

7.1 Enregistrement des données et données sources
Les données spectroscopiques, chromatographiques et statistiques seront stockées sous forme de données codées.

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c. is life-threatening or results in death.

Les données non codées, telles que les formulaires de consentement, l'assignation de code et les informations personnelles des donneurs, seront conservées séparément des écouvillons de prélèvement dans un coffre verrouillé à l'École des sciences criminelles de l'UNIL et ne seront accessibles qu'aux chercheurs directement impliqués dans cette recherche (Prof. Massonnet et MSc. Burnier).

7.2 Confidentialité et encodage des données

La génération de données, la transmission, le stockage et l'analyse de données personnelles relatives à la santé et le stockage d'échantillons biologiques dans le cadre de ce projet respecteront strictement les exigences légales suisses actuelles en matière de protection des données et seront effectués conformément à l'ordonnance HRO Art. 5

Les données personnelles saisies lors de ce projet et les échantillons biologiques des participantes sont strictement confidentiels et la divulgation à des tiers est interdite ; le codage préservera la confidentialité des participants.

Les données du projet seront traitées avec la plus grande discrétion et ne sont accessibles qu'au personnel autorisé qui a besoin des données pour remplir ses fonctions dans le cadre du projet de recherche. Sur les autres documents spécifiques au projet, les participantes ne sont identifiées que par un numéro de participant unique.

Le consentement contiendra une déclaration selon laquelle l'accès direct aux documents sources sera autorisé aux fins de surveillance, d'audits ou d'inspections et indiquera qui aura accès au plan de projet, à l'ensemble de données, au code statistique, etc. pendant et après le projet de recherche (publication, diffusion).

Tous les écouvillons recueillis durant de ce projet seront conservés au congélateur avant l'extraction (de l'échantillon total) et l'analyse. Les écouvillons seront codés par l'étiquetage avec le code alphanumérique de chaque donneur. Les données dérivées de ces échantillons (données spectroscopiques, chromatographique et tests statistiques) seront également codées et pourront être consultées par tous les chercheurs directement impliqués dans ce projet.

Les écouvillons de prélèvement seront codés en attribuant un code alphanumérique à chacun. Ce code sera la seule information utilisée pour étiqueter les échantillons selon HRO Art. 25 et 26. Les données papier telles que les formulaires de consentement, l'assignation de code et les informations personnelles des donneurs seront stockées séparément des écouvillons un coffre verrouillé à l'École des sciences criminelles de l'UNIL (Prof. Massonnet) et ne seront accessibles qu'aux chercheurs directement impliqués dans ce projet (MSc. Burnier).

7.3 Conservation et destruction des données d'études et du matériel biologique

Tous les écouvillons seront dégradés pendant le projet, en raison de la nature des procédures d'extraction et d'analyse. Les données personnelles de tous les donneurs seront stockées jusqu'à 10 ans après le début du projet avant d'être détruites. Tout le matériel analysé (écouvillons, extraits) sera détruit à la fin du projet, soit 4 ans après le début du projet. Tous les résultats relatifs à l'étude et à l'information codée seront conservés pendant au moins 10 ans.

8 Publication/ Declaration d'interet / Fonds

8.1 Publication des résultats

Les résultats de cette recherche seront publiés dans des revues scientifiques pertinentes et sous forme de présentations lors de conférences, le cas échéant. Au moins deux publications dans des revues évaluées par les pairs peuvent être attendues du projet proposé, basées sur la mise au point, la méthodologie et l'application des méthodes analytiques aux traces recherchées. L'application des méthodes analytiques en conjonction avec des analyses statistiques multivariées sera présentée dans 1 ou 2 autres publications.

8.2 Partage de données

Les résultats analytiques seront publiés sans aucune référence à l'identification du donneur et le partage des données suivra la même procédure. Les données de projet codées (chromatogrammes

et données statistiques) seront accessibles au personnel autorisé directement impliqué dans ce projet (par exemple les étudiants au cours d'un travail de master).

8.3 Fonds et support

Ce projet entre dans le cadre d'une thèse réalisée à l'Ecole des Sciences Criminelles. Aucun fond externe ne soutient ce projet.

9 REFERENCES

Annexe 1 : Formulaire de consentement pour l'étude de transfert/persistance

TITRE DE L'ETUDE :

Analyse des traces de préservatifs, application aux cas de viols

Consentement éclairé écrit de la participante à une recherche scientifique

Avant d'accepter de participer à ce projet de recherche, veuillez prendre le temps de lire et de comprendre les renseignements qui suivent. Ce document vous explique le but de ce projet de recherche, ses procédures, avantages, risques et inconvénients. Nous vous invitons à poser toutes les questions que vous jugerez utiles à la personne qui vous présente ce document.

Titre de la recherche scientifique	Analyse des traces de préservatifs dans les cas de viols
Promoteur	Ecole des Sciences Criminelles
Lieu de réalisation de la recherche scientifique	Ecole des Sciences Criminelles
Investigateur principal BURNIER Céline **Directrice de thèse** Pr. MASSONNET Geneviève	
Participante Nom et prénom : Date de naissance :	

- Je déclare avoir été informée, oralement et par écrit, des objectifs et du déroulement de l'étude.
- Je certifie avoir lu et compris l'information écrite qui m'a été remise sur l'étude précitée. J'ai reçu des réponses satisfaisantes aux questions que j'ai posées en relation avec ma participation à cette recherche. Je conserve l'information écrite et reçois une copie de ma déclaration écrite de consentement.
- J'ai eu suffisamment de temps pour prendre ma décision en mon âme et conscience.
- Je sais que mes données personnelles ne seront transmises que sous forme anonyme à des institutions externes à des fins de recherche. J'accepte que les spécialistes compétents du mandataire de l'étude, des autorités et de la Commission d'éthique cantonale puissent consulter mes données brutes, afin de procéder à des examens et à des contrôles, à condition toutefois que leur confidentialité soit strictement assurée.
- Je prends part de façon volontaire à cette recherche. Je peux à tout moment et sans avoir à fournir de justification, révoquer mon consentement à participer à cette étude, sans pour cela en subir quelque inconvénient que ce soit.
- Je suis consciente du fait que les exigences et les restrictions mentionnées dans l'information aux patientes devront être respectées pendant la durée de l'étude. L'investigateur peut m'exclure à tout moment de la recherche.

Lieu, date	Signature de la participante

Annexe 2 : Description Kit étude transfert/persistence

ANALYSE DES TRACES DE PRÉSERVATIFS DANS LES CAS DE PÉNÉTRATION VAGINALE

CONDITIONS DE PRELEVEMENT :
AFIN DE POUVOIR ETUDIER LE MAXIMUM DE PARAMÈTRES, NOUS DEMANDONS AUX VOLONTAIRES DE BIEN VOULOIR RESPECTER LES CONDITIONS EN FONCTION DU SCENARIO QUI LEUR EST ATTRIBUÉ ET LE TEMPS DE PRELEVEMENT DEMANDÉ !

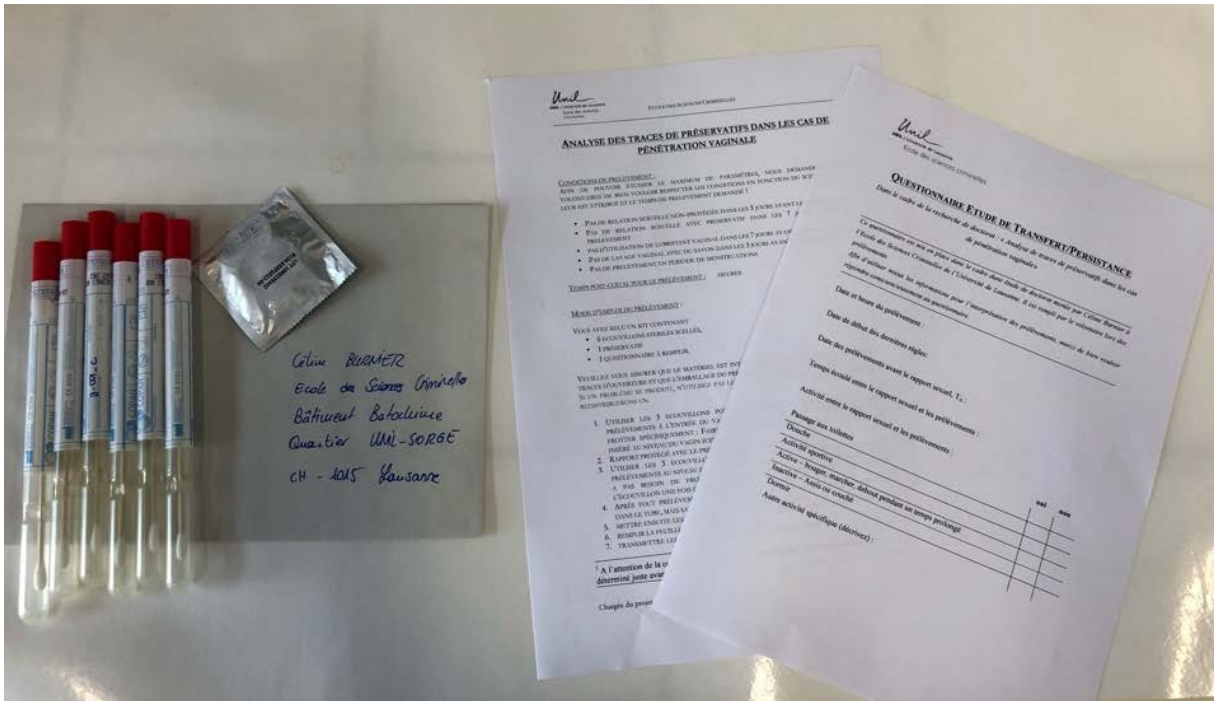
- PAS DE RELATION SEXUELLE NON-PROTÉGÉE DANS LES 3 JOURS AVANT LE PRELEVEMENT
- PAS DE RELATION SEXUELLE AVEC PRESERVATIF DANS LES 7 JOURS AVANT LE PRELEVEMENT
- PAS D'UTILISATION DE LUBRIFIANT VAGINAL DANS LES 7 JOURS AVANT LE PRELEVEMENT
- PAS DE LAVAGE VAGINAL AVEC DU SAVON DANS LES 3 JOURS AVANT LE PRELEVEMENT
- PAS DE PRELEVEMENT EN PERIODE DE MENSTRUATIONS

TEMPS POST-COITAL POUR LE PRÉLÈVEMENT : HEURES

MODE D'EMPLOI DU PRÉLÈVEMENT :

VOUS AVEZ REÇU UN KIT CONTENANT

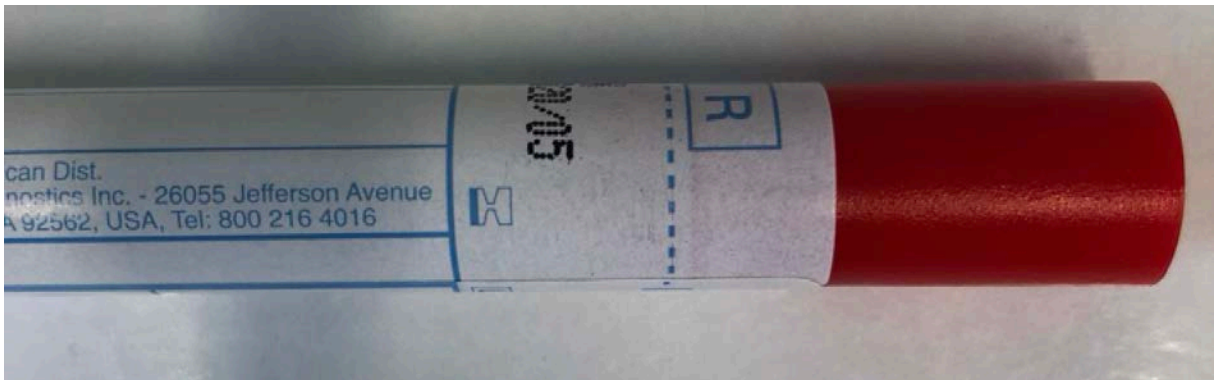
- 6 ECOUVILLONS STERILES SCELLÉS ET CODÉS,
- 1 PRÉSERVATIF
- 1 QUESTIONNAIRE À REMPLIR, PORTANT LE MÊME NUMÉRO DE CODE QUE LES ÉCOUVILLONS.
- LE PRÉSENT FORMULAIRE



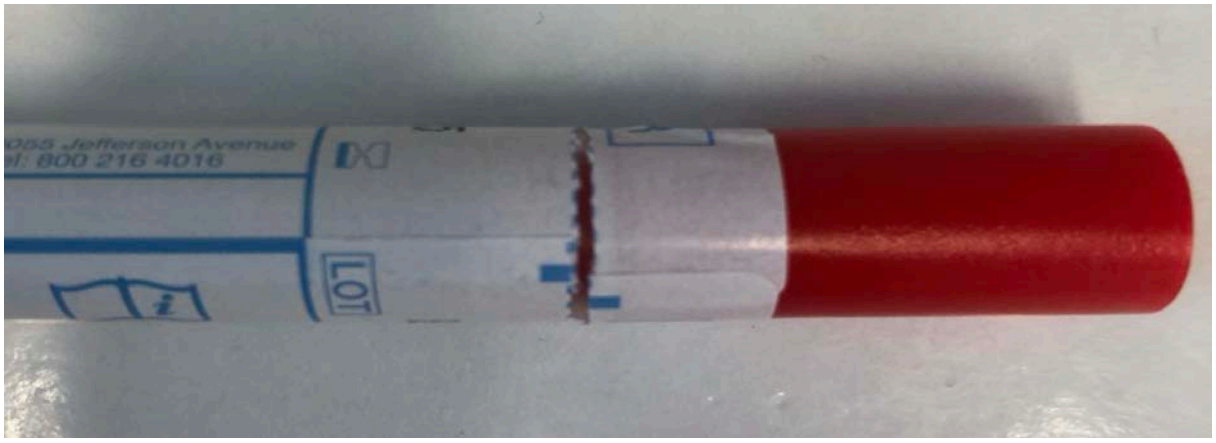
VEUILLEZ VOUS ASSURER QUE TOUT LE MATÉRIEL EST PRÉSENT ET INTACT, QUE LES ECOUVILLONS N'ONT PAS DE TRACES D'OUVERTURE (CF. IMAGES CI DESSOUS) ET QUE L'EMBALLAGE DU PRESERVATIF N'EST PAS ENDOMMAGÉ.

SI UN PROBLÈME SE PRODUIT, N'UTILISEZ PAS LE KIT MAIS RETOURNEZ LE ET NOUS VOUS EN REDISTRIBUERONS UN.

ECOUVILLON SCELLÉ ET STERILE UTILISABLE

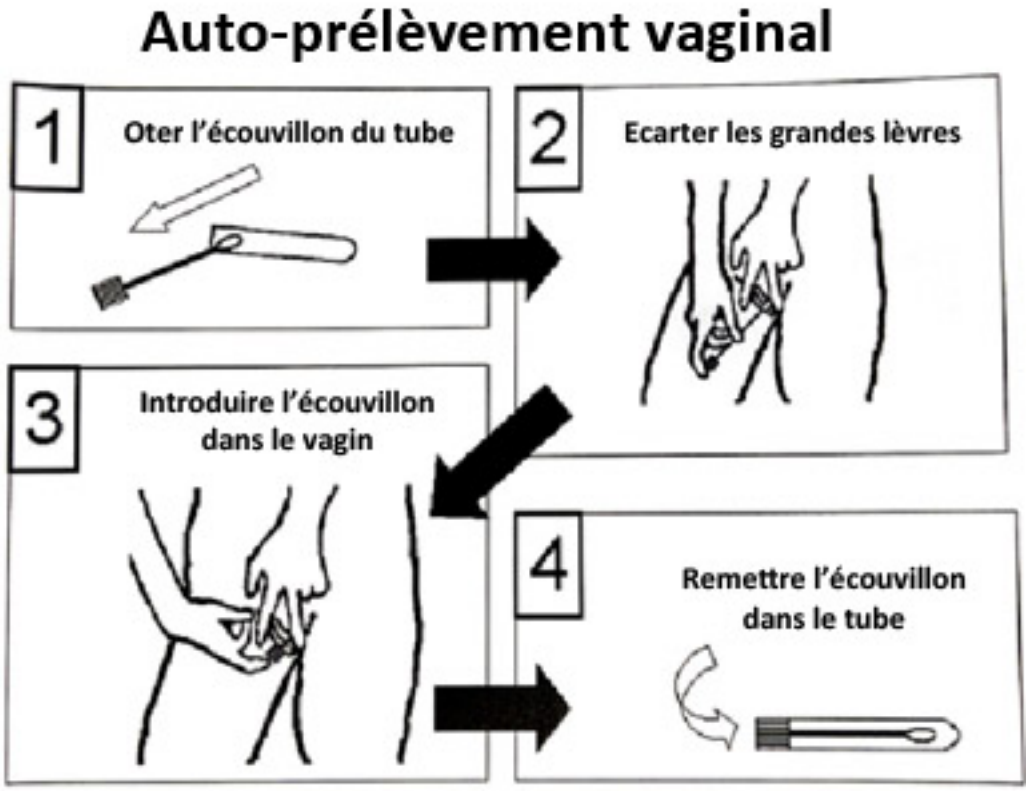


ECOUVILLON NON STERILE CAR OUVERT (TRACES D'OUVERTURE)



PRÉLÈVEMENTS

1. UTILISER LES 3 ECOUVILLONS PORTANT LA MENTION T_A POUR RÉALISER DES PRÉLÈVEMENTS À L'ENTRÉE DU VAGIN AVANT LE RAPPORT. IL N'Y A PAS BESOIN DE FROTTER SPÉCIFIQUEMENT : FAIRE TOURNER LÉGÈREMENT L'ÉCOUVILLON UNE FOIS INSÉRÉ AU NIVEAU DU VAGIN SUFFIT.



SOURCE : HTTP://WWW.CHU-NIMES.FR/MANUEL-PRELEVEMENTS/MANUEL-DU-PRELEVEUR.HTML

2. RAPPORT PROTÉGÉ AVEC LE PRESERVATIF FOURNI DANS LE KIT
3. UTILISER LES 3 ECOUVILLONS PORTANT LA MENTION T_x[8] POUR REALISER LES PRÉLÈVEMENTS AU NIVEAU DU VAGIN. L'ENTREE DU VAGIN CONVIENT TRÈS BIEN. IL N'Y A PAS BESOIN DE FROTTER SPÉCIFIQUEMENT : FAIRE TOURNER LÉGÈREMENT L'ÉCOUVILLON UNE FOIS INSÉRÉ AU NIVEAU DU VAGIN SUFFIT.

[8] A l'attention de la commission d'éthique : le x sera remplacé par le temps post-coïtal déterminé juste avant

4. APRÈS TOUT PRÉLÈVEMENT, LAISSER SECHER LES ÉCOUVILLONS EN LES REMMETANT DANS LE TUBE, MAIS SANS LE FERMER (VOIR IMAGE CI-DESSOUS) (ENVIRON UNE HEURE).

5. METTRE ENSUITE LES ÉCHANTILLONS AU REFRIGERATEUR AVANT DE LES TRANSMETTRE.
6. REMPLIR LA FEUILLE ANNEXEE AUX ECOUVILLONS.
7. TRANSMETTRE LES PRÉLÈVEMENTS DANS L'ENVELOPPE RETOUR.

Annexe 3: Questionnaire pour l'étude de transfert/persistance

QUESTIONNAIRE ETUDE DE TRANSFERT/PERSISTANCE

Dans le cadre de la recherche de doctorat : « Analyse de traces de préservatifs dans les cas d'agressions sexuelles »

Ce questionnaire est mis en place dans le cadre d'une étude de doctorat menée par Céline Burnier à l'Ecole des Sciences Criminelles de l'Université de Lausanne. Il est rempli par la volontaire lors des prélèvements. Afin d'utiliser mieux les informations pour l'interprétation des prélèvements, merci de bien vouloir répondre consciencieusement au questionnaire.

CODE :

Date et heure du prélèvement :

Date de début des dernières règles:

Date des prélèvements avant le rapport sexuel, T_A :

Temps écoulé entre le rapport sexuel et les prélèvements :

Activité entre le rapport sexuel et les prélèvements :

	oui	**non**
Passage aux toilettes		
Douche		
Activité sportive		
Active – bouger, marcher, debout pendant un temps prolongé		
Inactive – Assis ou couché		
Dormir		

Autre activité spécifique (décrivez) :

Annexe 4: Feuille d'information pour l'étude de bruit de fond (consentement implicite)

Change history

Version Nr	Version date	Valid and binding as of :	Modified without version change	Description, comments	Control
1.0	04.04.18	28.07.18		Rédaction de la première version du formulaire	CB
1.1	14.05.18			Corrections selon recommendations de la CER-VD	CB
1.2	25.09.18			Corrections selon CER-VD	CB

ANALYSE DES TRACES DE PRÉSERVATIFS DANS LES CAS DE PENETRATION VAGINALE : ETUDE DE PRÉVALENCE

Ce projet est organisé par l'Ecole des Sciences Criminelles de l'Université de Lausanne

Madame,

Nous vous proposons de participer à une étude qui a pour but général de rechercher la présence de traces vaginales de préservatifs après une relation sexuelle avec préservatif. Dans ce document vous trouverez toutes les informations concernant le déroulement de cette étude pour que vous puissiez prendre votre décision d'y participer ou non en étant parfaitement informée.
Cette étude est ouverte aussi bien à des participantes qui utilisent régulièrement des préservatifs qu'à des participantes qui n'en utilisent pas.

Information détaillée

1. Objectifs du projet de recherche

Dans les cas de viols, un lien biologique entre la victime et son agresseur est recherché. Le sperme est le liquide biologique le plus souvent recherché dans ce type de cas (agression d'un homme envers une femme,) et les supports de traces sont des échantillons gynécologiques ainsi que d'autres supports tels que les vêtements de la victime ou d'autres objets reliés au cas.
Lorsque du sperme n'est pas détecté, le lien physique entre l'agresseur et la victime et la gravité de l'acte ne peuvent alors pas être confirmés. Ceci peut être expliqué par plusieurs scénarios, dont notamment l'utilisation d'un préservatif.
Dans ce cas de figure, le seul moyen de pouvoir prouver la pénétration serait de détecter la présence de traces de préservatifs sur les écouvillons des prélèvements vaginaux. Or à l'heure actuelle, les tests permettant de prouver la présence de ces traces n'ont que peu été réalisés. De plus, les études réalisées ont été réalisées avec un petit nombre de volontaires (de l'ordre de 2 ou 4) ce qui permet difficilement de tirer de conclusions.

Le but de la recherche est d'optimiser des méthodes de détection des traces de préservatifs sur des écouvillons de prélèvements vaginaux. La recherche va donc se diviser comme suit:

- recherche d'une molécule spécifique des traces de préservatif (ici le PolyDiMethylSiloxane)
- mise au point d'une technique de révélation de cette molécule sur l'écouvillon de prélèvement.
- Etude du bruit de fond, du transfert et de la persistance des traces de préservatifs dans la matrice vaginale.

Pour cela, nous avons besoin de la collaboration de volontaires pour permettre dans un premier temps d'observer la prévalence de ces traces dans la population générale et dans un deuxième temps la capacité de transfert des préservatifs et de leur traces, le temps après lequel il est toujours possible de retrouver ces traces ainsi que l'impact de la matrice vaginale sur les traces recherchées.

2. Informations générales sur le projet

Ce projet s'inscrit dans le cadre d'une thèse de doctorat à l'Ecole des Sciences Criminelles. Il s'agit de mettre en place et optimiser une méthodologie pour la détection des traces de préservatifs dans une matrice vaginale. Cette méthode sera appliquée sur des traces obtenues

à l'aide de volontaires. Une fois les traces analysées, les résultats seront analysés à l'aide de statistiques afin de déterminer s'il est possible de différencier les traces provenant de préservatifs de celles provenant de produits intimes ou de lubrifiants intime. Parallèlement, nous prévoyons d'étudier, sur la base des analyses réalisées, s'il est possible d'évaluer la trace de préservatif selon une approche d'interprétation logique qui est préconisée lors de la présentation de la preuve au tribunal. Ceci nécessite de prendre en compte 3 paramètres : la prévalence dans la population (bruit de fond), le transfert et la persistance.

La durée de ce projet est de 42 mois, temps de la thèse de doctorat. Nous recherchons 100 participantes pour cette étude, dans une population locale et représentative de la population réelle ainsi que 50 autres participantes pour la partie transfert et persistance.

Nous effectuons ce projet dans le respect des prescriptions de la législation suisse. La commission cantonale d'éthique compétente a contrôlé et autorisé le projet.

3. Déroulement pour les participantes consentantes

Vous avez reçu un kit contenant 3 écouvillons de prélèvements, un questionnaire, un rappel des modalités de prélèvement ainsi qu'une enveloppe de retour d'échantillon.
Si vous acceptez de participer de manière anonyme à cette étude, nous vous demandons de bien vouloir réaliser 3 prélèvements vaginaux (1 par écouvillon). Vous n'avez aucune contrainte de temps ni de participation. Nous considérons que vous acceptez de participer à l'étude susmentionnée si vous nous renvoyez le kit.

Remettez les écouvillons et le questionnaire dûment rempli dans l'enveloppe réponse jointe, et renvoyez-nous les prélèvements.
***Attention** : ne vous faites pas mal lors du prélèvement. Il suffit d'insérer l'écouvillon à l'entrée du vagin et de frotter légèrement la paroi vaginale.*

Conditions de prélèvements

Afin de ne pas fausser les résultats, nous vous demandons de bien vouloir ne pas réaliser de prélèvement en période de menstruations.

Conservation & Envoi des échantillons

Une fois les prélèvements réalisés, veuillez les laisser sécher en les remettant dans le tube mais sans le fermer, pendant environ 1heure et conserver les au frais en attendant de nous les renvoyer.
Pour le retour des échantillons, remettez les écouvillons et le questionnaire dans l'enveloppe réponse adressée et affranchie jointe, et renvoyez-nous les prélèvements.

4. Bénéfices pour les participants

Votre participation au projet ne vous apportera aucun bénéfice. En revanche, les résultats obtenus au cours de ce projet fourniront potentiellement une orientation utile pour la recherche future de ces traces de préservatifs chez des victimes d'agressions sexuelles ou de viol.

5. Droits des participants

Vous êtes libre d'accepter ou de refuser de participer au projet. Si vous choisissez de ne pas participer ou si vous choisissez de participer et revenez sur votre décision pendant le déroulement du projet, vous n'aurez pas à vous justifier. Vous pouvez à tout moment poser toutes les questions nécessaires au sujet de l'étude. Veuillez-vous adresser pour ce faire à la personne indiquée à la fin de la présente feuille d'information.

6. Obligations des participants

En tant que participant au projet, vous serez tenue de suivre les conditions imposées pour les prélèvements tels qu'illustrés dans les instructions jointes avec le kit de prélèvement.

7. Risques

Bien que vous réalisiez les prélèvements par vous-même, un petit risque de blessure au moment du prélèvement ne peut être exclu. Afin de limiter les risques de blessures, nous vous recommandons de suivre et de respecter le protocole de prélèvement qui vous est transmis avec les écouvillons.

8. Confidentialité des données

Vos données sont intégralement anonymes. Nous n'avons pas de données permettant de vous identifier (p. ex. le nom, la date de naissance, etc.). Personne ne peut donc lier les données à votre personne. Dans le cas d'une publication, les données agrégées ne vous sont donc pas imputables en tant que personne. Toutes les personnes impliquées dans l'étude de quelque manière que ce soit sont tenues au secret professionnel.

Durant son déroulement, le projet peut faire l'objet d'inspections. Celles-ci peuvent être effectuées par la commission d'éthique qui s'est chargée de son contrôle initial et l'a autorisé, mais aussi être mandatées par l'Ecole des Sciences Criminelles. Toutes les personnes impliquées sont tenues au secret professionnel. Nous garantissons le respect de toutes les directives de la protection des données et ne ferons apparaître votre nom dans aucun rapport ou publication, imprimé ou en ligne.

Nous nous engageons également à ne faire aucune analyse ADN des prélèvements.

9. Conservation des données

Tous les écouvillons seront dégradés pendant le projet, en raison de la nature des procédures d'extraction et d'analyse.
Les questionnaires et tous les résultats relatifs à l'étude et à l'information codée seront stockées jusqu'à 10 ans après le début du projet avant d'être détruites. Tout le matériel analysé (écouvillons, extraits) sera détruit à la fin du projet, soit 4 ans après le début du projet.

10. Rémunération des participants

Si vous participez à ce projet, vous ne recevrez pour cela aucune rémunération.

11. Réparation des dommages subis

En cas de dommages éventuels qui seraient causés aux participantes dans le cadre de cette étude, l'UNIL répondra de ces derniers en sa qualité de promoteur, conformément aux dispositions légales applicables.

12. Financement du projet

L'étude est financée par l'Université de Lausanne dans le cadre d'un projet de doctorat.

13. Interlocuteur(s)

En cas de doute, de craintes ou d'urgences pendant ou après l'étude, vous pouvez vous adresser à tout moment à l'un des interlocuteurs suivants :

Doctorante:
Céline Burnier
Ecole des Sciences criminelles
Quartier UNIL-Sorge
Bâtiment Batochime
CH-1015 Lausanne
Phone : 079 814 50 37 (joignable en tout temps, en cas de problème)
Mail: Celine.Burnier@unil.ch

Directrice de thèse :
Professeure Geneviève Massonnet
Ecole des Sciences criminelles
Quartier UNIL-Sorge
Bâtiment Batochime
CH-1015 Lausanne
Phone : 021 692 46 16
Mail: Genevieve.Massonnet@unil.ch

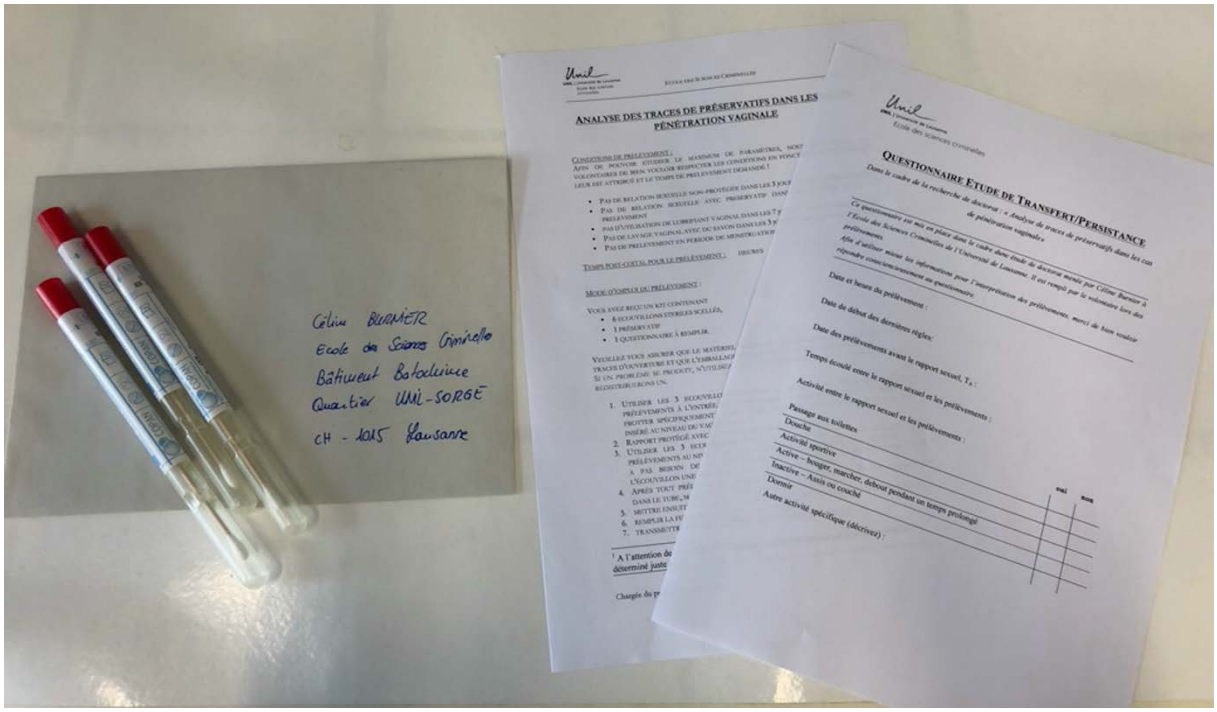
Annexe 5: Background Kit Description

ANALYSE DES TRACES DE PRÉSERVATIFS DANS LES CAS DE PÉNÉTRATION VAGINALE

MODE D'EMPLOI DU PRÉLÈVEMENT :

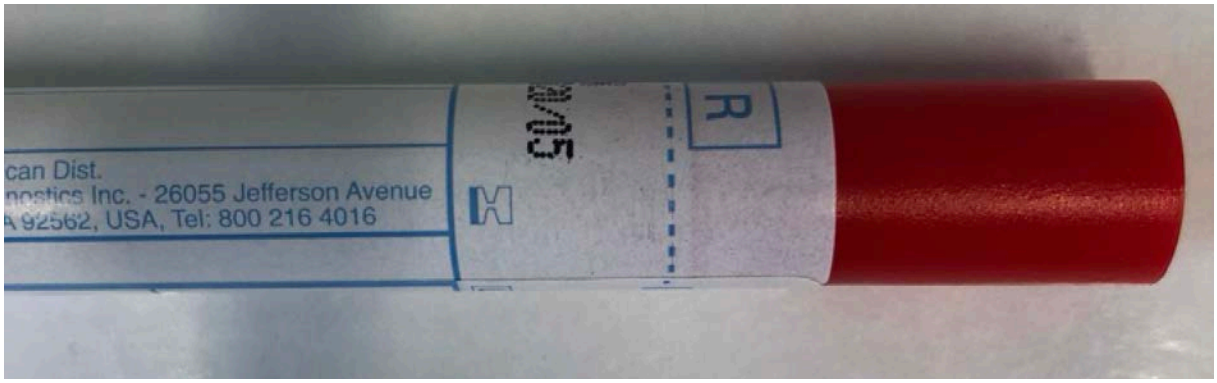
VOUS AVEZ REÇU UN KIT CONTENANT

- 3 ECOUVILLONS STERILES SCELLÉS,
- 1 QUESTIONNAIRE À REMPLIR.
- LE PRÉSENT FORMULAIRE

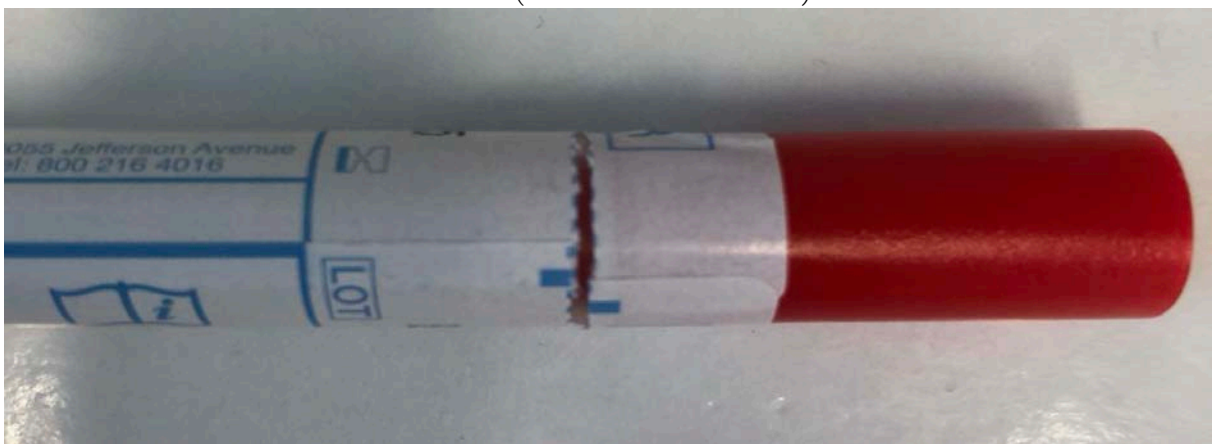


VEUILLEZ VOUS ASSURER QUE TOUT LE MATÉRIEL EST PRÉSENT ET INTACT, QUE LES ECOUVILLONS N'ONT PAS DE TRACES D'OUVERTURE (CF. IMAGES CI DESSOUS). SI LES ECOUVILLONS NE SONT PAS FERMÉS, RETOURNEZ LE KIT ENDOMMAGÉ SANS L'UTILISER.

ECOUVILLON SCELLÉ ET STERILE UTILISABLE



ECOUVILLON NON STERILE CAR OUVERT (TRACES D'OUVERTURE)



PRÉLÈVEMENTS

8. UTILISER LES 3 ECOUVILLONS PORTANT LA MENTION T_A POUR RÉALISER DES PRÉLÈVEMENTS À L'ENTRÉE DU VAGIN AVANT LE RAPPORT. IL N'Y A PAS BESOIN DE FROTTER SPÉCIFIQUEMENT : FAIRE TOURNER LÉGÈREMENT L'ÉCOUVILLON UNE FOIS INSÉRÉ AU NIVEAU DU VAGIN SUFFIT.
9. APRÈS TOUT PRÉLÈVEMENT, LAISSER SECHER LES ÉCOUVILLONS EN LES REMMETANT DANS LE TUBE, MAIS SANS LE FERMER (VOIR IMAGE CI-DESSOUS) (ENVIRON UNE HEURE)

10. METTRE ENSUITE LES ÉCHANTILLONS AU REFRIGERATEUR AVANT DE LES TRANSMETTRE.
11. REMPLIR LA FEUILLE ANNEXEE AUX ECOUVILLONS.
12. TRANSMETTRE LES PRÉLÈVEMENTS A L'AIDE DE L'ENVELOPPE RETOUR

Annexe 6 : Questionnaire pour l'étude de bruit de fond.

QUESTIONNAIRE ETUDE DE BRUIT DE FOND

Dans le cadre de la recherche de doctorat : « Analyse de traces de préservatifs dans les cas d'agressions sexuelles »

Ce questionnaire est mis en place dans le cadre d'une étude de doctorat menée par Céline Burnier à l'Ecole des Sciences Criminelles de l'Université de Lausanne. Il est rempli par la volontaire lors des prélèvements.

Afin d'utiliser mieux les informations pour l'interprétation des prélèvements, merci de bien vouloir répondre consciencieusement au questionnaire.

CODE INTERNE:

Date et heure du prélèvement :

Date de début des dernières règles:

Y a-t-il eu un rapport sexuel dans la semaine précédant le prélèvement :

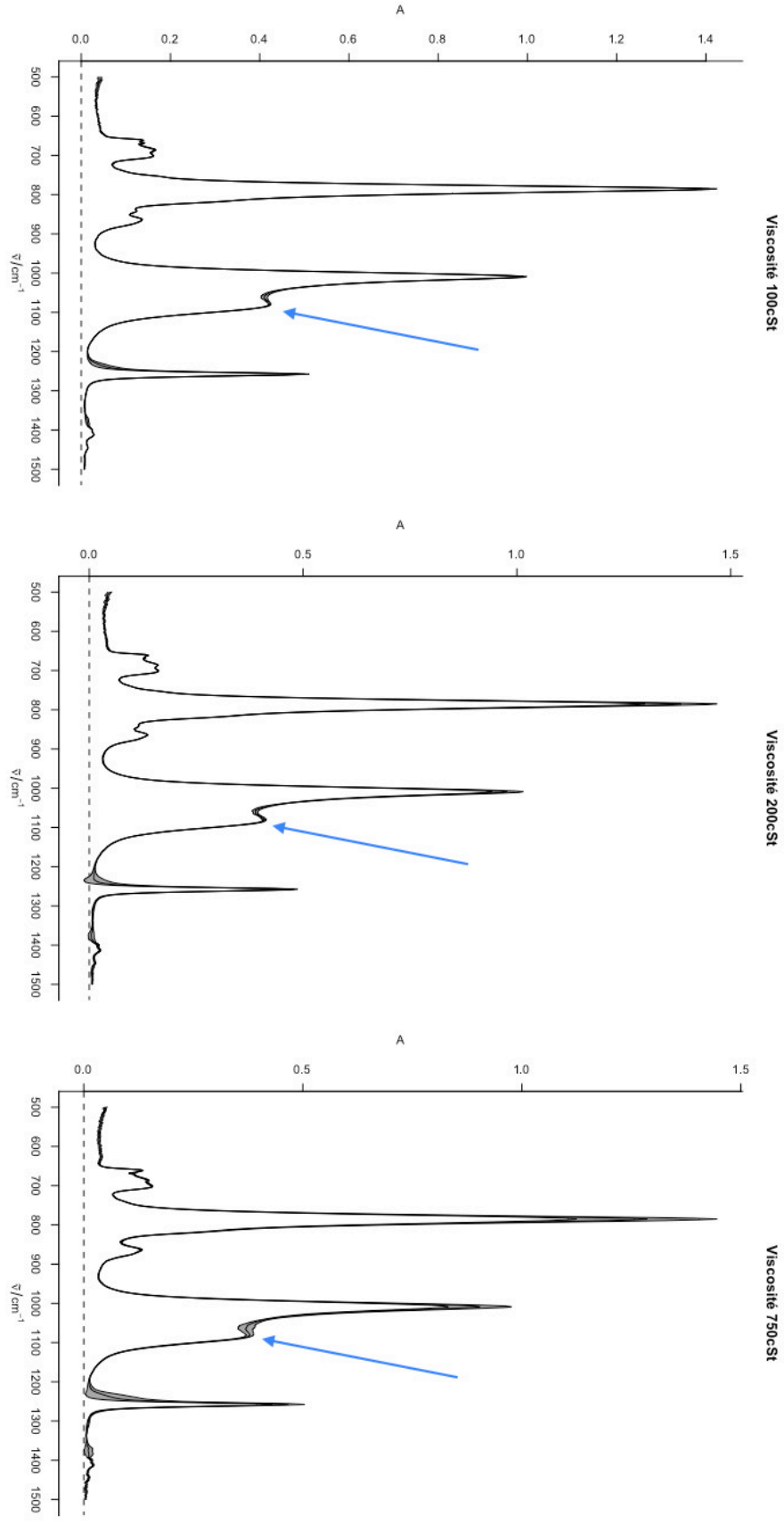
Oui Non

Si oui, quelle était la date du dernier rapport :

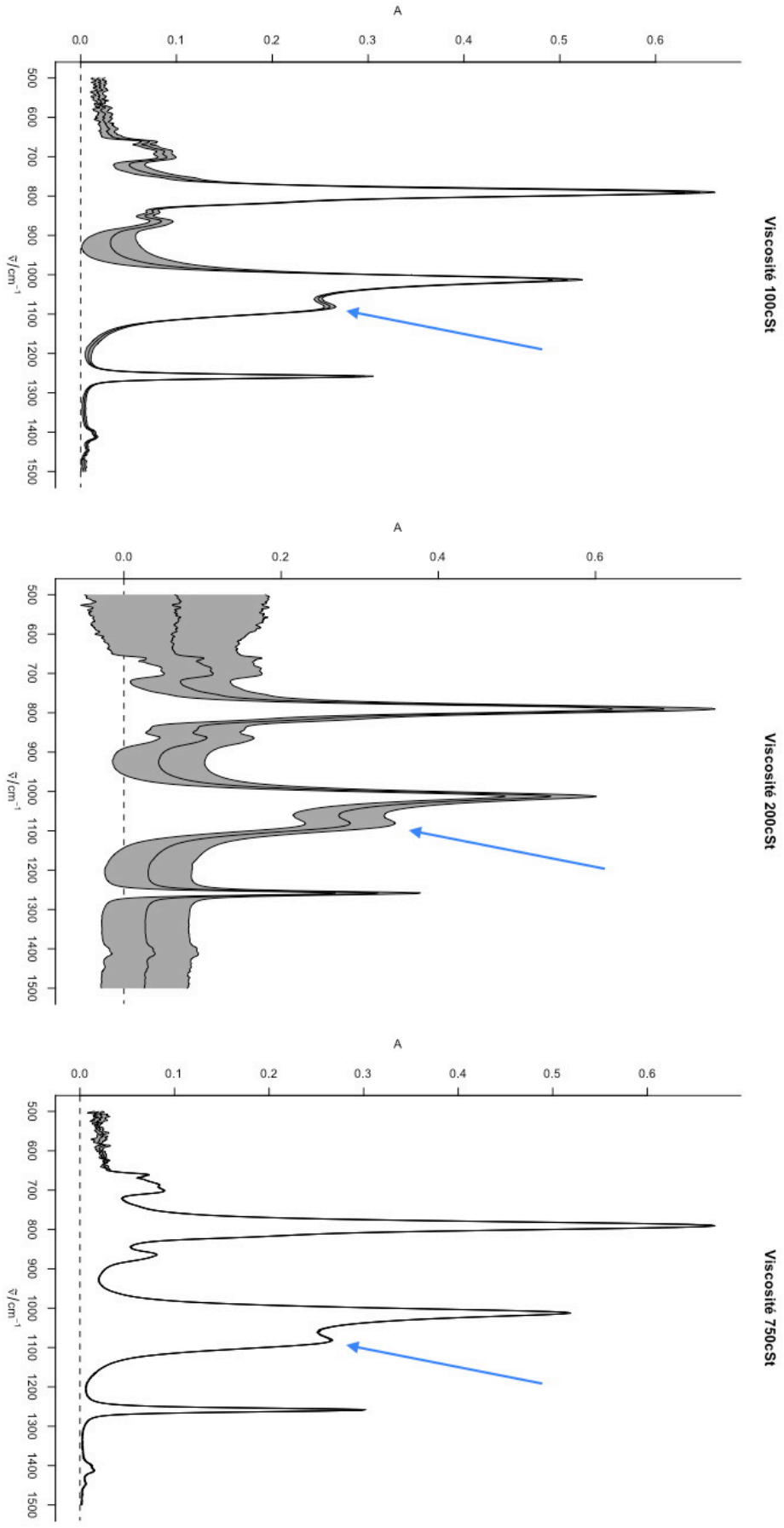
Ce rapport était-il protégé ?

Oui Non

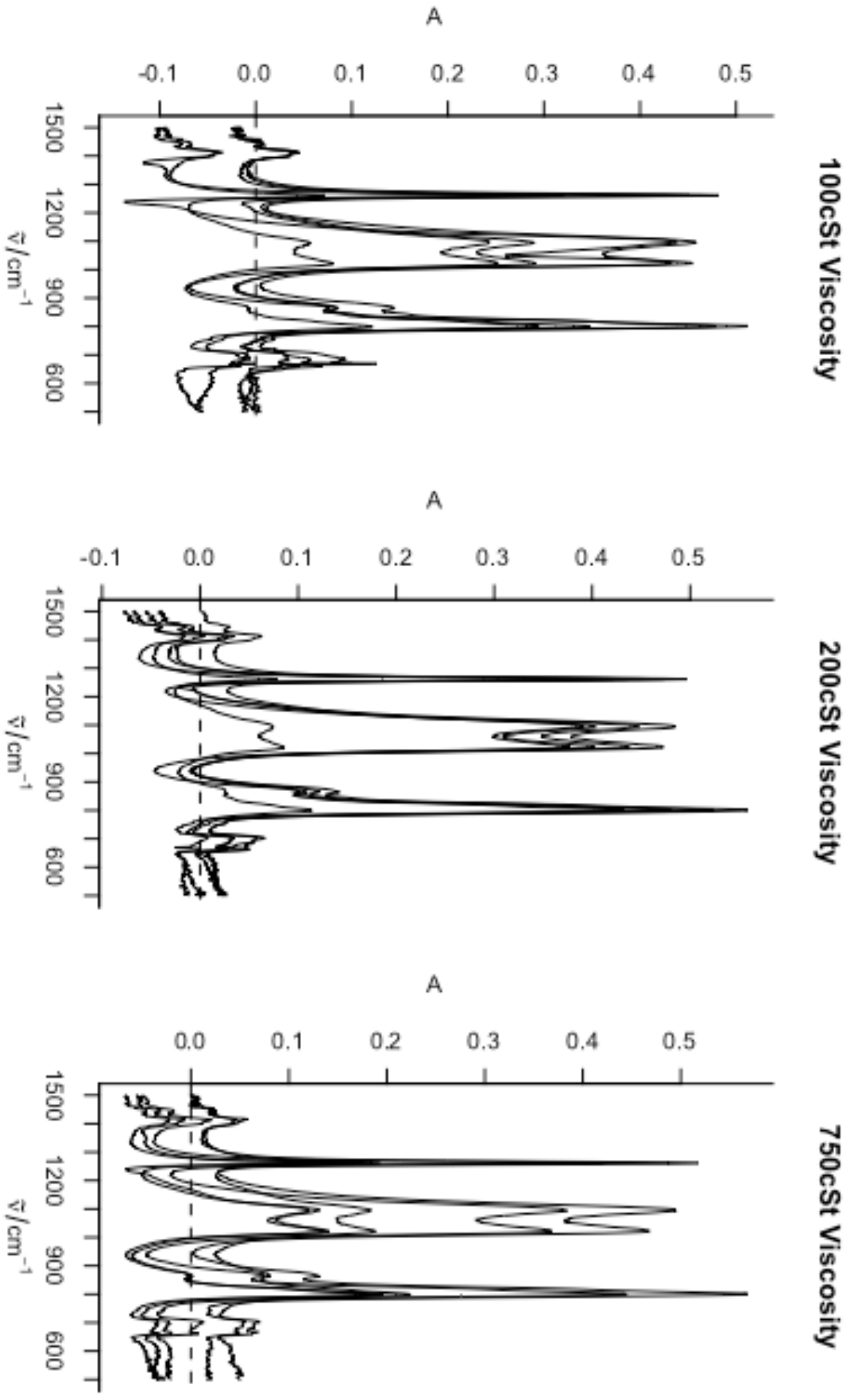
Appendix V: FTIR spectra obtained on different viscosities



ATR spectra obtained on PDMS of different viscosities



Micro-ATR spectra obtained on PDMS of different viscosities



Transmission spectra obtained on PDMS of different viscosities

Appendix VI: Experimental Design – Model selection

First cycle of experiments

Model 1: Linear regression, without interaction

$$Y = a_0 + a_1X1 + a_2X2 \text{ (Eq. 1)}$$

Regression statistics	
Multiple determination Coefficient	0.8014
Adjusted R2 Coefficient	0.7021
F-Statistics	8.071
Model significance (p-value)	0.03944

ANOVA Table

	DF	Sum square	Mean square	F	F-critic value
Variable X1	1	3.076e-05	3.076e-05	7.530	0.0517
Variable X2	1	3.518e-05	3.518e-05	8.611	0.0426
Residues	4	1.634e-05	4.090e-06		
Total	6				

	Coefficient	Error	T-Statistics	Probability
Intercept	0.941221	0.000764	1232.013	2.6e-12
Variable X1	-0.002773	0.001011	-2.744	0.0517
Variable X2	-0.002966	0.001011	-2.934	0.0426

Full statistics for the first regression model

Model 2: Linear regression, with interaction

$$Y = a_0 + a_1X1 + a_2X2 + a_3X1X2 \text{ (Eq. 2)}$$

Regression statistics	
Multiple determination Coefficient	0.9497
Adjusted R2 Coefficient	0.8994
F-Statistics	18.88
Model significance (p-value)	0.01886

ANOVA Table

	DF	Sum square	Mean square	F	F-critic value
Variable X1	1	3.076e-05	3.076e-05	22.295	0.0180
Variable X2	1	3.518e-05	3.518e-05	25.496	0.0150
Variable X1X2	1	1.220e-05	1.220e-05	8.843	0.0589
Residues	3	4.140e-06	1.380e-06		
Total	6				

	Coefficient	Error	T-Statistics	Probability
Intercept	0.9412207	0.0004440	2119.910	2.31e-10
Variable X1	-0.0027733	0.0005873	-4.722	0.0180
Variable X2	-0.0029657	0.0005873	-5.049	0.0150
Variable X1X2	0.0017466	0.0005873	2.974	0.0589

Full statistics table for the second regression model

Corrected cycle of experiment

Model 1b: Linear regression, without interaction based on the new FFD planification

$$Y = a_0 + a_1X1 + a_2X2 \text{ (Eq. 1)}$$

Regression statistics	
Multiple determination Coefficient	0.6788
Adjusted R2 Coefficient	0.5182
F-Statistics	4.227
Model significance (p-value)	0.1032

ANOVA Table

	DF	Sum square	Mean square	F	F-critic value
Variable X1	1	0.0001595	1.595e-04	3.299	0.1435
Variable X2	1	0.0002492	2.492e-04	5.154	0.0857
Residues	4	0.0001934	4.835e-05		
Total	6				

	Coefficient	Error	T-Statistics	Probability
Intercept	0.942060	0.002628	358.454	3.63e-10
Variable X1	-0.006315	0.003477	-1.816	0.1435
Variable X2	-0.007893	0.003477	-2.270	0.0857

Full statistics for the new model

Model 2b: Linear regression with interaction, based on the new FFD planification

$$Y = a_0 + a_1X1 + a_2X2 + a_3X1X2 \text{ (Eq. 2)}$$

Regression statistics	
Multiple determination Coefficient	0.9445
Adjusted R^2 Coefficient	0.889
F-Statistics	17.01
Model significance (p-value)	0.02183

ANOVA Table

	DF	Sum square	Mean square	F	F-critic value
Variable X1	1	1.595e-04	1.595e-04	14.32	0.0324
Variable X2	1	2.492e-04	2.492e-04	22.37	0.0179
Variable X1X2	1	1.600e-04	1.600e-04	14.36	0.0322
Residues	3	3.342e-05	1.114e-05		
Total	6				

	Coefficient	Error	T-Statistics	Probability
Intercept	0.942060	0.001262	746.714	5.3e-09
Variable X1	-0.006315	0.001669	-3.784	0.0324
Variable X2	-0.007893	0.001669	-4.729	0.0179
Variable X1X2	-0.006324	0.001669	-3.789	0.0322

Full statistics for the second regression model.

Model 3: Full second-degree model

$$Y = a_0 + a_1X1 + a_2X2 + a_3X1X2 + a_4X1^2 + a_5X2^2 \text{ (Eq. 3)}$$

Regression statistics	
Multiple determination Coefficient	0.8666
Adjusted R2 Coefficient	0.7332
F-Statistics	6.495
Model significance (p-value)	0.03041

ANOVA Table

	DF	Sum square	Mean square	F	F-critic value
Variable X1	1	3.634e-04	3.634e-04	15.903	0.0104
Variable X2	1	1.928e-04	1.928e-04	8.438	0.0336
Variable X1X2	1	1.600e-04	1.600e-04	7.001	0.0456
Variable $X1^2$	1	2.500e-05	2.500e-05	1.094	0.3435
Variable $X2^2$	1	9.000e-07	9.000e-07	0.039	0.8513
Residues	5	1.142e-04	2.28e-05		
Total	10				

	Coefficient	Error	T-Statistics	Probability
Intercept	0.942	0.0024521	384.515	2.26e-12
Variable X1	-0.0077821	0.0019514	-3.988	0.0104
Variable X2	-0.0056685	0.0019514	-2.905	0.0336
Variable X1X2	-0.0063240	0.0023900	-2.646	0.0456
Variable $X1^2$	-0.0031853	0.0030032	-1.061	0.3435
Variable $X2^2$	0.0005926	0.0030032	0.197	0.8513

Full statistics for the third regression model

Model 4 :

$$Y = a_0 + a_1X1 + a_2X2 + a_3X1X2 \text{ (Eq. 3)}$$

Regression statistics	
Multiple determination Coefficient	0.8364
Adjusted R2 Coefficient	0.7662
F-Statistics	11.92
Model significance (p-value)	0.003856

ANOVA Table

	DF	Sum square	Mean square	F	F-critic value
Variable X1	1	3.634e-04	3.634e-04	18.152	0.00374
Variable X2	1	1.928e-04	1.928e-04	9.631	0.01724
Variable X1X2	1	1.600e-04	1.600e-04	7.991	0.02552
Residues	7	1.142e-04	2.28e-05		
Total	10				

	Coefficient	Error	T-Statistics	Probability
Intercept	0.941449	0.001349	697.887	<2 e-16
Variable X1	-0.007782	0.001827	-4.261	0.00374
Variable X2	-0.005668	0.001827	-3.103	0.01724
Variable X1X2	-0.006324	0.002237	-2.827	0.02552

Full statistics for the fourth regression model

Appendix VII: Impact of the variable selection on the sample discrimination – PCA plots

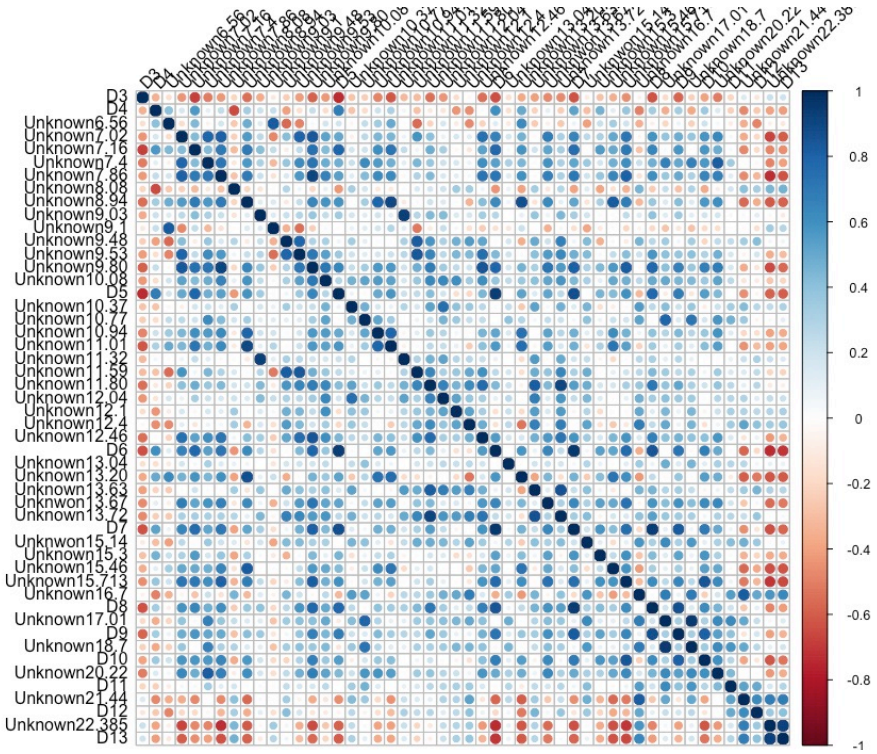
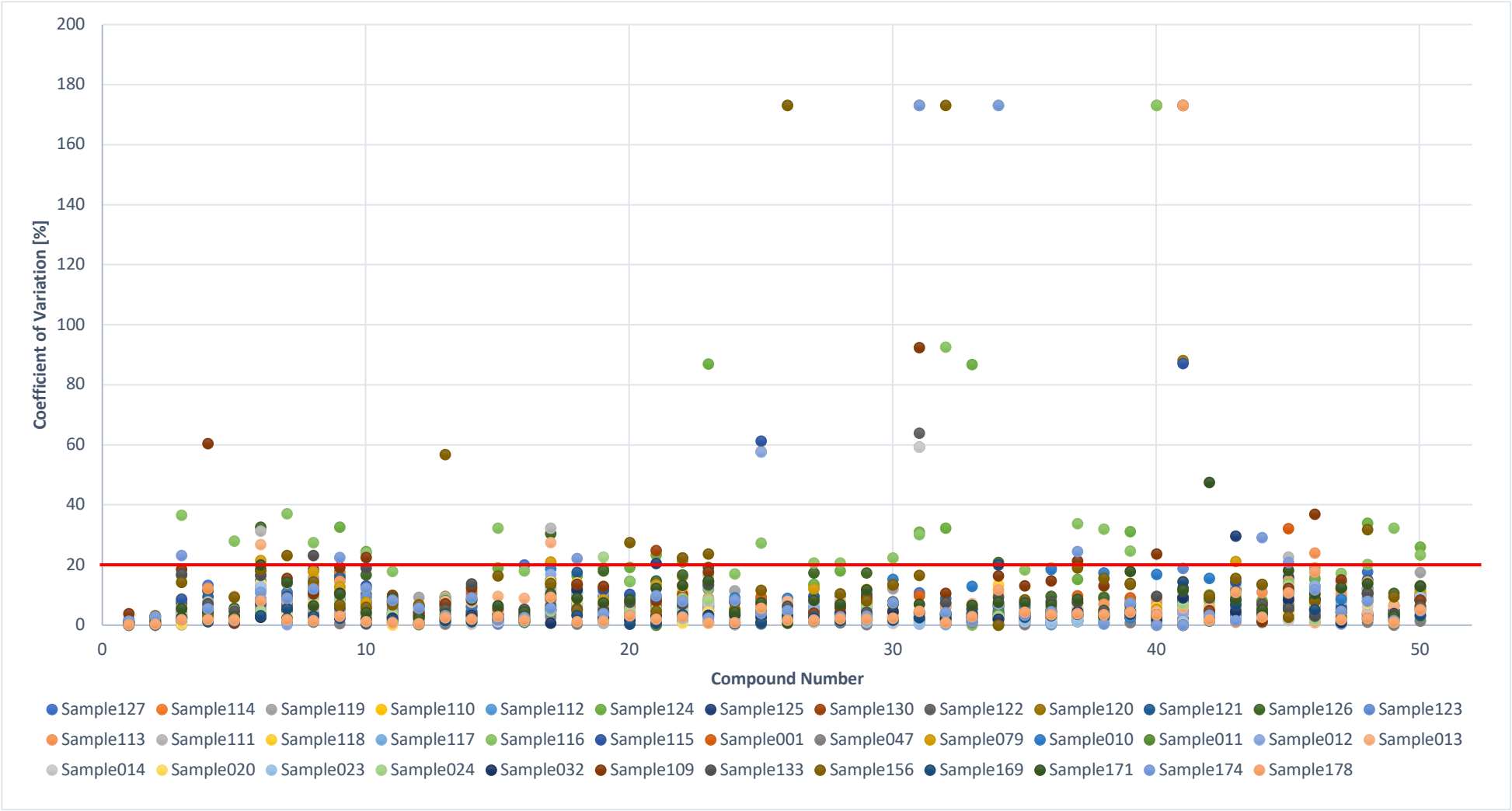
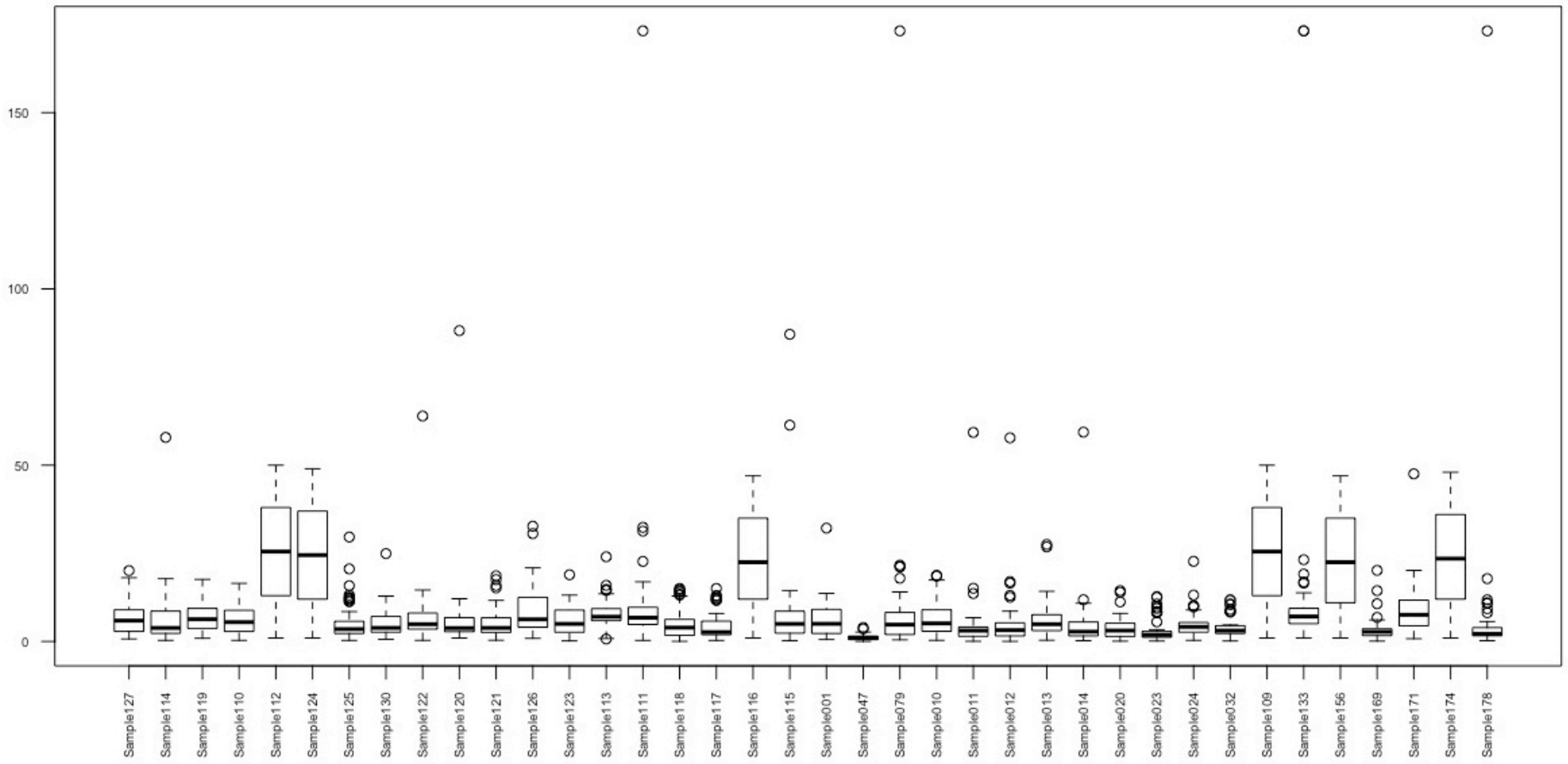


Figure 139: Correlation Plot of the variables

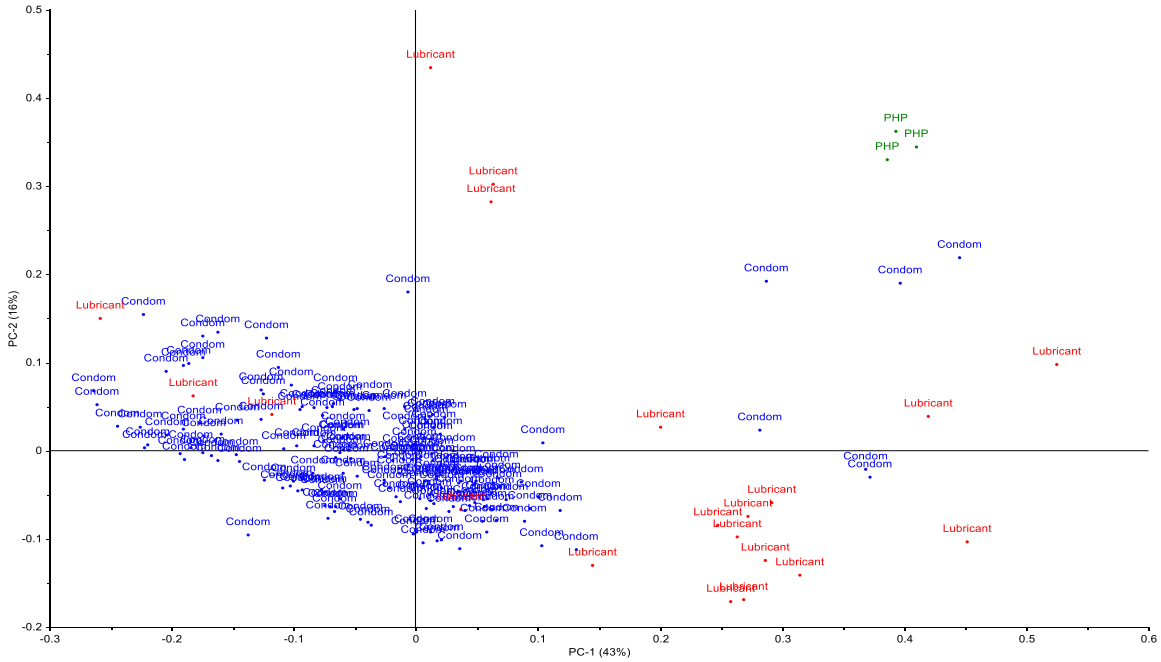


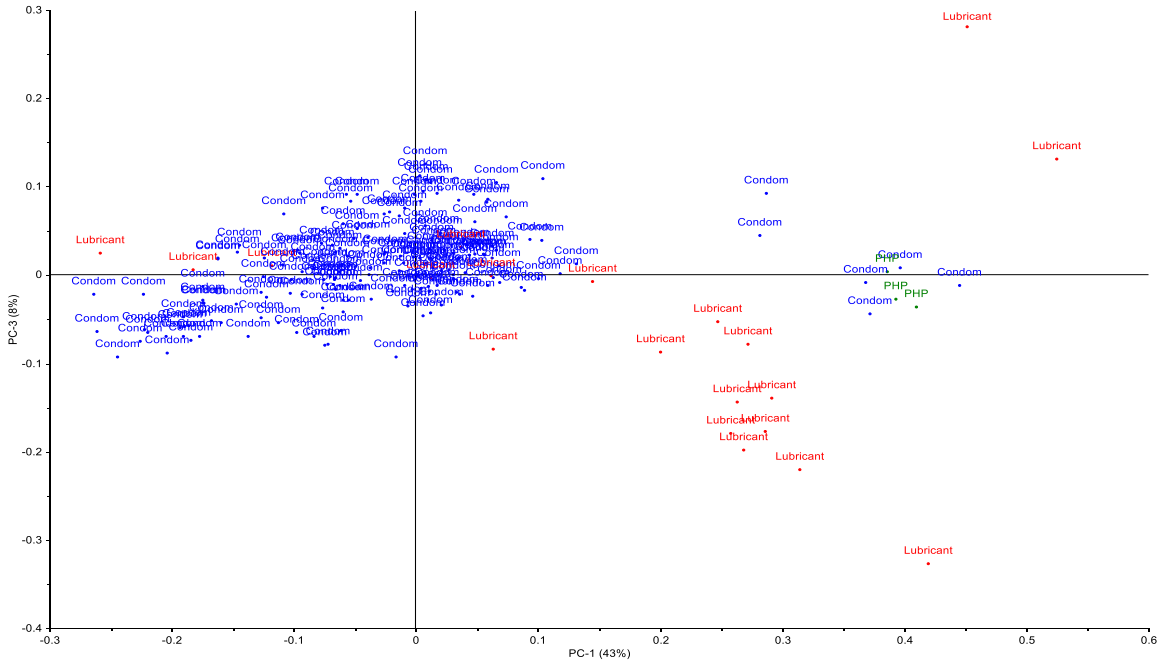
Distribution of the coefficient of variation (%) of the 50 compounds selected for each of the 38 samples considered, processed data

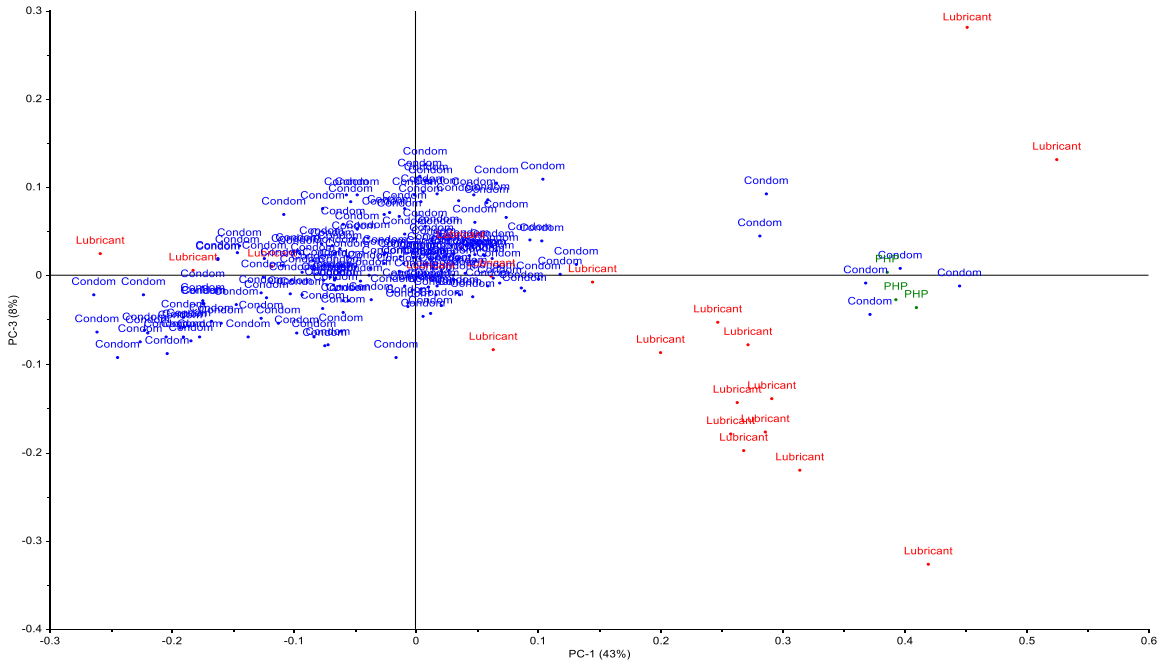


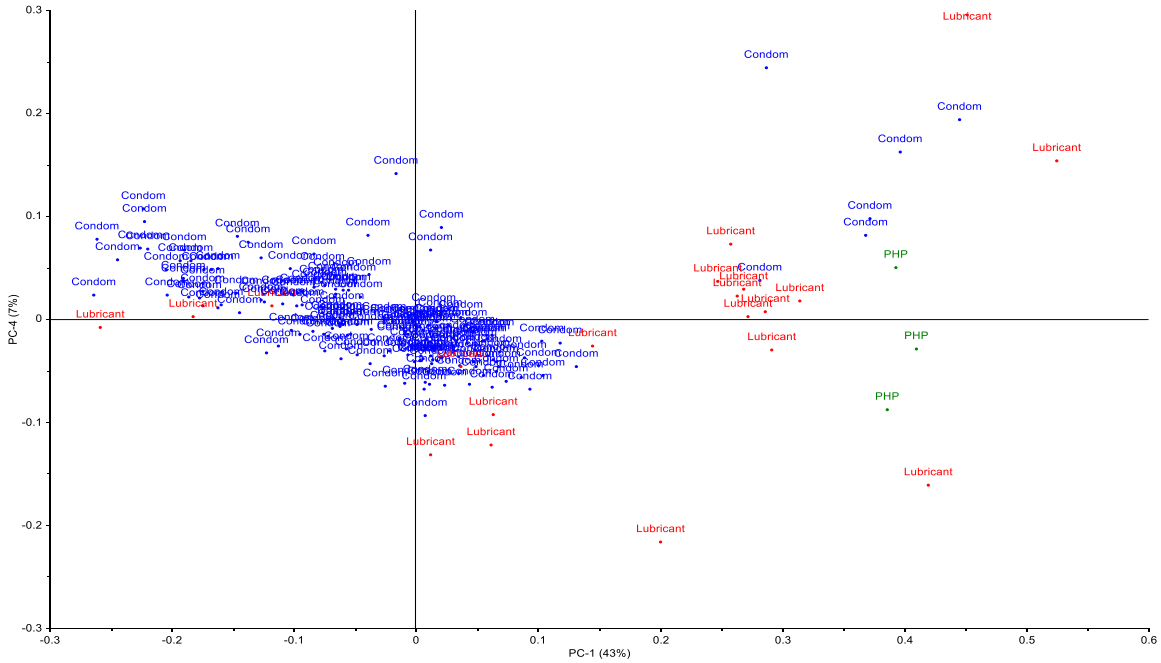
Boxplot of the coefficient of variation (%) of the 50 compounds for each sample tested, processed data

Variable selection 0 – All variable selected

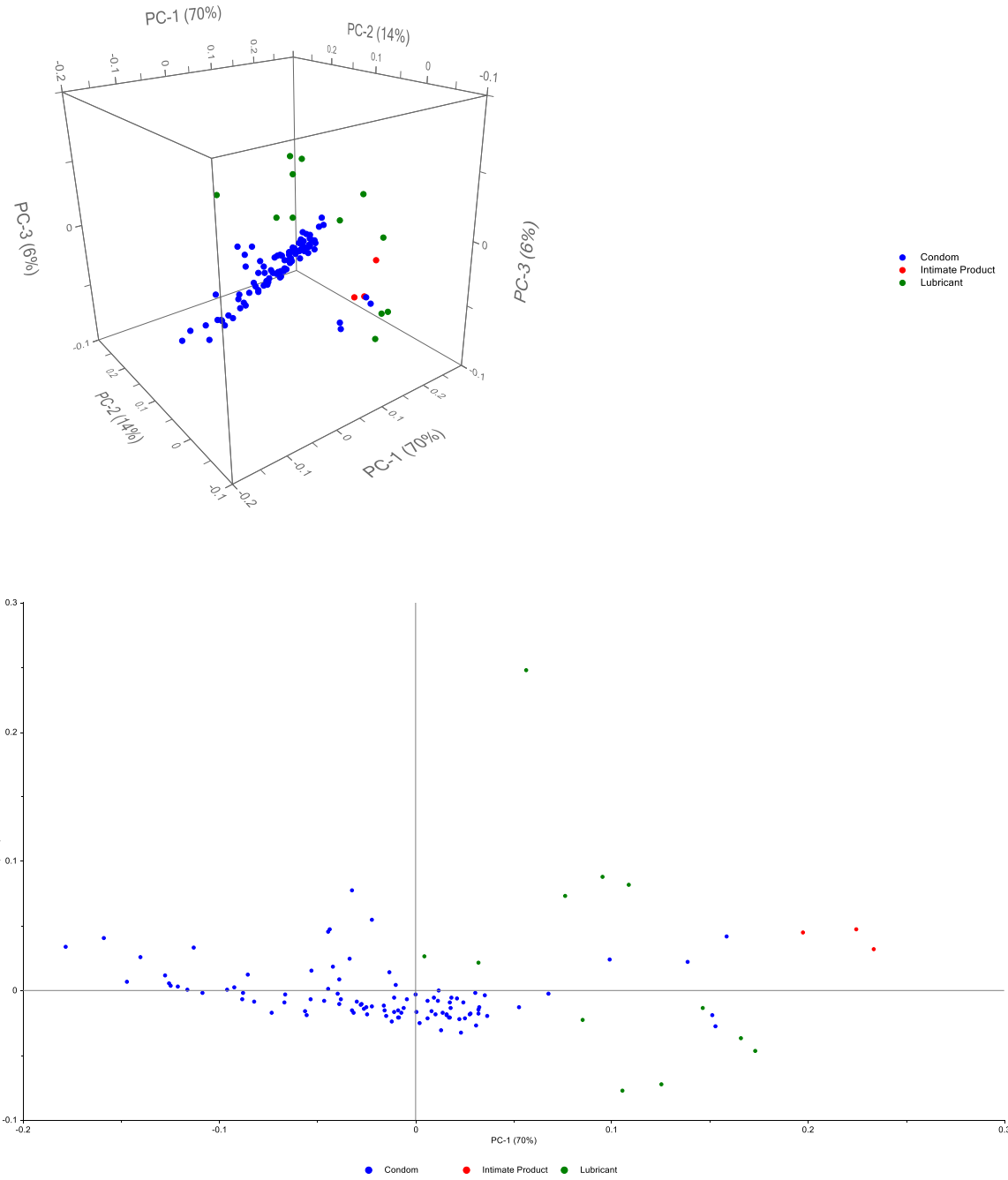


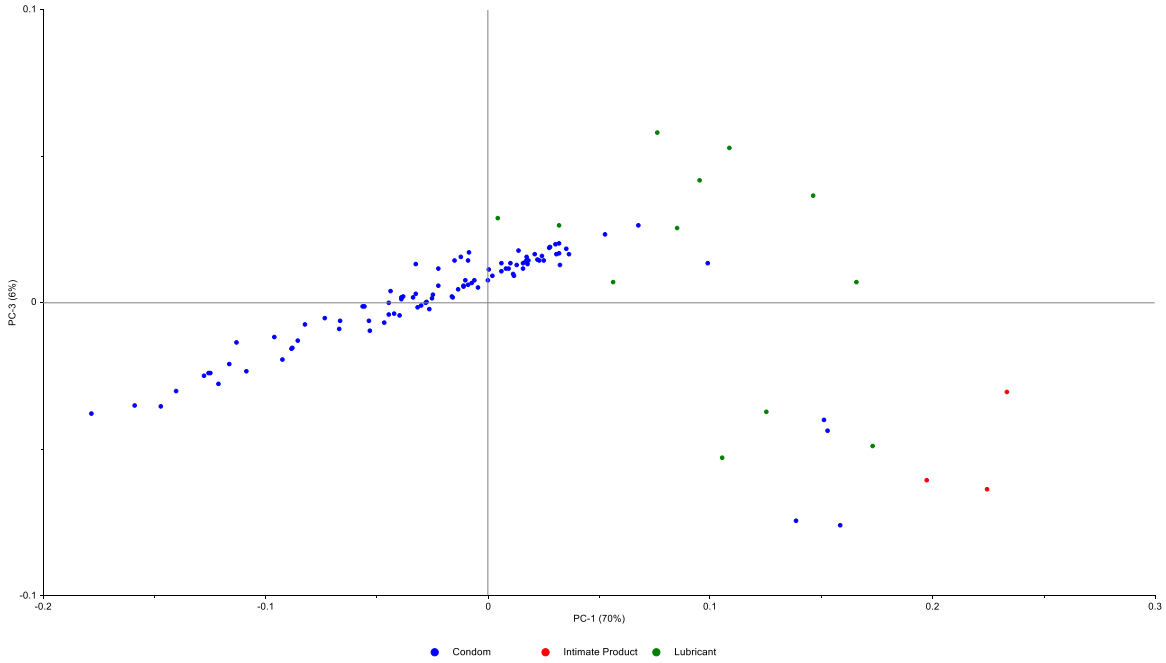


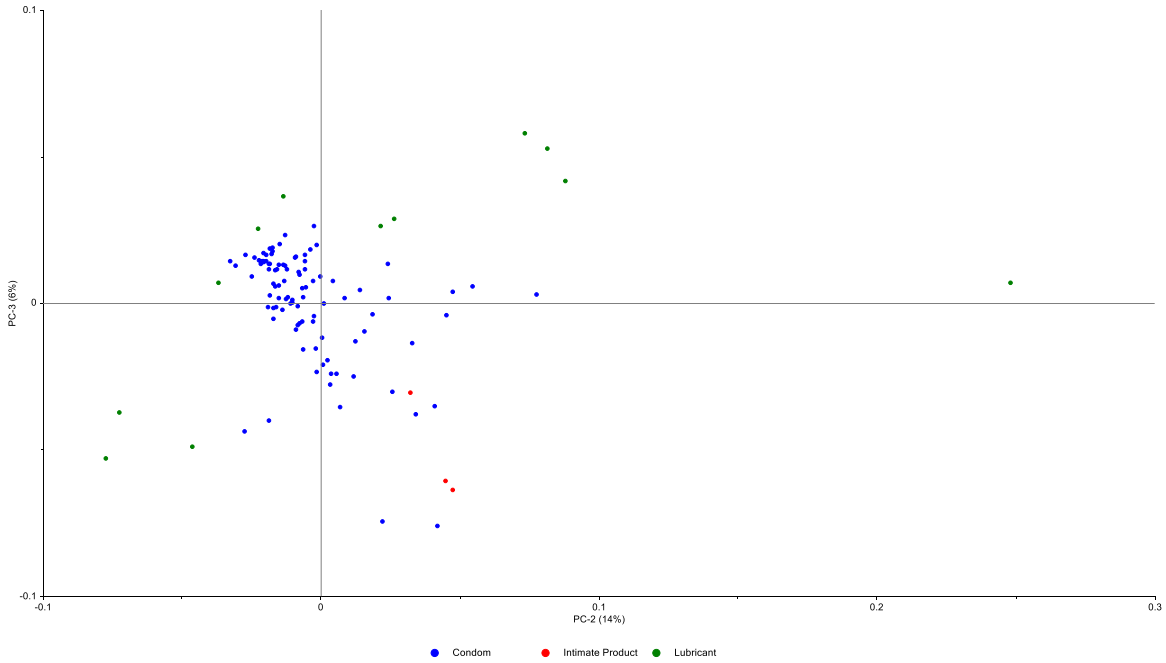

PC-3 (8%)
PC-1 (43%)
Lubricant
Condom
PHP

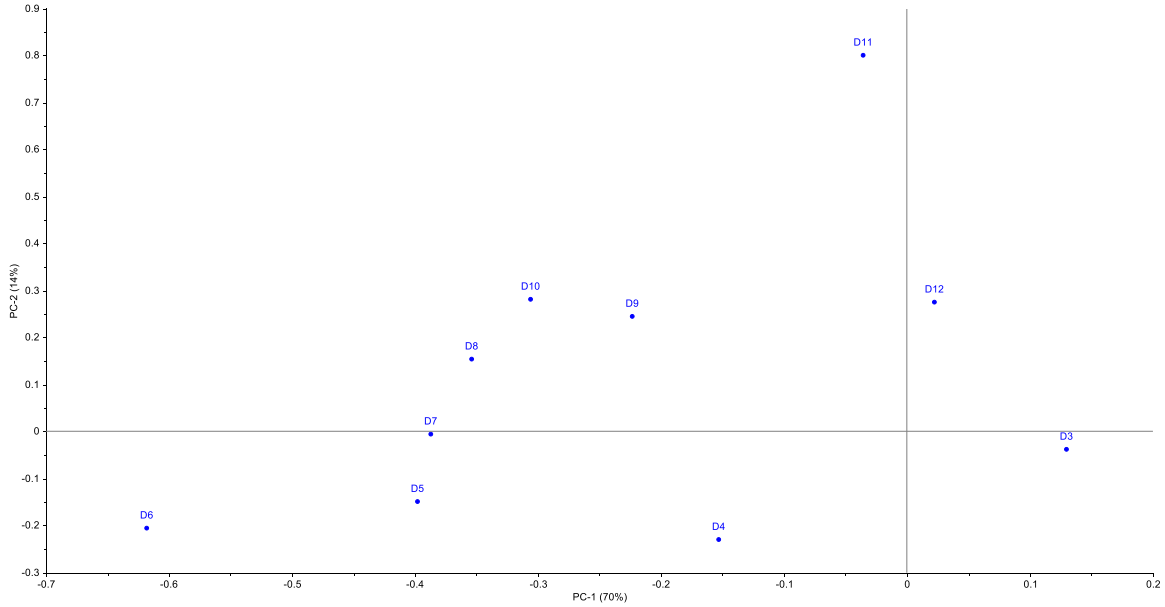

PC-4 (7%)
PC-1 (43%)
Lubricant
Condom
PHP

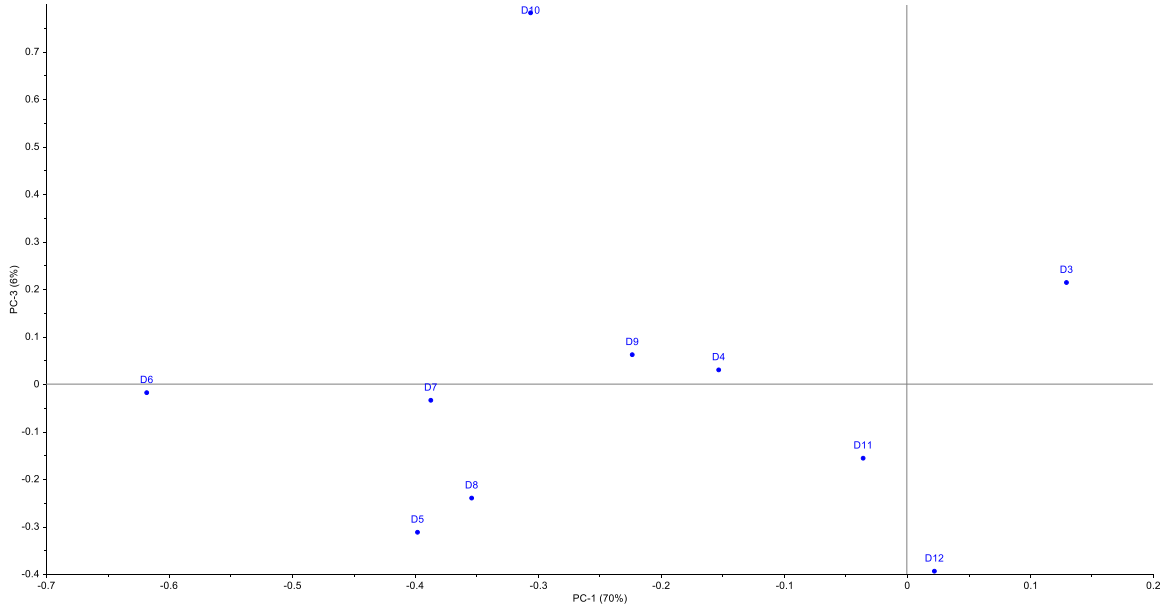
Variable selection 1 – Cyclic Oligomers D3-D13

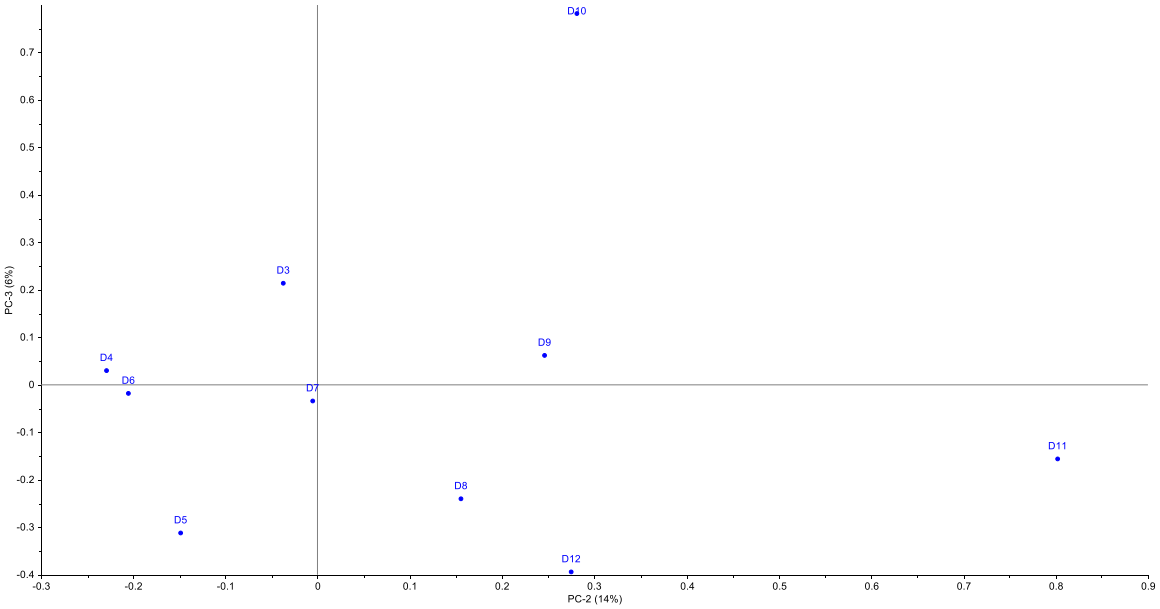



0.1
0
-0.1
PC-3 (6%)
-0.2
-0.1
0
0.1
0.2
0.3
PC-1 (70%)
Condom
Intimate Product
Lubricant

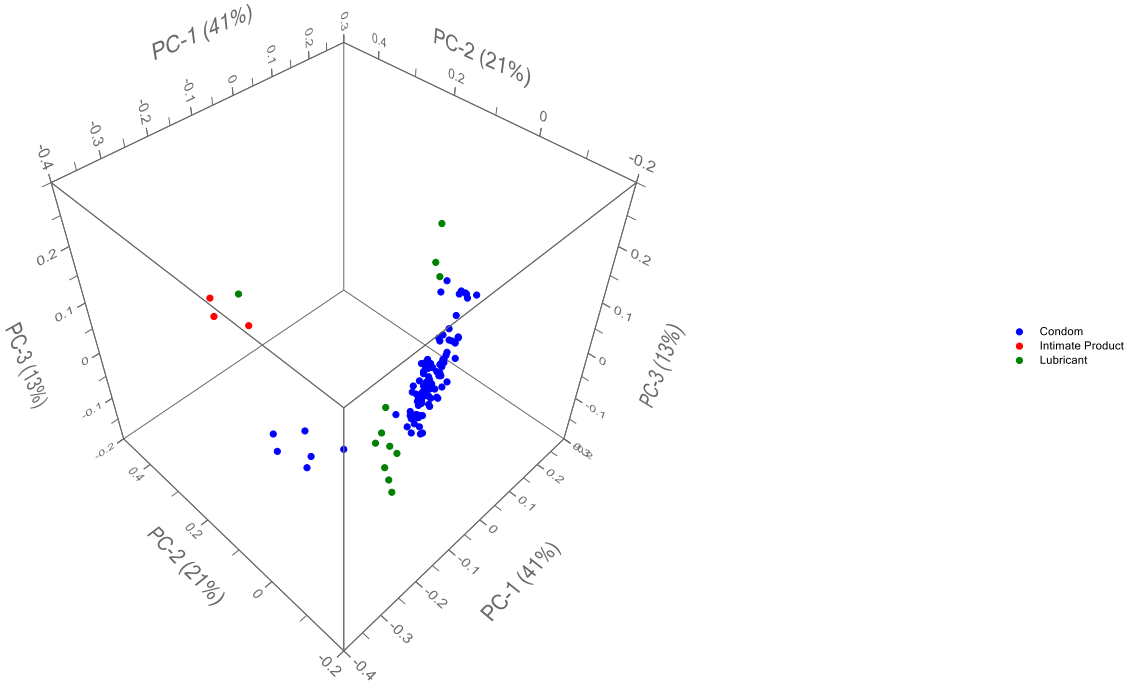

0.1
0
-0.1
PC-3 (6%)
-0.1
0
0.1
0.2
0.3
PC-2 (14%)
Condom
Intimate Product
Lubricant

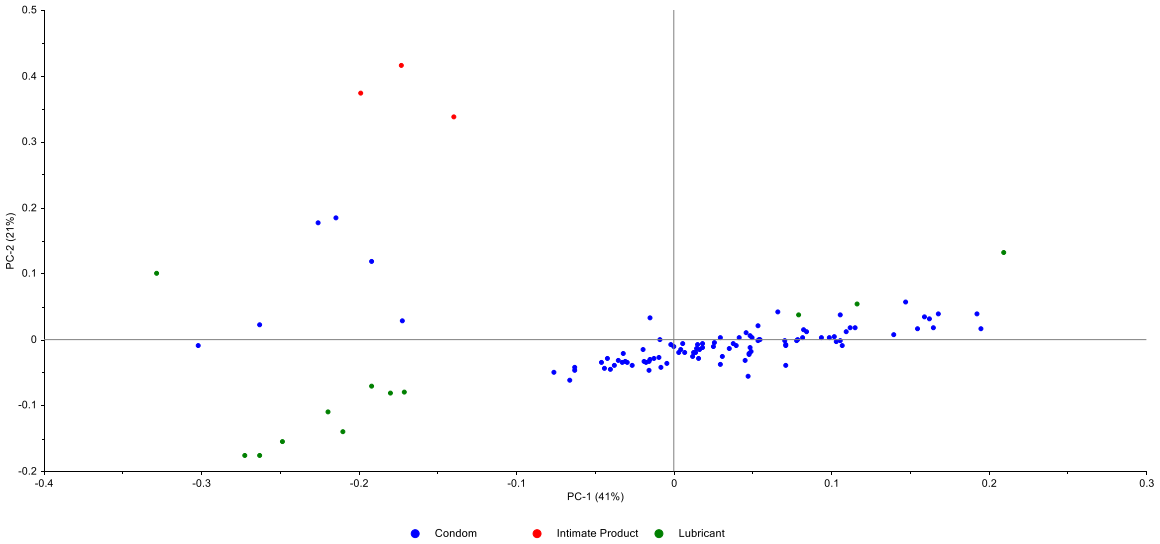

PC-2 (14%)
PC-1 (70%)
D11
D10
D12
D9
D8
D7
D3
D5
D6
D4

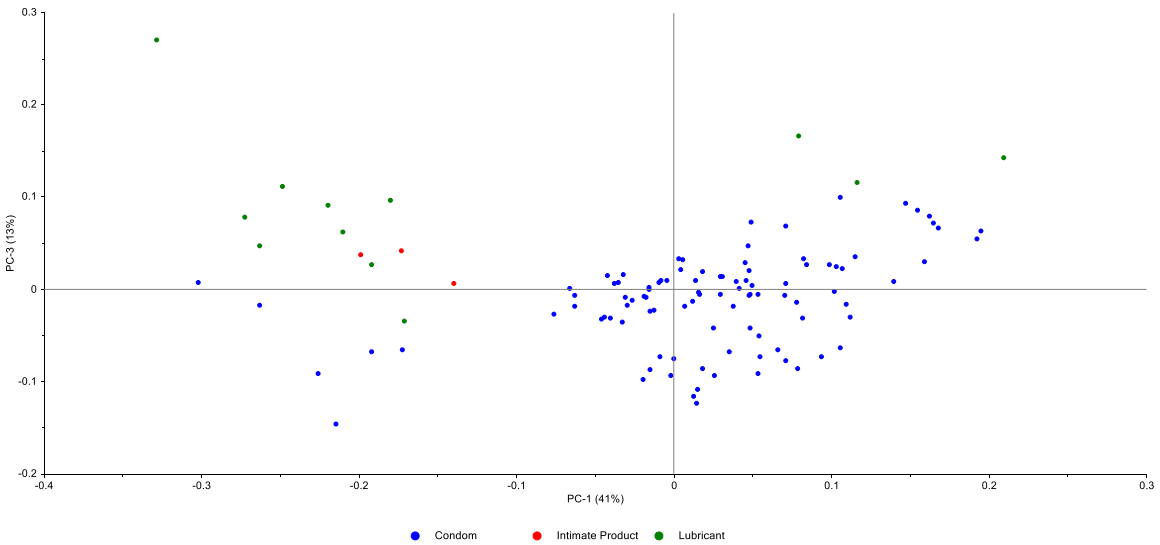

PC-3 (6%)
PC-1 (70%)
D10
D3
D9
D4
D6
D7
D11
D8
D5
D12

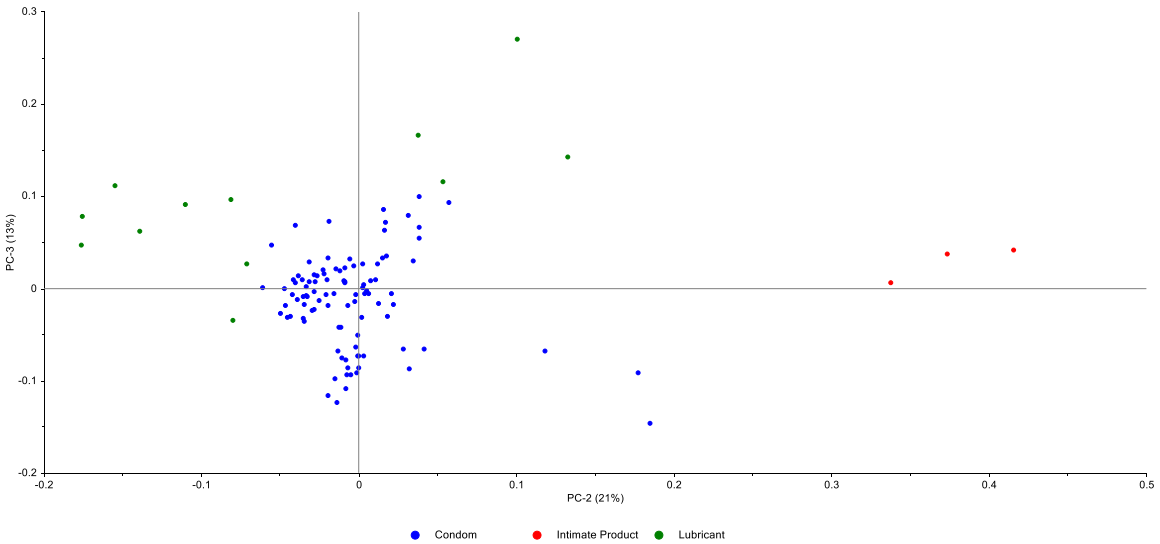


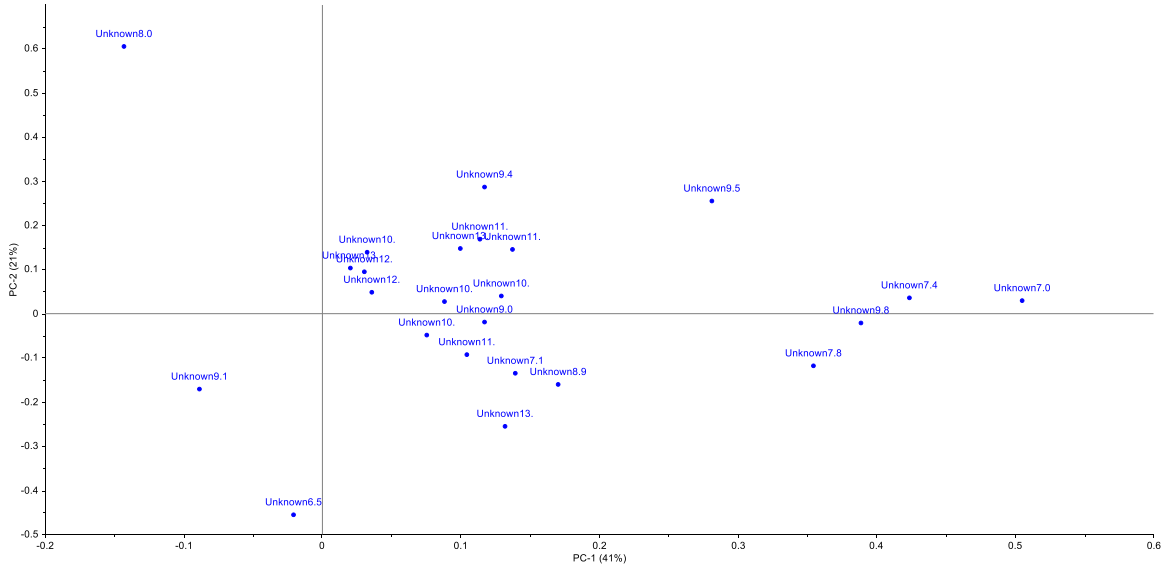
Variable selection 2 – Minor compounds only

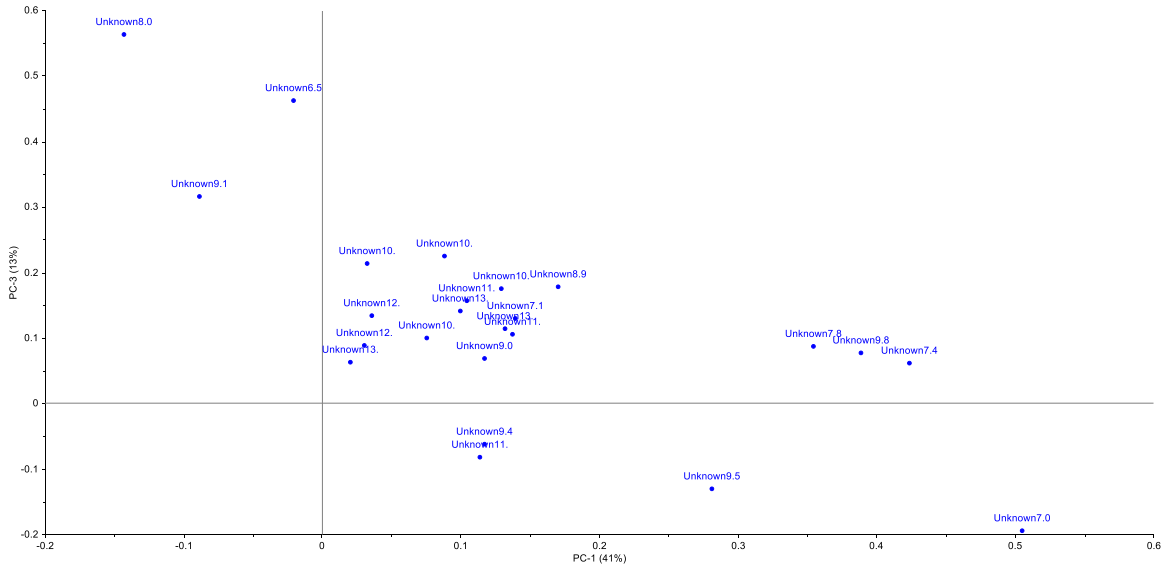



PC-2 (21%)
PC-1 (41%)
Condom
Intimate Product
Lubricant


PC-3 (13%)
PC-1 (41%)
Condom
Intimate Product
Lubricant


PC-3 (13%)
PC-2 (21%)
Condom
Intimate Product
Lubricant


Unknown8.0
Unknown9.4
Unknown9.5
Unknown11.
Unknown10.
Unknown11.
Unknown12.
Unknown12.
Unknown10.
Unknown10.
Unknown7.4
Unknown7.0
Unknown9.0
Unknown9.8
Unknown10.
Unknown11.
Unknown7.8
Unknown7.1
Unknown9.1
Unknown8.9
Unknown13.
Unknown6.5
PC-2 (21%)
PC-1 (41%)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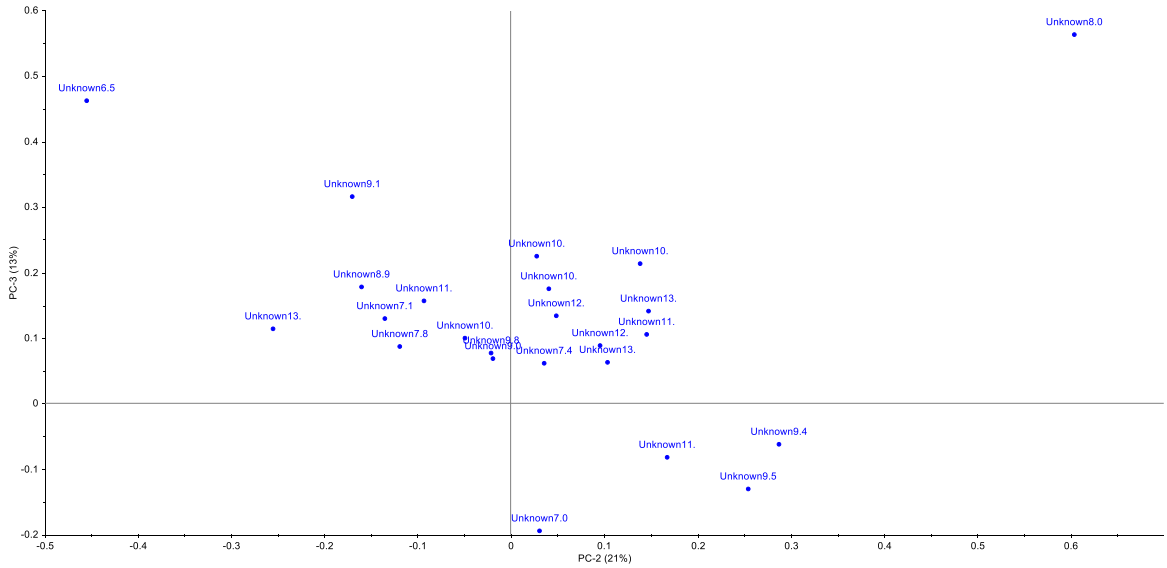
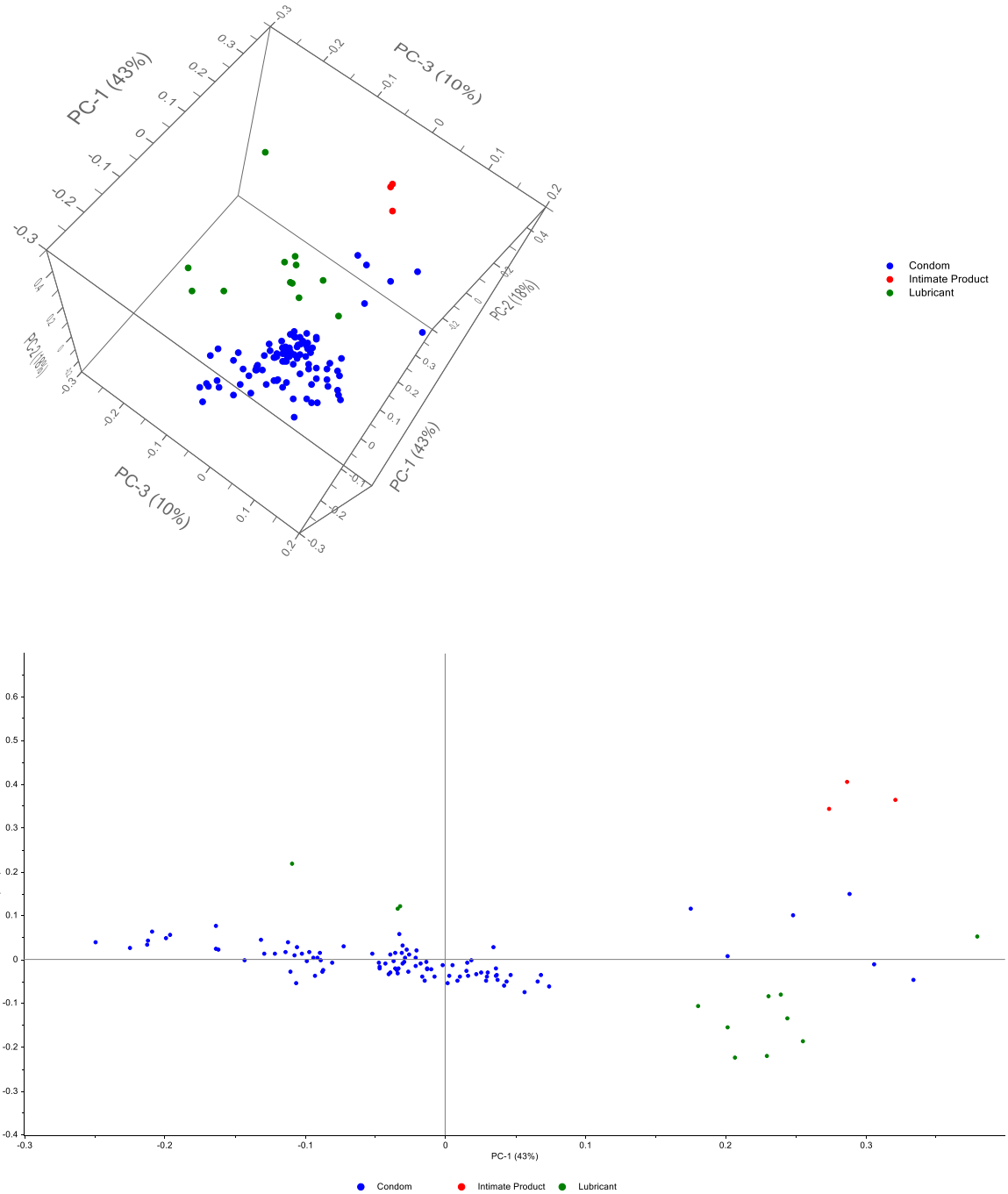
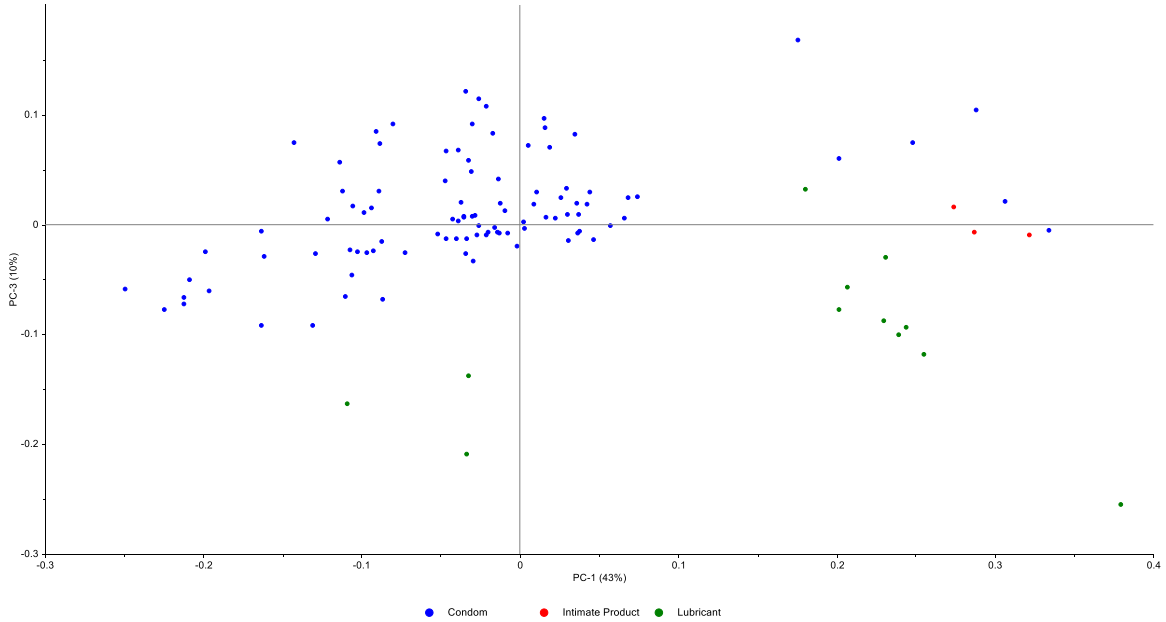
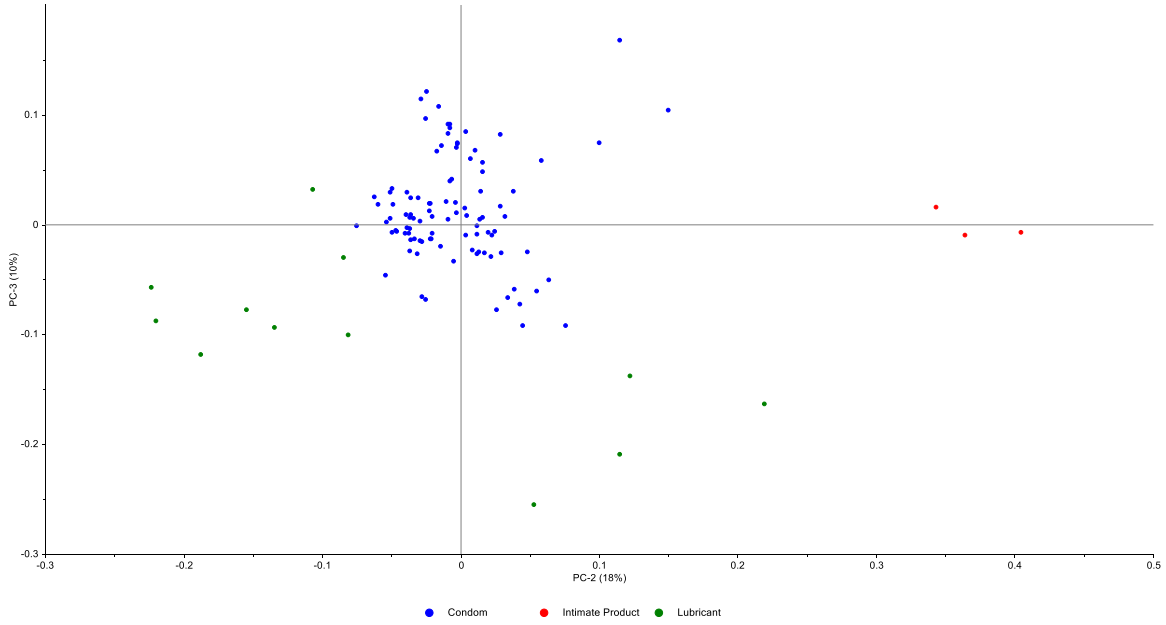
Unknown6.5
Unknown9.1
Unknown10.
Unknown10.
Unknown10.
Unknown8.9
Unknown11.
Unknown12.
Unknown13.
Unknown7.1
Unknown10.
Unknown12.
Unknown13.
Unknown11.
Unknown9.0
Unknown7.8
Unknown9.8
Unknown7.4
Unknown9.4
Unknown11.
Unknown9.5
Unknown7.0
PC-3 (13%)
PC-1 (41%)

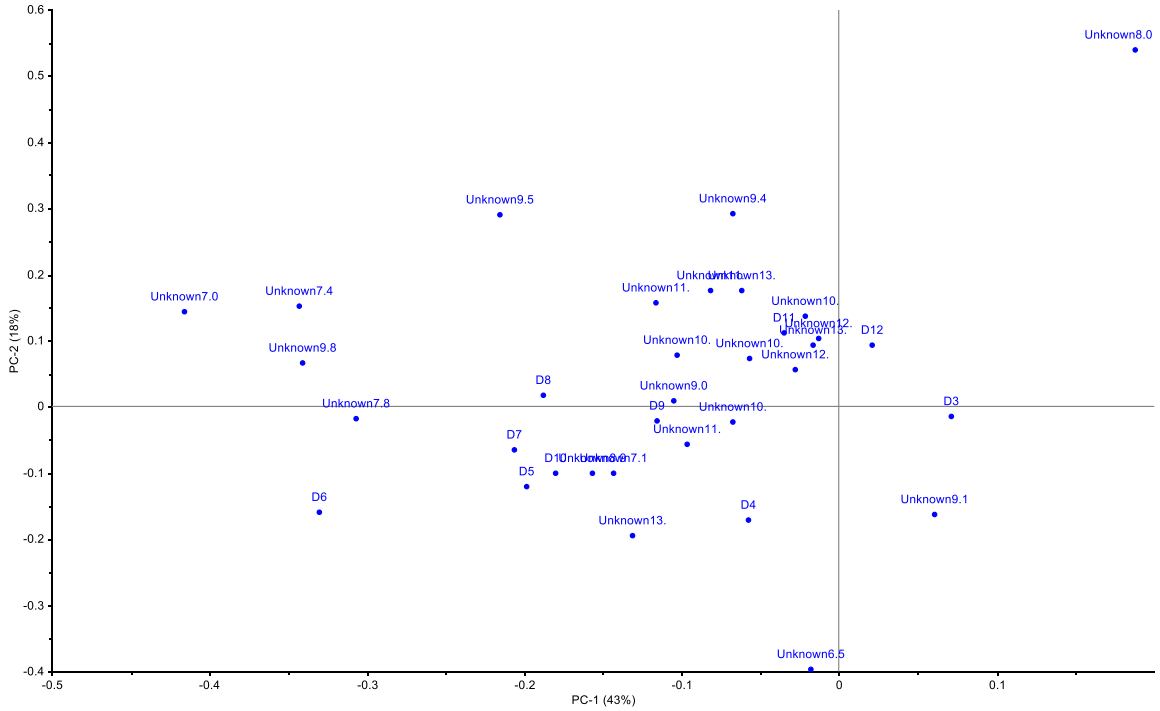

Unknown8.0
Unknown6.5
Unknown9.1
Unknown10.
Unknown10.
Unknown8.9
Unknown10.
Unknown11.
Unknown12.
Unknown13.
Unknown13.
Unknown7.1
Unknown11.
Unknown10.
Unknown12.
Unknown7.8
Unknown9.8
Unknown9.0
Unknown7.4
Unknown13.
Unknown9.4
Unknown11.
Unknown9.5
Unknown7.0
PC-3 (13%)
PC-2 (21%)

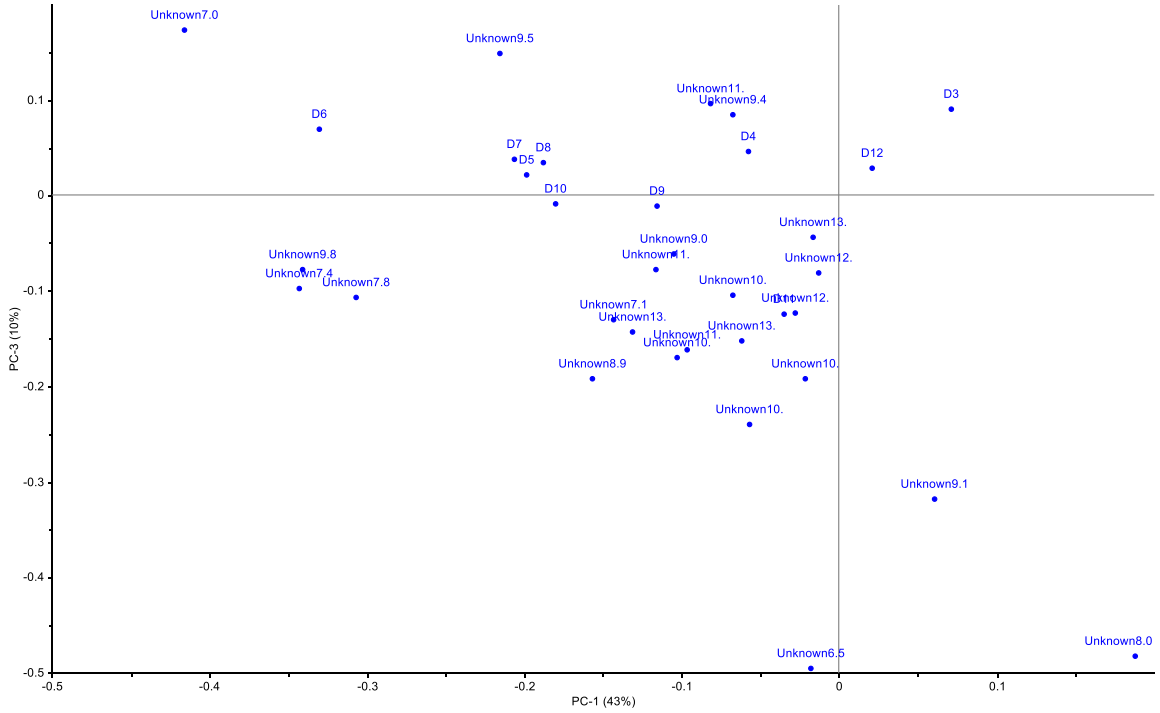
Variable selection 3 – Removed low percentage variables (28 variables)

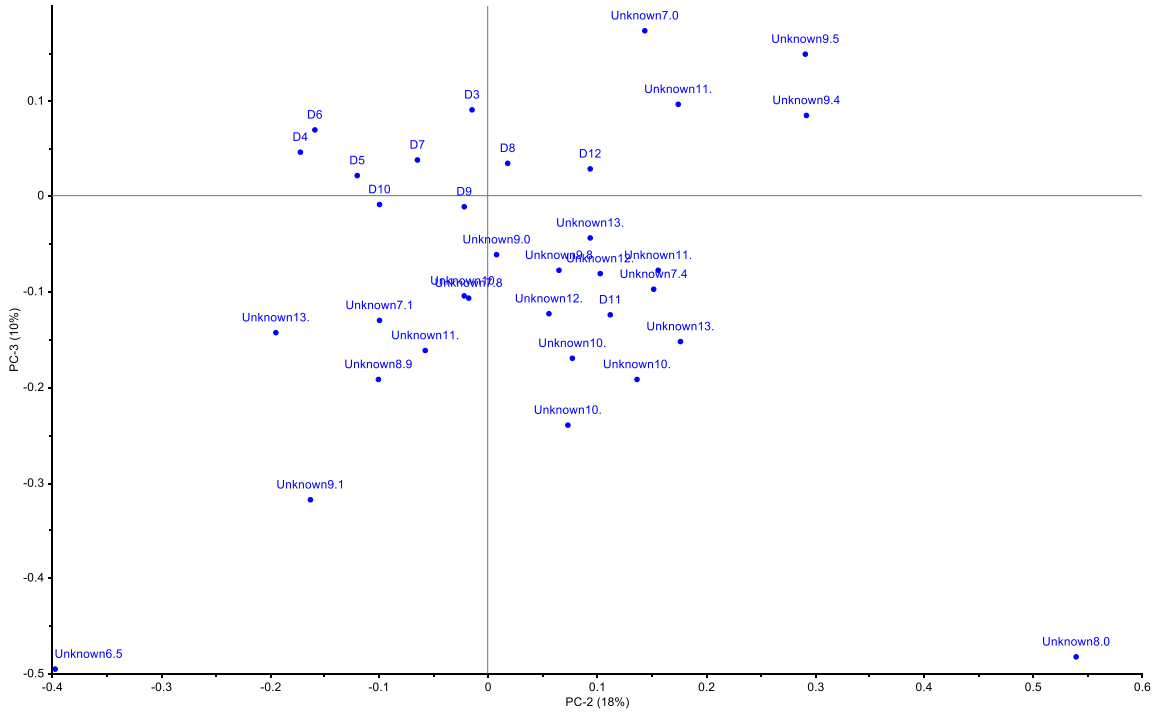



PC-3 (10%)
PC-1 (43%)
Condom
Intimate Product
Lubricant

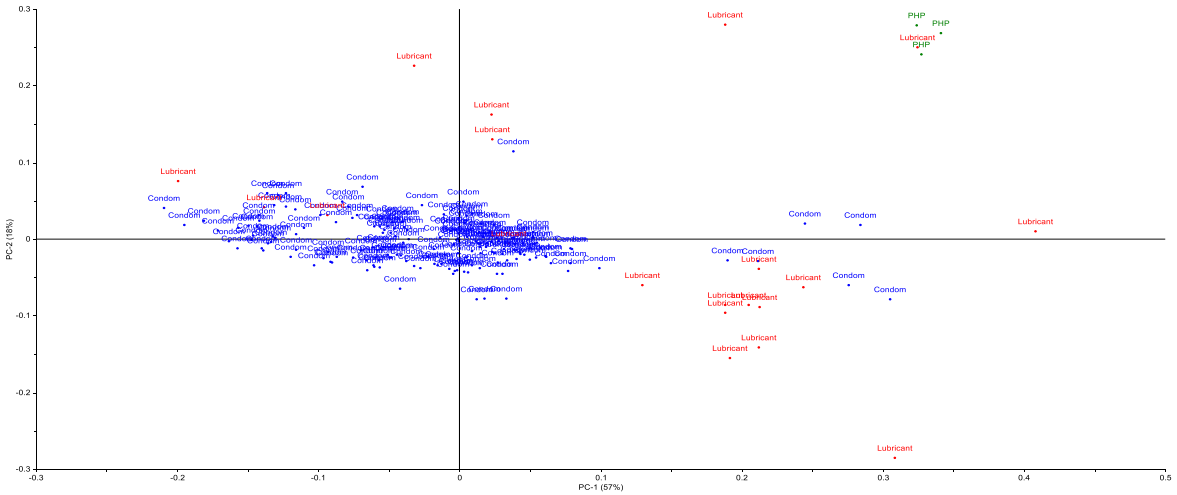

PC-3 (10%)
PC-2 (18%)
Condom
Intimate Product
Lubricant

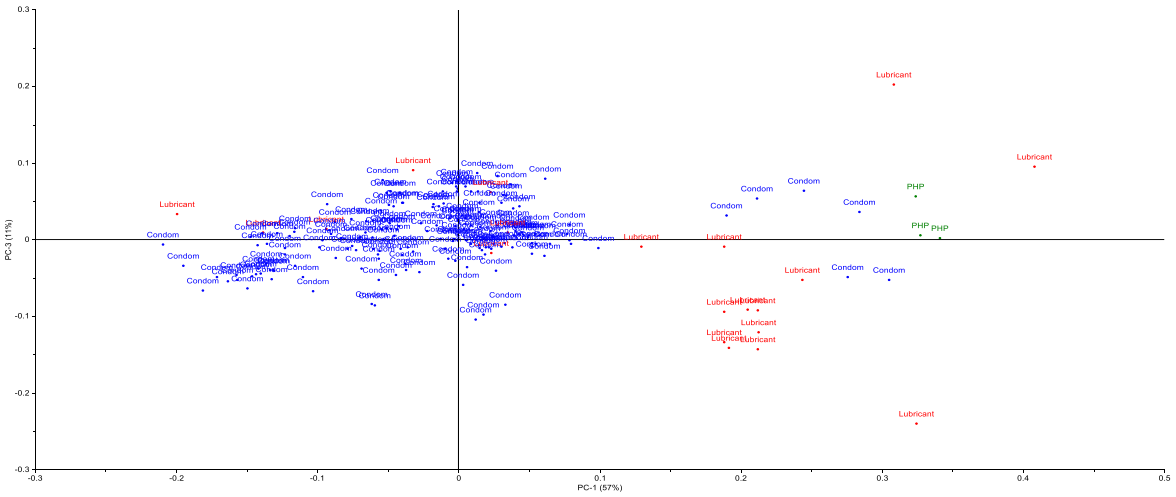

PC-2 (18%)
PC-1 (43%)
Unknown8.0
Unknown9.5
Unknown9.4
Unknown7.0
Unknown7.4
Unknown9.8
Unknown7.8
D8
D7
D5
D6
D4
Unknown13.
Unknown9.1
Unknown6.5
D3
D12
Unknown9.0
Unknown11.
Unknown10.
Unknown12.

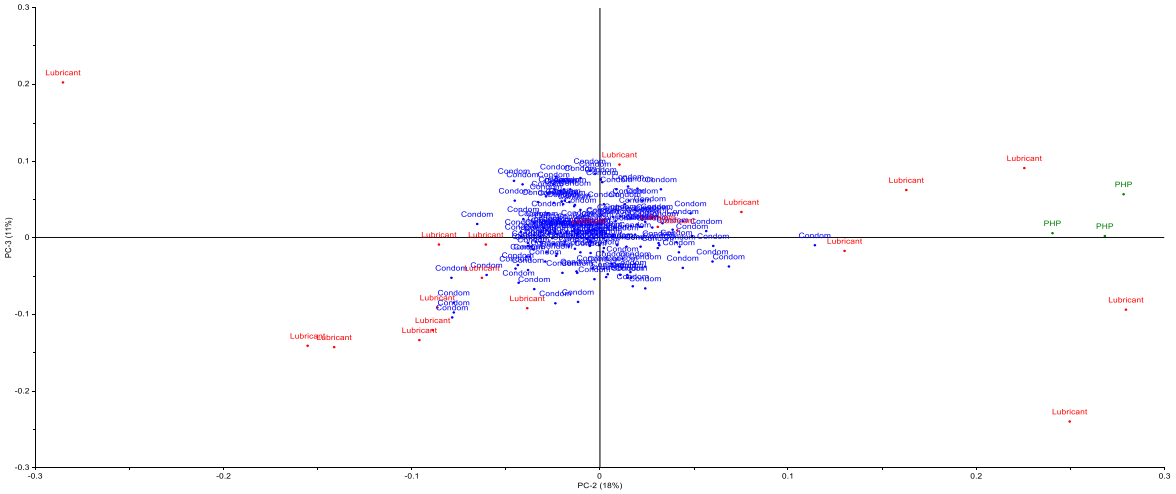

PC-3 (10%)
PC-1 (43%)
Unknown7.0
Unknown9.5
Unknown11.
Unknown9.4
D3
D6
D4
D7
D8
D5
D12
D10
D9
Unknown13.
Unknown9.0
Unknown9.8
Unknown7.4
Unknown7.8
Unknown12.
Unknown10.
Unknown7.1
Unknown8.9
Unknown9.1
Unknown8.0
Unknown6.5

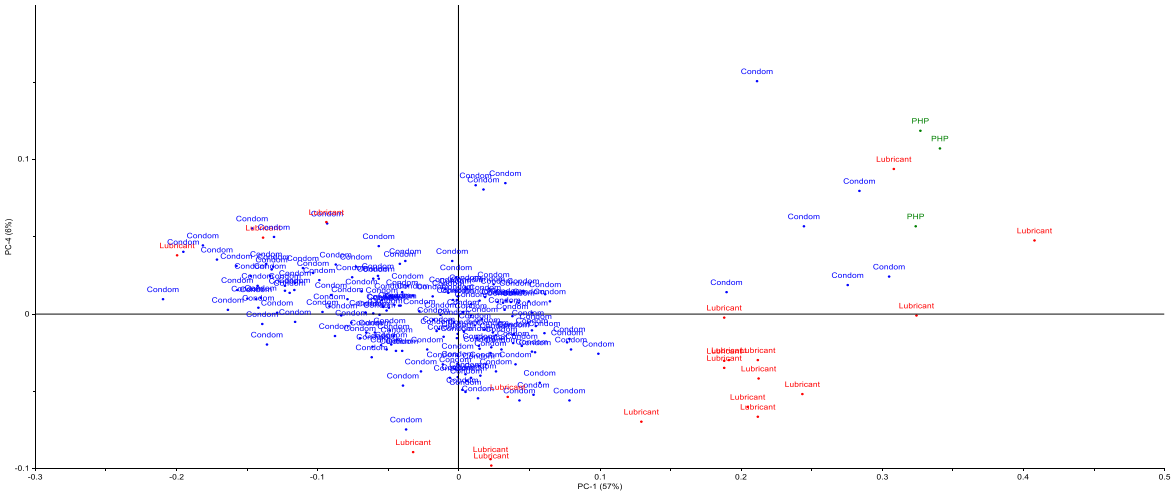


Variable selection 4 – 10 variables model

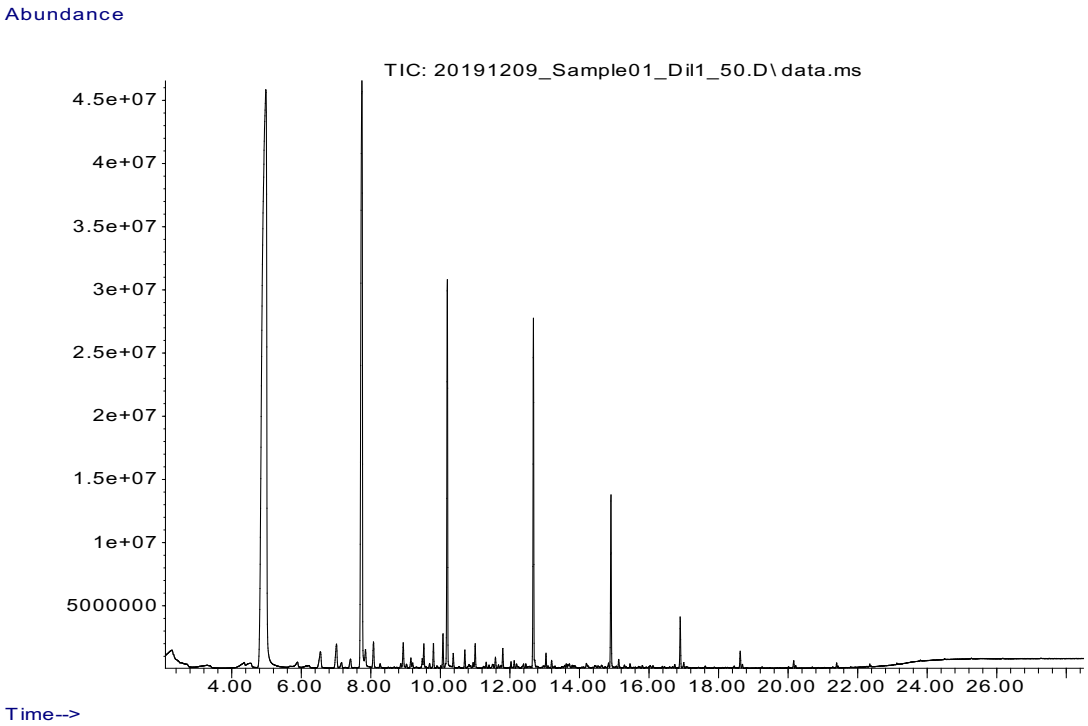


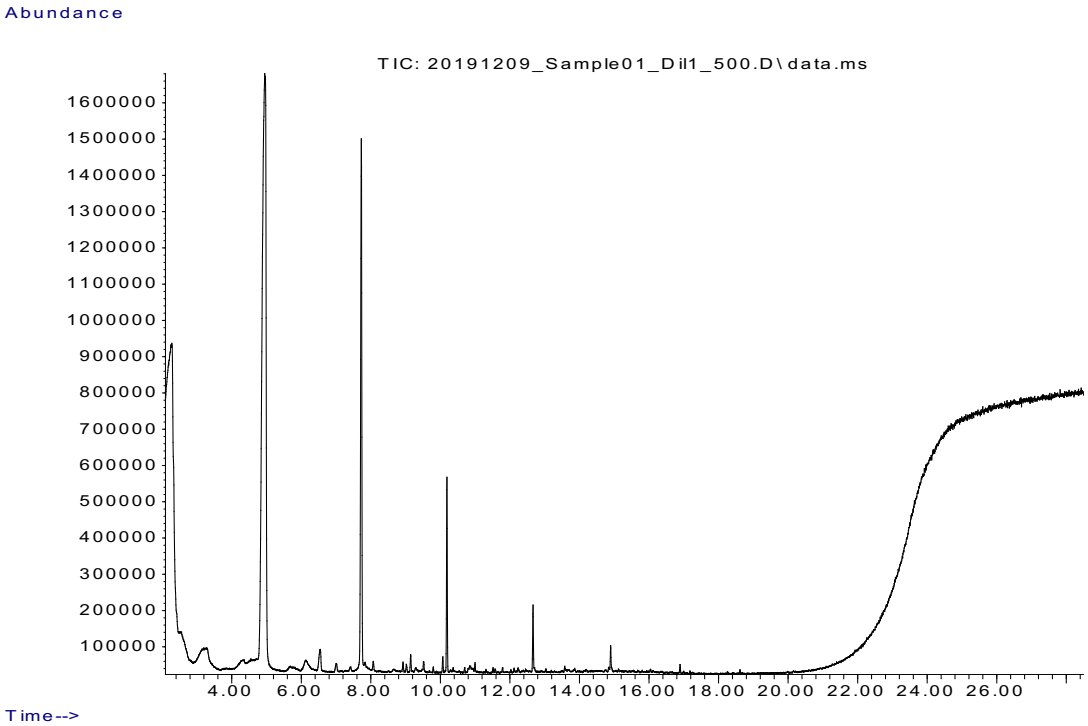

PC-3 (11%)
PC-1 (57%)
Lubricant
Condom
PHP

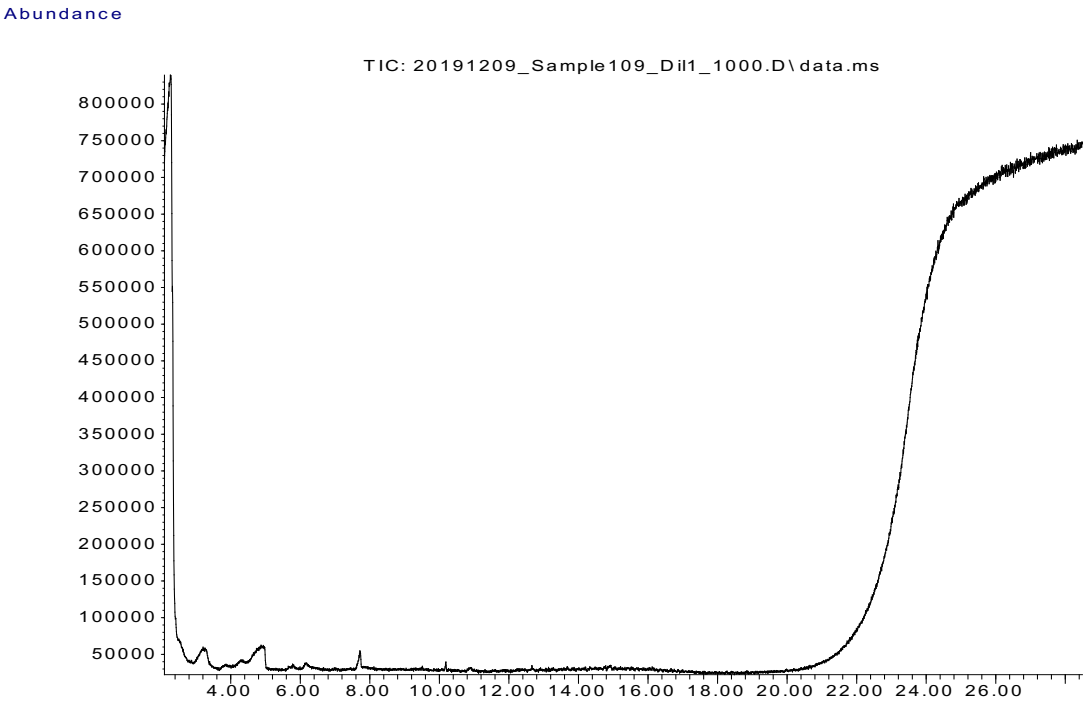

PC-3 (11%)
PC-2 (18%)
Lubricant
Condom
PHP


PC-4 (6%)
PC-1 (57%)
Lubricant
Condom
PHP

Appendix VIII: Impact of the dilution- Chromatographic patterns



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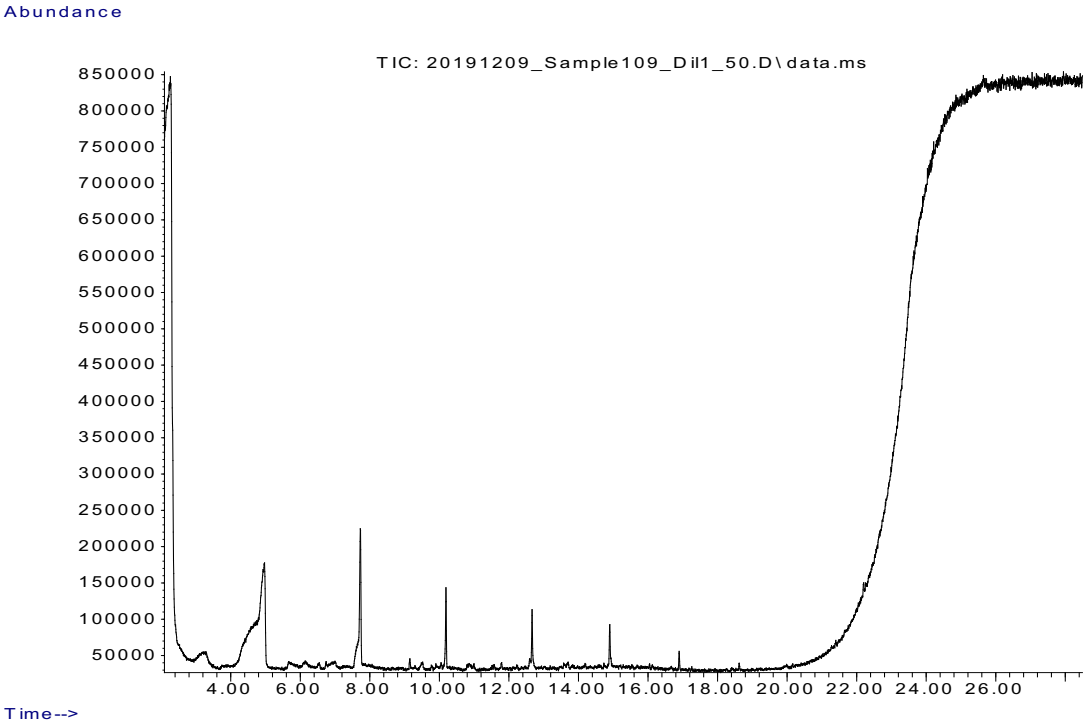
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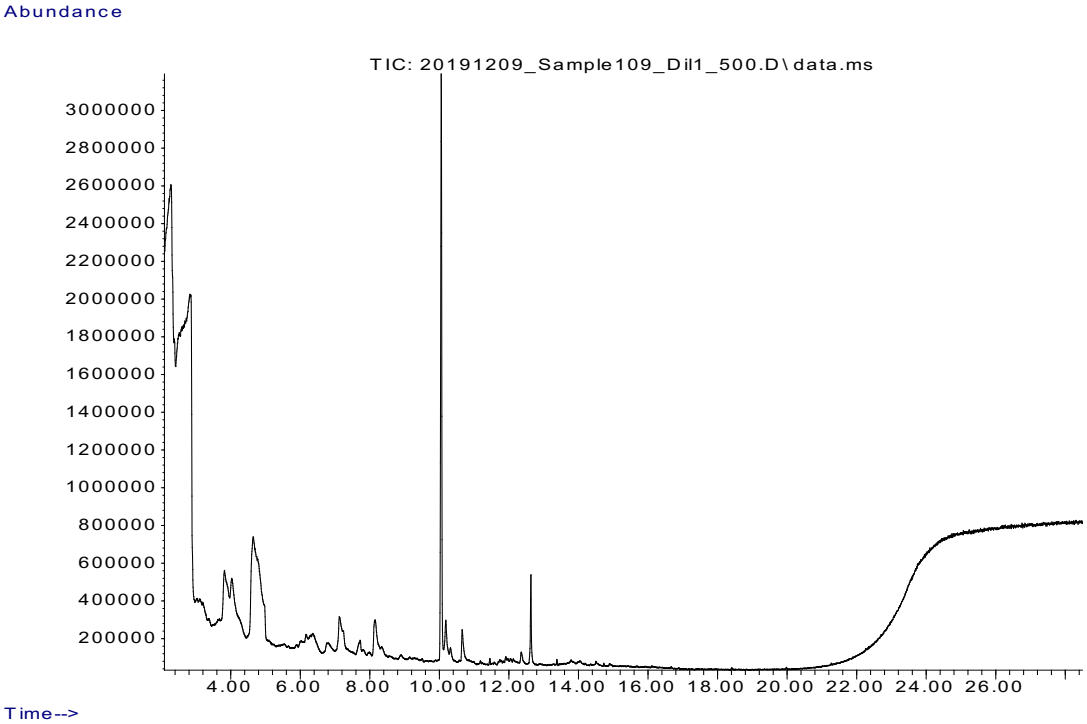
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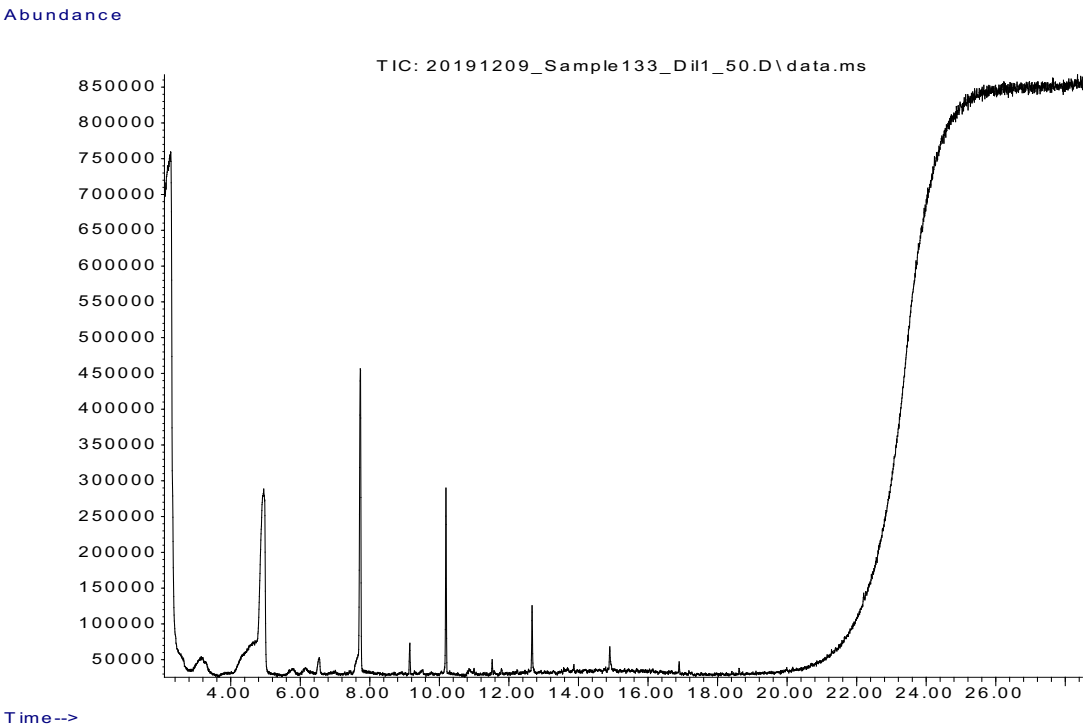
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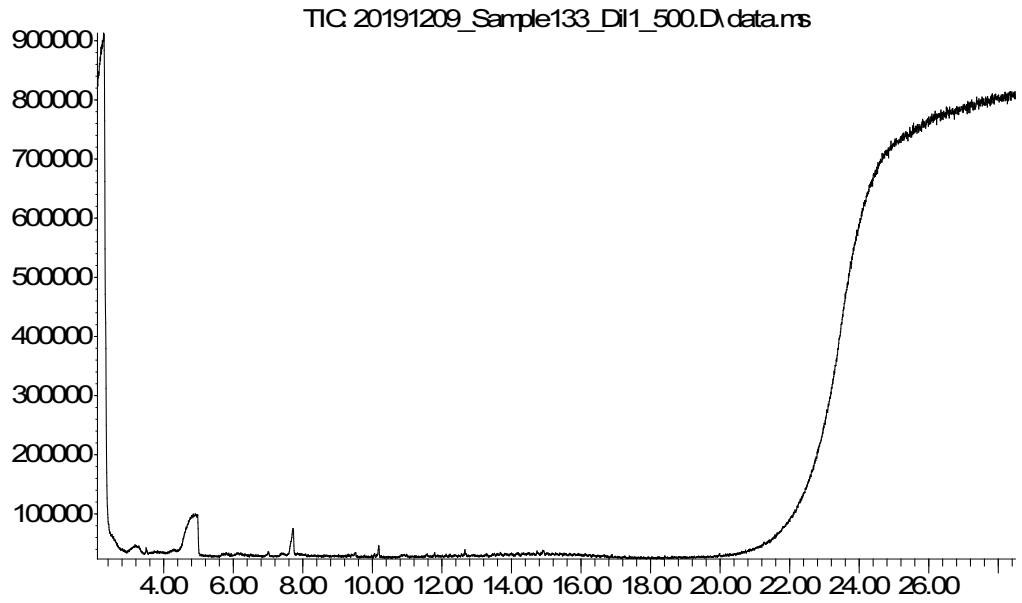

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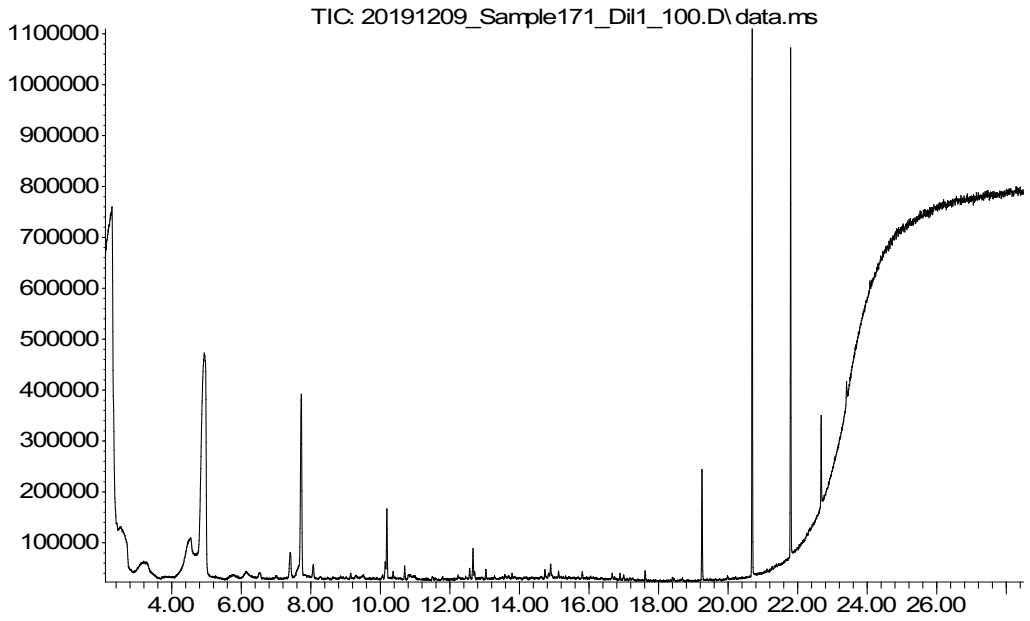

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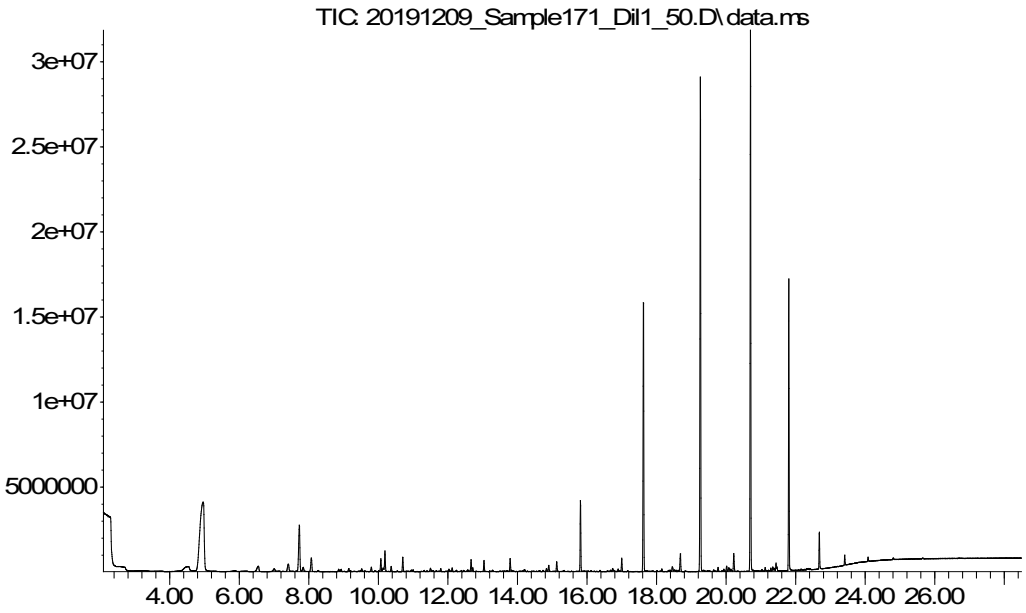

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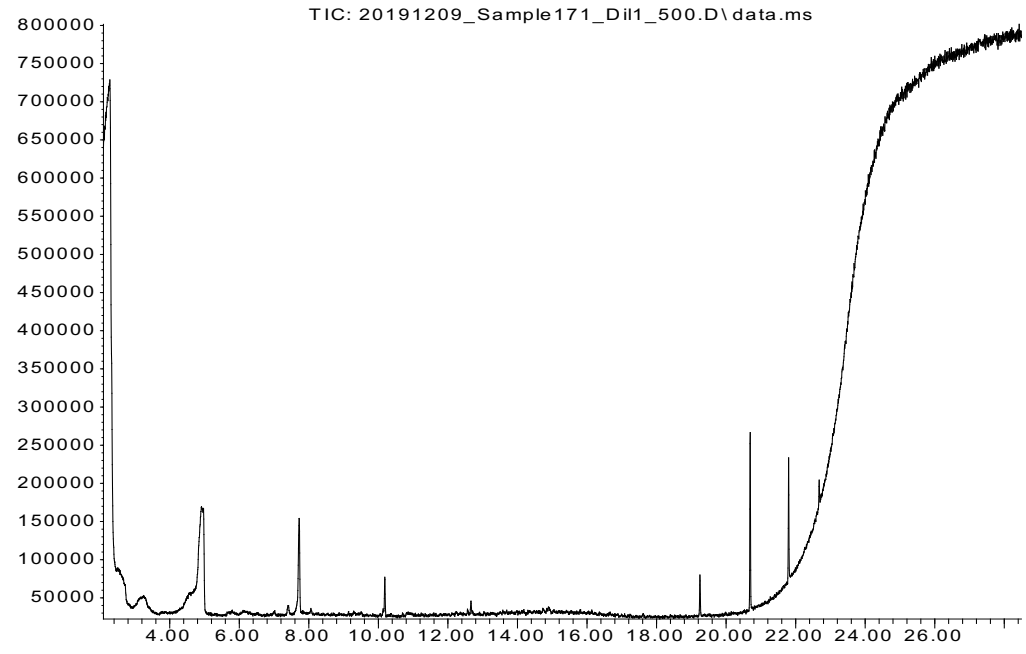

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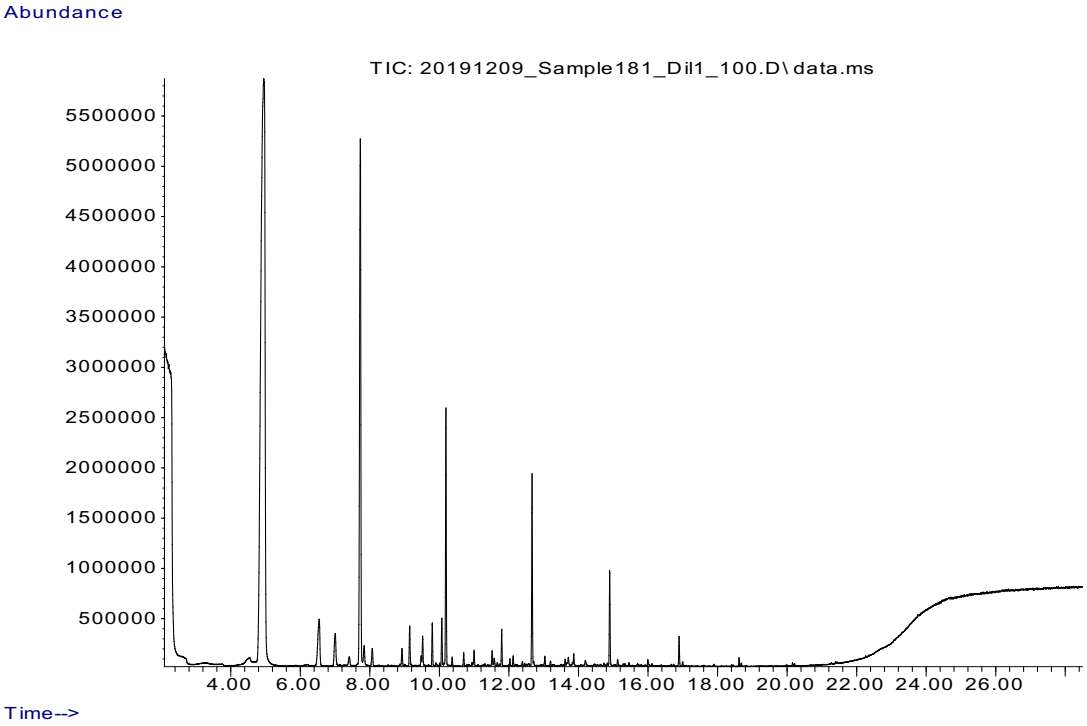
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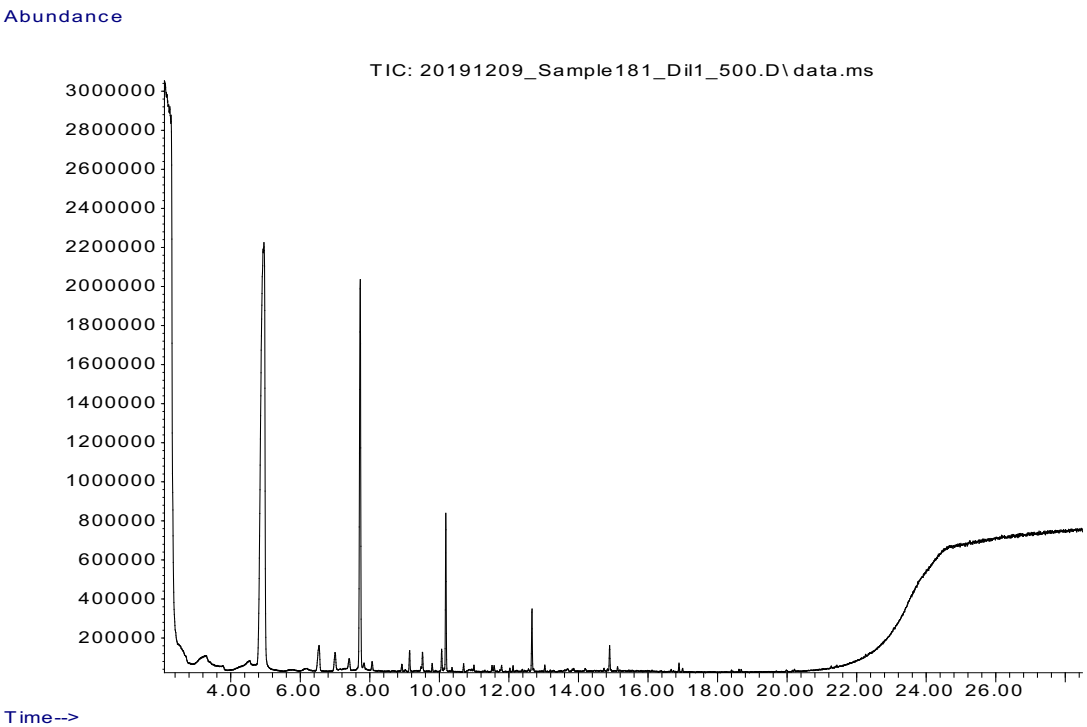
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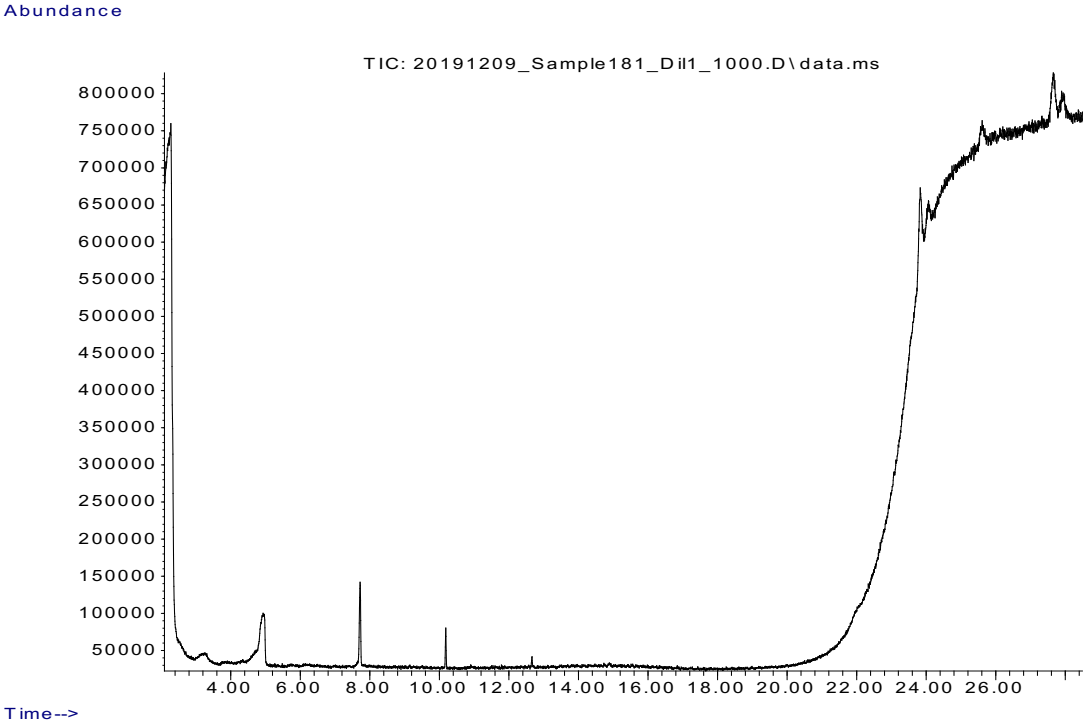
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Appendix IX: ATR-FTIR: Preprocessing variation and Multivariate Classification

IX-1 Model creation and variance variation according to the preprocessing used

	Percentage of explained variance			
Pre-processing	**PC1**	**PC2**	**PC3**	**Total**
Raw data	90	3	3	96
SNV	84	6	5	95
MSC	91	8	0	99
Der1	63	19	5	87
Der2	46	17	12	75
SNV + Der1	61	21	6	88
Der1 + SNV	59	13	8	80
SNV + Der2	45	19	10	74
Der2 + SNV	53	13	6	72
MSC + Der1	95	2	1	98
Der1 + MSC	82	14	2	98
MSC + Der2	78	10	3	91
Der2 + MSC	95	4	0	99

Table B: preprocessing methods applied on the dataset to enhance the discrimination of the samples. Legend: PC = principal component; SNV = standard normal variation; MSC = Multiple Scattering Correction; Der = derivative

IX-2 Classification and multivariate models

Supervised classifications were used on each of the pre-processed datasets presented in section 2.2 to evaluate the quality of each discrimination model. All the discrimination models were constructed using the first 3 PCs, treating each replicate of each sample as a separate sample. Discrete classes were attributed to each replicate based on observations of the chemical profile and knowledge of the samples (i.e. sample type, sample content, brand, model). Five classification techniques were used: LDA, QDA, SVM1 (linear), SVM2 (Polynomial, 3rd degree) and SVM3 (radial basis function). Confusion matrices were obtained, and model performances were calculated for each individual discrimination model created. Data were separated into a training set and a validation set, from data belonging to the model (2/3 of the samples used to build the model, 1/3 to test the model). The results of the validation using the test set are gathered here using performance analysis parameters, such as classification error rate, false positive and false negative rate. The main focus of the classification model is to adequately classify samples as coming from the condom population or another population.

Based on all these observations, classification based on the qualitative spectrum of the spectra would set 25 analysis (i.e. 5 samples) as false negative, i.e. the condoms being mixed up with lubricants and 25 analysis (i.e. 5 samples) as false positive, i.e. the lubricants being mixed with condoms. The question to answer can be formulated under the form of two mutually exclusive hypotheses which are:

H_1: A condom is the source of the chemical profile observed

H_2: Another product is the source of the chemical profile observed

Results of the classification models to compare for the raw data are presented in Table 44.

Table 44: Comparison of the performance of the 5 simple-class classification methods, after cross validation on the raw dataset. Results are presented for the validation using samples present in the model. Realised on the whole dataset. SVM a linear function, SVM2 uses a second-degree polynomial one and SVM3 uses the radial basis function to compute the data. All the replicates were considered as individual analysis, thus leading to n = 831.

Measure		LDA	QDA	SVM	SVM2	SVM3
Total samples	n	831	831	831	831	831
True Pos.	a	604	605	629	605	604
False Positive	b	25	24	0	24	25
False Negative	c	30	24	7	50	39
True Negative	d	172	178	195	152	163
Correct Classification Rate	(a+d)/n	0,934	0,942	0,992	0,911	0,923
Misclassification Rate	(b+c)/n	0,066	0,058	0,008	0,089	0,077
Sensibility	a/(a+c)	0,953	0,962	0,989	0,924	0,939
Specificity	d/(b+d)	0,873	0,881	1,000	0,864	0,867
False Negative Rate	c/(a+c)	0,047	0,038	0,011	0,076	0,061
False Positive Rate	b/(b+d)	0,127	0,119	0,000	0,136	0,133
Positive predictive Power	a/(a+b)	0,960	0,962	1,000	0,962	0,960
Negative predictive Power	d/(c+d)	0,851	0,881	0,965	0,752	0,807
General diagnostic Power	(b+d)/n	0,237	0,243	0,235	0,212	0,226
Training accuracy		93,38	94,22	99,16	91,1	92,3
Validation accuracy		-	-	98,55	91,09	92,05

Observations of the prediction accuracy for all the different combinations (65 in total) highlighted the same trend for all the different preprocessing tested. The SVM2 discrimination model did not provide proper discrimination, independently from the preprocessing, the general accuracy for this discrimination method being usually around 75%, except the raw dataset, the SNV set and the MSC set, with a maximal value for the SNV set, which presents an accuracy of 97% using SVM2 modelisation. The main changes generated by the different classification techniques are observed in the specificity rate which varies between 0.8 and 1, as the number of false positive and false positive were found to vary with the classification technique. LDA classification provided results from the raw data identical to classification of the samples based on the visual observation of the qualitative chemical profile. SVM was found to provide enhanced discrimination and offered the best discrimination model from a general point of view as it presented the lowest numbers of false positives and false negatives.

Observations on the overall data showed that most of the preprocessing methods did not significantly affect sample discrimination compared to the raw data classification (data gathered in supplementary information). The general accuracy of the raw data was found to be

respectively of 93.38% for the LDA, 94.22% for the QDA, 99.16% (training set) and 98.55% (validation set) for SVM, 91.1% (training set) and 91.09% (validation set) for SVM2, 92.3% (training set) and 92.05% (validation set) for SVM3 models. Only 3 models showed improvements over these numbers: SVM applied on SNV dataset (99.88%, training accuracy), SVM applied on Der1+ SNV dataset (100%, training accuracy) and SVM applied on Der2 + SNV dataset (100%, training accuracy). The most accurate prediction was observed on SVM applied on Der1 + SNV, with of 99.03% of correct classification using the validation set. However, given the distribution of the data, the classification of the samples would be more accurately achieved using the SVM3 model, as it also provides the most representative results of the data. Indeed, each of the tested classification methods have conditions to fulfil to be able to apply it to a sample batch. LDA assumes that the two populations tested fit a Gaussian multivariate distribution and present the same covariance. QDA considers the populations as having multivariate Gaussian distribution but assumes that the populations are neither homogenous nor equidistant. SVM learns the distribution of the data directly from the data and the separation limit is the best curve allowing proper separation of the two populations.

As previously highlighted in the discrimination study, it might be more relevant to be perform classification in two steps, as the silicone content affects the discrimination of the samples and may thus similarly affect the classification of the samples. Therefore, classification models were designed to inform on two different hypothesis that are consecutive to each other, and which defined as follows:

1. If the chemical profile contains silicones: This first classification step is mandatory to identify the proper cluster to subsequently project the sample. The aim of this first step is to identify whether silicones are present in the sample. One could state that visual observation could be sufficient to separate the samples, especially when considering that the visual classification of the samples was confirmed by chemometrics.
2. If the silicone-based chemical profile observed could come from a condom.

Discrimination models obtained using the raw data to answer these two questions are gathered in Table 45.

Table 45: Performance of the classification algorithm after cross validation on the raw dataset. Results are presented for the validation using samples present in the model. Realised on the whole dataset using SVM3 uses the radial basis function to compute the data. All the replicates were considered as individual analysis, thus explaining n = 831 for the overall dataset (classification according to the content), and n = 633 for the silicone dataset (classification according to the origin)

Measure		Overall dataset Classes: silicone or other	Silicone dataset Classes: condom or other
Total samples	n	831	633
True Pos.	a	633	603
False Positive	b	1	0
False Negative	c	1	6
True Negative	d	196	24
Correct Classification Rate	(a+d)/n	0,998	0,991
Misclassification Rate	(b+c)/n	0,002	0,009
Sensibility	a/(a+c)	0,998	0,990
Specificity	d/(b+d)	0,995	1,000
False Negative Rate	c/(a+c)	0,002	0,010
False Positive Rate	b/(b+d)	0,005	0,000
Positive predictive Power	a/(a+b)	0,998	1,000
Negative predictive Power	d/(c+d)	0,995	0,800
General diagnostic Power	(b+d)/n	0,237	0,038
Training accuracy		99.76	99.05
Validation accuracy		99.75	99.05

Training accuracy was found to be over 99% for most of the preprocessing methods, except for the first derivative, which presents an accuracy of 98%. Best validation accuracy was observed for the Der1+SNV preprocessing, with an accuracy of 99.36%. Interestingly this preprocessing was the one that systematically gave best results for all the hypotheses set previously presented. Therefore, this will be the preprocessing methods used with SVM classification for all the subsequent validation procedures, as well as the for the raw data.

4.5.3 Exploring multivariate classification

Sample classification under the form of two major hypotheses was found to be quite accurate for most of the samples. This was expected as the spectra of the two groups were significantly different visually. However, initial groups contained more than one sample category, either based on the initial type of sample or the visual spectra. Visual classification of the samples showed differences in the chemical profiles of the non-silicone-based samples, mainly based on the presence or the absence of certain peaks or based on the shape of the main peaks in the spectra. The operator could therefore easily classify the samples into 9 different categories, based solely on the appearances of the chemical profiles. Multivariate classification of the 5 classes known to be present in the dataset was then investigated.

LDA, QDA and SVM classification were used on the 3 first principal components obtained from the raw dataset. Classification were performed 1) as a function of the visual classification (i.e. silicone based or other type of chemical profile), and 2) as a function of the initial sample

type (i.e. condom, lubricant, cream, oils or intimate products). SVM was not considered suitable as it classified samples in only two categories, which were lubricants or condoms. Differences were definitely observed and reported on a visual point of view. If SVM classification was found to be quite accurate for bivariate classification, it is not adequate for the multivariate purposes found here. LDA is still not recommended for the classification purposes in this study as the different groups definitely do not share the same variance, although the accuracy was slightly better than that of QDA (85.8% for the LDA against 84.7% for QDA). QDA was found to be the most representative classification model, knowing the sample real classification. Table 46 present the results of the confusion matrix obtained for the discrimination of samples using QDA.

Table 46 : QDA confusion matrix of 84.7 % accuracy, showing predicted class of sample vs actual class of samples. Classes are labelled as sample types.

Predicted/Actual	Condom	Lubricants	Massage Oils	Intimate Products	Creams
Condom	605	23	0	0	0
Lubricant	23	55	0	0	0
Massage Oil	0	0	5	0	0
Intimate Product	0	5	0	5	2
Creams	1	47	0	26	34

Table 46 highlights several misclassifications of samples that are worth discussing. Although most of the condoms were classified correctly, 23 of them were classified as lubricants. This is due to the 5 samples that were previously highlighted as presenting significantly different chemical profiles. One of the condom samples was classified as a cream. This sample was the outlier from FairSquared Sensitive Dry sample, that was already highlighted as presenting two different profiles across 10 replicates. This condom contained almost no lubricant but a significant amount of solid particles. Other chemicals may also be found on this condom leading to this misclassification. However, all the other replicates were correctly identified and clustered. The observation of the chemical profiles led to the conclusions that this sample was an outlier and was not considered for the rest of the analysis.

All massage oils were properly classified, as they present a significantly different chemical profile and peak patterns. These oils already clustered together when processing PCA.

Misclassifications occurred between the intimate products and creams samples. Most of the intimate products were classified as being creams. The correlation matrix for the questioned samples was examined, and Table 47 shows the mean correlation value of three misclassified samples calculated on the five replicates obtained for each sample. Correlation values were found to be close for both intimate products and cream products, thus explaining the

misclassification: the distance to the centroid of each group was not clearly different and therefore replicates of these samples were misclassified.

Table 47: Discriminant values for misclassified samples showing the mean correlation between the spectra and the centroid of each groups

Sample	Condom	Lubricant	Massage oil	Intimate products	Creams
154	-76,67197	-5,7741798	-713503,38	-4,8082102	-4,4251296
160	-55,303863	-5,8326261	-557581,21	-4,8378709	-4,0866171
166	-73,497846	-5,6540652	-714940,64	-4,847604	-4,7681422

To understand why the correlation values were so close, it was necessary to consider the sample composition. All the samples contained glycerin except one intimate product. 3 cream samples out of 7 also contained silicones (indicated as dimethicone or cyclopentasiloxane). Intimate products were found to contain mainly water and other types of common chemicals such as sodium laureth sulfate, triglycerides, propylene or pentylene glycol or lactic acid. The five samples correctly classified as intimate products were from a product (FemFresh Feminine deodorant spray) containing silicones but no other lubricant (i.e. no glycerin or propylene glycol). The two creams incorrectly classified as intimate products were found to be only one of 5 replicates each acquired for the two considered samples (*Rosken Intensive Moisture Hand Cream* and *Love My Ink Tattoo Cream*), and were therefore not considered These observations suggest that samples containing glycerin and siloxanes are all classified together and that the main difference between the groups is the difference of the minor components or the relative abundance of each of the main lubricants used and the purpose of their use. Indeed, glycerin is used as an emulsifier or stabilizer and therefore will be more abundant than siloxanes which are usually used as anti-foaming agents. This easily explain the lack of discrimination between the samples coming from creams and from intimate products.

Lubricants was the class which presented the highest rate of misclassification. Among the 130 replicates that in this category, 42.3% were correctly classified, 17.6% were classified as condoms, 3.8% as intimate products and 36.1% as creams. The lubricants classified as condoms were respectively *Ansell Lifestyles Luxe Silicone-based* lubricant (Sample 109), *Ansell Skyn Maximum Performance* lubricant (Sample 133), *Astroglide Diamond Silicone Gel Personal Lubricant* (Sample 171), *Astroglide Waterproof Silicone Liquid* (Sample 174), *Durex Play Perfect Glide* (Sample 181). These samples are silicone-based lubricants and were clustered with the biggest group fitting their chemical profile, which contained 121 condom samples containing silicones. Interestingly, the correlation variables obtained for Sample 174 were found to be very close between condom and lubricants, and some of the replicates of this sample

were found to be clustered adequately with lubricants, as illustrated by the discriminant values gathered in Table 48.

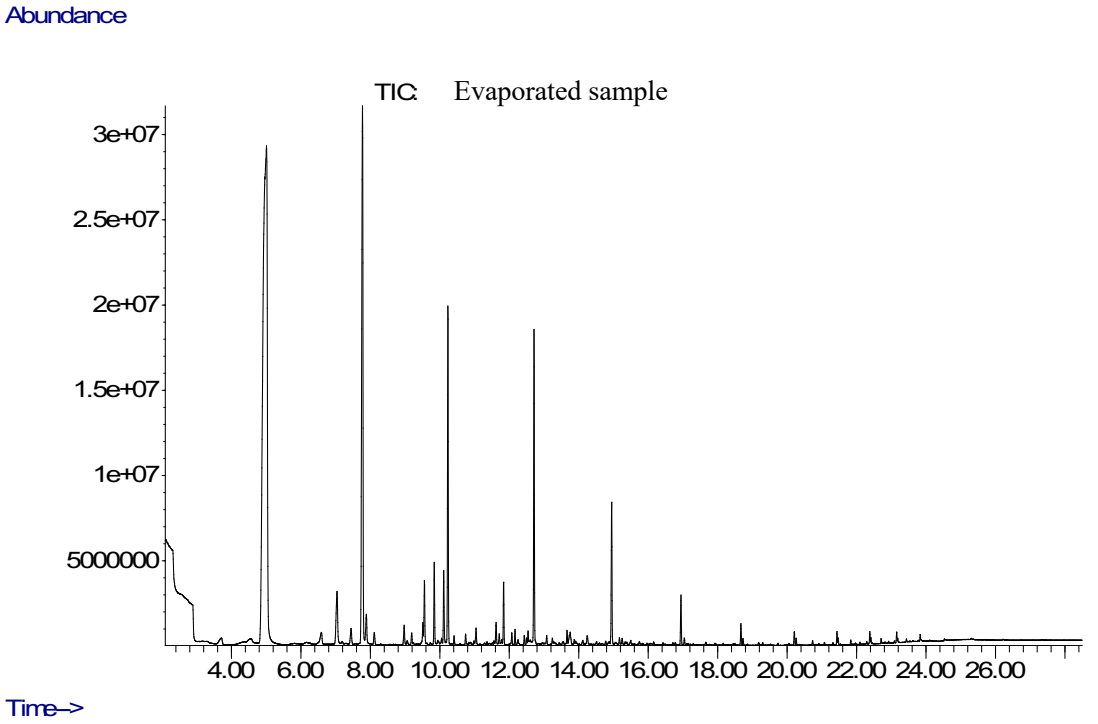
Table 48: Discrimination values of Sample 174.

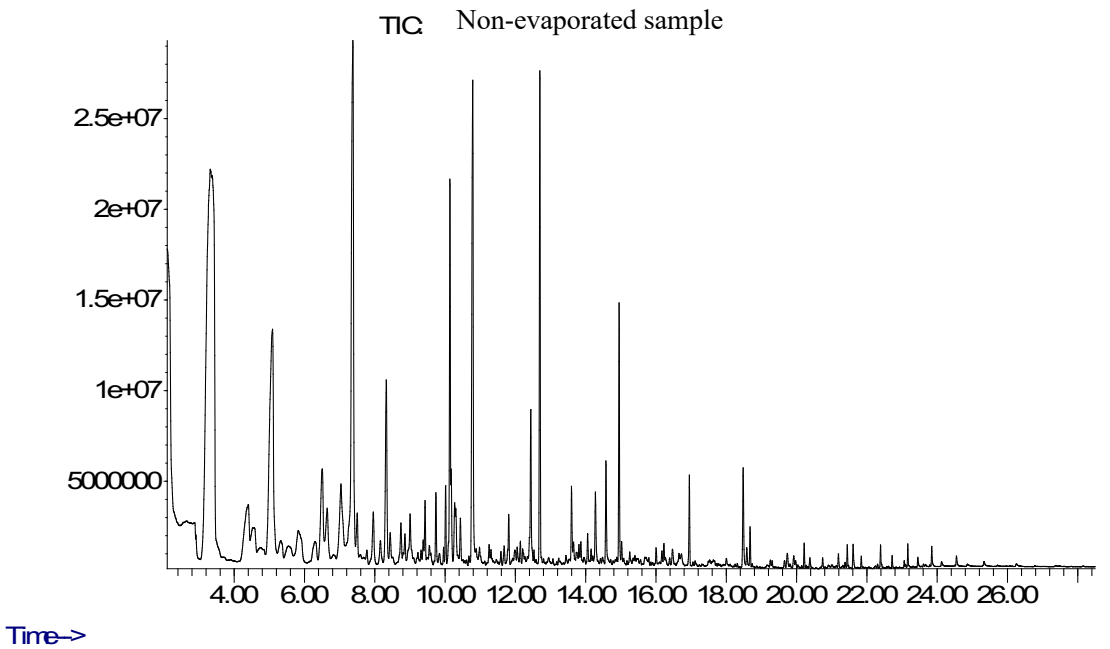
Sample	Condom	Lubricant	Massage Oils	Intimate products	Creams	Predicted Class	Actual Class
Sample174_01	-5,32432	-7,062156	-2012495	-15,42609	-175,7591	Condom	Lubricants
Sample174_02	-5,491717	-7,064705	-2012721	-15,67692	-175,7901	Condom	Lubricants
Sample174_03	-5,557473	-7,070156	-2010983	-15,59801	-176,0958	Condom	Lubricants
Sample174_04	-7,362357	-7,09257	-1936273	-14,4904	-175,2129	Lubricants	Lubricants
Sample174_05	-14,04444	-7,185297	-1742961	-12,75779	-167,8928	Lubricants	Lubricants

Examination of the discriminant values belonging to the incorrectly assigned spectra of replicates from Sample 174 show they have different correlation values within the categories and the replicates. Replicate 4 was found to show similar correlation to both condom and lubricant categories in comparison to the large distances with other categories. Replicate 5 presents a correlation value twice bigger for the condom than for the lubricant. These observations suggest variations can be observed in the different chemical profiles. However, no significant qualitative variation was observed, although the peak around 1100 cm^{-1} seems to present a slightly different shape between the two replicates. This suggests the algorithm is able to detect variations the operator is not able to visually assess.

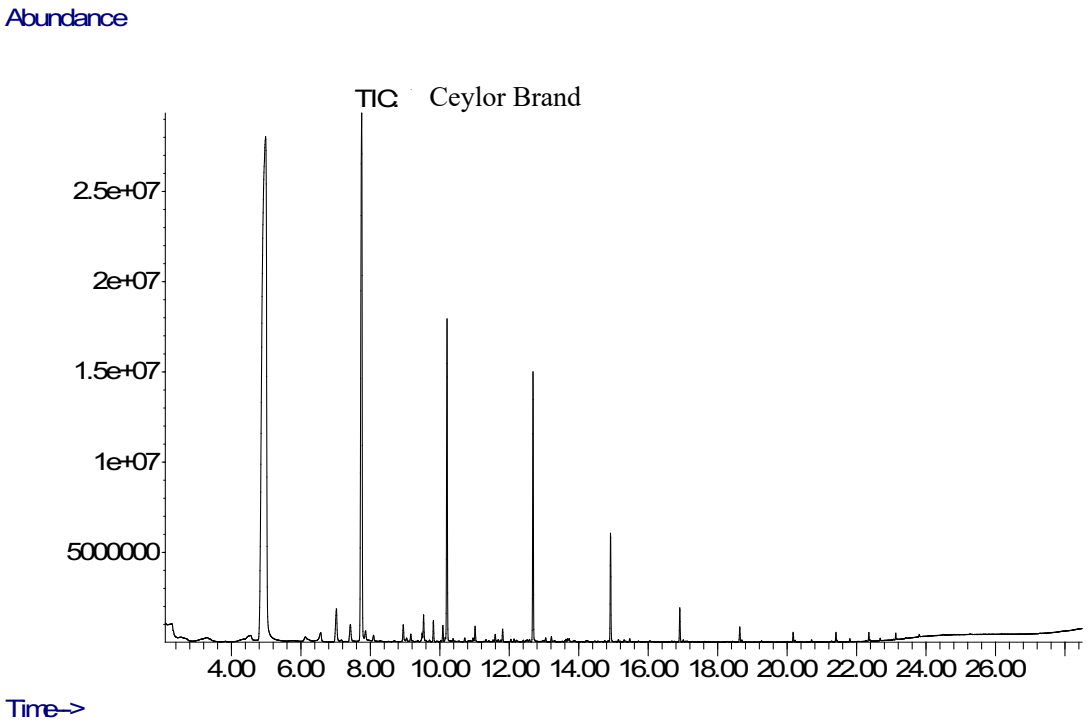
Appendix X: py-GC/MS chromatograms of multiple condom brands

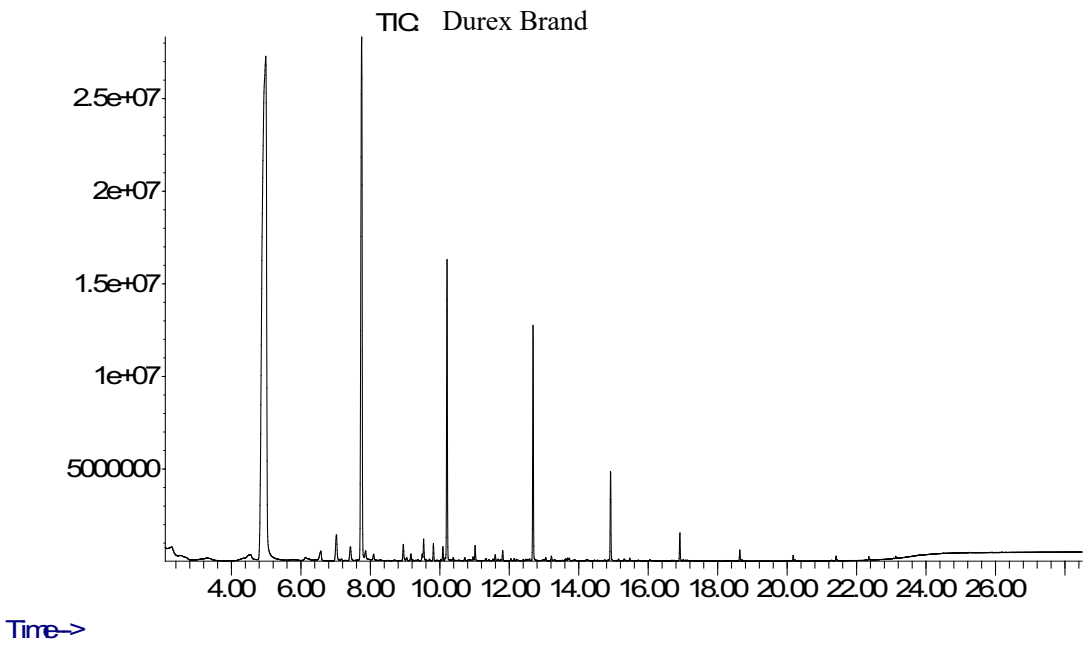
X-1 Comparison of evaporated and non-evaporated chemical profiles

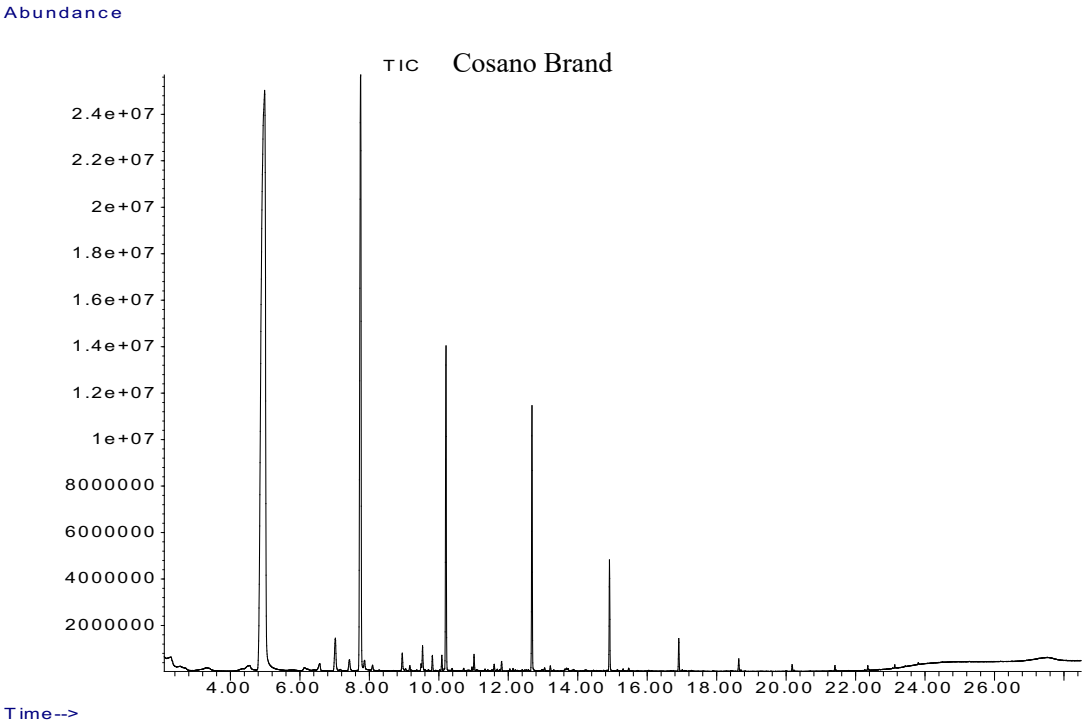


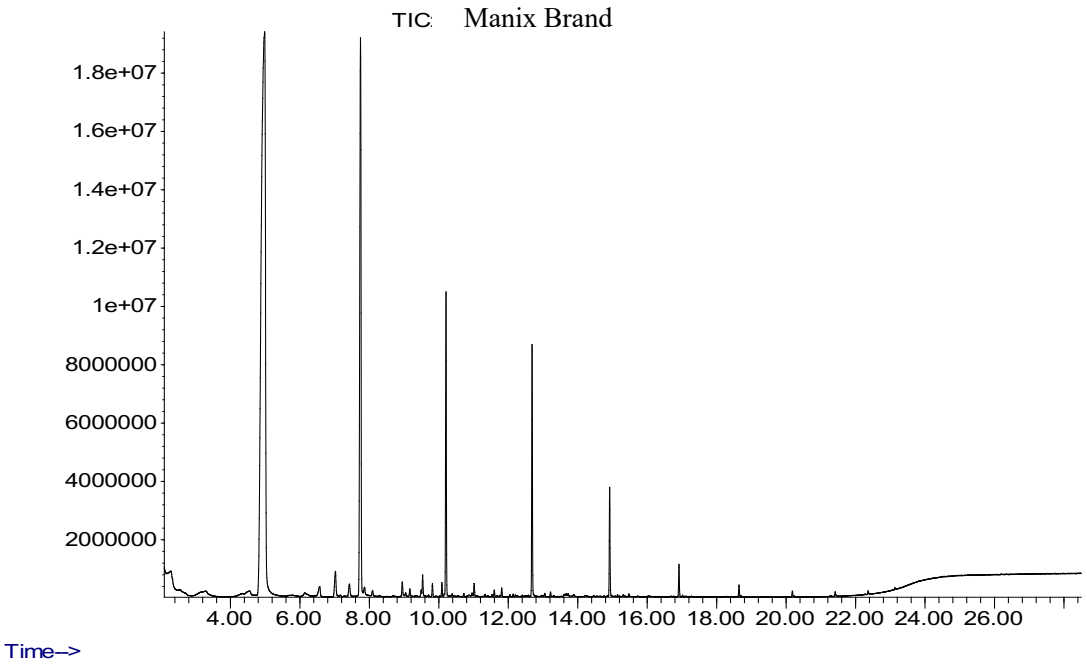


X-2: Multiple condom brands chromatograms

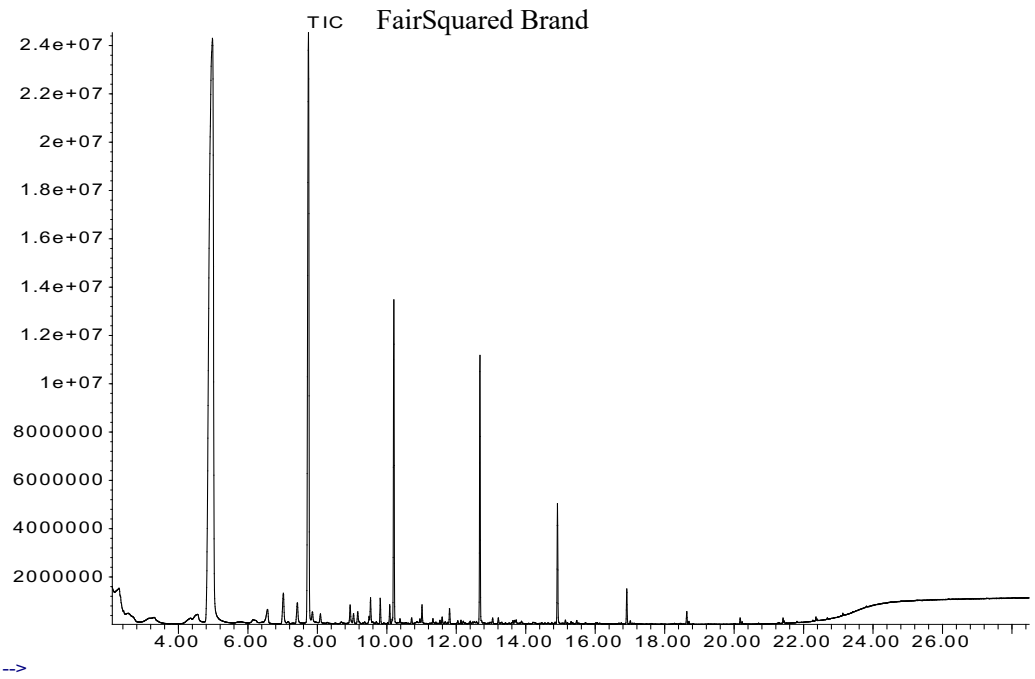




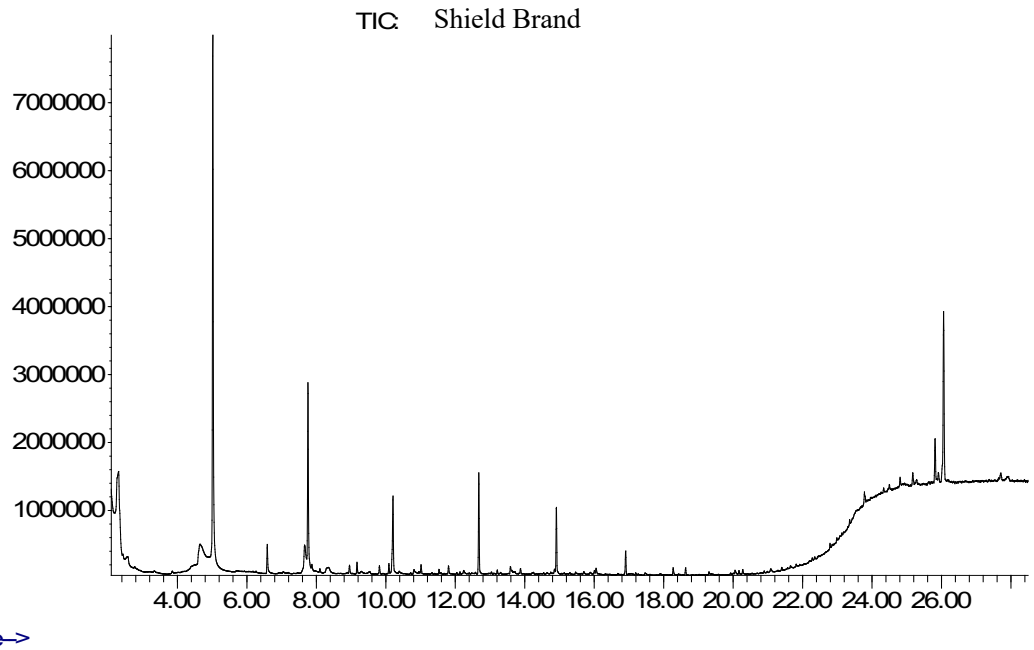

Abundance
TIC Cosano Brand
2.4e+07
2.2e+07
2e+07
1.8e+07
1.6e+07
1.4e+07
1.2e+07
1e+07
8000000
6000000
4000000
2000000
4.00 6.00 8.00 10.00 12.00 14.00 16.00 18.00 20.00 22.00 24.00 26.00
Time-->

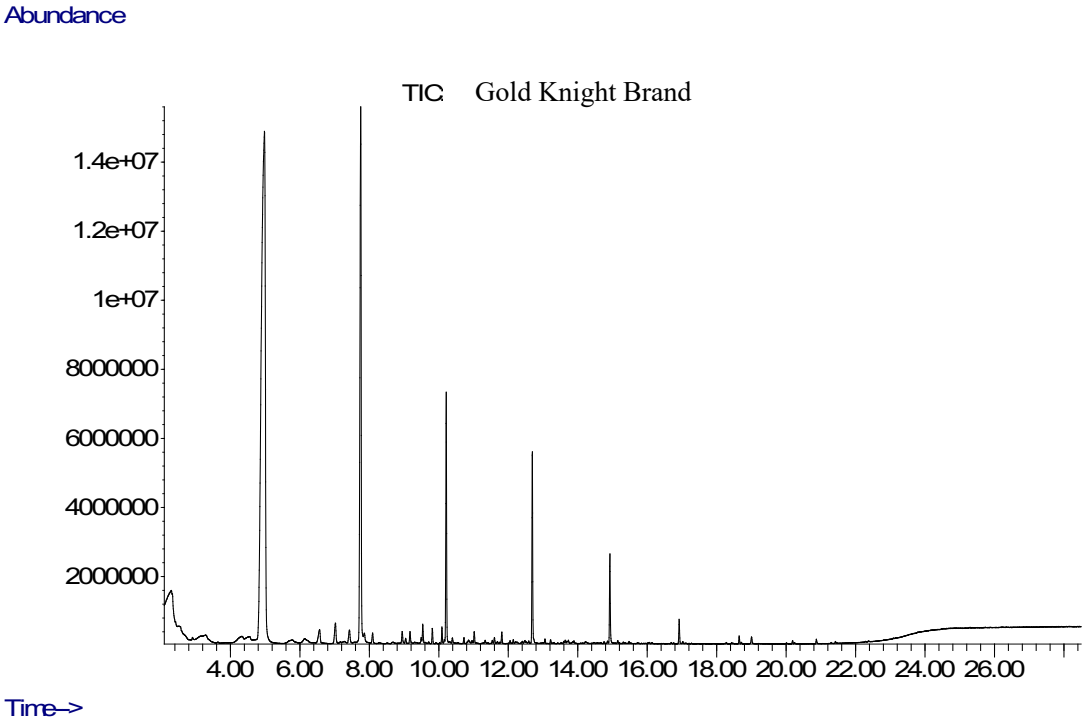

Abundance
TIC: Manix Brand
1.8e+07
1.6e+07
1.4e+07
1.2e+07
1e+07
8000000
6000000
4000000
2000000
4.00 6.00 8.00 10.00 12.00 14.00 16.00 18.00 20.00 22.00 24.00 26.00
Time-->

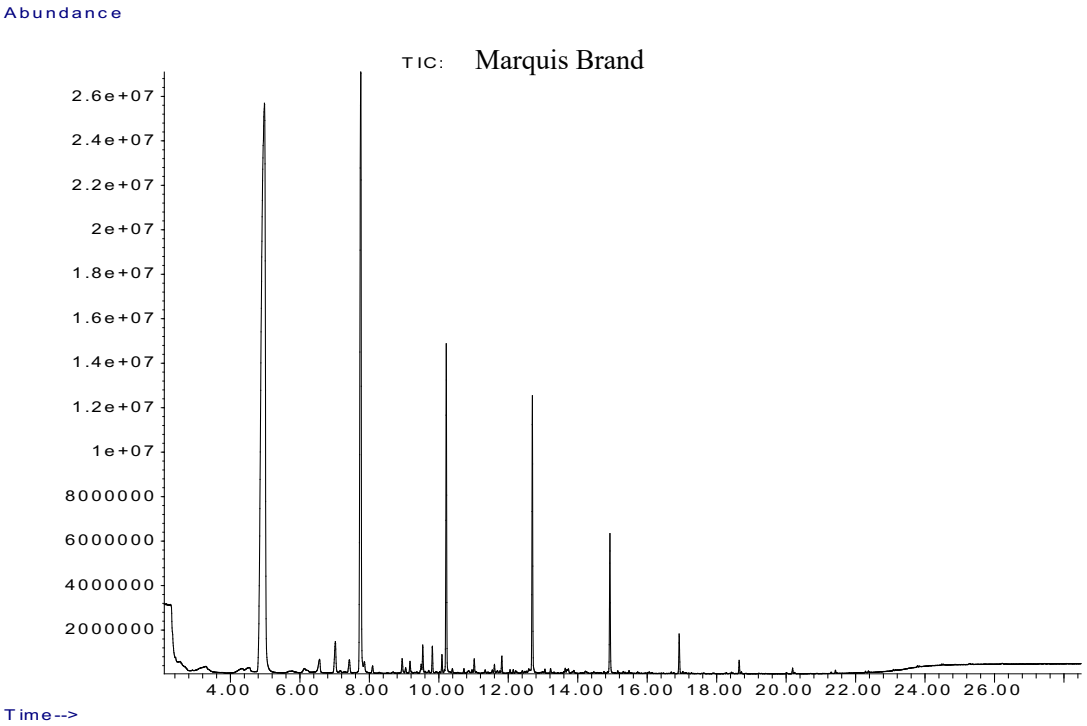
Abundance



Abundance




Abundance
TIC: Gold Knight Brand
1.4e+07
1.2e+07
1e+07
8000000
6000000
4000000
2000000
4.00 6.00 8.00 10.00 12.00 14.00 16.00 18.00 20.00 22.00 24.00 26.00
Time-->


Abundance
TIC: Marquis Brand
2.6e+07
2.4e+07
2.2e+07
2e+07
1.8e+07
1.6e+07
1.4e+07
1.2e+07
1e+07
8000000
6000000
4000000
2000000
4.00 6.00 8.00 10.00 12.00 14.00 16.00 18.00 20.00 22.00 24.00 26.00
Time-->

Analysis of Condom Evidence in Rape and Sexual Assault Cases

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Development of an analytical and interpretative framework and application

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APPENDIX 1: Publications

Céline A. Burnier

2021

Forensic Science International 302 (2019) 109861

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Review Article

Pre-analytical considerations of condom traces: A review of composition, background, transfer and persistence

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Activity
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ABSTRACT

Today, rape and sexual assault cases are mainly solved using evidence such as medical evidence or DNA analysis. Condom traces have been found to be present in 10% of assaulted women, when no DNA is found [1]. Numerous studies have emphasized the interest of analysing the composition of male condoms and their traces, and developing specific methods for the analysis of this type of evidence. However, transfer and persistence of condom traces in a specific matrix are rarely referenced. Therefore, forensic scientists have no complete knowledge of the trace and what could be expected in a real case. The purpose of this article is to review the literature addressing the composition of condoms and their traces as well as its influence on the transfer and persistence from a forensic point of view.

Peer-reviewed literature, patents, professional literature, data from international administrations and international organisations' reports have been used to track the composition and the problematics of transfer and persistence of condom traces.

The results of this review show that the composition of male condoms and their traces are complex systems, with numerous compounds originating from the condom at the moment of the transfer and evolving over time according to specific persistence patterns. Although numerous types of analyses have already been proposed and tested for condom traces, forensic evidence considerations have not been fully studied yet.

Considering the fact that sexual assaults without the detection of DNA are increasingly frequent, there is a definite medical and forensic need to improve our knowledge of the processes involved in the development of condom traces in order to better understand analytical results.

Contents

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0379-0738/

1. Introduction

The composition of condoms is heavily regulated by international norms [1–5]. However, information available online or provided by manufacturers or industries is insufficient for forensic scientists working on condom traces. The original chemical composition of condom varies both qualitatively and quantitatively when transferred into the matrix (i.e. the vaginal matrix) and then interacting with that matrix. Therefore, the composition of condom traces may differ substantially from the original condom chemical composition. Furthermore, in rape or sexual assault cases, condom traces, like DNA, will seldom be collected immediately after deposition. Therefore, chemical, physical and biological alterations over time will affect the condom traces left in the vagina at the moment of intercourse and hence will modify not only their initial composition but also the quantity and the persistence of these traces.

There has been no recent review covering the chemical composition of condoms since Maynard et al.'s review in 2001 [6]. Therefore, this article aims to provide an up-to-date review of the literature regarding the qualitative and quantitative analysis of compounds identified as condom components, which may be detected in condom traces. Research concerning condoms, which began in the 1990s, reported the possibility to detect condom or lubricant residues but did not allow proper discrimination of different samples [7–9]. In the last decade, improvements in analytical instrumentation and automated statistical analyses have enabled differences to be observed between chemical profiles and the classification of samples. Condom samples are now detected with a better sensitivity and selectivity, but this has led to new questions on condoms and their traces for the forensic scientist, such as the possibility to link a trace to a reference profile in a database, with high confidence and low error rate.

However, despite the large amount of research carried out on this topic, advanced knowledge on condom differentiation based on their composition has not been completely achieved yet. This is mainly because of the technical difficulties linked to the targeted compounds, despite continuing efforts to overcome these [10–16]. The detection of condom traces in a vagina is a strong analytical challenge, for two reasons. Firstly, the complex chemical properties of the condom components can be highly problematic. This is due to polar and non-polar compounds, with low and very high molecular weights which are present together in the sample. Secondly, the composition of the condom and its traces may vary as follows:

1 Original condom composition: This corresponds to the condom composition as it comes out of the packaging. All the compounds are present in defined quantities.
2 Pre-coïtal state of the matrix (background): This corresponds to the presence of traces for legitimate purposes. Use of personal hygiene products or recent coitus with a condom can affect it and should be considered.
3 Initial post-coïtal composition (transfer): This corresponds to the transferred condom residues immediately after the contact between the condom and the vaginal matrix. All compounds identified on the condom are taken into consideration.
4 Unknown delayed post-coïtal composition (persistence): This corresponds to the evolution of the initial composition over time. Compounds' disappearance and relative proportional changes in the amount of residues have to be considered.

When considering condom traces persistence, it is therefore necessary to take into account the role of these factors, which include time, activity of the victim or the vagina itself, as well as the initial and unknown post-coïtal composition, which will influence the amount of traces.

Condom traces are complex and their analysis are a real challenge for forensic scientist. Indeed, a large variety of analytical procedures have been tested, from simple microscopic methods to very complex mass spectrometric methods, resulting in an expansive, expensive and complex combination of analytical procedures. A significant variability of the transfer of the traces in the vaginal matrix is expected to happen due to uncontrolled and unknown factors such as individual human variations (e.g. menstrual cycle). In addition, the persistence process adds variability as it strongly affects the quality of the recovered traces. It is also affected by the elapsed time as well as the victim's activity (e.g. washing), or the trace's protection (e.g. avoid contaminants).

This paper will begin by presenting the condom composition and a detailed description of the compounds identified on different condom types. The condom traces will then be considered, as well as the transfer, the persistence and the background, and the factors influencing the latest. Finally, perspectives in the field of chemical analysis of condom traces will be outlined and personal consideration will be added. The issues of analytical procedures used to analyse condom traces as well as the operationalization of forensic analysis of condom will be discussed in a separate publication.

2. Article search strategy and study selection

An article search was conducted using the following electronic databases: PubMed, Scopus and Web of Knowledge, for articles published between 1980 and 2019 using predefined search terms (i.e. condom, analysis, forensic, spermicide, lubricants, nonoxynol, PDMS). The bibliographies of the published articles were further explored to identify new relevant papers. For the current review, an update of the original literature searches was conducted in April 2019. Studies with forensic purposes were considered as main articles. We included in the analysis only papers written in English and French. Only full articles were considered. No other publications were excluded from the analysis. Legal articles or considerations as well as medical papers were then added and used to better understand problematic issues that could affect the forensic analysis. All the published papers found were used for this review, though this paper offers no pretention of being fully exhaustive.

3. Condom composition and other use of target compounds

The composition of condoms is regulated by international standards (ISO4074, ISO 157) [1] as their use for contraceptive purposes has to be ensured, as well as the biocompatibility of the constituents. There are four main constituents: (1) the body of the condom, (2) solid particles, (3) the lubricants and (4) the spermicide. A fifth category (5) of constituents might be considered as all other compounds added for heating, aroma or other specific purposes. All the compounds are described below.

3.1. Body of the condom

The body of the condom is the main constituent of the condom and is usually made of latex, also known as 1,4-cis-polyisoprene [17–19]. To ensure good mechanical resistance, the latex is vulcanized, generating small quantities of dithiocarbamates and nitrosamines [18]. The latex itself is not of forensic interest as explained by Causin: "No traces are shed from the bulk polymer material used for producing condoms, which is thus quite irrelevant from a forensic point of view" [19].

As latex proteins and nitrosamines are allergens [20], manufacturers have developed condoms without any vulcanization residues or proteins. Synthetic polyisopren can be used in case of mild allergies to latex or if the allergy is due to the latex proteins [20]. In other cases, polyisopren is replaced by polyurethane, polyethylene or sheep caecum, with polyurethane shown to be the most commonly used [17,18,20,21]. Polyurethane presents numerous advantages, such as not being sensitive to degradation with time, or exposure to oil-based lubricants. Rosenberg et al. have also shown that polyurethane condoms are preferred by users [21].

In addition, it is necessary to consider the current uses of latex and of nitrosamines. Nitrosamines are carcinogenic compounds, found in numerous cosmetics, phytopharmaceuticals and latex-based products. The species present in condoms are not considered to be toxic for humans [22]. As nitrosamines are usually not present in a vagina, its presence may be a strong indicator of condom use [18,23,24]. These compounds originate primarily from condoms but can also come from other sources of latex and nitrosamines, such as feminine condoms or menstrual cups, which come into contact with the vaginal matrix.

3.2. Solid particles

Solid particles are added to the latex to avoid it sticking to itself and to enable easy unfolding for use [19,25]. These particles must be bioabsorbable, to be in accordance with ISO regulations. Four different types of particles are usually found in condoms:

- Corn starch is used in about 80% of powdered condoms [18,19];
- Polyethylene powder is also used in a mixture with corn starch, in a proportion of 1–5 with corn starch [18];
- Lycopodium spores are separating agents combined with starch. In 1990, 80% of condoms on the German market contained lycopodium spores [18]. They were present at very low concentrations. These spores are no longer used because they can cause allergic reactions [18,25]. In addition, they adhere to the serous surfaces of the vaginal matrix and can generate granulomas in the soft tissues [25].
- Talc has long been used but has been banned because it is not bioabsorbable and also produces granulomas [18,26].

The solid particles described above are also used in common products as summarized in Table 1.

Sources of solid particles can be multiple and there may be interferences, particularly with gloves used by medical examiners, during sampling or sexual examination [27,28]. Given this potential for contamination, positive analytical results should be interpreted carefully [29], as these particles are not necessarily specific to condoms [30].

Table 1
Solid particles and their current use in everyday life.

Compound	Current use
Corn starch	• Food additive • Component of cosmetics or some pills • Powdering of medical examination gloves
Polyethylene powder	• Food additive (E914) • Powdering of medical examination gloves

3.3. Lubricants

The lubricant is a viscous substance added to the condom during manufacture to facilitate penetration [31]. Lubricants for condoms are silicone- and polyethylene glycol-based. The chemical structures of polydimethylsiloxane (PDMS) and polyethylene glycol (PEG), found on condoms, are presented in Fig. 1.

As shown in Table 2, the various market studies presented in the literature indicate that more than 85% of condoms contain PDMS, a silicone-based lubricant [6,10,31,32]. PEG is found in less than 10% of condoms [6,32].

However, both PDMS and PEG are very frequently used in everyday products as shown in Table 3.

Two other types of lubricants can be found in the vaginal matrix, as they are used in daily care products or personal hygiene products: water-based and oil-based lubricants. Oil-based and greasy lubricants (glycerin, petroleum) are not used on condoms because they are not compatible with rubber and can make it porous [2], thus reducing the efficiency of the barrier. But water-based and oily lubricants are instead used as personal/intimate lubricants. Hollenbeck et al. indicate that some manufacturers use propylene glycol for lubricant gels [36].

Even though lubricants are used in daily feminine care products, PDMS is not supposed to be present in the vagina for natural purposes, except the use of a condom. PEG is more likely to be found in the vagina if the woman had a treatment using vaginal ovules (inserted medications). Lubricants are a typical type of trace where victim's allegations as well as their medical history are necessary to determine the reason(s) for the presence of PEG or PDMS. If the victim has never used any condoms or external lubricants nor had medical vaginal treatment, it is unlikely that those lubricants would ordinarily be present in the genital zones. Therefore, in the case of a sexual assault, if lubricants are properly identified and differentiated when analysed with an adequate method [37], the hypothesis that a condom or a lubricant was used is strongly supported as there would be no other legitimate reasons for their presence in the genital environment. In the case of the identification of PDMS lubricant in the vagina, the probability that those traces would originate from cosmetics is very low, as it is not likely to be used inside the vagina. In the case of the identification of PEG lubricant in the vagina, the probability that they originate from cosmetics or other lubricant formulation is higher than for PDMS, and more careful interpretation should be led, using case information and victim statement. However, identification of PDMS or PEG is a highly relevant evidence if the victim's statements do not indicate any regular use of lubricants or condoms [18].

3.4. Spermicide

Spermicide provides an additional protection [18] as it acts in twenty seconds to neutralize sperm. It can be found on the condom [38] or be used by women within spermicidal creams, gel capsules

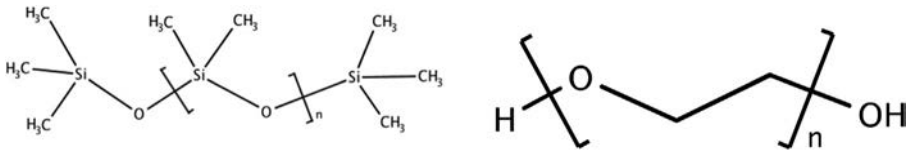

Fig. 1. Chemical structure of PDMS (left) and PEG (right).

Table 2
Number of condoms studied in different studies and proportion of PDMS and PEG lubricated condoms.

Ref.	Number of condoms types	Containing PDMS	Containing PEG	Other
[6]	56	46.4%	5.3%	–
[7]	40	72.5%	12.5%	15% non lubricated
[31]	25	92%	8%	–
[33]	53	88.6%	NA	6 other lubricants than PDMS
[34]	35	91.5%	5.7%	2.8% glycerin based
[35]	204	87.8%	6.4%	3% non-lubricated 2.8% non identified

Table 3
Lubricants and their current use in everyday life.

Compound	Current use
PDMS	• Topical ointments • Shampoo • Liquid soaps • Glue • Cosmetics • Food additive • Anti-lice product
PEG	• Liquid soaps • Shampoo • Food additive • Laxative • Excipiens in vaginal ovules

or foam as a contraceptive. Two types of spermicides are frequently found as contraceptives: nonoxynol-9 and benzalkonium chloride [19]. Benzalkonium chloride is found mainly in contraceptive creams, gels, capsules or foams. Nonoxynol-9 is the most commonly used spermicide [6], and is the only one authorized on condoms. It can also be found in some spermicidal contraceptives, such as creams or gels.

Nonoxynol-9 is a non-ionic detergent [7] with an amphiphilic nature as shown by the chemical structure in Fig. 2. Therefore, it is able to rupture the cell membrane, thereby causing cell death [39–41].

Since the 1960s, these cell lysing properties have been used in the prophylaxis of HIV and some sexually transmitted diseases to lyse the virus and/or the affected cells [42,43]. Since the 2000s, medical studies have examined the possible toxicity of nonoxynol-9 on the vaginal epithelium [40]. Nonoxynol-9, even in small quantities, modulates the structure and integrity of the plasma membrane [44], thus generating irritations, infections, ulcerations,

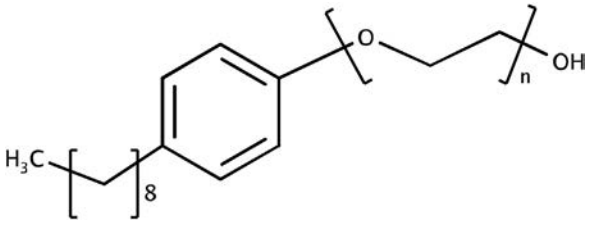

Fig. 2. Chemical structure of nonoxynol-9.

vaginal flora disturbances or granulomas [42,43,45,46]. The damaged vaginal epithelium is more susceptible to infection with HIV or papillomavirus. Since these studies, several European nations have banned nonoxynol-9 for cosmetics and pharmaceutical companies stopped using it on condoms [38,47]. Nonoxynol-9 has not been forbidden as a spermicide for humans by the European Union, as condoms are considered as non-cosmetic products [48]. The American Food and Drug Administration (FDA) stated that nonoxynol should remain on the market until they have completed their review of data related to nonoxynol-9 efficiency as a spermicide [49–51]. Research on new types of spermicides is ongoing [38,44,47] but for the moment nonoxynol-9 can still be found on condoms until international recommendations are submitted.

Current uses of spermicides are presented in Table 4. Possible confusion, leading to false positive detection, was found to be possible with surfactants present in detergent products [36].

Unlike lubricants, the use of contraceptive spermicides is widespread. Therefore, the presence of this type of product in a vaginal matrix alone is not a reliable indicator for ascertaining condom use, in case of daily or recurrent use of contraceptive spermicide. Information collected from the victim's daily life will help to determine whether or not the trace is relevant. This type of trace must be interpreted cautiously. The degree of confidence may be higher when other compounds of the condom composition are identified simultaneously in the same sample.

3.5. Other compounds

Other types of compounds can also be found on condoms: antioxidants and preservatives, aromas or anaesthetics.

Antioxidants and preservatives are added to the condom to prevent latex degradation and proliferation of bacteria, which is regulated by the World Health Organization (WHO) [3]. In a Swiss-Radio interview, manufacturers (i.e. Ansell, Durex and Lamprecht) reported using butylhydroxytoluene (BHT) as a protection agent [52]. BHT is also used as a food additive under the name E321,

Table 4
Spermicide and its current use in everyday life.

Compound	Current use
Nonoxynol-9	• Contraceptive creams and gels • Shaving foams • Heating balms • Surfactant in detergent products

which can be found in many cosmetics or plastic objects as it is used for polymer protection.

Aromas and flavours can be added to the lubricants. They are selected in accordance with ISO 10,993 and WHO recommendations, and must be biocompatible [3]. Thus, the compounds used are the same as food flavourings, since they are not or only slightly dangerous for the human body. They are regulated by the standards issued by the European Food Safety Authority (EFSA).

Finally, anaesthetics reported in the literature or at condom sales sites are benzocaine and lidocaine [10,18,19,52]. These anaesthetics assist in delaying ejaculation, and are also found in gels applied to the penis for the same purposes. It was not indicated whether these compounds were found outside or inside the condom but considering the intended purpose, it seems more likely that they are inside the condom, in contact with the penis. As the condom is unrolled immediately prior to use, transfer of these substances from the inside to the outside of the condom is possible. Due to the very low concentrations of these products on condoms, very few traces are expected, and we expect them to have a limited impact when studying traces in the vagina.

4. Transfer, persistence and background considerations

4.1. Transfer

Through analogy with DNA transfer, when a condom is used, the components present on the external surface will be transferred in the vagina whereas those from the internal surface will transfer on the penis. Condom also becomes a trace support, containing DNA from both persons implied in the coïtus.

The intensity of the action affects the transfer and thus, condom traces might contain specific components (qualitative) that would transfer during the sexual act in specific amounts (quantitative). As shown in Table 5, except the latex itself, all the components found on the condom may transfer in the vaginal matrix, but some are more likely to be recovered by the forensic examiner.

Lubricants are the most expected trace component and are the most studied regarding their composition, transfer to the vaginal matrix, the collection and sampling of the traces in the vagina and the development of methods for detecting their presence in the vaginal matrix [6–9,13,14,31–33,36,37,39,54,55]. The maximal quantity that can be applied on a condom, is around 800 mg and the minimal around 400 mg, although the mean is rather around 500 mg [2,3]. Assuming the hypothesis that the transfer is complete (100%) during the coitus and considering the dilution due to the vaginal matrix, the maximal concentration of the trace would be around 200 mg/ml (based on secretion production of 4 ml per day [58,59]).

Spermicides constitute the second most important marker of condom use and its detection in in the vaginal matrix was studied [14,17,36,39,56,57]. The maximal quantity applied on a condom is 10% (w/w), which is around 50 mg when considering a total quantity of 500 mg for the other compounds [36]. Thus, the maximal concentration of spermicide in the trace would be around 12.5 mg/ml based on secretion production of 4 ml per day [58,59]).

Solid particles are present on the surface of the condom, and are transferred during a contact such as sexual intercourse. It is a specific trace of condom use [60] although contaminants could come from external sources as discussed previously (cf. Table 1).

Other components, such as dithiocarbamates, nitrosamines, flavourings, aromas, etc. may also be present in traces in the vagina, given their affinity with the acidic aqueous vaginal matrix. Dithiocarbamates and nitrosamines have been relatively under-investigated as traces in a living matrix [5,22,53]. They are of limited interest for forensic purposes as they are not specific to condom use [18,19], and can originate from other latex-based material. No studies found during this review reported quantities of flavourings or aromas nor the possibility to detect them in the vaginal matrix. Due to their solubility in aqueous matrices it is highly likely that they become absorbed in the vagina or eliminated through washing.

4.2. Persistence of the traces

Persistence is defined by Campbell and Gordon as "[. . .] the period of persistence is from the time of the alleged act to the time of detection by standard laboratory protocol" [31]. For the authors, persistence in this paper is considered as *the loss of the trace (qualitatively and quantitatively) between the time of the criminal activity (i.e. sexual assault/rape) and the time of the sample collection*. The persistence of each compound is different as it depends mainly on their chemical properties, but variations in persistence indicate that several other parameters could influence the persistence (see Section 5) [7,31].

As shown in Table 6, PDMS lubricant was found to offer the best persistence, which is expected as it is a polysiloxane non soluble in polar solvents, thus not supposed to be absorbed by the vaginal matrix. It is also interesting to note that PDMS is also known to be less sensitive than PEG to the microorganisms present in the vagina [31].

Concerning PEG-based lubricants, a significant difference was noticed in the reported persistence by Maynard et al., and Tonkin et al. This is due to the type of support used, as one study analyzed vaginal swabs [6] whereas the other used skin as a support [55]. The difference in cell type between the vaginal matrix and the skin cannot be neglected, in addition to exposure to liquids (e.g. vaginal secretions). This also applies to oil-based lubricants, which have been found to have a good persistence on the skin, but the result in the vaginal matrix may be different and lower persistence would be expected.

Due to its chemical structure, nonoxynol-9 spermicide has a level of persistence partway between silicone-based and water-based lubricants, up to 8 h in the vaginal matrix [61], although 4 h seemed to be the maximal limit post-coïtus [36].

Table 5
Transfer possibilities, original quantities on condoms and forensic interest.

Compound	Transfer	Quantity expected on the condom	Studied as a trace in living matrix	Forensic interest
Latex	No	–	No	No
Dithiocarbamates	Yes	Not indicated	No	No
Nitrosamines	Yes	Not indicated	Few [5,22,53]	Limited
Solid Particles	Yes	Not indicated, mixed with lubricants	Yes [25,26]	High
Lubricants	Yes	500 ± 50 mg [2] 550 ± 150 mg [3]	Yes [6–9,13,14,31–33,36,37,39,54,55]	Very high
Spermicides	Yes	5-10% (w/w) of the whole other added compounds [36]	Yes [14,17,36,39,56,57]	High
Other components	Expected	Not indicated	Not found	Likely

Table 6
Persistence of the different compounds from the condoms.

Compound	Studied	Persistence [h]	Surface	Reference
Solid particles	No	Estimated: 24 h		–
PDMS	Yes	24 h	Vaginal matrix	[7]
		Up to 12 h	Vaginal matrix	[6]
		4.5–12 h	Vaginal matrix	[31]
		24–48 h	Vaginal matrix	[13]
		Up to 48 h	Vaginal matrix	Unpublished, mentioned in Ref. [6]
PEG	Yes	7-8 h	Vaginal matrix	[6]
		24 h	Skin	[55]
Oil-based lubricants	Yes	24 h	Skin	[55]
Spermicides	Yes	12–24 h	Simulated vaginal fluid	[41]
		4 h	Vaginal matrix	[36]
		8 h	Vaginal matrix	[61]

No study was found which analysed the persistence of solid particles in the vaginal matrix. Nevertheless, considering the physico-chemical properties of the potential transferred compounds, persistence can be roughly estimated. As they cannot be solubilised in water [62–64] or organic solvents [63] a persistence of over 24 h can be expected from corn starch and polyethylene, though possible mechanical adherence of the particles to the matrix may allow them to persist longer. Considering other compounds, no information was found in the literature.

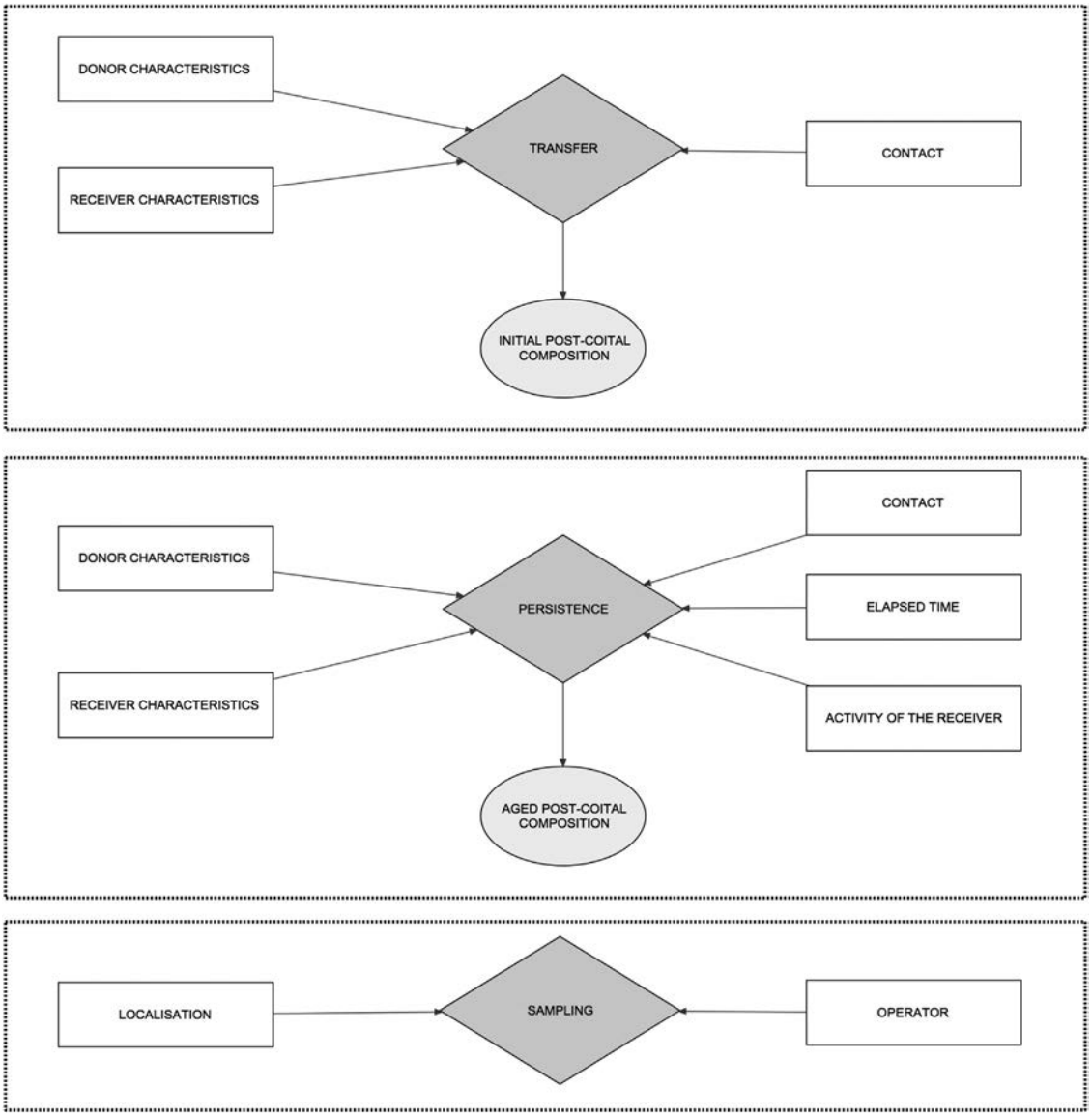

Fig. 3. Schematic representation of the variability induced by numerous influence factors occurring during the transfer (first table), persistence (second table) and sampling (third table).

4.3. Background

When interpreting evidence, transfer and persistence parameters are strongly linked to the background parameter. Background is described by Aitken and Taroni as "(. . .) the presence by chance (. . .)" [65]. In the case of condom traces, the presence by chance means that the trace does not come from the questioned action. Based on Refs. [66,67], the background will be here defined as *a combination of traces present naturally in the matrix and traces present for legitimate purposes in the matrix*. All those traces are present just before the criminal activity, but are not linked to the latest. Background was not studied in the articles found for this paper but is an important issue since Campbell and Gordon reported PDMS in their blank swabs analysis, when investigating the background matrix [31]. Therefore, is it possible that, depending on the elapsed time between a consensual sexual intercourse and a rape, condom traces from both events might be detected. This indicates that contextual information obtained during the interview of the victim is crucial and can explain or help interpreting some of the obtained analytical results.

4.4. On the use of interpretation in caseworks

In 1994, Blackledge reported two cases in which condom traces were used as evidence [7]. In the first case, the victim was examined at a hospital after she regained consciousness. The investigation led to a suspect who confessed to the rape and condom residues were used to corroborate the statement. In the second case, the victim reported having been raped by a man who had AIDS. The suspect admitted to the intercourse but said he wore a condom, a statement that was contested by the victim. The result of the examination for condom traces was negative, thus corroborating the victim's statement. In the case *Regina v. Andrew Nicholas Malkinson* (2006), PDMS traces were detected in the vagina and the anus. The expert initially concluded that a condom had been used, before realising the swabs were contaminated with PDMS. The victim's knickers were analysed and PDMS traces were found. Based on the victim's statement who "(. . .) never used cosmetics, hand creams or other ladies' toiletries" [68], the court stated that "(. . .) if her evidence is correct, it is very unlikely that the PDMS oil found on her knickers came from a source other than a condom." [68]. After considering the expert's testimony, the court finally concluded that "(. . .) evidence makes it even more likely that what was found in her knickers was oil from a condom." [68].

In the above cases, expert conclusions were mainly based on the presence and/or absence of traces in the vagina or underwear. However, as outlined by Coyle and Anwar [33], a positive result could be explained by the recent use, for legitimate purposes, of products containing PDMS, which is not unique to condom lubricants, thus resulting in a false positive. Moreover, an unpublished study by Monzò showed that PDMS traces can persist on underwear even after laundering at 60 °C [35]. Similarly, a negative result can eventuate from many factors, such as the period of time elapsed since the intercourse (see Section 5), differences in sampling or the sensitivity of the analysis. Therefore, studies into prevalence as well as transfer and persistence are critical to the rational and informed interpretation of evidence.

5. Factors influencing condom traces

Campbell and Gordon observed variations in persistence that were not only due to the chemical modification of the initial composition [31]. The interaction with the matrix generates a post-coïtal composition of condom traces which can be, from a qualitative and quantitative point of view, defined as the result of chemical, biological and physical processes (i.e. degradation, metabolism, oxidation, or absorption) occurring over time on the initial composition of the trace, leading to variable persistence of the compounds. Alteration or disappearance of the initial compounds will occur over time in a continuous process, thus generating problems for detection, analysis and interpretation of the recovered trace. For example, activity of the victim as well as the menstrual cycle, individual variation and the contact surface were identified as major influential parameters making analytical quantification difficult [41].

As shown in Fig. 3, five main factors have been demonstrated to influence transfer and persistence: the donor characteristics (e.g. condom composition, condom type, transfer capacity), the contact (e.g. intensity, contact duration, contact surface, contact type), the receiver characteristics (e.g. menstrual cycle, human variations), the elapsed time and the activity of the receiver. All these factors are known to significantly differ between cases, thus leading to variability of the initial composition (i.e. the quantities transferred through the deposition of the condom trace in the vagina, following sexual intercourse) and the aged post-coïtal composition. Thus, the variability of the condom trace is the sum of the variability of the initial composition (starting point of the persistence) with the variability of the influence factors occurring over time [54]. Each factor will be described hereafter. Although sampling is not defined in Fig. 3 as a factor influencing transfer and persistence, it is necessary to discuss it as Maynard et al. also highlighted the importance of taking several samples at different locations on the victim [6].

5.1. Donor characteristics: the condom

The condom characteristics include composition, such as latex or non-latex body, type of lubricants, or aromas, as well as more specific parameters such as the quantity of the components, the size of the condoms, and its form (ribbed and/or stubbed).

The composition characteristics of the condom impact the transfer and persistence on two main points: the ability of the components to transfer from the condom to the matrix and the quantity of compounds found on the condom itself. Most condom components are known to transfer, as few research studied the condom residues in the vaginal matrix [6,7,13,31]. But there has been, up to now, no report of how much of a compound will be transferred, which is an important parameter as a compound present at a trace level on the condom itself will transfer in lower quantity and disappear quicker than a compound present in high concentrations. The polarity of the compounds should also be considered as a source of variability as polar compounds were observed to disappear much more rapidly after deposition in the vagina than non-polar compounds (see Table 6) [6,41,56]. This is due to their absorption in the matrix, which is polar, or the dilution in the vaginal secretions [31].

Considering physical characteristics of the condom, they impact the contact surface area. The latest is highly variable, as it does not depend only on the condom but also from other factors (i.e. vaginal matrix, penis length). Ribbed and/or stubbed condoms or XL-sized condoms may contain more lubricants, or with a different distribution on the condom, thus inducing variability of the transfer and persistence. There has unfortunately been no study focusing on those physical aspects and their importance on the transfer of condom components in the vagina.

5.2. Contact

The contact condition refers to duration and intensity, as well as the surface area [54] and affects the quantity of traces transferred. Indeed, is expected that the higher the intensity, the higher the transfer.

Moreover, the pressure and the duration of the intercourse should be considered as it is linked to the action intensity. It might be considered that after a given time the surface compounds have fully transferred to the matrix and a steady state (saturation level) is reached. If the intercourse persists after the time the steady state is reached, material removal coming from reverse transfer should be considered and could be expected. Considering the contact surface, it is expected that more traces would be transferred with increased surface area. It is though very difficult to control this as both the penis length is not controlled and the vagina is an elastic surface that can vary between 80 and 400 cm^2 [41].

5.3. Receiver characteristics: the vaginal matrix

The influence of the receiver characteristics depends on the vagina's parameters. The vagina is an organ subject to constant regeneration and evolution following the menstrual cycle, as shown by Wagner and Levin "(. . .) the vaginal fluid of women has a distinct ionic characteristic throughout the menstrual cycle (. . .)" [69].

The vagina is constantly lubricated by cervical mucus, which flows between 1 and 4 ml per day. Mucus and transudate are produced to ensure adequate lubrication of the vagina [58,59]. The total production of vaginal lubricant is 6 g per day and 0.5–0.75 g are present in the vagina at a given time [59,70]. These parameters generate a variable dilution of the target compounds in the vagina. Moreover, physiological variations, notably due to the period of the ovulatory cycle, the hormones intake (contraception) or even sexual arousal affect the secretions [46,58,69–72] and modify the retention ability, as the matrix gets thicker with the menstrual cycle [41,46,58,69]. Blood may also be present in the vagina from the menstruation or in cases of vaginal lesions resulting from deep, unprepared intercourse, thus affecting the persistence of traces [31]. In particular cases of infections (e.g. mycoses or vaginitis) the composition of vaginal secretions can be affected leading to a modification of the retention and absorption capabilities of the matrix [69].

Finally, it is important to note that the initial amount of condom compounds in the vaginas of different women has not been reported by the literature yet. It is expected that each woman would present an individual vaginal matrix with its specific trace retention ability and contact surface thus affecting diversely the amount of transferred compounds. Forensic research investigation has focused mainly on the post-coïtal time [6,7,31,54] to determine the window of time following deposition in which condom trace detection was still possible.

5.4. Elapsed time and activity between the intercourse

These two factors will be discussed together. As for DNA or fibres, the longer the elapsed time between transfer (i.e. the intercourse) and sampling, the more likely the evidence will disappear. This was confirmed by the identification of time as an important factor affecting classification of condom traces in a given model [16].

Two types of activities can be differentiated: the natural activity of the receiver (i.e. modification of the matrix, microorganism activity, absorption of compounds) and human intervention. Daily activity, such as going to the toilet, showering, physical exercise (e.g. walking, sports) and periods of inactivity (e.g. sitting, lying down or sleeping) [41,55,56] may influence persistence. Victims of rape often feel dirty after the aggression and will wash themselves, including, for women, some vaginal washes, which will strongly affect any traces coming from the crime. Testing this hypothesis, Tottey et al. showed that PDMS in the vagina was not much affected by these activities but did PEG was not investigated in this study [13].

5.5. Sampling

Maynard et al. showed the importance of sampling, as they did not detect lubricants in samples taken from the vulva after 8 h post-coitus, but could be identified in vaginal samples collected at the same time [6] although Proni et al. reported that sampling did not affect significantly detection [61].

Condom traces collection follows the same principles as DNA sampling, with additional recommendations described by Blackledge [27]. The first recommendation is to wear non-lubricated latex or plastic examination gloves, to avoid contamination with lubricants or solid particles, which would be indistinguishable from the ones found on condoms. Therefore, sampling should be performed with cotton swabs, before gynaecological examination [27]. Samples should be collected from inside and outside the vagina, anus and mouth. Finally, DNA kits usually consist of at least 4 swabs, with 4 targeted regions: vagina, endocervix, anus, and vulva [29,73–75]. The use of an additional swab for condom traces is recommended [27] although it is possible to detect condom traces on the vaginal swab even after DNA extraction [13]. Used medical gloves should also be recovered and properly packaged [27].

Although standardized protocols for DNA sampling or for traces sampling in rape cases exist, variability is introduced by the operator. Variability in the sampling is then expected.

6. Conclusion and perspectives

Condom traces are a complex and variable system described by an initial composition (i.e. the condom composition, before use), a post-coital composition (i.e. transferred condom traces right after the sexual intercourse) and evolution of the initial composition over time. Moreover, six main types of influence factors influence this system: the donor characteristics (i.e. the condom), the contact, the receiver characteristics (i.e. the vaginal matrix) will affect the transfer while the time and the activity of the victim as well as the sampling techniques.

Numerous compounds have been identified on condoms and in their traces and different analytical procedures are available for their analysis. However, more information is needed, such as quantitative data, transfer and persistence studies. Effects of influence factors should be studied although this might encounter several problems, as most of them are difficult to control. The lack of knowledge regarding the transfer and persistence process seems to be due to two main causes:

Condom traces can be very interesting additional evidence in rape or sexual assaults cases knowing the allegation of the victim and the suspect. Moreover, it may be the only alternative physical evidence in the absence of DNA. It is thus important to improve our fundamental knowledge of condom traces and it is a new area to consider in forensic analysis and trace research. Nevertheless, in this type of cases, more conventional traces (i.e. DNA, medical evidence) are generally used as main forensic evidence when author is unknown. Therefore, there has yet been no urgent operational interest to know more about condom traces, their evolution over time or the effects of influence factors.

Technical difficulties involved with such a study. Modelling of transfer and persistence should be conducted with the help of volunteer subjects, under controlled conditions and taking influence factors into account. The number of samples necessary to obtain significant quantitative results as well as significant model of transfer and persistence is very high. These aspects complicate and slow down research, resulting in a lack of data in this particular field. Moreover ethical problems can be faced when trying to recruit human participants.

However, a better understanding of transfer and persistence of condom traces would prove to be important for advances in condom traces analysis and for the development of a reliable decision model following Bayesian evaluation of the scientific evidence. Moreover, information on condom traces, their content and their detectability are needed in order to be able to analyse them in rape or sexual assault cases. Research concerning the analysis of condom traces could be a complement to DNA analyses and both research fields could take advantage of the information concerning condom traces and their persistence. A promising approach for condom traces may rely on the use of transfer, background and persistence curves built by quantifying condom compounds (or their ratios) over time. Thus, the initial condom traces composition and its degradation kinetics are of primary importance for this specific application.

In conclusion, this review provides an update on the compounds that have been studied in condoms and in their traces. Furthermore, it highlights the missing knowledge on condom traces transfer and persistence and the need to conduct future research on this topic to help developing the condom traces analysis field. Quantitative data should be collected on the most interesting compounds identified in condom traces and transfer and persistence should also be studied in more details, in order to provide a Bayesian decision model and to consider real cases, using victim's and suspect's allegations to assess likelihood ratios for the scientific evaluation of the evidence.

Conflict of interest

The authors declare that they have no conflict of interest.

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Review Article

Forensic analysis of condom traces: Chemical considerations and review of the literature

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ABSTRACT

The analysis of condom traces has recently been added to the standard forensic examination protocol of sexual assault and rape cases. Several recent studies have thus focussed on the detection of condom components and classification of the chemical profiles using statistics, obtaining very promising results. The purpose of the present article is to critically review the literature regarding condom chemical analysis. A large analytical panel of both destructive and non-destructive methods has been proposed for the analysis of condom traces, each offering completely different analysis type and thus complementary information. However, few studies have considered these traces within a human matrix, which is necessary to establish an accessible protocol for forensic laboratories to allow this type of analysis. Additionally, issues remain concerning reproducibility, sensitivity, and the validation of analytical parameters. Considering that the demand for condom residue analysis is increasing, there is a definite need for further research on the forensic analysis of condom traces in order to offer quality services to the criminal justice system.

Contents

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1. Introduction

Condom evidence is particularly crucial in cases where a victim reports a sexual assault, but no DNA is recovered. According to [1], up to 25% of the cases handled by the Las Vegas Metropolitan Police Department Forensic Laboratory over 4 months indicated the use of a condom. The Centre Universitaire de Médecine Légale (CURML) in Lausanne, Switzerland also reported that in up to 10% of the cases analysed in 2018, no DNA was detected (Personal communication, Frédéric Grosjean, Biochemist). In Western Australia, doctors collect sample almost every day (Personal Communication, Dr Maire Kelly, Sexual Assault Resource Centre (SARC), Perth, WA) but only 20% are sent to the forensic laboratory for condom testing (Personal Communication, Dr. David De Tata, ChemCentre, Perth, WA). DNA is usually the main informative evidence in rape cases, especially where the perpetrator unknown to the victim, which is estimated to represent up to 35% of cases involving condoms [2]. In the United States, DNA profiles are generally stored in the Combined DNA Index System (CODIS) database. Repeat offenders in particular, who have previously been convicted based on their DNA, may therefore be tempted to hide their traces. Therefore, in a forensic context, a main motivator for condom use by a sexual offender is the prevention of DNA evidence [3]. These considerations also help in understanding why some offenders have reportedly force their victim to wash themselves, to remove potential DNA evidence [4]. Condom traces can therefore be found in a variety of forensic investigations, such as rape, sexual assaults or murder, and forensic laboratories may need to undertake chemical or microscopical analyses of this evidence type.

Another type of cases where forensic analysis of condom might be required are rapes where the offender is HIV positive. Latex condoms were initially designed to avoid the transmission of sexually transmitted diseases (STDs) [5] and are currently the only effective protection against HIV transmission [2,6]. Given that various international laws criminalise the transmission of human illnesses, especially HIV, it is hence of high relevance in these cases to determine whether the offender used a condom [1].

Berkefeld was the first to propose the forensic exploitation of condom traces in the 1920s. Condom traces are used as associative evidence to establish the *corpus delicti*, to verify penetration [1,7,8] and can also be used to validate the allegation(s) of the prosecution or defence; as it is not necessarily the sexual act itself but the way it happened that is under question [9]. According to [2], condom use in rape/sexual assault cases represent less than 15% of reported cases and trace analyses have been implemented in few cases in the United States. However, the question of the interpretation of the analytical results raises some issues amongst the scientific community because there is a lack of information concerning the chemical diversity between condom traces, potential error rates, and the effects of contextual factors such as transfer and persistence, which may affect interpretation. These interpretation considerations have rarely been investigated. This shows that the development and validation of a reliable condom trace methodology would be important in forensic practice.

Therefore, this article aims to outline the “best-practice” condom trace analysis currently in the forensic literature, show the limits of the current approaches, and assess the potential of sequential protocol for condom trace analysis. Whilst the first part focuses on highlighting the chemical considerations for the analysis, while the second part reviews condom trace analysis methods that are proposed in the literature. Finally, the third part illustrates the use of a sequence of analytical techniques for forensic purposes targeting those traces.

2. Article strategy and study selection

Electronic databases such as PubMed, Scopus and Web of Knowledge, were used to locate articles published between 1980 - March 2019 using pre-defined search terms (*i.e.* condom, analysis, forensic, spermicide, lubricants, nonoxynol, PDMS). The reference lists of the published articles were further explored to identify recent additions to the literature. Only full articles written in English and French, with a focus on condom analysis for forensic purposes, were considered. While all published papers meeting these criteria were used for this review, this paper offers no pretention of being fully exhaustive.

3. Physico-chemical properties of target compounds

This section considers only the chemical and physical properties of potentially transferable material. Although condoms are made of latex and may also contain nitrosamines and dithiocarbamates, the latter do not transfer and are considered in the literature as not being of forensic interest [10,11]. Additives such as flavours or aromas can be found on condoms as well, but as there has been no study concerning a potential transfer, the quantity is not known and they have also never been studied as a trace in a

biologically-active matrix [12]. Therefore, they are not discussed in this paper. This section discusses instead three categories of condom components: solid particles, lubricants and spermicides, as these are the components that are of highest forensic interest [12].

3.1. Solid particles

Currently, only two types of solid particles may be routinely found on condoms and therefore in condom traces: corn starch and polyethylene powder [12].

3.1.1. Corn starch

The biochemical definition of corn starch is given by Horton et al. (2006) as *a homopolymer of glucose residues that is a storage polysaccharide in plants. There are two forms of starch: amylose, an unbranched polymer of glucose residues joined by α-(1–4)-linkages; and amylopectin, a branched polymer of glucose residues joined by α-(1–4)-linkages with α-(1–6)-linkages at branch points* [13]. Figs. 1 and 2 show the chemical structure of amylose and amylopectin as described by Horton et al. (2006).

Despite the presence of multiple —OH groups in their chemical structure, starch grains are not soluble in water or in most organic solvents [14]. Heating the solvent or agitating with an ultrasonic bath dissolves the starch, but leads to a change in the physical properties of the starch. It then forms a paste or gel [14]. The only known solvent for solubilizing starch is dimethyl sulfoxide (DMSO) [15]. From the point of view of the physical properties, the starch presents as a solid structure with a variable arrangement of the constituent molecules. It is opaque and exhibits birefringent characteristics when observed *via* microscopy [14]. When gelatinized, it loses its optical properties [14]. Starch was found not to be subject to digestion by bacterial enzymes present in the vaginal vault [16,17]

3.1.2. Polyethylene powder

Polyethylene is a polymer of which there are several forms. Its chemical structure is shown in Fig. 3 below.

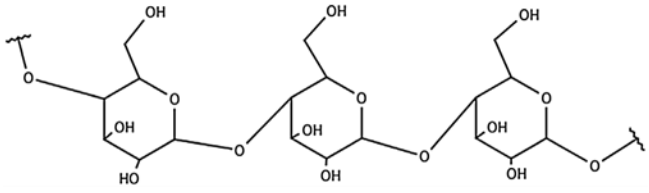

Fig. 1. Chemical structure of amylose according to [13].

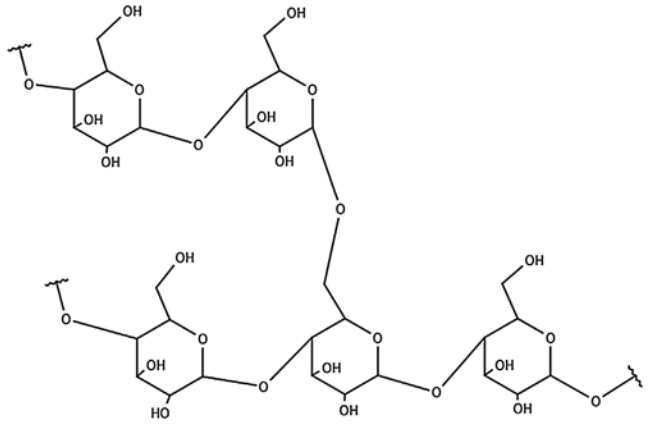

Fig. 2. Chemical structure of amylopectin according to [13].

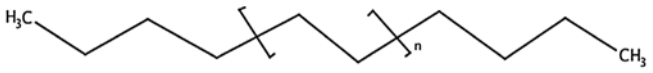

Fig. 3. Chemical structure of pure polyethylene, where n represents the number of polymeric repetition according to [18].

Polyethylene powder is comprised of granules ranging from 40 μm to more than 350 μm in diameter [18]. The powder may then be heated or converted to form high or low-density polymers for other uses. Due to its chemical structure, polyethylene is not soluble in polar solvents.

3.2. Lubricants

3.2.1. Polydimethylsiloxane (PDMS)

PDMS, also called dimethicone or simeticone [1], is an inorganic polymer whose chemical structure is based on an alternation of silicon and oxygen atoms. PDMS synthesis occurs in two stages: monomer synthesis and polymerization. These steps are detailed in the literature [19] and so will not be reproduced here. The repetition of monomeric units $(CH_3)_2$-Si-O is variable in the PDMS molecule, due to the reversible polymerization reaction. At equilibrium, there is therefore a Gaussian distribution of molecular weights. As the number of monomeric units increases, the viscosity of the polymer becomes greater [19–21]. The PDMS molecule has a stable conformation, in which all CH_3 groups are in the trans position (see Fig. 4), because it is the lowest energy conformation [19].

The absence of substituents on the oxygen atom makes this long chain very mobile. It is also protected from intermolecular interactions thanks to the apolar methyl groups, which are present on both sides of the Si-O chain. Therefore, PDMS is soluble in apolar solvents such as hexane, octane or toluene [22].

The mobility of the chain influences its physicochemical properties. The physical properties of PDMS change little during increases in temperature. Its solubility and gas permeability are high (especially with water vapour). Finally, the polarity of the Si-O bond induces an amphiphilic character [19,20,23,24]. According to the literature [1,9,25,26], the polymer predominantly used as a lubricant in condoms is PDMS with methyl terminations as shown in Fig. 4. Maynard et al. [26] indicated that hydroxyl-terminated PDMS can also be used in condoms that contain nonoxynol-9. Fig. 5 shows the chemical structure of the hydroxyl terminated PDMS.

3.2.2. Polyethylene glycol (PEG)

Polyethylene glycol (PEG), (IUPAC: poly (ethylene oxide)), is a linear polyether (see Fig. 6). If its molecular weight is less than 600 g/mol, the PEG polymer is in the form of a viscous colourless liquid, while if it is greater than 800 g/mol, it will be in the form of a waxy solid. The hydroxyl and oxygen groups that compose PEG enable it to be miscible and soluble in water, ethanol or acetone. It is also soluble in toluene or dichloromethane thanks to the prevalence of apolar groups (—CH_2). However, it is not soluble in aliphatic hydrocarbons, e.g. hexane. According to the literature,

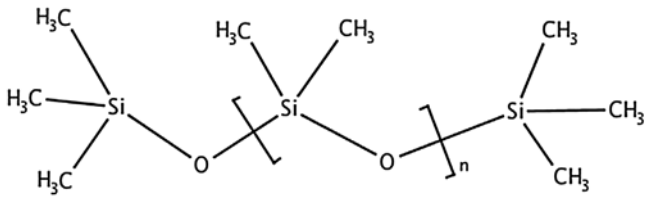

Fig. 4. *trans* configuration of PDMS molecule, according to literature [31].

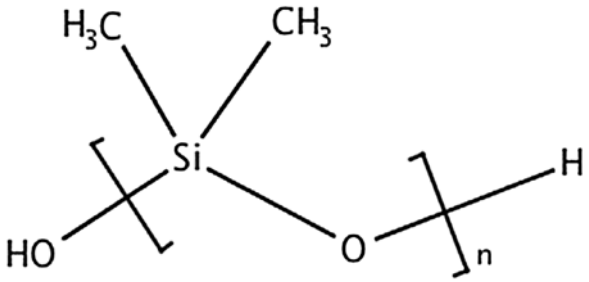

Fig. 5. Chemical structure of hydroxylated PDMS.

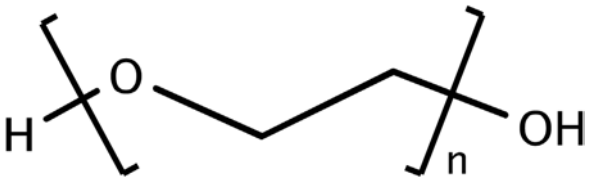

Fig. 6. Chemical structure of polyethylene glycol.

low molecular weight liquid PEG is used for condoms, most frequently PEG300 or PEG400 [25,27].

3.3. Spermicide

Nonoxynol-9 is a polyethoxylated nonylphenol with a molecular weight of 615.81 g/mol. It contains ethoxy units, -O(CH_2)$_2$- repeated a different number of times. Fig. 7 shows an instance where the units are repeated 9 times, although repetitions can vary from 5 to over 10 units, generating a mixture of ethoxymers whose molecular weights differs by ethoxy units (44 a.m.u)) [1,28]. The chemical structure of nonoxynol demonstrates its amphiphilic character, specific to non-ionic detergents. It has a significant apolar component by the aliphatic chain C_9H_{19} as well as a polar component by the presence of the chain - [O-(CH_2)$_2$]-OH. Thus, this compound should be soluble in both polar and apolar solvents.

4. Literature review of the analytical methods used for condom traces analysis

It is possible to separate the research concerning condom traces analysis into multiple categories, such as the analytical methods, the type of traces, and the type of methodology. In this paper, research studies were classified by the function of the analytical method and the total analytical procedure. Five categories are presented:

(1) Sample preparation
(2) The use of microscopy
(3) Spectroscopic analysis (e.g. FTIR, Raman, NMR)
(4) The use of separation methods (e.g. GC/MS, py-GC/MS, LC/MS)

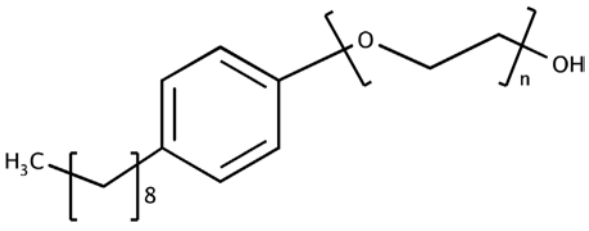

Fig. 7. Chemical structure of nonoxynol-9.

(5) Mass spectrometric analysis (e.g. ESI-MS, MALDI-MS, DART-MS, DIOS-MS)

4.1. Sample preparation

Sample preparation is a key step in any analysis. The quality of the analyses and the results obtained depends heavily on this phase. It is therefore necessary to carefully consider the target compounds, the analytical method, and whether it is necessary to extract the molecules from their support, or whether the sample can be analysed *in situ*.

4.1.1. Extraction procedure

Vaginal swabbing is commonly performed using cotton swabs. Thus, the most appropriate extraction approach is a solid-liquid extraction. There are several forms of solid-liquid extraction, ranging from simple techniques (decoction, maceration, infusion) to more complex techniques, such as Soxhlet extraction or supercritical fluid extraction [29].

Several authors report the use the maceration process [1,25,26,28,30–32]. The swab is put in a vial and then immersed with the extraction solvent. The vial may go through several classic steps such as sonication, vortex mixing or centrifugation, in order to improve the extraction yield. In 1988, Fujimoto suggested recovering the polysiloxane-containing fraction of oil by dissolving it in acetone and precipitating the organic phase with ethanol [33]. Dissolving the siloxanes to extract them was also used in 2003 by Keil et al. as they diluted the swab in a mixture of ethyl acetate, diethyl ether and ammonium hydroxide buffer [34]. Finally, a concentration step can be performed by evaporation of the extraction solvent.

The choice of the extraction solvent depends on the targeted compounds. Among the polar solvents, methanol and water are the most commonly used solvents in the literature, comprising 16% and 11% of reported methods, respectively. Other common polar solvents (acetonitrile or ethanol for example) are used in less than 5% of studies. In the case of non-polar solvents, 13% of the studies described in the literature utilise hexane and dichloromethane and a further 10% report using chloroform.

In 2001, Maynard et al. tested 8 different solvents to determine which ones are most suitable for extracting target compounds (apolar solvents for PDMS; polar solvents for PEG and nonoxynol) [26]. The extraction efficiency was studied by weighing the swabs before and after extraction. Thus, chlorinated solvents (chloroform and dichloromethane) were shown to result in an incomplete extraction of apolar compounds. Finally, hexane was recommended for the extraction of apolar compounds and methanol for the extraction of polar compounds [26].

4.1.2. In situ *Analysis*

The challenge of *in situ* analysis is to successfully analyse the compounds directly on their support. *In situ* trace analysis is desired because of two main advantages from a forensic perspective:

(1) It is not necessary to separate the trace from its support, which reduces the risk of losing targeted compounds (especially important in the case of traces) or to dilute them so that they are no longer detectable.
(2) Since no extraction phase is required, the analysis time may be reduced, and storage of samples or extracts does not need to be considered.

In situ analysis of condom traces was only reported in 5% of the articles studied. It was proposed by Coyle and Anwar (2009) in the

context of Raman analyses [35]. However, this is a methodology also applicable with mass spectrometry, especially when the analytical method allows the direct desorption of the analyte from its support. Examples of this include Desorption electrospray ionization mass spectrometry (DESI-MS) or desorption/ionization on silicon (DIOS)-MS [36–38].

Finally, smearing a cotton swab on a microscopic slide, as it is often the case for microscopic analysis, may be classed as a form of *in situ* analysis in that it does require a transfer but is not a full extraction procedure. This type of sample preparation is carried out for solid particles in [34,39]. Other types of extraction for solid particles could also be used based on preparations reported in food science, pharmaceutical or illicit drugs analysis.

4.2. The use of microscopy

Optical microscopy and scanning electron microscopy (SEM) have been used since the 1990s to analyse solid particles, namely corn starch and polyethylene, since the importance of these particles in determining contact with a condom was highlighted [1].

Microscopy has been used to investigate solid particles on condoms in studies using 10–54 samples. The particles were extracted either with a solvent [31] or *via* smearing [34,39]. Starch was detected in all cases [31,34,39] and at least four different mixtures of particles were observed [34]. The detection limit detailed for a starch/talc mixture was reported as 0.2% (w/w) [31].

Applied to casework, microscopy has found a considerable amount of corn starch in real sample [34]. Haematoxylin-eosin staining which stains cells pink was one method proposed as it allows better differentiation of the uncoloured solid particles from the now pink epithelial cells [34]. Alternatively, a Lugol solution (I_2KI) may also be used to visualize corn starch because iodine interacts with the structure of polysaccharides and stains them blue [39].

4.3. Spectroscopic analysis

Spectroscopic methods are the most frequent methods reported in the literature for the analysis of condom traces, specifically the condom lubricants and spermicides. Spectroscopic examinations are commonly conducted at the beginning of the analytical sequence, as they are predominantly non-destructive methods.

4.3.1. Fourier transform infrared spectroscopy (FTIR)

Infrared spectroscopy is a recognized method for both the identification of polymers, and also to study the structure of these polymers [19,40,41]. In the case of condom traces, the compounds targeted by infrared spectroscopy are mainly lubricants, more specifically PDMS and PEG. Less commonly, it can also be used for the analysis of spermicides.

The FTIR method has been implemented for the analysis of condoms and intimate lubricants on sample sets ranging from 6 [42] to more than 60 samples [26]. FTIR detects PDMS, PEG and nonoxynol-9 in very small quantities (traces) and sometimes reveals other compounds, such as unspecified additives [9] or benzocaine [43]. It has also demonstrated the ability to determine whether cyclic or linear siloxanes are present and in what relative quantity, although the exact quantity is not reported [44].

FTIR spectroscopy may be carried out in diffuse reflectance (DRIFTS) or attenuated total reflectance (ATR) modes. Both were performed by Maynard et al. (2001), who considered the DRIFTS approach preferable to ATR because it allows the analysis of liquids (extracts) and offers better spectral quality [26]. Despite this, both techniques highlight the possibility of differentiating brands of condoms based on the viscosity of their lubricant [1]. This can be done in a simple way based on the comparison of IR spectra [26], especially considering that small differences between PEGs can be detected (though this is more difficult for PDMS) [9]. Blackledge (1995) proposed sorting PDMS-containing samples by viscosity, using Fourier Self Deconvolution to separate two peaks (dimethyl (2ME) and trimethyl (3ME)) that are in the same absorption zone (at about 800 cm^{-1}) [30]. The ratio between the two peaks indicates the viscosity and thus makes it possible to sort the samples into different categories accordingly. It was noted that the majority of condoms fell into the category "200 cSt" [30]. Databases have been created, particularly in Taiwan, although the method may lack the capacity for discrimination [43]. FTIR is mainly used as a screening method [26], but has also been used to confirm the results obtained by other methods such as Raman or mass spectrometry [9,42,44].

Applied to simulated cases, no interference was reported between vaginal secretions and PDMS [1,26]. Interference with polar compounds was reported, due to their polarity and therefore their presence in polar extracts [26]. A dilution effect was noted when sperm was present, affecting the PDMS detection limit to a point of not being detected [1].

4.3.2. Raman spectroscopy

Raman spectroscopy is considered a relatively new method of characterizing condom traces [45]. This method can be used for the analysis of solid particles, lubricants and nonoxynol-9 [35,45]. However, most studies have focused on lubricant (PDMS/PEG) analysis.

Raman has been demonstrated to be capable of analysing PDMS, PEG and nonoxynol-9, as both pure standards and mixtures [45]. PDMS and nonoxynol-9 could be differentiated primarily on the basis of the C—H stretching band, for which the two compounds present different characteristics [45].

Condom analyses were conducted *in situ* (53 samples) and after extraction of lubricants (6 samples) [35,45]. Fluorescence has sometimes been observed and was attributed to the additives used in the fragrant additives of condoms [35]. Condoms could be classified on the basis of their lubricant in at least three categories (PDMS, PEG, other lubricants) [35]. Chemometric modelling using principal component analysis (PCA) highlighted the possibility of differentiating the various brands of condoms, but not samples within the same brand [42].

In simulated cases, no interference was observed between the vaginal matrix and the target compounds [35]. DNA analyses subsequent to in situ Raman analysis showed no degradation due to the previous analysis [35].

4.3.3. Nuclear magnetic resonance (NMR)

NMR spectroscopy is a very powerful method for polymer analysis, especially polysiloxane, because they have atoms whose nuclei can react in a magnetic field [41]. In the context of condoms, the compounds sought in the context of NMR analyses are mainly lubricants (PDMS/PEG) and nonoxynol-9.

Applied to PDMS and nonoxynol-9 standards, the NMR technique has shown very good sensitivity for these compounds, around 0.04 ppm [46,47]. For nonoxynol-9, various oligomers are visible and NMR results are consistent with those obtained through high performance liquid chromatography [47]. PDMS was consistently detected in the samples [46] and it was possible to estimate the ratio between cyclic and linear siloxanes [44]. The history of the trace and the dilution effect do not seem to hamper the analysis nor the likelihood of detection [46]. *Solid state* ^{13}*C-NMR* and ^{1}*H-NMR* were applied on a set of 38 condoms [27]. The *solid state* ^{13}*C-NMR* detects lubricant and latex (which is consistent since the analysis is done in-situ on the condom support) and ^{1}*H-*

NMR detects the other compounds [27]. Condoms could be differentiated on the basis of their physical properties (colour, smell, *etc*.) and their NMR spectra [27]. The discriminating power of the method has been reported to be as high as 0.98. However, no analysis on real or simulated cases has been reported.

4.3.4. Critical perspectives on spectroscopic methods

FTIR seems to be the most recommended method for the analysis of condom lubricants. Reflection (ATR, DRIFTS) is the most widely used method, although in the United States, the Naval Criminal Investigation Service laboratory reported using transmission methods to analyse lubricant extracts from condoms. The analyses presented in the literature mainly used samples extracted directly from condoms or from swabs scrubbed on condoms, though there were also samples from two reported real cases [1] and several simulated case samples for research [25,26]. The detection capability, simple and inexpensive implementation and the possibility to use databases make FTIR one of the most suitable techniques for screening purposes in terms of detection of evidence and leading forensic investigation. However, it is also evident that there is a lack of consensus regarding the different analytical parameters used for each FTIR technique. Therefore, more research addressing the potential application to either condom samples or real samples and adequately comparing these techniques is needed.

Raman spectroscopy is less represented in the literature than infrared spectroscopy but could be a promising technique, as it has the advantage of detecting most of the targeted compounds from condom analysis. However, only one simulated case has been studied and the number of condom samples analysed was limited. Although the results show potential, the lack of experiments using this technique do not provide adequate evidence of its feasibility, and further investigations into its capabilities in real cases are needed. A comparison with FTIR spectroscopy would be of great interest to evaluate the potential of Raman as complementary to IR.

Concerning NMR, it is interesting to note that proton and carbon NMRs have been mainly studied. However, it would make sense to use the NMR of silicon-29, given the presence of silicon in the target compound, despite the low natural abundance of this isotope. Preliminary results obtained by Blackledge and Vincenti reported using ^{29}*Si-NMR*, but not obtaining enough sensitivity to allow the differentiation of the PDMS used by the various manufacturers [1]. Finally, since the analytical method has not been tested on real or simulated case samples, it is difficult to determine the relevance of NMR when detecting condom traces. Moreover, this method can detect compounds only present in large quantities and is mainly used when unknown compounds are sought. Knowing the target compound, this technique may not be suitable for trace research because the concentrations would be too low to be detected. The use of NMR does not yet appear to be a very useful forensic analytical tool for this purpose.

Based on these considerations, spectroscopic techniques were mainly used for detection of the compounds present on the condoms and in real cases. This is a good starting point for research, as it can definitely be used by forensic laboratories when this type of evidence is encountered. The interpretation of evidence was mainly related to a binary inclusion/exclusion-type analysis. Several gaps were found in the use of spectroscopy, such as lacking the specificity and sensitivity of the analysis, insufficient discrimination studies, which were only processed on a given set of samples with NMR technique or in one case with FTIR spectroscopy, as well as no transfer and persistence studies and a lack of interpretative model. These observations suggest that spectroscopy would be a good screening method to detect evidence but should be followed by a more specific method (*i.e*. confirmation method) to allow proper interpretation of the results.

4.4. The use of separation methods

Separation methods, such as gas or liquid chromatography, are very commonly used in forensic trace analyses. However, some precautions need to be considered before chromatographic analysis of condom residues, as some lubricant compounds have a high molecular weight, non-volatile properties or need specific detection parameters.

4.4.1. Gas chromatography–mass spectrometry (GC–MS)

GC may be used for the analysis of PEG [25,48], nonoxynol-9 [32] and nitrosamines [49,50] as well as other types of hydrocarbon-based lubricants, such as petroleum jelly [26,46,51]. PDMS analysis by GC–MS is not recommended as its molecular weight and volatility exceed the maximum temperature limits of most conventional instrumentation. A derivatization step is otherwise necessary [52]. Moreover, PDMS is also a constituent of capillary columns, particularly in GC. It is therefore necessary to ensure that what is detected originates from the sample and not column bleed [1]. When applied to PEG standards using separation followed by electrospray or chemical ionisation (EI or CI) MS detection [48,53], sensitivity increases with molecular weight. The EI source is often preferred because it allows the use of library databases for the characterization of the separated compounds.

Applications to condoms (between 2 and 54 samples) have demonstrated the ability of GC to detect nitrosamines [49], PEG [25], nonoxynol-9 [32] and even PDMS [34,39]. The chromatograms of PEG and nonoxynol-9 also reveal the various oligomers of the two molecules [25,32]. The detection limits for these compounds are on the order of 1 μg [25,32]. For PDMS analysis, the outer surface of the condoms was mainly targeted [34]. Various methods of sampling have been tested, including solid phase microextraction (SPME) [39]. Chromatograms of different condoms showed similarities [34] but siloxanes exhibited low peak height and were poorly separated in the chromatograms [39]. Other compounds were however visible, such as hydroxytoluene or hexa- and octadecanoic acids [39]. The results are therefore inconclusive for PDMS analysis and swabs of traces have not been tested [39]. The GC method was also used to analyse lubricants containing petrolatum (16 samples) [51], oily compounds or glycerol (6 samples) [52]. The authors note the importance of derivatizing samples to obtain a result [35]. The results highlight a possible differentiation between manufacturers, especially in petrolatum. The method is reproducible and has a detection limit of 0.5 mg for the analysis of petrolatum [51]. Maynard et al. (2001) employed GC–MS as a confirmation method when infrared analyses indicate petroleum residues [26].

Six simulated cases were studied and highlight the absence of interference between target compounds and biological fluids. As the surface characteristics observed on condoms are found in vaginal samples, the authors conclude that with the GC–MS method "obvious condom use can be proven" [34]. Nitrosamines have also been shown to be detectable in simulated biological fluids, although the proportion extracted from these fluids is less than 10% of the total amount of nitrosamines present on condoms [50].

4.4.2. Pyrolysis-gas-chromatography–mass spectrometry (Py-GC/MS)

To enable the analysis of PDMS through GC–MS, pyrolysis can be performed to convert the linear PDMS into cyclic oligomers of low molecular weight. Those oligomers (monomers, dimers or trimers) are volatile and they can be analysed through the capillary column and mass spectrometer without any problems. Pyrolysis is therefore often used for the analysis of polysiloxane [1,44,54–56]. This method is very popular as the degree of polymerization of the molecule can then be inferred and organic additives can be

detected. The pyrograms of at least 11 different PDMS standards present a composition mainly containing cyclic oligomers such as dimethylsiloxanes (DMS), which are characteristic of PDMS degradation [25,33,56]. The preferred conformation is that of the cyclic hexamer [33]. It turns out that the viscosity affects the size of the area of the peaks in the pyrogramm: the lower the viscosity, the smaller the area [56]. The effect of the pyrolysis temperature is influential since the number of pyrolysis products varies if the temperature increases or decreases [44]. A fairly high temperature (over 300 °C) must be applied to obtain the degradation of PDMS [25,33], but not over 400 °C, otherwise PDMS degradation is impaired [55]. Nevertheless, researchers targeting PDMS coming from condoms previously reported the use of a pyrolysis temperature of 600 °C [25,57], allowing proper detection with a limit of detection on standards of 0.1 ng [56].

Applied to a set of 25 condoms, the py-GC–MS analysis detects the PDMS with no additional chemical residue is observed [25]. The detection limit is of the order of 1 μg. The results do not show differences between brands and models of condoms [25]. In an unknown sample, py-GC–MS allowed an inference on viscosity and the absence of other organic compounds [44]. Moreover, Tottey et al. [57] managed to use the methodology to study the persistence of PDMS in different types of biological areas (penis, vagina, skin, mouth, *etc.*).

4.4.3. Liquid chromatography (LC)

LC analysis is often used for molecules that cannot be analysed through GC (such as non-volatile compounds). It is necessary to carefully consider the detection methods, as ultraviolet detection might not be adequate for all kind of molecules. Generally, LC–MS is presented in the literature, rather than LC-UV/Vis [26,28,58].

LC is used to analyse nitrosamines [59] and spermicides. For spermicide analysis, an appropriate column should be chosen after considering the amphiphilic properties of nonoxynol-9. Using a normal phase column may generate some compatibility problems between the solvent and an ESI source. In the literature, assays are usually performed in reverse phase [26,28]. PDMS can also be analysed in LC–MS, using an atmospheric pressure chemical ionisation (APCI) source instead of an ESI source for mass spectrometry. This would be the most appropriate and quickest technique for the characterization of unknown polysiloxanes [44].

Applied to nonoxynol-9, LC analysis shows the possibility of separating up to 17 oligomers present in the molecule [47]. Coupled with a UV–vis diode array detector (DAD), the LC is able to detect concentrations of the order of μg/mL and is applicable to residue of nonoxynol-9 [60]. When coupled with mass spectrometry using an ESI source, many adducts are observed in the mass spectrum and this is considered to be one of the main limitations of this method [28].

Condoms and vaginal contraceptives (2–5 samples) were analysed by LC and it was possible to detect nonoxynol-9. Moreover, differences between condoms and other contraceptives (e.g. gels) were identified [28]. An LC-DAD method has been fully validated for the analysis of nonoxynol-9 in mixtures such as foam, cream or contraceptive gel, or on residue found on various supports [61]. A second limitation was observed during the extraction, since selective extractions of ethoxymers with long ethoxylated chains may occur depending on the solvent used [28]. For Maynard et al. (2001), the LC is used as a confirmatory method when infrared analyses indicate the presence of spermicide [26].

In simulated case studies on humans, LC clearly demonstrated nonoxynol-9 on swabs of specimens [28]. Some interference from the swab constituents may be observed but it is less likely if it is cotton [60]. In nonoxynol-9 mixtures with vaginal fluid simulants or animal plasma, assays show no interference between matrix and analyte, analyte stability and natural absence in the animal body [58]. Results are promising when looking for nonoxynol-9, but PDMS has not really been extensively studied with this method.

4.4.4. Other methods

Two other separation methods, capillary electrophoresis (CE) and size exclusion chromatography (SEC), were used for condom traces analysis or PDMS analysis. As they are less common and are present in only one or two papers, the results are briefly described here under.

UV–vis-CE was used on 67 condoms and intimate lubricants [8]. All but two of the samples had at least 1 (but often several) peaks in the electropherogram. A statistical model was created using PCA and linear discriminant analysis (LDA). Real samples were also simulated by applying lubricants to the skin and clothing and several samples were taken. The 67 condom lubricants and personal lubricants were analysed and classified in the model correctly, if the sample was taken directly after application, though incorrectly if the sample was taken after 30 min. The authors conclude that their method is difficult to use in a routine analysis as the traces were not detected on skin after 30 min [8].

Gel permeation chromatography (GPC) is a method often used for the analysis of polymers, biopolymers or proteins [1]. As part of the analysis of traces of condoms, the GPC may be used for the analysis of lubricants; more specifically PDMS as it would be possible to differentiate the different PDMS, since the molecular weight or their molecular distribution can vary. Since 2011, it was attempted to identify PDMS in pharmaceutical formulations using a validated SEC method, according to ISO17025 standards [62,63]. The method makes it possible to differentiate PDMS standards according to their viscosity. No interaction with a white matrix (placebo) was observed. When applied to targeted pharmaceuticals, a matrix effect could be observed. The quantitative results obtained were still in agreement with the manufacturer's data, which confirms the precision of the method.

4.4.5. Critical perspective on separation methods

Concerning GC–MS, several authors arguably justify its use by the fact that it is used in a large number of laboratories. Although its use may be questionable in the analysis of condom traces, especially if the targeted molecule is PDMS, the results for PEG and nonoxynol-9 are promising. Tests on simulated cases should be performed to verify the applicability of the method to real cases.

Results provided by py-GC–MS are quite interesting, as it allows the use of GC–MS, which is one of the most used techniques in forensic laboratories, but with the advantage of PDMS detction. The detection limit is very low and that may be useful in real cases, as the low-level traces may therefore be detected.

LC has many advantages, especially in overcoming potential limitations to GC analysis. However, it remains less used than the GC method, perhaps because it cannot be coupled to ionization by electronic impact (and therefore reference databases are not necessarily available). Results are promising for the detection of spermicides in a simulated vaginal matrix but the question of PDMS has not yet been comprehensively studied.

The successful results presented using UV–vis-CE were surprising as UV–vis detection is used and the target molecules (lubricants) do not have any chromophoric groups. Moreover, no reference standard analyses were used to ensure that the peaks observed in the electropherograms originated from the target molecules. This method is therefore inadequate for forensic analysis of condom evidence at this time.

Finally concerning GPC, there exists at least one validated protocol for PDMS analysis by GPC but it has unfortunately not been applied to condom traces. There were to our knowledge, no studies of the application or applicability of GPC to condom traces,

although the current methods allows the detection of PDMS in a concentration range of 0.1–1%.

Based on these considerations, separation techniques were used in the majority of the articles for the detection of compounds as well as the application to real samples. Discrimination models were found to be built on a wide range of samples that were mainly personal hygiene products, creams or contraceptives. Only CE-UV–vis was used for the discrimination of condom samples, but the target compounds are not clearly identified and therefore, it is difficult to ensure the use of this model in caseworks. Py-GC/MS was found to be a very valuable tool for transfer and persistence studies, with a very good sensitivity, and a high potential for the use [25,57]. The same observation as with spectroscopy was made concerning the interpretation of evidence which was a binary inclusion/exclusion analysis based on the presence or absence of characteristics peaks known to originate from the sample. The specificity of the analysis and the validation of the different techniques were not clearly reported, and there's a definite need for further discrimination research as well as a broader set of samples for transfer and persistence studies. This will help building a strong and valid interpretation model for this type of evidence.

4.5. Mass spectrometric analysis

There are currently numerous mass spectrometric methods used to analyse the different compounds from a condom. Each method has a specific range of mass or sensitivity/specificity. All the methods found in the literature will be presented here under.

4.5.1. Electrospray ionisation (ESI)

Although usually coupled with a separation method, for example liquid chromatography, the ESI technique can also be used alone. Two conditions must be fulfilled: (1) the sample must arrive in liquid form so that the ESI can be used [44,64] and (2) the solvent of a compound must allow the ionization, either positive or negative.

In relation to condom traces, ESI and nanoESI-MS techniques were studied for the analysis of spermicides (nonoxynol-9) and lubricants (PDMS). Applied to 4 different PDMS standards ESI has shown its ability to characterize samples with good resolution, whether used alone [44] or coupled to GPC [65]. The ESI spectrum allows determination of whether the molecule is in cyclic or linear form, thus confirming the results obtained by py-GC [44]. Some limitations have been observed, especially in the detection of compounds with a mass over 4000 Da. Ion suppression effects make it difficult to detect these compounds [65]. A competition between the analytes could also be observed [66]. Results may be biased because of the selectivity of some compounds and this is a problem given the desired applications [66].

The application of ESI to condoms and personal lubricants targeted nonoxynol-9 [28]. The ESI technique has several adducts in the spectra obtained. The nanoESI method has also been tested for application on condom traces because it is very sensitive; a small amount of sample is sufficient for the analysis. Both methods allow for the identification of the spermicide in the mixtures analysed. The detection limit of the nanoESI is lower than 500 pg/μL [28].

4.5.2. Matrix –assisted laser desorption ionisation (MALDI)

MALDI is a soft ionisation technique allowing polymer, peptide or other macromolecules up to 300'000 Da to be analysed. It has thus been used to provide qualitative and quantitative information on condom lubricant (PDMS, PEG). Coupled with time-of-flight (TOF)-MS, it is an ideal method for this type of analysis [44,46,67]. It can also be coupled to a Fourier Transform MS to analyse spermicides (nonoxynol-9) [28]. MALDI was applied to 4 different PDMS standards and was able to detect large molar masses [65], but was not able detect whether cyclic oligomers were present [44]. The method is more stable and repeatable than the ESI [65]. The spectrum obtained is complex and minor compounds may therefore be difficult to detect [44].

When applied to actual condoms (between 6 and 26 samples), MALDI detected PDMS, PEG [9] and also nonoxynol-9 [28]. The method shows good sensitivity for PEG and nonoxynol-9 [28], despite the presence of adducts in the spectrum [68]. Unfortunately, PDMS is not always detected by MALDI [42,68]. The optimized method must take into account ion suppression, spectral quality and their effect on the detection limit [9], which was found to be 2.5 ng/μL for nonoxynol-9 [28] and of the order of 0.003–0.5% in biological fluids, depending on the class of lubricants [9]. PCA was performed on the data and allowed the separation of the condoms into at least 5 classes, based on the compounds present. Within the various classes, subclasses could sometimes be established but often this was difficult when the main lubricant was PEG [9]. Coupling the results with those obtained by FTIR or Raman is recommended in order to obtain further information and to increase the discriminatory power of the analytical sequence [9,42].

4.5.3. Other types of mass spectrometric analysis

Desorption chemical ionisation (DCI)-MS method was mentioned for the analysis of lubricants (PDMS) by Blackledge and Vincenti in 1994, as a confirmatory method after FT-IR [1]. From all the mass spectrometry techniques, it was chosen for its ability to detect nanogram traces. Applied to 40 condom samples, it allowed the detection of PDMS based on the presence of a regular sequence of characteristic peaks. A link between viscosity and reproducibility was observed: the lower the viscosity, the more reproducible the results are. It was then simple to work back to an average molar mass. DCI also made it possible to differentiate PDMS from a different origin and a different viscosity, if the differences are not too small. DCI-MS also allowed the analysis of nonoxynol-9. No interference with PDMS is observed. Applied in two simulated double-blind cases, it appeared that the PDMS was correctly detected and was not detected, if it was indeed absent [34].

Desorption electrospray ionisation (DESI)-MS was developed in 2004 [69] for the analysis of drugs, toxic industrial compounds, chemical war agents or explosives [70] at ambient temperature and directly from the support. It can be used for the analysis of condom lubricants and spermicide. However, other analytes may be detected, such as additives in condoms. The method was applied on 6–10 condoms; 7 molecules were found on the condoms (PDMS, PEG, nonoxynol-9 but also other less common compounds such as methylmorpholine, octylamine, isonox 132 and dibutylformamide) and each condom had a distinctive mass spectrum [37] suggesting a possible differentiation. Adducts were observed as in most other mass spectrometry techniques [37]. Statistical treatments, such as PCA and LDA, were applied to the DESI results [71]. A total of 87 variables were established suggesting that there are other types of lubricants. The PCA highlighted 11 useful variables with an explained variance percentage of 84–96%. The correct prediction rate of the prediction model created was 91% [71].

Direct analysis in real time (DART)-MS was developed in 2005 [72], for the same purposes as DESI-MS. It allows the instant ionisation of gases, liquids and solids under normal laboratory conditions (25 °C, 1 atm). This method was used to analyse nonoxynol-9 and water-based lubricants. Other compounds may also be detected, such as, for example, adjuvants (lidocaïne, benzocaine), aromas [73], or those already presented by Mirabelli et al. (2013) such as isonox 132, octylamine or dibutylformamide for example [37]. DART-MS method was used on condoms and

lubricants (between 3 and 110 samples) and was able to detect at least 3 major compounds, including lubricant and nonoxynol-9 [36,74]. Groupings were made on the basis of visual observations, such as presence or absence of certain compounds in the spectrum and detection of specific markers [36,74]. This suggests that the lubricant may be brand specific [36]. In order to differentiate visually similar samples, chemometrics analysis was used and models were examined. A total of 9 variables was required with approximately 90% of the variance explained [75,76]. The model created on this basis had a prediction capacity of 98% [75,76]. DART-TOF-MS offers low sample preparation requirements [36,76], good resolution, accurate mass determination and no ambiguity in assigning peaks to certain molecules [36]. In case work samples, DART-MS could detect nonoxynol-9 if it was present on the specimen [36]. No indication of PDMS detection or possible interactions with the vaginal matrix was mentioned, but Maric et al. [75] reported performing experiments on the application of their model to real cases.

The last presented mass spectrometric methods is desorption ionisation on silicon (DIOS)-MS which was developed as an alternative to MALDI-MS [77]. The targeted compounds within condoms are nonoxynol-9 and PEG. The silicone compounds (PDMS) are not analysed by this method since the samples are deposited on a plate containing porous silicone. Since the molecules present a chemical structure very close to that of PDMS, the results could be erroneous. Tests were undertaken on standards (2), condoms (2) and vaginal inserts (2). PEG and nonoxynol-9 were distinguished by separate ion series [38,78]. Other compounds, such as sodium bicarbonate or sodium citrate, were also detected [78]. The comparison between the results of condoms and vaginal inserts, shows that it is possible to differentiate PEG coming from the insert and condoms. Adducts were present in the spectra, but in a smaller quantity than other mass spectrometric methods as, in the case of this method, their presence depends on the quantity of sample [38]. In real cases, the method has shown that blanks present a different chemical profile than the targeted compounds [38]. It was also possible to determine whether vaginal insulin-type contraceptives, containing nonoxynol-9, had been used, both on samples from the victim and those from the suspect [38,78]. Possible interactions with the vaginal matrix were not mentioned. The results were confirmed by FT-IR analysis, although it was not as discriminant as DIOS-MS [78].

4.5.4. Critical perspectives on mass spectrometric methods

Mass spectrometric methods are numerous to have been used and the choice for analysis depends in particular on the mass domain, that one seeks to analyse. Although these methods have been shown to provide high discriminatory power and require minimal sample preparation, they are quite expensive and may not be readily available in forensic laboratories.

The ESI and MALDI techniques showed their complementarity in the domain of the analysed masses since each one allows the highlighting of target compounds but in different mass domains. MALDI has already been applied to a large batch of samples (up to 26 condoms), showing good sensitivity and performing data processing may provide useful conclusions.

DCI-MS was only applied in a single 1994 publication, suggesting that other more efficient methods were developed later. It will not be considered further in this review, as this method has been superceded by more recent approaches.

Other techniques using a direct desorption form (DESI-MS, DIOS-MS and DART-MS) have the advantage of not requiring sample preparation. The DESI is based on the principle of ESI, and DIOS on the principle of MALDI. DESI has a demonstrated ability to detect a large number of compounds and it is possible to perform statistical treatments with promising results for the classification of samples. However, no applications to real cases were noted. DART-MS differentiated large sample sets (up to 90 samples analysed) and detected nonoxynol-9 in real case samples. As the detection of other compounds was not mentioned, nor the possible interactions with the vaginal matrix, it is difficult to ascertain the effectiveness on real samples, but studies are in believed to be progress. As for the DIOS, it does not allow the analysis of silicone compounds since its initial matrix is made of silicone. It is not therefore a method considered further for the analysis of condom traces since it is not adapted to the targeted compounds required.

Compared to spectroscopic and separation techniques, mass spectrometric techniques cover a wider range of interesting points, as most of them were used for detection and characterization of samples, with reported limits of detection and validation procedure, but also for discrimination and classification of wide ranges of samples, from condoms to personal hygiene products. The interpretation of evidence shifts therefore from the binary inclusion/exclusion model reported with other techniques to the use of multivariate analysis with the help of chemometrics to classify samples and establish error rates, misclassification and issues encountered with the models, if any. Although the sensitivity of the instrumentation was not always described, researchers have described the specificity of their analysis and validated most of their analytical parameters. Unfortunately, these techniques were not tested on real sexual assault case wor samples yet, which makes it difficult to evaluate and compare with previous methods which were found to describe all the requirements for real sample analysis. Application of the previously described methods to reals samples, with transfer and persistence studies would enable to build a strong and valid interpretation model, therefore establishing them as a suitable and recognized techniques worldwide for this type of evidence analysis.

5. Analytical sequence

The success of a given analytical technique relies on the investigation of the nature of the selected target compounds. However, some criteria are appropriate for any technique, regardless of the compounds:

- The technique should be accessible and/or available in most forensic laboratories, at an affordable price and with a reasonable time of analysis
- The technique should be able to detect condom traces in the vaginal matrix
- The technique should ideally be able to differentiate condom lubricants from other possible lubricants and/or sources.
- The technique should allow quick, easy, and reproducible sample preparation and analysis, with good reliability and robustness

For the analysis of condom traces, and more specifically condom lubricant traces, numerous techniques could be used, but to date, none of them meet all the above-mentioned criteria. Hence, authors have developed analytical sequences to assess the presence of the aforementioned components.

Maynard et al. (2001) proposed a generic analytical sequence [26] for the global analysis of condom residues. The protocol contains at least 4 methods for the analysis of PDMS, PEG, nonoxynol, glycerin and petroleum residue. The workflow is reproduced in Fig. 8.

This is a common generic sequence that can be found in the literature. Authors usually propose two analytical procedures, the first used as a screening method and the second as a confirmation method, which would mainly be two complementary methods. The first example was illustrated by Blackledge and Vincenti [1] who in 1994 used DCI-MS after FT-IR analysis, targeting only PDMS.

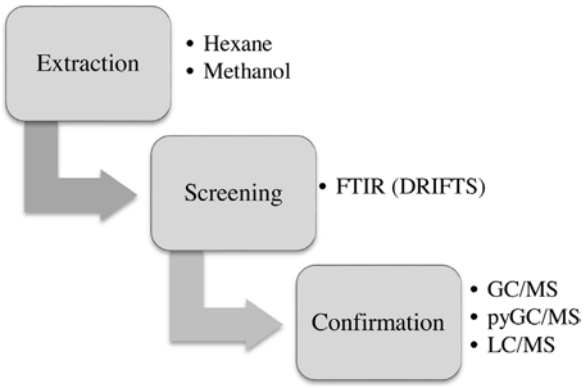

Fig. 8. Analytical sequence resumed as proposed by Maynard et al. (2001) [26].

Campbell and Gordon proposed a sequence for the detection of PDMS and PEG, using FTIR and py-GC/MS and described a full flowchart for the analysis process [25]. Few protocols integrate more than two methods. In 2013, Bradshaw et al. proposed to use FTIR, Raman and MALDI-MS for the detection of condoms residues, suggesting the integration of the sequence in a workflow protocol [42]. Flowcharts containing a screening and a confirmation method are in alignment with Scientific Working Group for Materials Analysis (SWGMAT) and European Network of Forensic Science Institutes (ENFSI protocols) [79–82]

6. Analysis summary

Table 1 summarizes the analytical methods discussed above, their target compounds and the media on which they were tested. Overall, all methods are applicable to condoms, with the exception of the GPC, for which no published data were found.

Among all techniques, FTIR, py-GC–MS and DART-MS offer the greatest potential for non-polar compounds such as PDMS. FTIR is more suitable as it is available in most forensic laboratories, but the methods are complementary. While DART-MS allows a good classification of samples with a high level of discrimination, py-GC–MS allows the confirmation of PDMS through the identification of specific pyrolysis products. By contrast, FTIR will detect only atomic bonds and is therefore considered to be less specific. On the other hand, FTIR and DART-MS require minimal sample preparation and can be performed in-situ (although this has not been reported yet for FTIR) and offer better repeatability and fewer analytical problems than py-GC–MS. According to the previous sections concerning these methods applied condom lubricants, FTIR and py-GC would both need sample extraction whereas DART-MS would not. It is necessary to keep in mind that the extraction efficiency can dramatically change depending on the solvent used and that it could be necessary to repeat the extraction, leading to material loss or dilution.

In regards to polar components (*i.e.* PEG and nonoxynol), methods such as LC–MS can be used as a complementary technique to FTIR, as its use has been reported for the analysis of PEG or spermicides [26]. More research is also needed here to investigate the potential use of LC–MS when applied to real case samples and to compounds mixed within a human matrix.

From the analytical point of view, Raman spectroscopy may also be used to detect condom traces, but it is necessary to check whether the information provided is complementary to FTIR or just a replicate. Hence, there is a need for research concerning the detection and analysis of PDMS (and lubricants in general) using Raman spectroscopy, but it is less common to find it in forensic laboratories, and this is the reason why it is not commonly presented in frameworks.

7. Forensic issues in condom evidence analysis

7.1. Analysis and interpretation issues

As illustrated in this paper, most methodologies are well established. Although the implementation of an analytical procedure is of great importance, the interpretation of the analytical results should also be enhanced. The analytical techniques presented were found to be adequate for the analysis of the different components of a condom, which is one of the main challenges for a forensic scientist working on cases where condom use is evidentiary. However, all of the reviewed articles appeared to be analytical chemistry articles with little application to forensic case work, though some authors have focused on the question of the interpretation of the results. These result interpretations aim to evaluate the question; what is the conclusion if the result is positive? or if it's negative?

If the result is positive, it therefore means that there has been contact with a product containing this type of molecule. It is necessary to take precautions when asserting that a condom has been used since other products may contain the targeted molecules targeted as a component of condoms [83]. False positives cannot be excluded but have not yet been specifically discussed in the literature.

It is generally difficult to draw conclusions from a negative result in forensic science because this may come from the absence of the sample but also from a lack of sensitivity of the method or from inadequate sampling. A potential exception is the DCI-MS method described by Blackledge and Vincenti (1994), which has high sensitivity [1]. Thus, if the victim has not undertaken an activity that can cause the loss of the condom traces and the PDMS was not detected by the DCI-MS method, it most likely means that there was no condom worn. However, as reported by different authors [14,17,30], several factors can influence negative results such as the time elapsed between the act and the sampling, or the sampling techniques.

In addition, the presence of a condom is determined if certain characteristic peaks of condoms are present in the various test results, without evaluating the relevance of these peaks or their specificity. Few authors have tried to verify their results with analytical standards. In addition, the possibility that other products may contain the same compounds as those targeted in the various studies has not been fully evaluated. The specificity of the target compounds as well as the possibilities of false positives and false negatives have therefore not been fully studied. Additionally, there does not appear to have been any evaluation of the analytical results using a likelihood ratio (LR) approach [84], considering at least two exclusive and competing proposals. This is despite LRs being a recognized approach in forensic science [85] that has been applied in the search for seminal fluid in cases of sexual assault [86] as per ENFSI recommendations [87].

7.2. Practical considerations for future researches

Condom evidence is in an emerging state of analysis, with a significant amount of analytical chemistry work already done. However, there is a lack of statistical validation and published error rates which could be useful for the court system. The key parameters on which research efforts should focus are described here.

Several compounds can be found and targeted, but they are not purely specific to condoms. PEG or PDMS for example may be found in a wide range of products. Researchers should therefore focus on identifying and studying the lubricants that are specific to condom residues and study the possibility of distinguishing condoms from other personal products. In addition, it would be

Table 1
Summary of the analytical methods with their target compounds and the tested material.

Analytical procedure	Target compound	Tested on
Optical methods		
Microscopy	Solid Particles	Condoms
		Simulated cases
Spectroscopic method		
FTIR	PDMS, PEG	Condoms
		Lubricants
		Simulated cases
		Real samples
Raman	PDMS, PEG, nonoxynol-9, human samples	Reference standard material
		Condoms
		Real samples
		Human samples
RMN	PDMS, PEG, nonoxynol-9,	Standards
		Condoms
Separation method		
GC	Petroleum Jelly, PEG, nonoxynol-9, PDMS	Real samples
		Lubricants
		Condoms
		Standards
LC	Nitrosamines, nonoxynol-9	Standards
		Condoms
		Personal lubricants
		Simulated cases
CE	Lubricants	Condoms
		Lubricants
		Simulations on skin
GPC	PDMS	Pharmaceutical products
Py-GC	PDMS	Standards
		Condoms
		Medical products
Mass spectrometry		
ESI-MS	PDMS, nonoxynol-9	Standards
		Condoms
		Personal lubricants
		Simulated cases
MALDI-MS	PDMS, PEG	Standards
		Condoms
		Lubricants
		Contaminated fingermarks
DCI-MS	PDMS	Condoms
DESI-MS	PDMS, PEG, nonoxynol-9	Condoms
DART-MS	PDMS, aqueous-based lubricants, Nonoxynol-9	Condoms
		Lubricants
		Contaminated fingermarks swabs smeared on condoms
DIOS-MS	Nonoxynol-9, PEG	Standards
		Vaginal inserts
		Condoms

of relevance to carry out quantification studies concerning condom traces in a living matrix, as similar processes have been carried out in other forensic areas such as fibre or DNA analysis, and have assisted in the interpretation of these evidence types [88,89].

The presence or absence of condom lubricants is currently assessed based on the presence of the compounds in infrared spectra or of their pyrolysis residues in py-GC–MS results. Persistence has been very rarely studied, and the available results are variable depending on the methodology and the operator. No reproducible transfer and persistence models have been established and the mathematical modelling is not yet available. Reproducible regression curves were not used and may be necessary for considering cases with multiple traces and illustration of former use of condoms. Furthermore, the persistence of lubricants under different influential factors has not yet been fully studied. Future research efforts should focus on investigating the impact of these factors on the trace composition, as well as on transfer and persistence, as most of these are rarely known with any level of confidence in real case scenarios [12]. When the nature of a factor is known, it can be integrated into the transfer and persistence interpretation model more easily than if it is completely unknown.

Mathematical and statistical tools such as chemometrics or Bayesian network-derived statistics can also be used on to evaluate the potential discrimination between different condoms and their traces, and to model transfer and persistence. For example, principal component analysis (PCA) and linear discriminant analysis (LDA) were used in many different studies to build a model allowing the correct groupings of condoms containing different components or different human fluids [9,71,75,76,90]. These chemometric tools are very efficient but have only been applied to two separate sets of condoms and water-based lubricants [74–76], with the classification and error rates presented using a specific instrumentation (*i.e.* DART-MS). This procedure has, to our knowledge, not been applied to a set containing a mixture of all types of samples. Classification of traces with associated error rates, or in linking the profile to a specific group as illustrated in other studies [91,92] were not reported nor were statistics used to model transfer and/or persistence based on data obtained in the literature (*i.e.* infrared spectra or pyrograms). These

chemometrics tools may offer preliminary results for transfer and persistence models and there is a need to undertake some research to identify reliable markers in condom traces and build strong models to understand the transfer and persistence of condom traces. The robustness of the parameters when exposed to different contextual factors should also be carefully studied and evaluated. It may also arise that statistical tools cannot help discriminating samples as expected in a forensic context, but this needs to be established, in which case a binary inclusion/exclusion analysis based on reproducible real-world samples should be considered.

7.3. Application to casework

Once robust procedures have been developed and potentially validated in accordance with ENFSI or SWGMAT recommendations [79–82], the application of the procedure to real case samples can be considered. The application of this procedure to real case samples may infer costs that should be carefully considered before ordering the necessary analyses. Ideally, condom traces analysis should only be conducted in specific cases where the identification of traces are of crucial importance. The casework context has to be carefully considered in order to account for the relevancy of such trace analysis. For example, if there are legitimate explanations for the presence of the condom trace in the victim's vagina, is the analysis still necessary? If the condom analysis is proven to be relevant, and if it is possible to analyse it in the specific given case, then the collected questioned trace should be analysed with the appropriate analytical techniques based on previous research results. The transfer and persistence parameters should be determined, and their values used in the interpretation model. It may appear that models built on research data will not be applicable to real samples as the variability of the trace can be very large and beyond the analyst's control. This represents the most important limitation of this framework. Future research should therefore give precise information about the relevance of such models. There exist few protocols for casework conditions, but up-to-date protocols should be established with more realistic and rigorous approaches for the analysis and evaluation of condom traces in real cases.

8. Conclusion

The literature review presented in this article highlights the lack of consensus and uniform protocols prevailing worldwide in the way questions about the presence and the analysis of condom traces are managed. In reality, whilst the literature generally states that condom traces analysis is possible using from spectroscopic to mass spectroscopic analytical procedures, few experts apply thesemethods to solve cases. Few cases were studied and most research studies were undertaking market studies, using large sets of condoms samples bought in the market of the country the research was performed in.

As such, condom analyses are not new, and this paper shows that research has already been conducted on the detection of condom traces. Different methodologies were proposed, principally based on the chemical characteristics of the condoms. The aims of the methodologies were essentially the same, *i.e.* to identify condom lubricants and to aim to be able to classify different condom brands and types. However, very few of these studies provided error rates of the proposed methods or limits of detection, nor was any quantification of the condom residues or condom components undertaken. This may be due to the early stage of development of the different methodologies or because practical considerations and access to samples are missing, preventing actual use in real forensic caseworks. Thus, much more research should be conducted in order to clearly assess the potential of developed methodologies and to prevent misunderstanding of the analytical results. Efficient research efforts should focus on the study of chemical characteristics that can be used as a guide to carry out the necessary research steps when considering condom traces analysis. However, due to the high variation potential of condom traces within a living matrix, it is important to highlight the potential, as well as the limitations of condom analysis methodologies, to the forensic community. It is important to keep in mind that the interpretation process may need some additional development before application to the Courts.

Authorship contributions

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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.

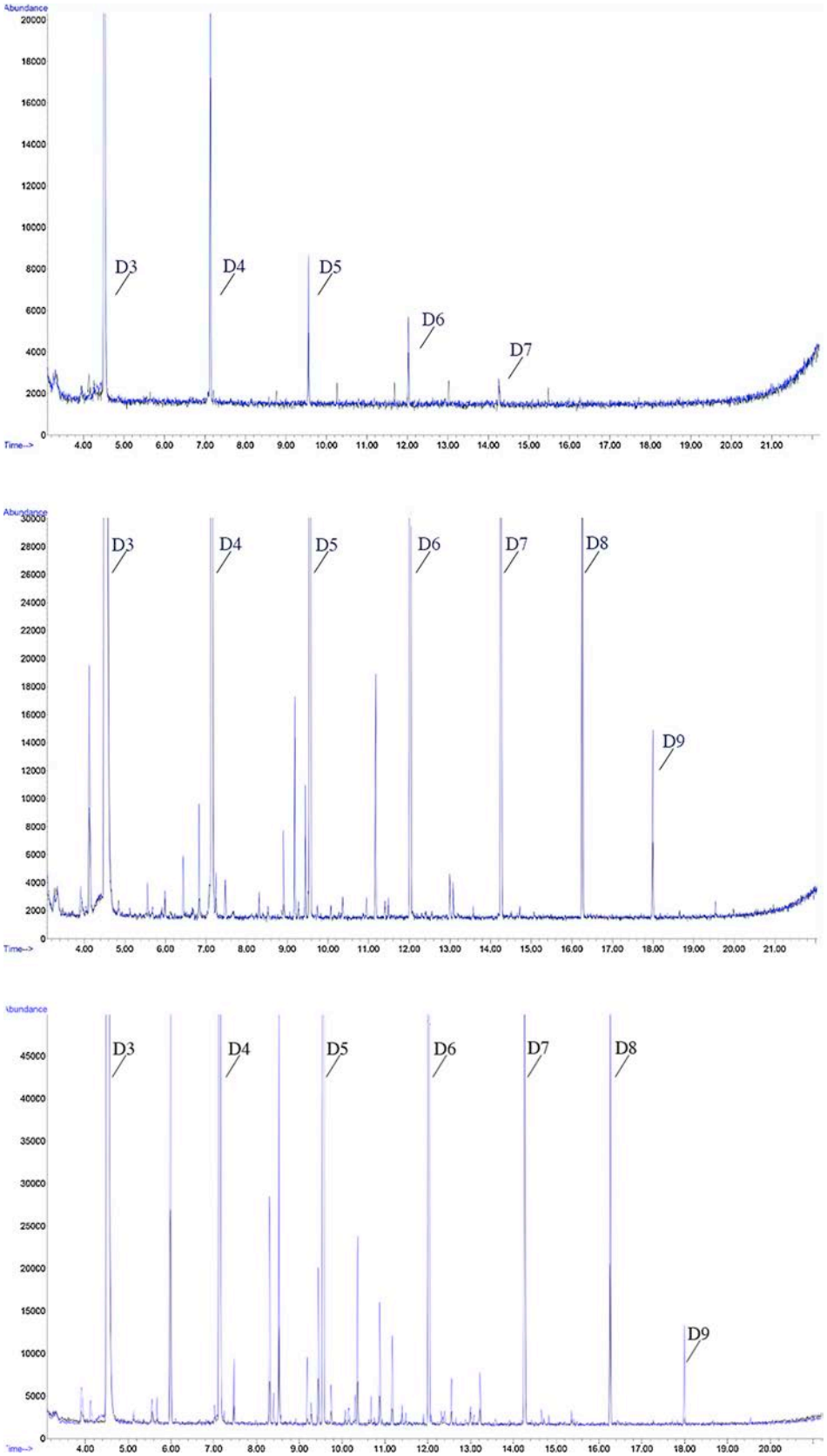

Fig. 1. Illustration of pyrograms acquired under different pyrolysis conditions (temperature/pyrolysis time), two replicates per design point are presented a) 420 °C/10 s, b) 620 °C/20 s, c) 920 °C/30 s. Variation in terms of number of peaks and their position as well as their abundance can be observed. 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

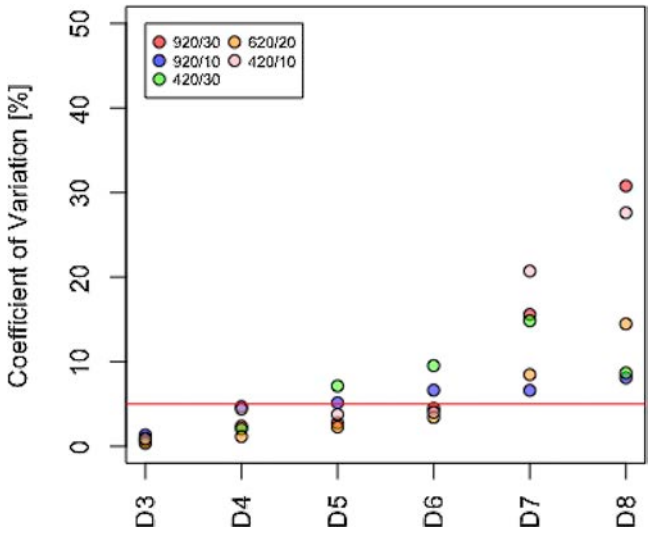

Fig. 2. Illustration of the distribution of the CVs according to the analysed compounds, after area sum normalization and double square root pre-treatment.

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.

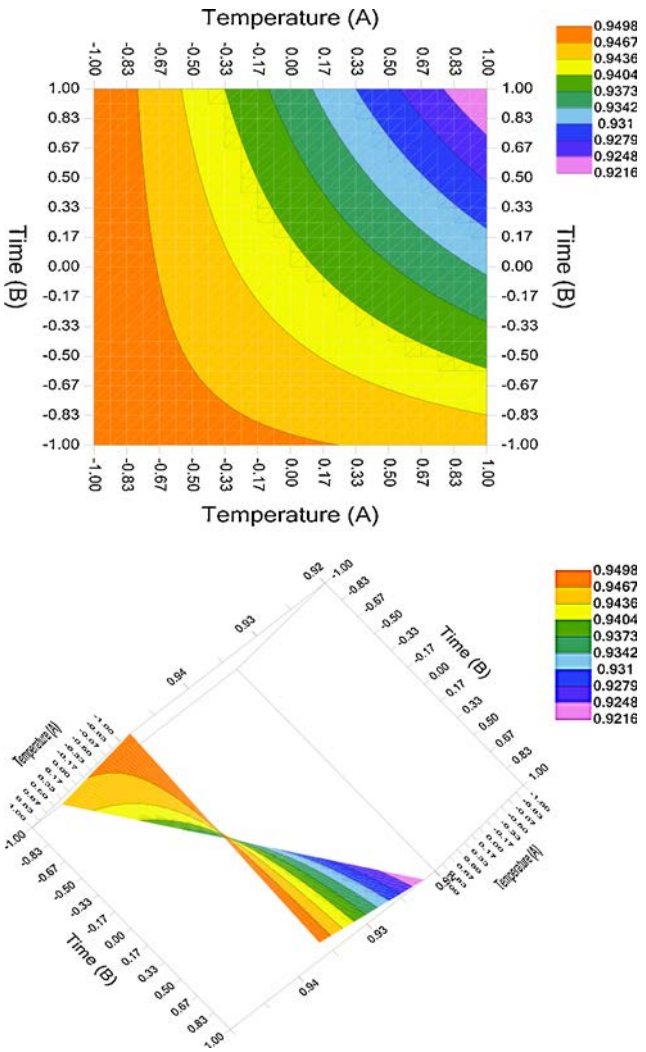

Fig. 3. Illustration of the response surface obtained from the FCCD, a) 2D surface, b) 3D surface. Axes contain the coded values used to draw the surface response. Interaction on the 3D space refers to the interaction between Time and Temperature. The numbers refer to the amount of styrene obtained along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

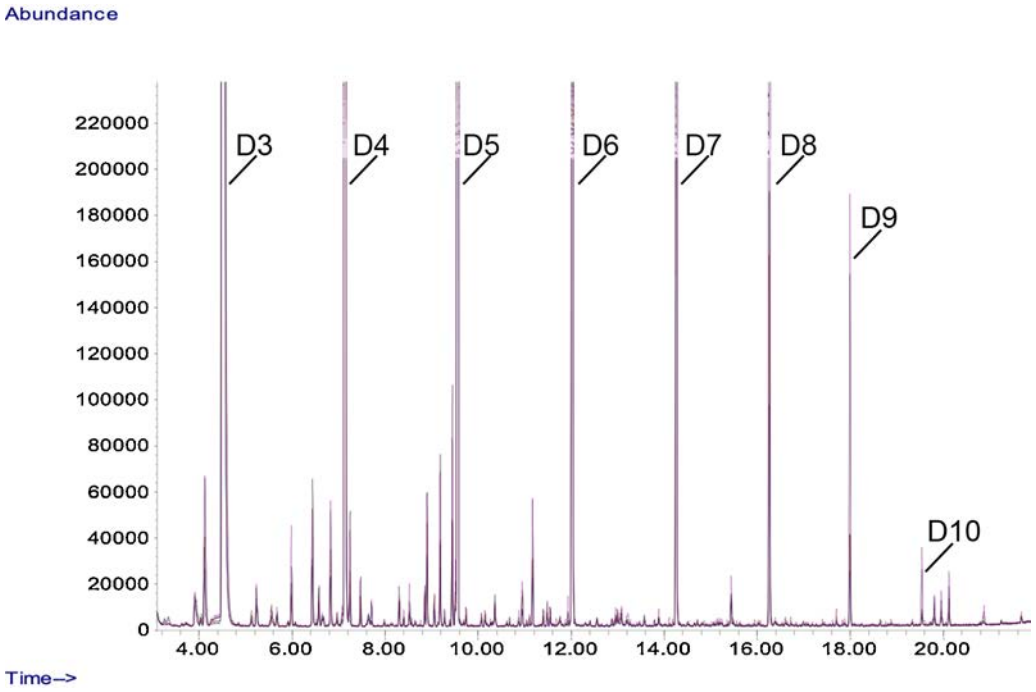

Fig. 4. Illustration of the repeatability of the pyrograms on the five replicates sample Manix Skyn. Displayed between 4 and 23 min for better readability. Cyclic oligomers D3 -D10 are indicated on the pyrogram after identification in the NIST database.

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

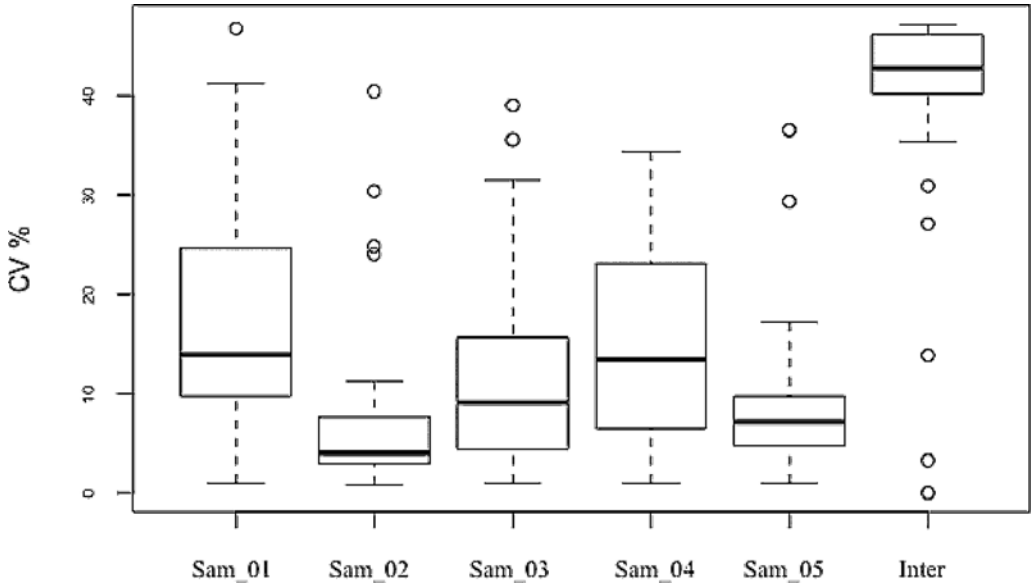

Fig. 5. Boxplots of the CV calculated within each sample and between the five condoms on 31 variables. Inter refers to between sample variability (intervariability).

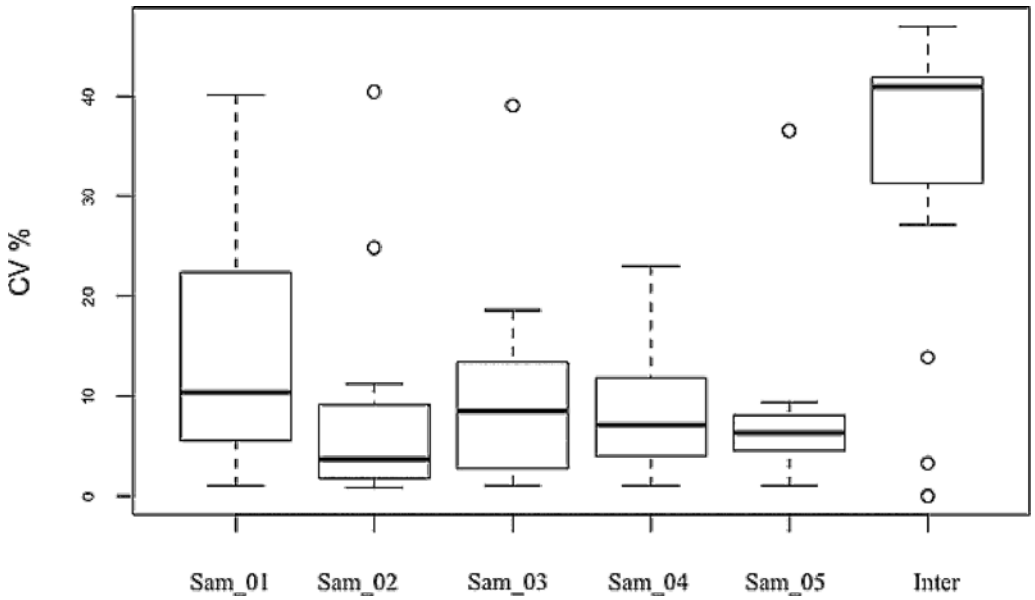

Fig. 6. Boxplots of the CV calculated within each sample and between the five condoms on 15 variables. Inter refers to between sample variability (intervariability).

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

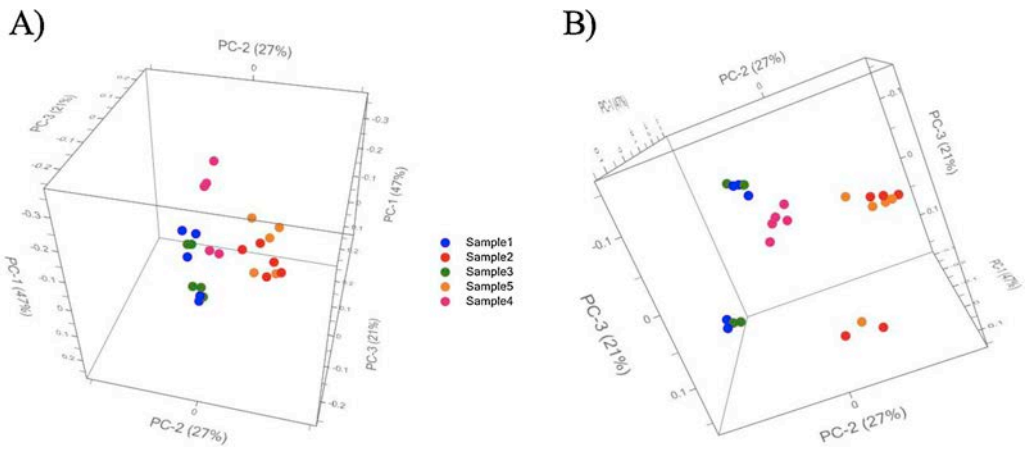

Fig. 7. PCA realised on the five studied samples, using the 15 compounds, illustrating the problematic of the clustering. Replicates coming from sample 1 and sample 3 (blue and green dots) are clustered together, although they are not from the same source. Same observations are outlined with sample 4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

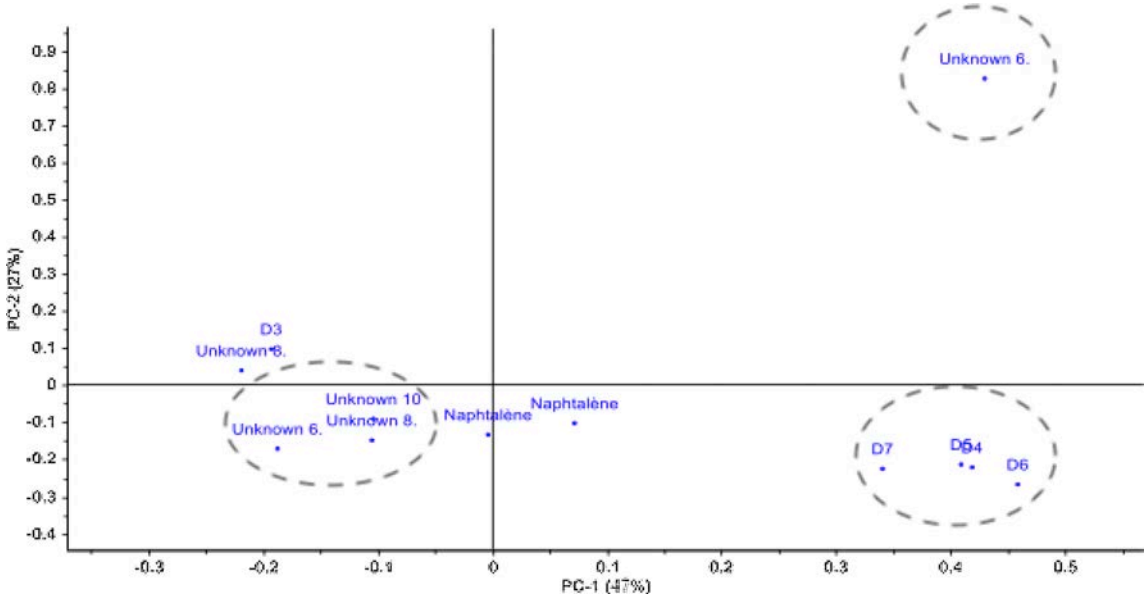

Fig. 8. Loading plots obtained from the PCA plots, indicating which variables describe PCs and are responsible for the separation of the samples. PC1 and PC2 are presented.

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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Comparison of spectroscopic methods in the detection of silicone-based condom lubricant evidence

Céline Burnier, *[a] Wilhelm van Bronswijk[b] and Geneviève Massonnet[a]

Spectroscopic techniques, such as infrared and Raman spectroscopy, are very powerful and valuable tools for analysing evidence in forensic sciences. In sexual assault cases, vibrational spectroscopy has been reported as a useful screening method to detect condom residues. However, there has been no study to identify which specific method could be the most suitable technique for the analysis of silicon-based lubricants as forensic evidence. This study looked at the different reported infrared and Raman techniques. The aim was to develop an adequate spectroscopic technique for polydimethylsiloxane (PDMS) analysis and detection. Five FTIR sampling techniques were assessed: ATR, micro-ATR, DRIFTS, transmission and micro-transmission. Raman spectroscopy was assessed with four lasers and included imaging/mapping techniques. The techniques were evaluated on the basis of sensitivity, analysis time, and sample preparation method and time. Although ATR techniques offer good reproducibility, the spectral quality was lower than that obtained with transmission and DRIFTS techniques. Raman spectra were found to be less informative and of lower quality than infrared spectra. Considering case work sample analysis, where material from swabs will need to be extracted, DRIFTS was found to be the best method for trace evidence analysis, although the sample preparation time is longer than for ATR.

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1. Introduction

A number of analytical techniques have been reported for forensic analysis of condom residue trace evidence, focusing on lubricants such as polydimethylsiloxane (PDMS) or polyethylene glycol (PEG).[1] PDMS is the most common target as it is used as a lubricant in more than 85% of the condoms found on the market.[1] A range of analytical instrumentation has been reported in the literature for the analysis of condom lubricants, such as spectroscopic techniques,[2–4] chromatographic techniques (mainly py-GC/MS, GC/MS and LC/MS)[5–7] and mass spectrometric techniques, *i.e.* DART-MS or MALDI-MS.[8–13] Of the analytical techniques, Fourier transform infrared spectroscopy (FTIR) and Raman spectroscopy techniques are the common screening methods that have been used in several research investigations and expert frameworks.[2,3,14] More specifically, in the existing framework, FTIR is recommended as a screening method to help determine which analytical technique would be the most suitable for the identification of specific compounds present in the sample.[4] These two techniques were chosen as screening techniques with potential application to the detection of PDMS evidence in the vaginal matrix. The criteria used for this selection included the information obtained from the spectra for screening purposes as well as the nature, quality and quantity of the analysed evidence. They are, *a priori*, suitable for the desired analyses as previous research showed good results on pure material and on real samples, *i.e.* condom lubricant traces in the vaginal matrix, with no overlap between vaginal secretion spectra and the PDMS spectra being observed.[2–4,14–17] However, though different infrared analysis and Raman analysis techniques have been used, none have been clearly identified as the best method for this type of analysis. Identifying the most suitable method for the analysis of condom residue trace evidence is therefore necessary to ensure that the chemical profiles obtained are exploitable and the method produces reproducible results as well as being sensitive enough to allow detection of trace evidence.

The parameters involved for the acquisition of spectra are different according to the method used for the analysis and can vary widely (Tables 1 and 2). Based on the information provided in Table 1, the various authors did not recommend a particular method that allows the adequate analysis of PDMS-type silicone lubricants by infrared spectroscopy. It is interesting to note that the number of scans accumulated and the spectral resolution used vary significantly between the authors. As these two points influence the reproducibility of the measurements, the presence of artefacts and the variability between the different measurements[15] the results reported in the literature are not

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Table 1 FTIR analysis parameters reported in the literature. NR = not reported

Mode	Resolution	Scans	Range of measurement	Notes	Ref.
Micro-transmission	4 cm^{-1}	100	NR	Use of a 3M IR card	2
DRIFTS	4 cm^{-1}	100	NR		2
ATR	4 cm^{-1}	256	950–3600 cm^{-1}	Imaging used	3
Micro-ATR	NR	256	650–4000 cm^{-1}		4
DRIFTS	NR	128	650–4000 cm^{-1}		4
DRIFTS	2 cm^{-1}	500	NR		16
ATR	4 cm^{-1}	256	600–4000 cm^{-1}	Germanium crystal used	17

Table 2 Raman analysis parameters reported in the literature, NR = not reported

Mode	Laser	Power	Scans	Resolution	Spot size	Ref.
Imaging/mapping	785 nm	10 mW	NR	1 cm^{-1}	1 × 10 μm	3
FT-Raman	1024 nm	NR	128	8 cm^{-1}	50 μm	14
Imaging/mapping	532 nm	NR	NR	NR	NR	18

directly comparable. Hence in order to be able to choose the most suitable method for the analysis of silicone-based condom lubricants, a comparison of all the methods used up to now needed to be carried out.

Raman spectroscopy for silicone-based lubricant evidence analysis was reported in three articles. The analysis parameters are shown in Table 2. Again, no comparisons were reported as to the efficacy of the excitation laser wavelength, spectral resolution and analysis area.

Despite being commonly used for condom residue evidence analysis and regularly applied in criminal investigations, IR and Raman spectroscopy remain little studied in comparative terms. No strategic choice of their use for silicone-based lubricant analysis has been stated. The lack of comparative data can be confusing and generate variable and unreliable analyses in investigations. Hence, the aim of this article is to evaluate the different infrared and Raman techniques for the analysis of silicone-based condom lubricants as forensic evidence, to compare the results and help determine which would be the best technique for trace evidence analysis.

The criteria used for selecting the most suitable method include the information obtained from the spectra for screening purposes, the quality of the spectra, the time taken for the analysis and the nature, quality and quantity of the material to be analysed. It needs to be kept in mind that in case work, extracts or smears from vaginal swabs will need to be assessed. Therefore, the most adequate technique was tested on real samples for validation.

2. Experimental

2.1 Chemicals and samples

Hexane (Sigma-Aldrich), ethanol 99% for GC (Sigma Aldrich), ethyl acetate (Sigma Aldrich), polydimethylsiloxane 100 cSt (Acros Organics), dimethylpolysiloxane 200 cSt (Sigma-Aldrich) and polydimethylsiloxane-hydroxyterminated 750 cSt (Sigma-Aldrich) were used as received. Microscope glass slides from VWR International were used. 36 real sample cotton swabs were obtained from 4 different volunteers who used self-sampling techniques. 12 swabs were vaginal secretions sampled before sexual intercourse (background); 12 swabs were obtained directly after protected sexual intercourse (transfer); 12 swabs were obtained at different post-coital times to evaluate persistence. All experiments were performed in accordance with Helsinki Declaration and the protocol was approved by the local institutional review committee (Ethical Committee of the Canton de Vaud, Switzerland). Study participants were fully informed regarding the purposes of the study and consent was obtained.

2.2 Solutions and extractions

Because of the viscous consistency of all the reference materials (100, 200 and 750 cSt), a solution of each reference material is required for DRIFTS. Moreover, this is also more representative of real case challenges, considering that most often an extraction procedure will be needed to remove the lubricant from swabs in case work. Solutions of PDMS in hexane were prepared at concentrations of 100 mg mL^{-1}, 10 mg mL^{-1} and 1 mg mL^{-1}. All the solutions were vortexed 1 minute before the analyses, to ensure a uniform dilution of the polymers in hexane. All solutions were prepared fresh and stored in a freezer at −18 °C.

Based on research led by Blackledge *et al.* in 1994 and 1995 and Maynard *et al.* in 2001,[2,4,16] real samples of cotton 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 triplicate.

2.3 Instrumental conditions

The following FTIR and Raman instrumentation and accessories were used for the analyses (Tables 3 and 4). FTIR acquisition parameters were fixed at 64 scans, a resolution of 4 cm^{-1} and a gain of 1 for all analyses, as a good compromise between the spectral quality and spectrum acquisition time.

Table 3 FTIR instrumentation and accessories

FTIR	Digilab FT 3000 Excalibur Series
Microscope	Digilab UMA600, objective 16×
Compartment accessories	Transmission: Diamond Compression Cell Kit (Thermo-Spectra-Tech)
	ATR: Golden Gate Single Reflection Diamond ATR System (Specac)
	DRIFTS: Spectra-Tech 0030-05 Collector II Diffuse Reflectance Accessory (Spectra Tech)
Micro-FTIR	Transmission: Micro Single Diamond Plate Cell (Thermo-Spectra-Tech)
	ATR: Slide-On ATR Objective [Germanium crystal] (Thermo Nicolet)

Table 4 Raman instrumentation

Raman	Renishaw RM1000
Microscope	Leica DML
	Objectives 5×, 20×, 20× LWD, 50×, 100×
Lasers	488, 514, 633 and 785 nm

2.4 Methodology

2.4.1 Transmission and micro-transmission FTIR. A blank of the support (diamond cell windows) was determined before spiking one droplet of the bulk material with a Pasteur pipette onto one diamond cell window. Once spectral acquisition of the sample was completed, the support was cleaned with ethanol and a new blank measurement was taken before the next analysis. Solutions were analysed with both methods with 5 minutes of drying time before the analysis so that all the solvent gets evaporated. For each bulk PDMS or solution a series of analyses consisting of 5 replicates analysed the same day using each analysis method were carried out.

2.4.2 ATR and micro-ATR FTIR. The same procedure as for transmission was used (see Section 2.4.1). Once the bulk reference material was deposited on the support (*i.e.* glass microscope slide for micro-ATR and diamond cell window for ATR), the crystal was then brought into contact. The contact pressure could not be standardised, but real-time visualization of the acquired spectrum was used to enable a satisfactory spectrum to be acquired. For each reference standard, a series of analyses made up of 5 replicates analysed the same day using each analysis method were carried out. No solution samples were analysed with these methods.

2.4.3 DRIFTS FTIR. For DRIFTS analyses, KBr was finely ground for about 15 minutes using an electric mechanical grinder. The finely ground KBr was placed in sample cups and dried for 15 minutes at 100 °C. When the bulk material was deposited on the KBr it was observed that it could not adsorb onto it. Hence solution samples of each PDMS were used and individually deposited on the dry KBr filled cups using an eVol® syringe. Four cups filled with just KBr were kept for blank measurements. After spiking with 10 μL of solution, the samples were put in an oven for 15 minutes at 100 °C to ensure solvent evaporation. The samples were left to cool down before analysis. A blank measurement was taken every 3 analyses. For each reference standard solution, a series of analyses made up of 5 replicates analysed the same day were carried out.

2.4.4 Raman. Bulk PDMS was deposited on a microscope slide covered with aluminium foil. Blank measurements were made just next to the droplet. Diluted solutions were also analysed. For these analyses, a drying time of 5 minutes was used before the analysis, so that all the solvent gets evaporated. For each reference standard or solution, a series of analyses made up of 5 replicates analysed the same day using each analysis method were carried out.

2.5 Data processing

2.5.1 Data processing. All infrared spectra were acquired using ResolutionPro v. 4.0 from Agilent, and Raman spectra using Wire 4.3 from Renishaw and then exported in the .spc format. Spectra were imported into RStudio using the hyperSpec package. All the spectra were subjected to a correction of the baseline using the Savitzky–Golay algorithm (spc.fit.poly.below).

2.5.2 Evaluation of the spectra and comparison. The evaluation of the spectra is based at first on the qualitative analysis of the PDMS standards, observing the various peaks found in the 5 replicates of each reference material. This analysis enabled us to determine which technique would be the most appropriate for the analysis of real samples. The techniques were evaluated on the basis of 4 characteristics, namely (1) the quality of the spectra, (2) the reproducibility, (3) the sample preparation time and (4) the analysis time. The best-performing techniques were applied to solution standards to verify their applicability to real samples.

3. Results and discussion

3.1 Preliminary considerations

Reference materials were chosen based on the work of Blackledge who showed that PDMS 100 and 200 cSt were commonly used on condoms.[16] Hydroxyterminated polydimethylsiloxane was chosen as it has been reported that it could be found in nonoxynol-9 containing condoms.[4] The presence of PDMS is evident when the 4 peaks linked to PDMS vibrations are present, *i.e.* Si–O–Si 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} (ref. 2, 4 and 17) (Fig. 1).

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. Therefore, we focused mainly on the zone between 1500 and 500 cm^{-1} for this study.

3.2 Infrared spectroscopy

3.2.1 Transmission and micro-transmission. Visual evaluation of the replicates obtained for compression cell

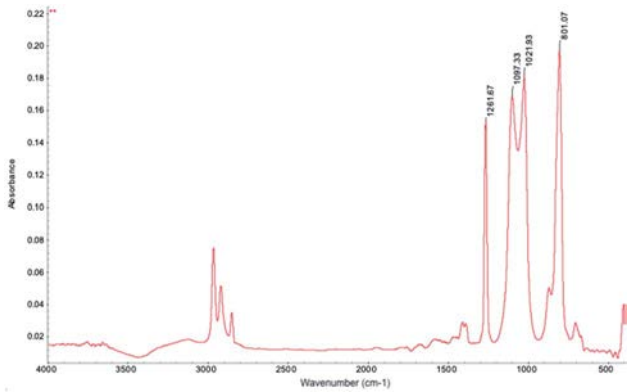

Fig. 1 Reference spectra for PDMS 200 cSt viscosity, acquired with DRIFTS.

transmission spectra showed that the 4 expected peaks for PDMS are present. The quality of the spectra is good, with well-defined peaks, absence of spectral saturation and good resolution for the three PDMS samples. From the point of view of reproducibility, Fig. 2 shows a significant variation visually with absorbance intensities varying between 0.2 and 0.5 for the same sample. This variation is due to the variation in the preparation of the sample for analysis since the amount of sample pipetted on the diamond cell (and its compression) is not necessarily the same between replicates. In terms of preparation time and analysis time, both are very short (less than 5 minutes). It is necessary to clean the diamond cell between each analysis. However, in view of the vertical mounting of the micro-diamond cell (with a beam collimator) and a design which is not designed to contain liquids, the analysis of solution samples from solid–liquid extractions of sample swabs seems compromised. Specifically designed liquid cells could be used but their cleaning presents a significant time penalty.

Micro-transmission was performed only on 4 samples, as saturation of the spectra was observed as the % transmission approached zero (Fig. 3).

Despite the saturation, spectra presented a semblance of profile that had the expected characteristic 4 PDMS peaks. Saturation is a function of the droplet of the bulk material

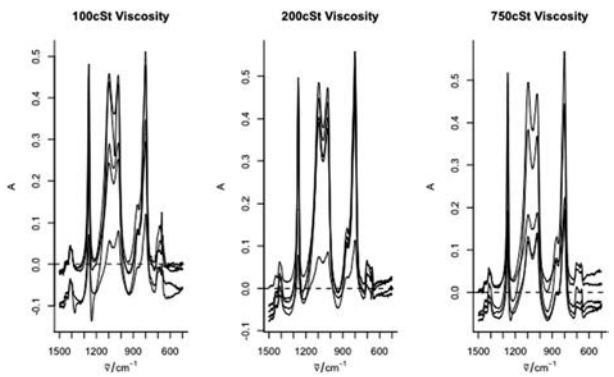

Fig. 2 Illustration of the variability of compression cell transmission spectra, between 500 and 1500 cm^{-1}.

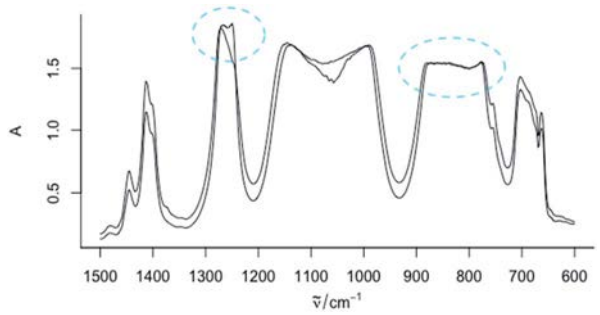

Fig. 3 Illustration of the saturation of micro-transmission spectra, between 500 and 1500 cm^{-1}, for PDMS 200 cSt.

deposited and it was not possible to control/limit the quantity of material deposited with a Pasteur pipette.

The possibility of determining samples of PDMS diluted in hexane and subsequently dried by transmission IR microscopy was also assessed. The effect of the sol–gel transition linked to the dissolution of a polymer in a given solvent determines the possibilities for such an analysis. At the time of deposition of the solution on a microscope slide the solution is homogeneous and the PDMS is uniformly distributed in the solvent. After deposition as the solvent begins to evaporate the capillary forces that come into play generate irregular agglomerates which are randomly distributed on the surface of the slide. At the time of analysis, all the solvent gets evaporated and only the PDMS aggregates remain (Fig. 4).

The aggregates can be difficult to find on the microscopy slide, especially if the concentration is low. Indeed, it is necessary to slowly cover the entire deposition zone using a very high magnification objective, in order to find one of these polymeric aggregates. Spectra were acquired on three aggregates and the profiles were found to be similar to the one obtained for the bulk material. However, the time penalty when using micro-transmission as a screening method for the analysis of condom lubricant residues limits its usefulness. Hexane can also damage the compression cell by dissolving the glue, generating unsealing of the diamond, and therefore a regular use is not recommended.

3.2.2 ATR and micro-ATR. The visual evaluation of the spectra showed the same spectral profile for all the samples (Fig. 5).

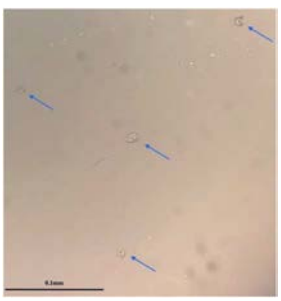

Fig. 4 Illustration of aggregates, transmission mode, Leica DMRX-P microscope, 40× magnification objective, bright field.

The doublet of the Si–O–Si bonds is not as well resolved as in the transmission spectra (see the blue circles in Fig. 3 and blue arrows in Fig. 5), which is the major difference between transmission and ATR spectra. The same observation was made for both germanium and diamond crystals. This observation suggests that there may be an interaction between the PDMS and the crystals, generating a relatively enhanced intensity of the antisymmetric vibration at 1090 cm^{-1} and the CH_2 and CH_3 deformations near 800 cm^{-1}. The changes are too great to be accounted for solely by the change in depth of the IR beam penetration as a function of wavelength.

In terms of repeatability, minimal variations were observed visually, despite the fact that the amount of sample pipetted on the analysis cell was not always the same between the replicates. This is not surprising as with ATR the depth of penetration is only a few microns and not the full thickness of the sample as with transmission. Preparation and analysis times are short (2–5 minutes). Micro-ATR was found to present the same qualitative findings as those for the ATR spectra.

From the reproducibility point of view, ATR results showed only a slight variation of the replicates whereas the micro-ATR showed greater variations according to the type of sample analysed (Fig. 6).

Indeed, the 5 replicates of the PDMS100 sample show a greater variation than those of the PDMS200 and PDMS750 samples. The variation is mainly a baseline offset variation, which is probably due to a droplet height disparity when loading the sample. This illustrates the impact of droplet size and spread on spectrum intensity, but it does not affect the ultimate spectral quality.

Both techniques were applied to PDMS solutions but were found not to be suitable for their analysis. Indeed, no acquired spectra showed the PDMS diagnostic peaks. As previously mentioned for transmission methods, polymeric aggregates are formed when solvent gets evaporated. In the case of ATR methods, it is very likely that the aggregates are randomly found on the crystal and are difficult to localize to target them for the analysis. Moreover, the use of hexane on ATR accessories is not recommended by the suppliers as it may unseal the ATR crystal from its position by dissolving the binding glue.

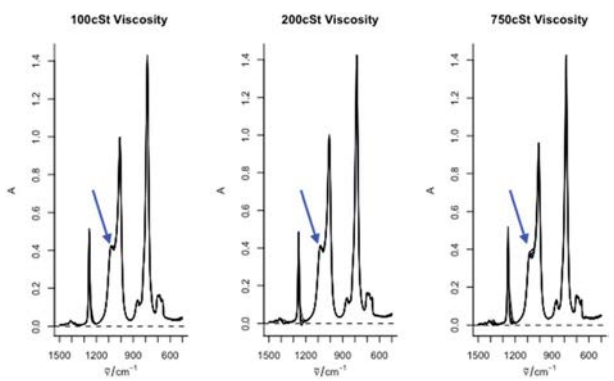

Fig. 5 ATR-FTIR spectra obtained on PDMS with different viscosities.

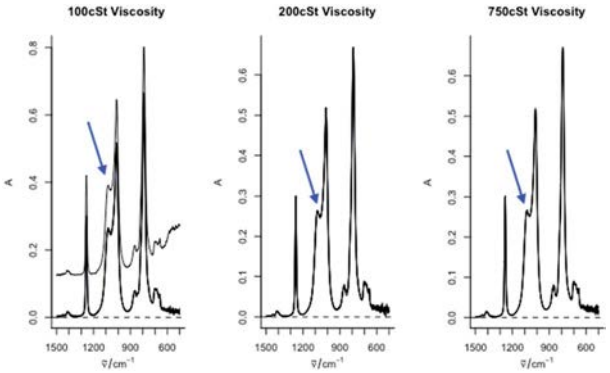

Fig. 6 Micro-ATR-FTIR spectra obtained on PDMS with different viscosities.

3.2.3 DRIFTS. Lesser known than other techniques, DRIFTS is however still widely used when it comes to liquids adsorbed on, or finely dispersed solids in, a KBr powder matrix.[1,16,17] Unsurprisingly no spectrum could be obtained for as-received standards applied to the top of the KBr powder because reflectance from essentially a 100% PDMS surface leads to spectral saturation. Dissolving PDMS in hexane, dispersing it on the KBr matrix in the sample cup and drying off the solvent generate a coated KBr matrix which is ideal for DRIFTS. Dilution of samples over a range of concentrations generated high quality spectra.

Fig. 7 shows the 4 peaks expected with a profile similar to the transmission spectra.

This is as expected as DRIFTS is essentially a transmission technique when the sample is adsorbed on the KBr. The beam penetrates the coating and is reflected from the multitudinous KBr powder surfaces. Slight indications of spectral saturation (noise) are visible for the doublet of the Si–O–Si vibrations for the 2.0 mg mL^{-1} concentration spectrum.

The reproducibility of the analyses was evaluated on three solutions of different concentrations. A significant variation in intensity was observed, *e.g.* for 0.1 mg mL^{-1} the Si–O–Si doublet has an absorbance of ~0.3 whilst the 0.5 mg mL^{-1} sample has an absorbance of ~0.4. This variation can be attributed to the variation in preparation of the sample for analysis: the KBr powder is prepared manually and variations in size

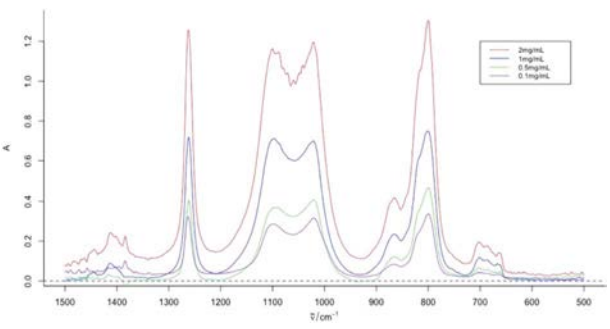

Fig. 7 DRIFTS analysis of diverse concentrations of PDMS 200 cSt dissolved in hexane and dried *in situ* on KBr powder.

distributions are to be expected. Particle size of the analyte, in this case coated KBr, has a significant effect on spectral intensity.[19] Hence the use of a controlled narrow size range KBr is recommended for maximizing reproducibility. More dilute solutions were analysed to assess the limit of detection, which was found to be around 0.025 mg mL^{-1}. In view of the results obtained DRIFTS is clearly a suitable technique for condom lubricant screening in case work.

3.3 Raman spectroscopy

The first spectrum was acquired using a 514 nm laser, to observe the chemical profile. The bands resulting from the symmetric and antisymmetric stretches of the CH_2 and CH_3 groups between 2800 and 3000 cm^{-1} were visible along with two smaller peaks between 400 and 800 cm^{-1} (Fig. 8). These peaks could be attributed to the deformation vibrations of CH_2 and CH_3.

An overview of the Raman spectra obtained shows similar profiles for the different excitation lasers used (Fig. 9).

Each laser used produced different relative scattering ratios between the band groups of interest. The spectra obtained with lasers in the visible region, namely 488 and 514 nm, are dominated by the CH_2 and CH_3 stretching bands (Fig. 9A and B). But these peaks are less relevant than the peaks from the Si–C and O–Si–O vibrations, since the C–H bonds are the most common molecular bonds and also present in semen and vaginal secretion Raman spectra.[20–22] Despite good reproducibility of the spectra acquired with these lasers, bands arising from the Si–O–Si and Si–C stretches are absent.

The spectra acquired with the 633 nm laser highlight better relative intensity between the two groups of peaks (Fig. 9C). The peaks that may be attributable to the Si–O–Si and Si–C deformations (450–750 cm^{-1} region) are better defined, but it is with the 785 nm laser that they have the greatest relative intensity (Fig. 9D). As Si–O–Si vibrations are the main components of the target compound, Raman peaks coming from these bonds are highly significant. However, whilst a peak clearly appears near

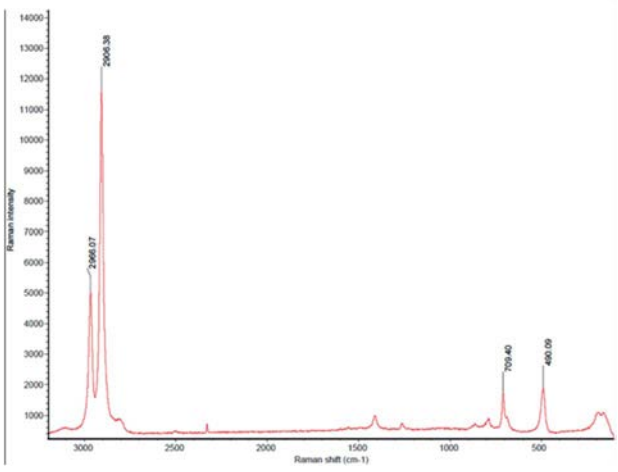

Fig. 8 Illustration of the chemical profile of PDMS obtained using a 514 nm excitation laser.

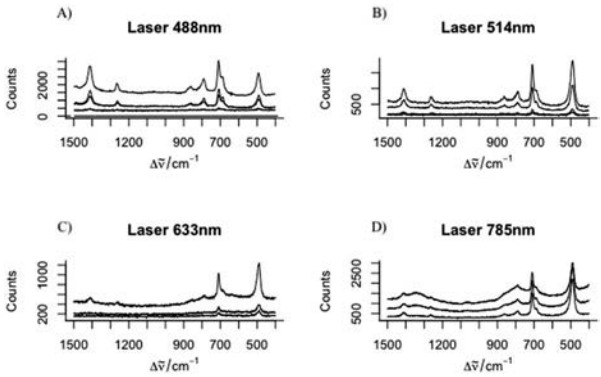

Fig. 9 Raman spectra obtained on 200 cSt PDMS with (A) 488 nm laser, (B) 514 nm laser, (C) 633 nm laser and (D) 785 nm laser.

450 cm^{-1} suggesting the presence of a Si–O–Si bond deformation,[23] the IR major diagnostic Si–O–Si doublet near 1000 cm^{-1} (ref. 23) does not appear to be Raman active (*i.e.* the polarizability change in the vibration is too low whilst the dipole change is significant). Raman spectroscopy is thus a less attractive technique for condom lubricant detection than infrared spectroscopy.

Nonetheless the potential application to dried hexane extracted samples was assessed using a reflectance microscope. Diffraction patterns are visible at the edge and inside of the dried deposited drop (10 μL of the 100 mg mL^{-1} solution, thus leading to an amount of 100 μg of PDMS on the slide), which were not evident in transmission (Fig. 10).

The concentration is large enough for clusters of PDMS to be visible at low magnification. The use of a 100× objective magnification was needed for a specific aggregate to be targeted, and a spectrum was obtained. In case work the sample would be expected to be heterogeneous and the entire deposit would need to be assessed.

Hence, Raman imaging/mapping of pure PDMS droplets with a 514 nm laser and a 20× LWD objective in the domain 200–2000 cm^{-1} and an evaporated solution of 100 mg mL^{-1} PDMS 200 cSt in hexane was also carried out. As shown in Fig. 11A, bright field mapping presents a uniform surface without any aggregation for pure PDMS, as would be expected. The evaporated solution of PDMS clearly showed aggregates as small green points (Fig. 11B).

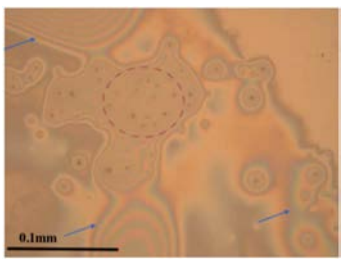

Fig. 10 Analysis using reflection microscopy, bright field, 10× magnification objective, dried solution 100 mg mL^{-1}. The blue arrows indicate diffraction fringes, and the red circle indicates what could be PDMS aggregates.

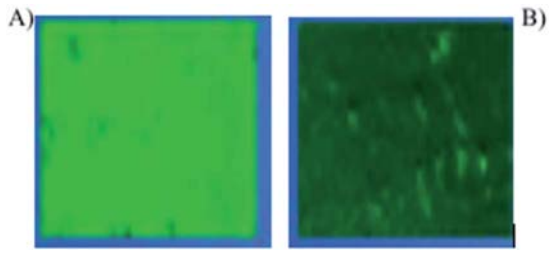

Fig. 11 Bright field mapping results of (A) pure PDMS and (B) evaporated solution of PDMS, concentration 100 mg mL^{-1}.

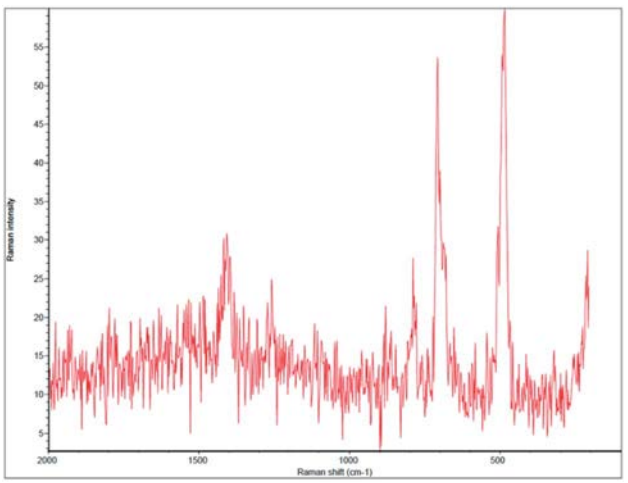

Fig. 12 Raman spectrum of identified PDMS aggregates, with a 100× magnification objective.

The PDMS aggregates highlighted with the visual mapping procedure were targeted for a more specific analysis, using the 20× LWD objective. However, no results were obtained from these aggregates under these conditions. Trials were made with 50× and 100× magnification objectives. Although both allowed us to locate where PDMS aggregates could be found using a 100× magnification objective, on a specific area known to contain aggregates of PDMS. Small diagnostic peaks from PDMS were observed but the spectrum was very noisy (Fig. 12).

The Raman technique seems, despite the imaging/mapping possibilities, tedious and possibly difficult to set up quickly and easily for any operator and did not generate acceptable spectra for forensic purposes. No fluorescence was observed in any of the Raman spectra acquired.

3.4 Comparisons

Comparison of the results obtained on neat and diluted PDMS by the infrared and Raman techniques that we have explored for the analysis of condom lubricants as forensic evidence demonstrated clear differences between the performances of each technique. These differences have their origin in part in the physical and chemical properties of the polymer in solutions but also in the fundamentals of the different spectroscopic phenomena.

Table 5 summarizes the observations of the 4 parameters considered important for the analysis methods presented as well as the potential application and relevance to case work.

The processes of infrared transmission, ATR and DRIFTS are fundamentally different and give rise to the different spectral profiles obtained for each of these techniques. In all the cases, a response was observed in the form of four vibrational peaks, at around 800, 1020, 1090 and 1263 cm^{-1}, with the peaks at 1020 and 1090 cm^{-1} being the most important ones as they originate from the Si–O–Si bonds in the target molecule. However, this doublet was not always well resolved in ATR spectra, whereas transmission and DRIFTS techniques offered a fully resolved doublet in their spectra. The micro-transmission spectra showed some saturation for the raw material as the detector response below 3% transmission and at lower frequencies was unsatisfactory.

No evidence of the Si–O–Si doublet was found in any of the Raman spectra, thus indicating that it was not an adequate technique for this type of analysis.

All the studied methods presented a short analysis time and an easy preparation, except for DRIFTS, as there is the need to prepare KBr powder, preferably of a narrow size range. However,

Table 5 Observation for all the spectroscopic methods used in this study

Method	Spectral quality (bulk material)	Reproducibility	Analysis time [min]	Preparation	Casework application
Transmission	Good Si–O–Si doublet well resolved	Medium	~1	Easy	Impossible on diluted samples
Micro-transmission	Spectral saturation Si–O–Si doublet well resolved	Good	~2–3	Easy	Impossible on diluted samples
ATR	Si–O–Si doublet not well resolved	Excellent	~2–3	Easy	Impossible on diluted samples
Micro-ATR	Si–O–Si doublet not well resolved	Good	~1	Easy	Impossible on diluted samples
DRIFTS	Good Si–O–Si doublet well resolved Not applicable to bulk material	Medium	~2–3	Needs narrow size range KBr	Good spectra obtained on diluted samples
Raman	Si–O–Si doublet not present	Medium	~1	Easy	Possible but difficult

this was found to be an acceptable balance between time and results when considering the quality of the spectra and the possibilities of application to real samples. It was also found that it was not too time consuming to prepare a batch of KBr filled sample cups and store them at a temperature of 100 °C before using them for analysis, with the sample preparation time being that required for spiking samples on the KBr.

Clearly, in order to choose the most adequate technique for the analysis of the sample of interest, it is mandatory to ensure good spectral quality not only on raw material but also on diluted/extracted samples, as would occur in case work. Although most of the presented techniques allow the detection of PDMS, significant differences were highlighted through the use of diluted samples. For example, all the techniques using microscopy were found to be unsuitable for real samples analysis as, due to the required solvent evaporation, it was challenging to find where PDMS aggregates could be found. The same issue was encountered with transmission and ATR techniques, where it has not been possible to target PDMS adequately after deposition on the crystals.

DRIFTS analysis can clearly detect PDMS when in solution, with a very low limit of detection. Our work supports the earliest use of condom evidence analysis in the 90s, when DRIFTS was the infrared technique used and applied in casework, although there was no real comparison with any other infrared techniques.[2] Even though the current technology offers a whole set of more "sophisticated" methods such as Raman spectroscopy, micro-ATR or micro-transmission, DRIFTS seems to remain the most efficient method to use as a screening method for the detection of condom residues in vaginal swabs collected from real casework.

3.5 Application to real samples

DRIFTS analysis was selected as the most effective spectroscopic technique for the analysis of condom lubricant residues in real cases, based on the study of the reference material. However, to ensure proper evaluation, real samples were analysed. Pre-coital swabs were used to determine if there were any residues of the vaginal matrix within the target zone where PDMS presents its diagnostic peaks. These peaks from silicone-based lubricants were found to be well resolved from vaginal secretion spectra as shown in Fig. 13. As a set they would be unambiguously indicative of PDMS in vaginal matrices.

The PDMS lubricant was found to transfer quite readily during sexual intercourse and recovery using the aforementioned extraction procedure was possible. Typical spectra obtained from real samples are presented in Fig. 14.

No interaction with the matrix was noted, and the spectra presented clear and well-defined Si–O–Si and Si–C stretching bands, thus confirming the efficacy of DRIFTS analysis for case work. Persistence was shortly evaluated on a small sample set, and the results obtained using DRIFTS analysis for four different time intervals, 6 h, 12 h, 18 h and 24 h post coitus, are presented in Fig. 15. As illustrated in Fig. 15, no significant impact of the matrix was found on the residues collected at 6 hours post intercourse. Importantly a decrease of the PDMS peaks was noted in the residues collected at 12 and 18 hours after intercourse, revealing a matrix effect, with peaks coming from the matrix being almost as intense as the ones coming from PDMS. In the residues collected at 24 hours after intercourse, PDMS peaks were not clearly evident, with only a small 800 cm^{-1} peak being present, and the spectrum is clearly dominated by the vaginal matrix residues.

DRIFTS analysis can clearly detect PDMS in the presence of vaginal residues. Persistence up to 18 hours post coitus was noted, whilst Tottey *et al.* (2019) found that PDMS traces could be found up to 24 h post coitus when using py-GC/MS analysis.[5] As DRIFTS is rapid and less expensive it would be of great interest to integrate this analytical method more regularly in the sequence of analyses suggested in the literature, with the use of infrared spectroscopy as a screening method.[4] Indeed, the chemical profile obtained using FTIR results will help define

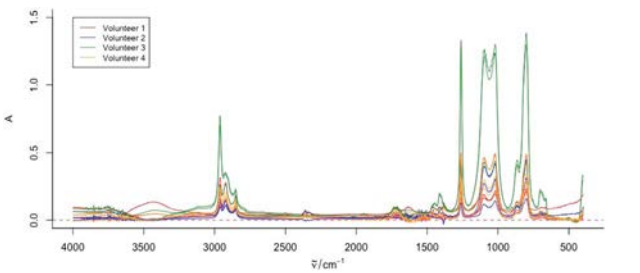

Fig. 14 Infrared spectra of post-coital transferred residues from 4 different volunteers.

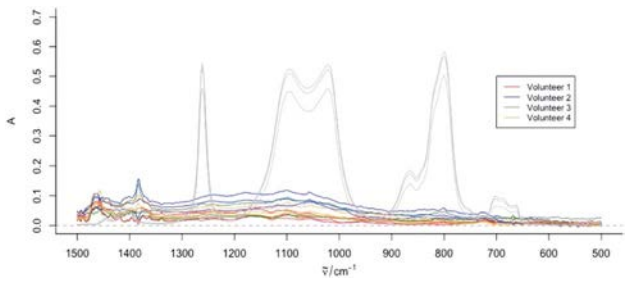

Fig. 13 Infrared spectra of vaginal secretions from 4 different volunteers and an overlay of PDMS reference spectra (in grey), between 1500 and 500 cm^{-1} to enhance readability.

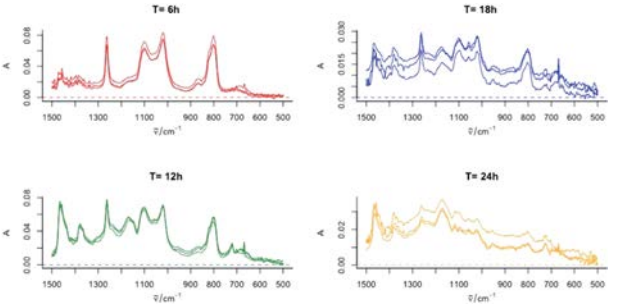

Fig. 15 Infrared spectra of post-coital transferred residues at four different time intervals.

which technique would be the most suitable for the confirmation of the presence of any condom component.[4]

4. Conclusions

PDMS is the most commonly used lubricant for condoms. It has been targeted in several studies as an indicator of the presence of condom use. In view of current cases, it is becoming increasingly important to broaden the knowledge concerning the modalities of rapid analysis of this compound in a living matrix. This requires the various analytical techniques to be compared and the potential advantages and disadvantages of each of them to be evaluated.

In comparing the spectroscopic potential of Raman and five FTIR spectroscopic sampling techniques for the analysis of PDMS, DRIFTS analysis was found to provide significantly better spectral results, especially when targeting real samples. DRIFTS clearly offered the four diagnostic peaks of PDMS and a very good limit of detection, around 250 ng, when applied to liquid extracted samples, used as simulation of real samples. Application to real samples was also successful. The other FTIR techniques were found to be successful only on bulk material and did not enable liquid extracted samples to be analysed. As for Raman spectra, it turned out that their quality and reproducibility depended on the laser used, but as they did not show the most characteristic (Si–O–Si) peaks of PDMS it is unlikely to be suitable for casework applications.

Ethics approval

Collection of human data was approved by the Swiss Ethics Committee (Study 2018-00690).

Conflicts of interest

There are no conflicts to declare.

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A forensic international market survey of condom lubricants and personal hygiene products using ATR-FTIR coupled to chemometrics

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ABSTRACT

Condom residues may be encountered in forensic investigations as traces in sexual assault or rape cases. Casework studies have shown the value of distinguishing condom residues from other types of personal products used by women. However, up to now, there has been no investigation of their chemical variability within an international context. This work employed attenuated total reflectance Fourier transform infrared spectroscopy with chemometrics to provide objective characterisation of condom lubricants and personal hygiene products from the international market. 166 samples were obtained covering five major classes of products likely to be used by women. Principal component analysis distinguished most major classes based on their spectral profiles, with subsequent support vector machine models yielding discrimination accuracies over 90%. A two-step approach was subsequently developed and enabled both classification and a discrimination accuracy of 100%. This could provide greater confidence in chemical discrimination of residues from these products when conducting investigations and help assess the origin of the chemical profile obtained. Further testing using three validation sets produced an identification accuracy of 100% for generic classes, which may allow investigative leads to be more readily obtained from recovered evidence.

1. Introduction

Studies have reported the use of condoms in sexual assaults and rape cases, leading to forensic trace evidence potentially left in the vaginal matrix, on the penis of the perpetrator or in the victim's underwear [1-8]. The recovery and characterisation of these traces may provide evidence of association which can corroborate, or contradict, the allegations of the parties involved.

When condom evidence is investigated, either because the victim has indicated that a condom was used or does not remember the exact circumstances of the offence, the detection of lubricant trace evidence is of primary interest. Any resulting evidence will then be evaluated to determine if its source was indeed a condom. This question is of great importance in casework, as reported in the literature [2,4,9,10] and described hereafter.

In the United States, attempts have been made to answer this question based mainly on analytical observations [2,5,10]. In these cases, positive (i.e. presence of silicone) [2,5,10] or negative (i.e. absence of silicone) [2] profiles were obtained, and in one case a comparison between a trace and a reference condom performed [5]. When the profile was positive, it was concluded that a condom was present, while a negative profile was taken to indicate the absence of the use of a condom [2]. Similarly, in the *Regina v. Andrew Nicholas Malkinson* case (2006) in the United Kingdom,[10,11] PDMS traces were detected on the victim's underwear. The victim's statement was that she never used personal products. Therefore, the court stated that if the victim's statement was correct, it was more likely that the traces originated from a condom [10,11].

However, indistinguishable profiles between the trace and the source does not mean that the reference sample is the source of the trace evidence. At the time of the publication of these cases, i.e. between 1994 and 2006, there was no published model that identified the error rates

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related to these analyses, nor that allowed interpretation of a positive nor a negative result. False positives generated by hygiene products or alterations due to textiles have not been investigated, and these cases could have benefited from more research regarding the discrimination of condom chemical profiles using similar instrumental analysis, i.e. Fourier transform infrared spectroscopy.

The aforementioned casework in addition to earlier studies reported the use of vibrational spectroscopy as a non-destructive means to characterize a trace. Several such studies have used infrared spectroscopy as a screening method to examine condom lubricants [2,9,12-15] and classify them as a function of their viscosity [13], while Raman spectroscopy has also been used for trace evidence detection [3,16]. As these methods are recommended for identifying potential condom residues in casework [2,9], it is of prime importance to define the discriminating capabilities of the technique to face issues of sample differentiation or source identification. One common way to evaluate discriminatory performance is to conduct a statistical exploratory study on a large population set. Although a recent study found that DRIFTS-FTIR [15] showed greater potential for characterising lubricants, ATR-FTIR was chosen for this study as it readily available in most forensic laboratories and requires minimal sample preparation.

This preliminary study made use of bulk samples (i.e. personal hygiene products, condom lubricants and intimate lubricants) found on the Australian, New Zealand and Swiss markets, in order to first evaluate any structure in the market and assess the ability of ATR-FTIR to distinguish bulk lubricants based on their chemical profile. The spectra obtained were qualitatively analysed based on visual observation before being subjected to chemometrics. As highlighted in both the National Academy of Sciences (NAS) [17,18] and more recently in the PCAST [19] report, visual interpretation of forensic evidence can suffer from operator bias or subjectivity. The use of chemometrics can minimise this bias as it uses mathematical and statistical methods to explore differences in a given dataset. Chemometrics is also known to be useful for pattern recognition and may help to reveal trends not clearly visible otherwise. This is useful in observing the structure in the data and understanding the structure of the market.

Assertions on the content of the samples present on the market have never been verified and the discrimination of similar chemical profiles have been verified only on a small set of samples. Previous papers [9,12,20-22] offered an overview of data acquired on a small set of samples, rarely going over 100 samples. The present paper offers conclusions drawn on one of the biggest known datasets regarding condom and personal hygiene products. The significant amount of data and their variable provenance allows more accurate conclusions to be drawn, as a benefit of a more thorough representation of the market. This paper offers an international perspective, which may help the understanding of the worldwide market.

2. Material and methods

2.1. Samples

100 condoms representing 8 brands present on the New Zealand market were purchased from major distributors and manufacturers. 20 condoms representing 6 brands present on the Swiss market were purchased from Swiss supermarkets and pharmacies; and 6 condoms and representing 4 brands present on the Australian market were purchased from Western Australian supermarkets and pharmacies, for a total of 127 condoms. 6 creams, 5 personal hygiene products, 1 massage oil and 26 lubricants that may be used by women on a daily basis, representing 9 brands from the Australian and international market, were purchased from pharmacies. The samples obtained were considered representative of the market share of the major condom and personal hygiene brands and sub-brands available to consumers. In total, 166 samples were analysed. The entire listing of the samples is available in Appendix A.

Reference material was used for the identification of the main expected compounds: PDMS 200cSt and and Polyethylene Glycol (PEG) 400 were purchased from Sigma-Aldrich, Glycerin from Univar, Ajax Chemicals and used as received.

2.2. Instrumental conditions and data acquisition

Infrared spectra were collected using a Nicolet iS50 FTIR spectrometer equipped with single-bounce diamond crystal ATR accessory (Australian samples), Smart Orbit attachment (New Zealand samples) or Golden Gate Single Reflection Diamond ATR system (Swiss samples). Data collection was carried out using the OMNIC software. Spectra were collected over the 4000 to 400 cm^{-1} range with 4 cm^{-1} resolution and 32 co-added scans. ATR correction was performed on all spectra to account for variations in penetration depth based upon wavelength.

Condoms were rubbed directly on the ATR crystal and analysed with no further preparation. All other products were applied as thin films to cover the ATR crystal and analysed with no further preparation. The sampling window was thoroughly cleaned using ethanol and lint-free tissue before each sample, and a background scan of the clean crystal obtained between each replicate acquisition. For each sample, 5 replicates were acquired, to be able to statistically consider the sample variation. In a single sample, if there was variation amongst the 5 replicates and more than one chemical profile could be observed, an additional 5 replicates were run to ensure adequate representation of this variability. A total of 830 analyses were used for the present study.

2.3. Data analysis

2.3.1. Qualitative analysis

Qualitative observations were carried out using OMNIC software and samples were visually classified into different groups according to their chemical profiles. Data pre-processing and chemometric analysis were performed using the Unscrambler® X 10.5 software (Camo Software AS, Oslo, Norway). Spectra were truncated to omit the 2340–1880 cm^{-1} region due to interference from the diamond crystal. Then range normalisation was applied to remove variation related to the amount of sample deposited and therefore the sample layer thickness on the crystal. Before performing the complete exploratory study, it was important to know whether consistent results were obtained when analysing the same types of condoms. Consistency was evaluated using the replicates and visually assessing that peak position, peak shape and abundance were similar within a same condom (same brand, model, lot number). Tests, using discriminant analysis, were performed to evaluate if different lots of the same brand presented different chemical profiles.

2.3.2. Chemometrics

Using the Unscrambler X (v. 10.5), principal component analysis (PCA) was carried out using mean-centered spectra and non-linear iterative partial least square (NIPALS) algorithm, with 1000 iterations. Samples were plotted using up to the first three principal components (PCs) to visualise the distribution within the samples and identify any clustering. 12 different pre-processing methods (available in Appendix B) were applied and the resulting PCA plots compared to see which combination allowed the best visual discrimination of the samples.

2.3.3. Discrimination model creation and comparison

Given that visual observation of principal component clustering can still suffer from operator's subjectivity, supervised classification was used to provide more objective discrimination. Models were constructed using the first three PCs, treating each replicate of each sample as a separate sample, as they were analysed as if they came from different samples. Discrete classes were attributed to each replicate based on the observations of the chemical profile and the knowledge of the samples (i.e. sample type, sample content, brand, model). Five classification algorithms; linear discriminant analysis (LDA), quadratic discriminant analysis (QDA), and support vector machine (SVM) with linear,

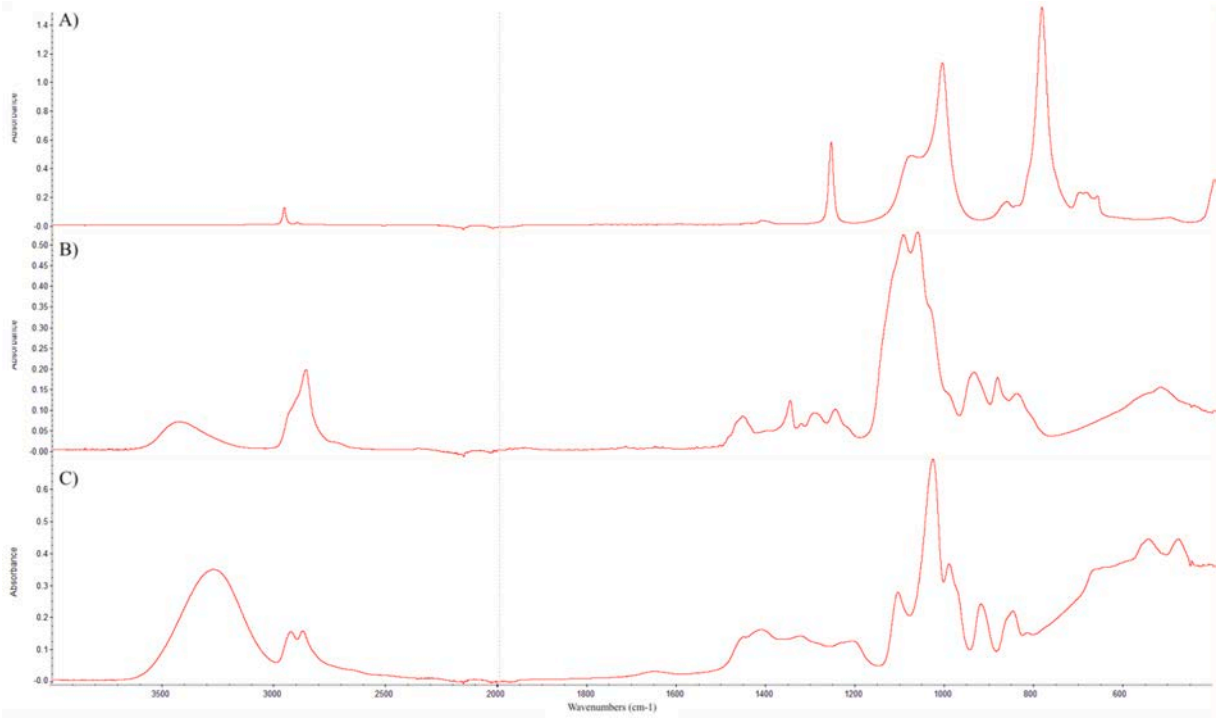

Fig. 1. Reference FTIR chemical profiles used to visually discriminate spectra from the overall dataset, a) silicone-based, b) PEG-based c) Glycerine-based.

polynomial (3rd degree) and radial basis function algorithms, were used and compared. Confusion matrices showing the actual and predicted classes were obtained, and model performances were calculated for each discrimination model created. The predicted classes using the test set were compared to the actual class, to evaluate the accuracy of the model using performance analysis parameters, such as classification error rate, false positive and false negative rate.

2.3.4. Discrimination model validation

Performance on an external sample set is crucial to rigorous validation, as using the same data to build and test a model can result in overly optimistic discrimination and classification accuracies. Therefore, the different models were evaluated using an external sample set and the performances were calculated. The models were then tested on three external sample batches involving different analysts and different instruments: a known-matching samples set (i.e. a batch of samples already represented in the model); a known non-matching samples set (i.e. a batch of samples not yet represented in the model); and a blind validation consisting of samples unknown to the analyst. Regarding the blind validation procedure, and a second operator acquired new data, from selected samples taken in the dataset, and the results were interpreted by another operator. Such an approach reinforces the quality of the model as experimental factors may significantly affect the observation, due to instrumental and operator variation. Therefore, the higher the correct classification, the stronger the model is for further applications. Samples were subjected to the same data pre-processing as the samples used to build the model and were then projected and classified using each model.

3. Results and discussion

3.1. Preliminary considerations

Suitable replicates are essential in a forensic context. In the case of this study, replicates not only allow consideration of sample or instrumentation variability, but also operator variation such as in depositing samples on the ATR crystal. It was observed during the analysis that, when analysing non-condom samples, if the droplet on the crystal was too thick then minimal spectral variation was seen between samples. Water-based chemical profiles would be observed, and the profile would change with water evaporating. Therefore, the deposition strategy to ensure proper qualitative observation of a full and adequate chemical profile was to deposit the sample on a gloved finger and then rub it on the crystal to ensure that the sample was applied as a thin film.

As previously reported in the literature, [9,12,23] at least 3 major classes, i.e. silicone-based, water-based, or oil-based (vegetable or mineral oils), are most likely to be encountered on the market. Within the water-based class, the most common lubricants are PEG and glycerine. Reference materials were run to observe the differences between the different lubricants. Chemical profiles from PDMS, PEG and glycerine are presented in Fig. 1. PEG and glycerine were distinguished based on the peak number and position, as well as the OH peak shape. For the oil content, comparison with ATR spectra found in the literature and NIST webbook online database (https://webbook.nist.gov/chemistry) was used.

Any sub-groupings within these main categories could be due either to variation in the deposition and analysis approach or to variation in the sample composition. The use of replicates, in this case, is needed to ensure that any observed characteristics are consistent in all the profiles and hence differences are attributable to compositional changes rather than variation in the analysis.

These experiments aimed to study if samples coming from condoms and other sources could be distinguished, as well as to evaluate the potential discrimination of different types of condoms.

Chemical profiles of 10 Durex Extra Safe condoms were not found to present distinguishable chemical profiles, and the observation of the PCA scores plot (Appendix C) did not highlight differences within the samples presenting different lot number. These observations give confidence that there is no observable difference between production lots.

3.2. Chemical qualitative classification

Qualitative spectral identification was carried out to determine whether different chemical profiles were observed in the overall data

Table 1
Categories of chemical profiles obtained from qualitative analysis of the dataset, along with the number of samples in each group or subgroup, their characteristic infrared absorption frequencies and corresponding components identified through comparison with reference materials.

Group	Number of samples	Sample no	Infrared Absorption Frequencies (cm^{-1})	Components
1	129		790, 1012, 1090, 1260, 2960	PDMS
1a	124	1–13; 15–18; 20–21; 23–56; 58–69; 71–100; 109–111; 113–123; 125–133, 169,171, 178–181	790, 1012, 1090, 1260, 2960	
1b	3	14, 19, 22	790, 865, 1012, 1090, 1260, 2960	
1c	1	174	700, 790, 1050, 1260, 2960	
1d	1	156	790, 1012, 1090, 1260, 2960	
2	15		850, 921, 992, 1029, 1108, 1410, 2879, 2936, 3280	Glycerine
2a	9	57, 70, 108, 175, 182, 165, 172,176, 177	799, 839, 922, 992, 1036, 1108, 1261, 2879, 2936, 3310	
2b	5	135, 137, 140, 141, 142	922, 992, 1040, 1112, 1414,1641, 2888, 2944, 3320	
2c	1	163	994, 1043, 1112, 1639, 2848, 2916, 3345	
3	2	138, 158, 159, 173	722, 1098, 1160, 1237, 1464, 1743, 2853, 2922, 3008, 3408	Oils
4	1	124	519, 840, 885, 935, 1063, 1095, 1248, 1294, 1348, 1459, 2865, 3430	PEG
5	3	112, 168, 170	802, 837, 921, 990, 1040, 1078, 1136, 1640, 2881, 2935, 2975, 3330	Unknown water-based component
6	13	160, 161, 162, 164, 157, 154, 134, 136, 166, 167, 155, 132, 139	1636, 2853,2924, 3350	Water-dominated spectra

and if it was possible to visually classify them. Spectra were overlaid in Omnic software, and evaluation was based on the presence of peaks as well as their number, position and shape. Systematic comparison with reference materials was performed to ensure correct identification of the composition.

Five main categories representing different chemical profiles were obtained based on the chemical composition. The first four were silicone-based, oil-based, glycerine-based, and PEG-based as expected. Category 5 is made of an unknown water-based component presenting a different chemical profile than glycerine and PEG. An additional sixth category gave a chemical profile of products dominated by water, which were hence unable to be reliably assigned to any of the specific water-based sub-groups. Spectra were then examined within each category, and sub-groups within the 2 major groups (silicone-based and glycerine-based), were identified (Table 1). Subgroups were defined based on peak presence or absence, peak position and peak shape. Table 1 lists the number of samples in each category, and the IR bands characteristic of each chemical profile.

Silicone-based samples (Group 1 in Table 1, Fig. 1a) were the main category observed with the presence of characteristic peaks associated with PDMS corresponding to symmetric and asymmetric Si-O stretching at 1020 and 1090 cm^{-1}, a Si-C stretching at 1263 cm^{-1} and a C–H dimethyl and trimethyl deformation around 800 cm^{-1} [15]. Group 1 samples only presented these four peaks, thus suggesting that either PDMS is not mixed with any other component when applied to a condom or in a lubricant, or PDMS is predominant in the spectrum and other components are present in too low a relative amount to be detected. Sub-clusters 1a to 1d were created based on the observation of variations within the chemical profiles (see Appendix D). Category 1a was the most commonly observed profile and was matching the reference spectrum of PDMS, without any noticeable differences. Other sub-clusters were distinguished by the presence of additional peaks to the classic silicone pattern (1b), a single peak instead of a doublet for the silicone double bond (1c), or an inverted relationship between symmetric and asymmetric Si-O vibrations (1d). Differences observed in group 1b may be explained by the presence of other components:GC–MS analysis of the samples constituting group 1b revealed the presence of benzocaine and traces of PEG [24]. Regarding the differences within group 1c and 1d, a different viscosity of the PDMS are likely to generate such variations. This was confirmed by leading a pyrolysis-GC/MS analysis [data not shown].

Oil-based samples (Group 3) did not present any typical vibration of O–H or silicone bonds, whereas all other sample types presented a strong O–H peak between 3700 and 3000 cm^{-1}. This peak was found in all samples containing PEG (Group 4), glycerine (Group 2) or more generally water-based products (Groups 5–6). However, differences between the O–H peak shape as well as additional peaks allowed these samples to be separated into different categories (Appendix D). The presence of additional peaks as indicated in Table 1, for example peaks at 800, 1261, or 1640 cm^{-1} in the glycerine group, allowed the creation of sub-categories within this group. These peaks are potentially due to other water-based additives.

A more careful observation of the different groups highlighted that water-based type samples (Groups 2, 4, 5 and 6) did not present any strong vibrations linked to silicone-based products. Likewise, silicone-based samples did not present any peaks specific from glycerine- or PEG-based lubricants. These observations suggest that condom lubricants appear to be either silicone-based or water-based, but not a mixture of the two. This is surprising considering that some of the silicone-lubricated condoms are flavoured or known to contain additives dedicated to long-lasting pleasure or that some samples, such as Ceylor Gold samples contain both silicone and water-based compounds [24]. In addition, Maynard *et al* in 2001 [9] reported that some silicone-lubricated condoms or lubricants were also containing nonoxynol or glycerine. Such differences might be explained by the difference of the spectroscopic instrumentation used for the study as well as a modification of the market within the last 20 years.

3.3. Statistical discrimination of the samples

12 pre-processing methods were considered in this study, based on previous studies reporting the importance of pre-treatment on the discrimination of samples [25-29]. PCA was carried out for each set of pre-processing, but none were found to improve sample discrimination compared to the initial corrected dataset (baseline correction and range normalisation).

PCA was performed on the entire dataset without further pre-processing to visualise any clusters. The scores plot obtained using the first three PCs (accounting for 93% of the variation) is shown in Fig. 2. Silicone-based samples were separated from the water-based samples along PC1 and the oil-based samples along PC2. This clustering pattern reinforces the main categories identified during the qualitative examination of the spectra. Water-based samples were found to offer a strong variation along PC1 and PC2, revealing at least 2 sub-clusters. The use of PC3 showed that water-based samples were clustered together, but PEG

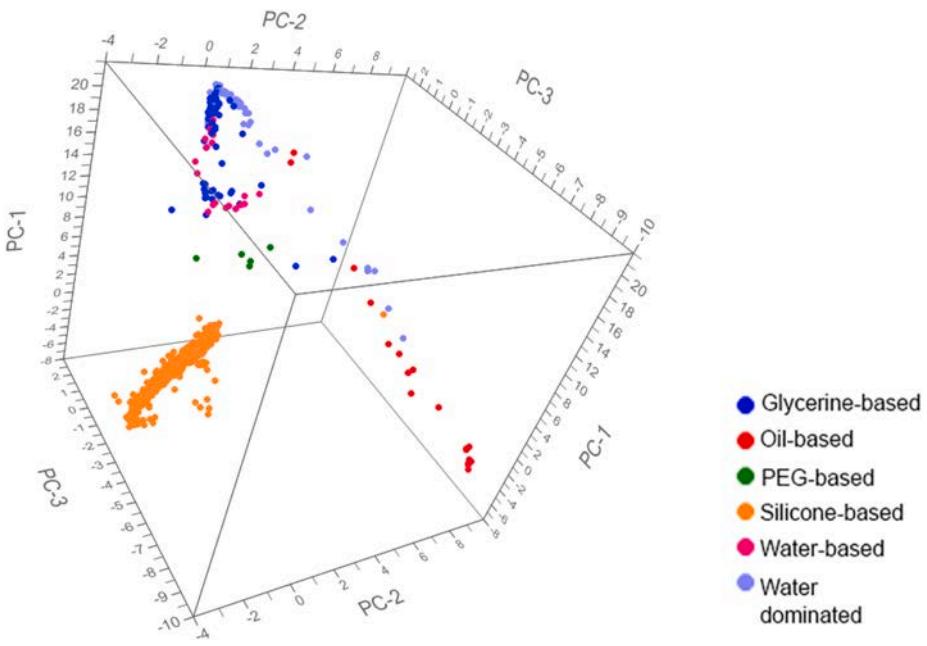

Fig. 2. 3-dimensional PCA score plot showing the distribution of the dataset according to the detected content obtained with ATR-FTIR spectra. Silicone-based samples (Group 1) are represented by the orange dots, glycerine-based (Group 2) samples in blue, Oil-based (Group 3) in red, PEG-based (Group 4) in green, water-based (Group 5) in pink and Water-dominated spectra (group 6) are in light violet. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

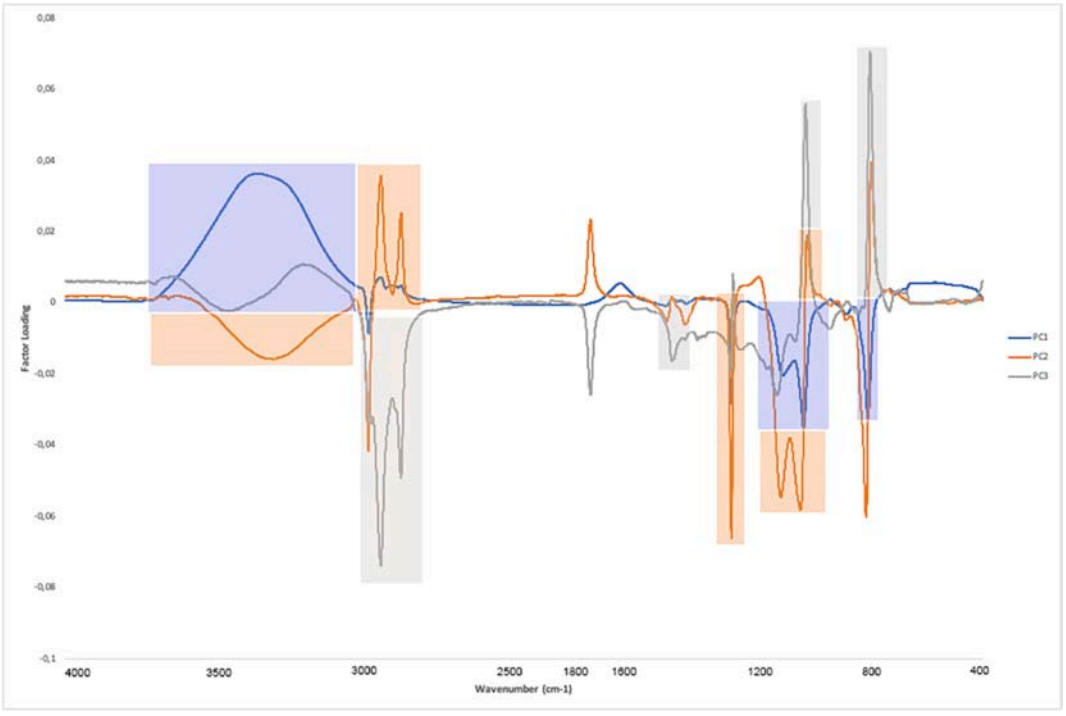

Fig. 3. Factor loadings of PCs 1–3 for PCA conducted on the entire dataset, based on their ATR-FTIR spectra.

containing samples were separated from the rest of the water-based samples.

Interestingly, visually distinguished samples were clustered in the same groups along these PC1 and PC2, and samples that were visually indistinguishable based on their spectra were found to be visually clustered in different groups in the scores plot. Additionally, different sub-groups could be observed compared to the visual examination. The minor peaks enabling visual differentiation between specific groupings were likely dismissed by PCA as having low significance and thus incorporated into later PCs that weren't examined. These observations demonstrate the importance of visually examining the data, and not just relying on chemometric interpretation.

The factor loadings for these PCs (Fig. 3) were used to identify the spectral regions, and thus specific chemical components, contributing to sample discrimination. PC1 was found to be negatively correlated with peaks at ~ 790, 1020, 1090, 1260 and 2963 cm^{-1}, which are characteristic of the silicone backbone from PDMS [9,12]. A highly positively correlated peak at ~ 3000–3700 cm^{-1} was also observed, consistent with the O–H stretching bond attributed to water and water-based components [9,12]. Consequently, the discrimination between classes across PC1 is due to the presence or absence of silicones and water containing components. The samples containing silicone bonds attain

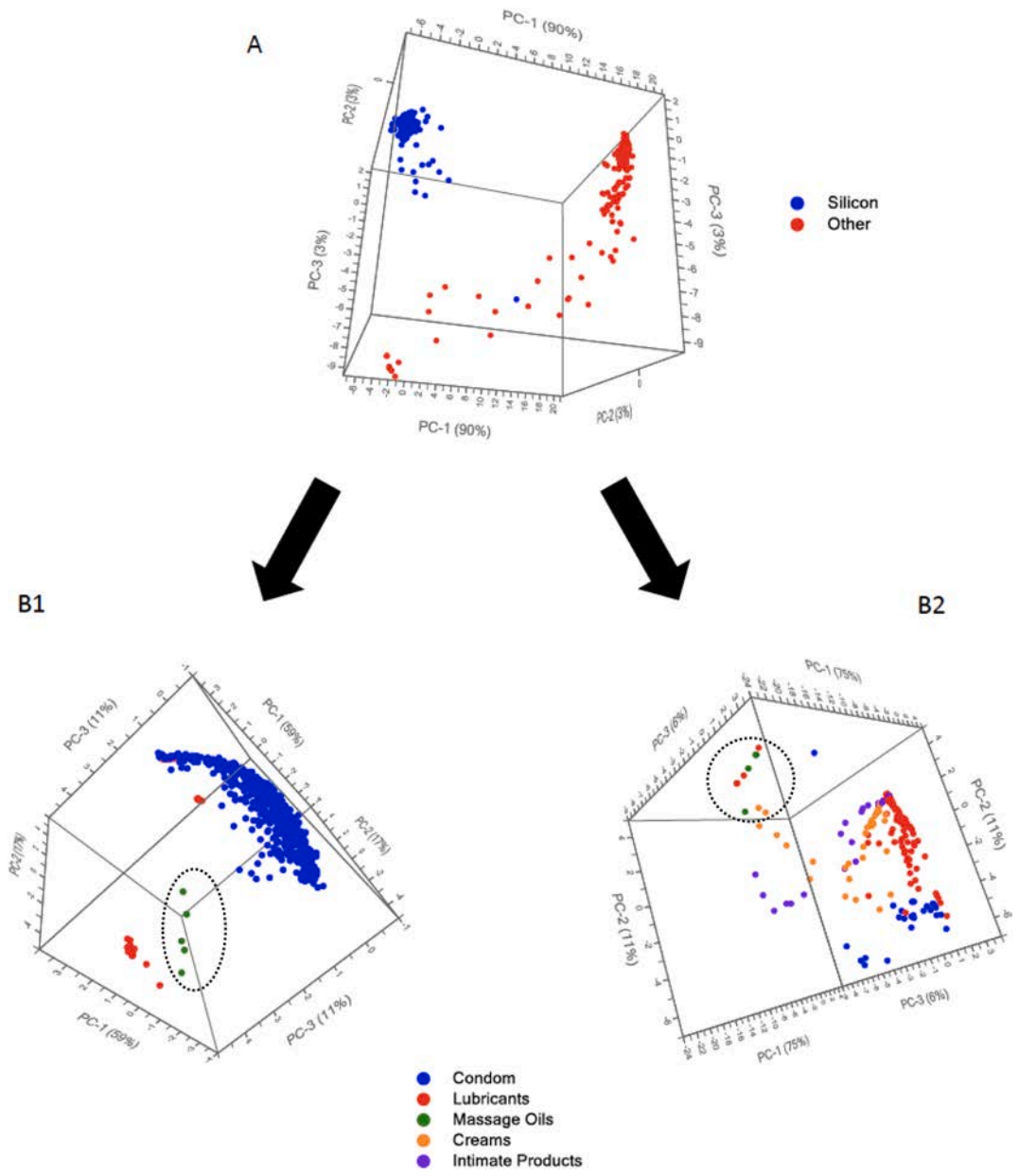

Fig. 4. 3-dimensional PCA score plot showing the distribution of the samples A) according to the chemical profile (i.e. silicone vs other), B1) within the silicone-containing cluster, B2) within the water-based containing cluster. Dotted circle highlight massage oils clusters in both populations.

large negative values on PC1 while the remaining samples attain significant positive score on PC1. These observations match the clustering that was observed in Fig. 2.

The same correlations appeared along PC2 (Fig. 3), suggesting that variations were detected within the silicone content of the different samples. Several other peaks were found to contribute to separation along PC2. A peak at 1000 cm^{-1} was linked to glycerine, while those at 3700–3000 cm^{-1} (O–H stretching), 2920 cm^{-1} (CH_2 asymmetric stretching), and 2850 cm^{-1} (CH_2 symmetric stretching) could be attributed to PEG, glycerine and other water-based components. This explains why water-based containing samples are all within the same interval along PC2 in Fig. 2. The peak at 1740 cm^{-1} (C = O stretching) could not be attributed to any of the major compounds or other water-based components, suggesting it could be coming from an oily compound in some of the oil-based samples, as this vibration is usually associated with a carboxylic acid. This explains the strong variability of the water-based and oily samples. Finally, along PC3, peaks at 1254 cm^{-1} (CH_2 twisting) and around 1360 and 1460 cm^{-1} attributed to CH_2 wagging and CH_2 scissoring, allowed the discrimination of the PEG and glycerine containing samples on Fig. 2.

PCA scores plots showed that excessive leverage on the overall model was obtained due to silicone or non-silicone content (Fig. 4A), potentially reducing discrimination between the rest of the samples. Performing PCA separately on each of the two main categories was found to give additional information on the separation of the samples.

The silicone-based cluster was found to incorporate three classes of samples, including condoms, lubricants and massage oils. This observation highlights that silicones can be used in various other products that might be found in the vaginal matrix. Despite the improved separation of the silicone-containing samples, some overlap was observed between lubricants and condoms with a non-differentiated silicone chemical profile (Fig. 4B1). The present observations confirm previous assessments on the separation of condom lubricant types [9,13,23], as most of the condoms used in this study were found to share the same qualitative profile, i.e. silicone-based. Although lubricants were found to mainly contain water-based compounds, 18% (5 of 27 samples) of the

lubricant batch (*Astroglide Waterproof Silicone Liquid, Ansell Skyn Maximum performance, Ansell Lifestyles Luxe Silicone Based Lubricant, Astroglide Diamond Silicone Gel Personal Lubricant, Durex Play Perfect Glide*) presented a silicone-based profile that was clustered with the condom profiles. This was expected as these products specifically indicated the presence of silicone in their composition.

In the context of *R. v. Andrew Nicholas Malkinson* [8,10], this suggests that a silicone-based profile could originate from products other than condoms. It was also observed that 3.9% of the whole condom batch (5 of 126 samples) presented a significantly different chemical profile that clustered with water-based samples (Fig. 4B2). These condoms (*Ansell-Lifestyles Party Variety Warm/Cool, Ansell- Lifestyles Party Mix Warm Smooth, Manix OrgazMax Plus, Manix Endurance* and *Ceylor Gold*) represented 'warming' condoms from a single supplier (Manix being manufactured by Ansell) and potentially contained nonoxynol-9 (a spermicide) or other water-based additives, explaining their similarity to other water-based products. The other lubricants clustering with those special condom types were not warming lubricants.

Massage oils were found to present two different chemical profiles (Fig. 4B1 and Fig. 4B2), clearly separated from the other samples. It was interesting to note that a cluster was observed with other water-based lubricants (dotted circle on Fig. 4B1, and 4B2). It appeared that the lubricant in question was named as a Personal Lubricant and Massage Oil. It can be therefore assessed that massage oils, named as such, present a separate and specific profile, which can be separated from other types of samples.

Four of the six cream samples reported silicone in their composition but were not found to cluster close to silicone-based condoms. This may be explained by the complete composition being a mixture of glycerine and silicone-based components, with a predominance of glycerine, water, wax or paraffin wax leading to a specific profile. The two samples reporting no silicone in their composition were found to cluster very closely to the main lubricants class. This observation was expected, as the discrimination of the samples generated in the PCA was mainly dictated by the silicone composition.

Personal hygiene products, classified in Fig. 4 as intimate products, were generally grouped and close to cream and lubricant products. An exception was observed for one of the samples (*FemFresh Feminine deodorant spray*) which was found to be silicone-based. The initial composition of the product stated dimethicone as the major component of the product, dimethicone being another denomination for PDMS. Such differentiation was therefore not surprising. The dispersion on the water-based side of the clusters was due to only one sample (*FemFresh Daily Intimate Wash,*), whose profile was found to present a high variation within the replicates. Such variation can be explained by the variation of water evaporation after the deposition of a thin layer of a water-based product.

3.4. Structure of the international market

Studying the structure of the market aims to see if the clusters observed can be associated with particular characteristics of the samples. This is useful to generate investigative leads from a questioned sample by predicting what its origin might be. The main interest was to understand if it was possible to distinguish samples from different brands or models based on their infrared chemical profile.

The analysis revealed that no major differences were observed between the different markets in terms of the country of purchase. This was not surprising as the country of purchase does not necessarily correspond to a specific manufacturer.

To highlight potential variation between different brands, a smaller dataset containing only silicone-based condoms was used, as they were the biggest sample group in this study. Separate analysis did not highlight any evident clusters or separation of silicone-based condoms from the different brands. This suggests that different brands use the same type of chemicals for any given type of samples. These assumptions are in agreement with earlier research [13], which concluded that most lubricated condoms present in the market used the same type of lubricant, with chemical properties being too similar to allow visual or statistical differentiation.

Within-brand analysis and lot number production variation were investigated using samples from *Durex*, the most common brand found on the international market [9,12,13,22,30,31]. No evident clusters were formed from analysing the spectra from different condom models within this brand, nor were any clusters highlighted regarding different lot numbers from the same brand and same model condom type (Appendix D). It thus appears that individual brands use the same lubricant composition across a range of products, and that there do not appear to be substantial variations in this composition across production lots.

3.5. Discrimination model and classification procedure

Five different discrimination models (LDA, QDA, and SVM with three different algorithms: linear, 3rd degree polynomic and radial basis function) were constructed based on the sample class. SVM analysis with a radial basis function kernel (RBF), γ(kernel parameter) set at 0.01 and C (soft margin parameter) set at 0.001 was found to present the highest discrimination (calibration) accuracy (92.3%) and predictive (validation) accuracy (92.05%), as well as the best classification regarding sample content (Appendix E). The model was constructed using the training set containing 2/3 of the dataset, and then the validation set (1/3 of the dataset) was predicted to provide a more realistic estimate of model performance. Only 3 samples out of 101 were misclassified; 2 false negatives (6% rate) and 1 false positive (13.3% rate).

Regarding false positives and false negatives, a false positive indicates that a profile from a personal hygiene product has been associated with a condom profile. In contrast, a false negative indicates that a profile from a condom has been associated with a profile from another intimate product. The interpretation of these two results in a real case is important. Observations of the classification quality showed that samples are easily classified according to their chemical profile, but that the identification of the membership of a sample class is difficult, has a high error rate and is certainly related to the initial classification by chemical profile. This suggests that classification should be undertaken in a two-step approach to limit errors:

1. Identify the sample caegory according to the chemical profile of silicone or non-silicone type (Fig. 4A). This step aims to determine whether silicones are present in the sample.
2. Identify the class of origin separately within each population, i.e. within the silicone profile population or the other profile population (Fig. 4B1, Fig. 4B2).

Discriminant models were built using a two-step approach to classify the samples, with SVM again found to give the most accurate classification. This discriminant model gave 100% accuracies for both the calibration and validation sets. The model was then used to predict external test sets, including both samples present in the model and samples not yet represented in the model, analysed using different instruments and analysts. Finally, a blind validation test was carried out to validate and evaluate the robustness of the developed approach. From a forensic point of view, blind validations are mandatory before considering any application to casework, as it is representative of the simulation of a real scenario, where the source and the nature of the evidence are unknown. Also, it can minimise potential confirmation bias from a classic validation procedure.

In the present study, 100% correct classification of all external test samples was obtained using the two-step model. It was possible to classify samples according to their chemical content and their class. This is of great value for further application to casework as shown in [32], more specifically for investigative purposes, as it means that it is possible to infer the source of recovered forensic evidence. However, in

respect to casework application, further work is required to validate these findings using trace evidence samples, as characterisation may be affected by the presence of a biological matrix or other factors as discussed in previous studies [23,33].

4. Conclusion

This study investigated the distribution of condoms, lubricants, massage oils, creams and personal hygiene products from an international perspective through the analysis of 166 samples purchased between 2018 and 2019 from three different international markets.

At least 6 different chemical profiles could be visually and qualitatively differentiated. However, different clustering patterns were observed using unsupervised statistical analysis, highlighting this as a complementary method to qualitative examination. It is thus important to still visually examine the chemical profile of the sample rather than relying solely on statistical analysis. Condoms, lubricants, creams and other intimate products could be distinguished based on their qualitative profiles, although some condoms and lubricants were found to be atypical for their category. Nevertheless, the results indicate that condoms and other personal products can present distinct chemical profiles. As expected, no differentiation was observed according to the international market, brand, specific product or production lot.

ATR-FTIR spectroscopy with SVM was found to be a valuable tool for the discrimination and classification of samples according to their chemical profiles. The presented model allowed discrimination of samples according to whether they contained silicone or not, and whether it was a condom or another type of sample. It was observed that over 96% of the condom samples contained silicones as lubricants, but silicones were also detected in some personal hygiene products. As stated in the introduction, a key forensic question in cases of rape or sexual assault is whether an observed profile comes from a condom or another source. The observations in the present paper highlighted that it is possible that a silicone-containing trace did not originate from a condom. These findings have significance in the context of cases such as the *R. v. Andrew Nicholas Malkinson* case (2006) [8,10], where the ability to distinguish condom lubricants from other personal products could affect the interpretation of evidence within the context of the case. Interpretation of the absence of evidence should be handled with great care, and the present paper does not allow a position on this precise point. False positives due to silicone-containing personal products may appear in casework samples. Victim interviews are necessary to interpret the evidence and complementary analysis, such as py-GC–MS, might be necessary to confirm the results of the FTIR analysis.

Initial discrimination and classification based on a single model presented a high level of false positive errors (up to 15%). Therefore, a two-step approach is recommended for the classification of infrared spectra; the first step is to identify if the sample contains a silicone-based profile or not, and the second step is to further classify the sample in the suitable model based on the initial profile. This methodology was successfully applied to predict the classes of known match samples, known non-match samples and blind validation samples representing unknown casework samples. ATR-FTIR spectroscopy is hence a powerful screening method for the investigation of condom or lubricant in forensic casework. Further research should investigate the classification of real traces, to see whether they classify the same way pure lubricant samples do.

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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Appendix A:. List of the samples used in the study

Abbr. Sign: NZ = New Zealand, AUS = Australia, CH = Switzerland

Sample	Brand	Product	Lot	Purchased
1	Durex	Extra Safe	1,000,026,318	NZ
2	Durex	Extra Safe	1,000,010,957	NZ
3	Durex	Extra Safe	1,000,106,077	NZ
4	Durex	Extra Safe	1,000,049,207	NZ
5	Durex	Extra Safe	1,000,046,754	NZ
6	Durex	Extra Safe	1,000,041,595	NZ
7	Durex	Extra Safe	1,000,049,636	NZ
8	Durex	Extra Safe	1,000,041,595	NZ
9	Durex	Extra Safe	21,306,074	NZ
10	Durex	Classic	1,000,063,396	NZ
11	Durex	Unknown	1,000,010,958	NZ
12	Durex	Pleasure Me	10,939,364	NZ
13	Shield	XL	PN29803	NZ
14	Durex	Mutual Climax	1,000,026,346	NZ
15	Durex	Unknown	1,000,015,117	NZ
16	Durex	Unknown	1,000,011,869	NZ
17	Durex	Unknown	1,000,012,759	NZ
18	Durex	Classic	10,121,993	NZ
19	Durex	Mutual Climax	1,000,039,272	NZ
20	Durex	Intimate Feel	1,000,044,392	NZ
21	Durex	Intimate Feel	10,837,252	NZ

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Sample	Brand	Product	Lot	Purchased
22	Durex	Mutual Climax	1,000,039,272	NZ
23	Durex	Confidence	1,000,059,628	NZ
24	Durex	Banana	14F2456B	NZ
25	Durex	Pleasure Me	1,000,039,189	NZ
26	Durex	Pleasure Me	1,000,042,518	NZ
27	Durex	Confidence	1,000,045,624	NZ
28	Durex	Unknown	22B09482	NZ
29	Durex	Unknown	1,000,049,211	NZ
30	Durex	Unknown	1,000,064,796	NZ
31	Durex	Classic	10,135,039	NZ
32	Durex	Strawberry	14F1293S	NZ
33	Durex	Apple	14F1293A	NZ
34	Ansell	Contempo-Rough Rider	1,011,081,616	NZ
35	Durex	Strawberry	14F2456S	NZ
36	Durex	Orange	14F2456O	NZ
37	Durex	Classic	21,306,193	NZ
38	Durex	Thin Feel	10,847,195	NZ
39	Durex	Orange	14F1293O	NZ
40	Durex	Confidence	1,000,179,693	NZ
41	Gold Knight	Chocolate	PC29801	NZ
42	Marquis	Flavoured	PG1202	NZ
43	Durex	Extra Safe	1,000,136,970	NZ
44	Marquis	Regular	1,005,096	NZ
45	Gold Knight	Strawberry	PS2980	NZ
46	Durex	Extra Safe	1,000,174,930	NZ
47	Ansell	SKYN-Original	1,705,403,516	NZ
48	Ansell	Lifestyles- Ultra Thin	1,504,111,016	NZ
49	Ansell	Lifestyles- Ultra Thin	1,611,060,216	NZ
50	Ansell	Lifestyles- Regular	1,705,591,616	NZ
51	Ansell	SKYN Original	1,705,743,116	NZ
52	Ansell	Lifestyles- Regular	1,701,081,516	NZ
53	Ansell	Lifestyles- Zero	AK017A04	NZ
54	Ansell	Lifestyles-Party Variety- Snake Skin Textured	1,512,032,416	NZ
55	Ansell	Lifestyles – Party Variety - O'Max	1,603,780,316	NZ
56	Ansell	Lifestyles – Party Variety -Tutti Frutti	1,607,192,816	NZ
57	Ansell	Lifestyles – Party Variety -Warm/Cool	1,605,512,216	NZ
58	Ansell	Lifestyles – Party Variety – Glow in the Dark	AGP610A	NZ
59	Ansell	SKYN-Elite	1,702,093,216	NZ
60	Ansell	Lifestyles – Assorted – Banana Bump Studded	1,701,142,016	NZ
61	Ansell	Lifestyles – Assorted –Sonic Strawberry Ribbed	1,606,011,716	NZ
62	Ansell	Lifestyles – Assorted – Berry Blast Smooth	1,601,151,216	NZ
63	Ansell	Lifestyles – Assorted – Vanilla Thriller Smooth	1,512,101,016	NZ
64	Ansell	Lifestyles – Assorted – Choc Ripple Ribbed	1,610,022,416	NZ
65	Ansell	Lifestyles – Assorted – Mintensity Studded	1,512,050,316	NZ
66	Ansell	Lifestyles – Assorted – Sonic Berry Ribbed	1,702,111,116	NZ
67	Ansell	Lifestyles – Assorted – Banana Bump Studded	1,612,032,416	NZ
68	Ansell	Lifestyles – Party Mix – Choc Ripple Ribbed	1,611,602,316	NZ
69	Ansell	Lifestyles – Party Mix- Dynamint Studded	1,703,090,316	NZ
70	Ansell	Lifestyles – Party Mix – Warm Smooth	1,612,122,916	NZ
71	Ansell	Lifestyles – Party Mix – Tutti Frutti Smooth	1,608,452,116	NZ
72	Ansell	SKYN- Intense Feel	1,612,813,316	NZ
73	Ansell	SKYN-Extra Lubricated	14707PI16	NZ
74	Hero	Natural	7365	NZ
75	Hero	Ultra Thin	7367	NZ
76	GLYDE	Maxi	PN32503	NZ
77	GLYDE	Slimfit	PN31564	NZ
78	Durex	Intense Stimulating	1,000,214,762	NZ
79	Durex	Performa	1,000,170,528	NZ
80	Durex	Together	1,000,174,855	NZ
81	Durex	Love	1,000,033,421	NZ
82	Durex	Close Fit	1,000,065,555	NZ
83	Durex	Real Feel	1,000,243,647	NZ
84	Four Seasons	Naked – Pink Strawberry	X27150902	NZ
85	Four Seasons	Naked Bubble gum	X31150901	NZ
86	Four Seasons	Naked- Chocolate	X26150903	NZ
87	Four Seasons	Naked – Banana Yellow	X33150902	NZ
88	LELO HEX	Original	X35160502	NZ
89	Four Seasons	Extra Strength	X31141101	NZ
90	Four Seasons	Glow	16 N3253	NZ
91	Sir Richard's	Ultra-Thin	13 N3983	NZ
92	GLYDE	Vanilla	PV27711	NZ
93	GLYDE	Strawberry	PS21131	NZ
94	GLYDE	Wild Berry	PW25701	NZ
95	GLYDE	SuperMAX	PN26161	NZ
96	GLYDE	Blueberry	BB23801	NZ
97	GLYDE	Cola	BL28311	NZ

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Sample	Brand	Product	Lot	Purchased
98	GLYDE	Ultra	PN34381	NZ
99	GLYDE	Maxi	PN32503	NZ
100	GLYDE	SlimFit	PN31564	NZ
108	Wet Stuff	Gold Water based personal lubricant	9D729	AUS
109	Ansell	LifeStyles Luxe Silicone-based lubricant	19051507DR	AUS
110	Ansell	Manix Contact	1,606,380,216	CH
111	Ansell	Manix Natural	1,601,012,016	CH
112	Ansell	Manix Orgazmax Plus	1,507,200,316	CH
113	Ansell	Manix Endurance	1,409,151,416	CH
114	Ansell	Manix Fraise Gourmande	1,408,211,216	CH
115	Ansell	Manix Xtra Pleasure	1,411,082,316	CH
116	FairSquared	Sensitive dry	0953BA14228	CH
117	FairSquared	Original	0796IF14180	CH
118	FairSquared	Max Perform	1491PF13371	CH
119	Migros	M-Budget	IUR-010	CH
120	Migros	Cosano Regular	OUR-097	CH
121	Migros	Cosano Sensual	OUR-176	CH
122	Migros	Cosano Feeling 0.05 mm	OUR-149	CH
123	Coop	Prix Garantie	1594ZF16451	CH
124	Ceylor	Gold	160831P	CH
125	Ceylor	Thin Sensation	1626B2S	CH
126	Ceylor	Non-Latex UltraThin	660801U	CH
127	Ceylor	Strawberry	153332P	CH
129	Durex	Silver	1,000,176,796	CH
130	Durex	Strawberry	1,000,271,385	CH
131	GLYDE	Silver Lubricated Condom 53 mm	PN34261	AUS
132	GLYDE	Premier Water Based Lubricant	(B) 51,711	AUS
133	Ansell	SKYN Maximum Performance Lubricant	19051103JP	AUS
134	Durex	Play Massage 2 in 1	447X1	AUS
135	Four Seasons	Lush Lubricant with Aloe Vera	9B722	AUS
136	Four Seasons	Nature Lubricant Vegan Friendly	9A716	AUS
137	Durex	KY Jelly	488X2	AUS
138	Four Seasons	Massage Oil	BG668	AUS
139	Durex	Play Feel, Pleasure Gel	213X4	AUS
140	Ansell	LifeStyles Silky Smooth Water Based Lubricant	19,042,006	AUS
141	Durex	Naturals Intimate Gel	919Y3	AUS
142	Astroglide	Naturally Derived Liquide	A011874	AUS
154	Multi-Gyn	Active Gel	1,033,164	AUS
155	Summer's eve	Feminine Wash Sensitive Skin	0217H0161	AUS
156	Femfresh	Feminine deodorant spray	(B)1079351	AUS
157	Femfresh	Daily Intimate Wash	101,702,163	AUS
158	Vagisil	Soothing Oatmeal Cream	Z19D126	AUS
159	Canesten	Intimate Discomfort Cool Cream Gel	GPO1KPL	AUS
160	Vagisil	Intimate Wash Fresh Plus	B91653	AUS
161	Dermeze	Moisturising Cream	B15866	AUS
162	Essentials	Barrier Cream	1,081,235	AUS
163	Rosken	Intensive Moisture Hand Cream	B14519	AUS
164	Love My Ink	Tattoo Cream	30,789	AUS
165	Sasmar	Classic Personal Lubricant	160271C	AUS
166	Sensuous	Smooth & Warming Lubricant	8,433,821	AUS
167	Sensuous	Frenzy Extreme Pleasure Gel for Women	870	AUS
168	Ansell	Skyn Intimate Moments	19031301DR	AUS
169	Ansell	Skyn Intense Feel Non-Latex Condoms	1,905,013,316	AUS
170	Pjur Med	Vegan Glide Intimate Personal Lubricant	32965–01	AUS
171	Astroglide	Diamond Silicone Gel Personal Lubricant	A011819	AUS
172	Astroglide	Gel Personal Lubricant	A011395	AUS
173	Astroglide	Personal Lubricant and Massage Oil	A010316	AUS
174	Astroglide	Waterproof Silicone Liquid	A011415	AUS
175	Astroglide	Warming Liquid Personal Lubricant	A011326	AUS
176	Astroglide	Water based Personal lubricant	A011494	AUS
177	Astroglide	Strawberry Liquid Personal Lubricant	A011452	AUS
178	Four Seasons	Naked Black Condom	Z07180301	AUS
179	Four Seasons	Stubbed & ribbed Stimulating condoms	18 N0363	AUS
180	Checkmate	ExtraSensitive Lubricated Condoms	1,809,122,816	AUS
181	Durex	Play Perfect Glide	541X1	AUS
182	Durex	Climax Stimulating Gel	2231 W7	AUS
183	Durex	Comfort XL	1,000,417,937	AUS
184	Ansell	Lifestyles ribbed condoms	18,009,812,116	AUS

Appendix B: Preprocessing methods and effect on the percentage of explained variance

Table B1. preprocessing methods applied on the dataset to enhance the discrimination of the samples. Legend: PC = principal component; SNV = standard normal variation; MSC = Multiple Scattering Correction; Der = derivative.

	Percentage of explained variance			
Pre-processing	**PC1**	**PC2**	**PC3**	**Total**
Raw data	90	3	3	96
SNV	84	6	5	95
MSC	91	8	0	99
Der1	63	19	5	87
Der2	46	17	12	75
SNV + Der1	61	21	6	88
Der1 + SNV	59	13	8	80
SNV + Der2	45	19	10	74
Der2 + SNV	53	13	6	72
MSC + Der1	95	2	1	98
Der1 + MSC	82	14	2	98
MSC + Der2	78	10	3	91
Der2 + MSC	95	4	0	99

Appendix C: Investigation of brands, models and lot number discrimination (Figs. C1–C3).

Appendix D: Visual discrimination of infrared spectra and subgroups division (Fig. D1).

Appendix E: Comparison of the discrimination parameters obtained for the five discrimination algorithms tested.

Table E1. Comparison of the performance of the 5 simple-class classification methods, after cross validation on the raw dataset. Aims is to distinguish silicone and non-silicone-based samples. Results are presented for the validation using samples present in the model. Realised on the whole dataset. SVM a linear function, SVM2 uses a second-degree polynomial one and SVM3 uses the radial basis function to compute the data.

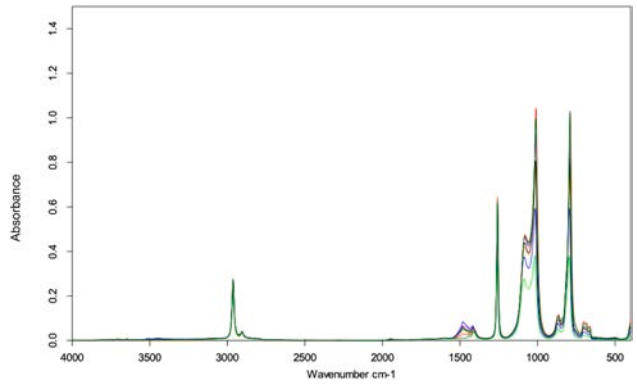

Fig. C1. Illustration of the variation within different models of condom from Durex brand.

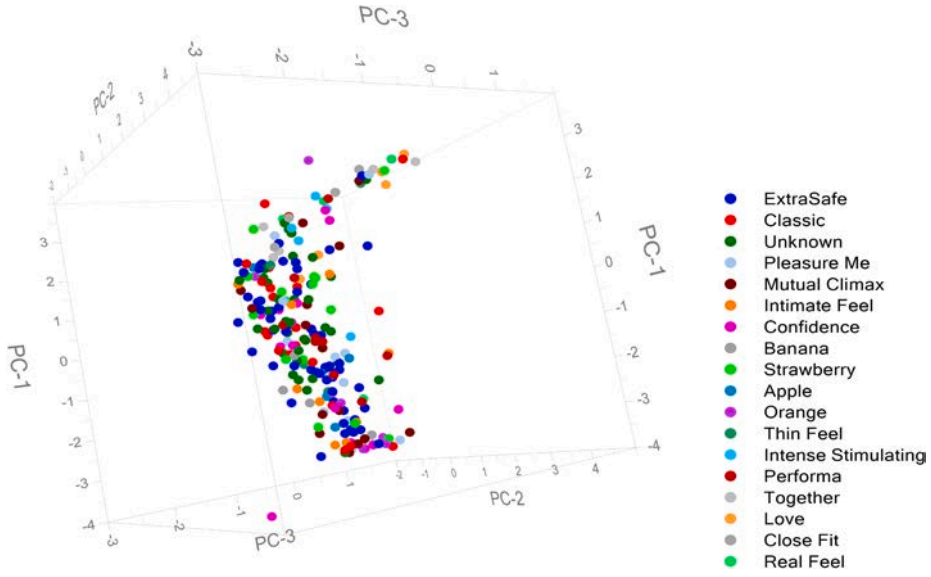

Fig. C2. 3-dimensional score's plot obtained on condom samples from Durex brand, classified according to the different models.

Measure		LDA	QDA	SVM	SVM2	SVM3
Total samples	n	831	831	831	831	831
True Pos.	a	604	605	629	605	604
False Positive	b	25	24	0	24	25
False Negative	c	30	24	7	50	39
True Negative	d	172	178	195	152	163
Correct Classification Rate	(a + d)/n	0,934	0,942	0,992	0,911	0,923
Misclassification Rate	(b + c)/n	0,066	0,058	0,008	0,089	0,077
Sensibility	a/(a + c)	0,953	0,962	0,989	0,924	0,939
Specificity	d/(b + d)	0,873	0,881	1,000	0,864	0,867
False Negative Rate	c/(a + c)	0,047	0,038	0,011	0,076	0,061
False Positive Rate	b/(b + d)	0,127	0,119	0,000	0,136	0,133
Positive predictive Power	a/(a + b)	0,960	0,962	1,000	0,962	0,960
Negative predictive Power	d/(c + d)	0,851	0,881	0,965	0,752	0,807
General diagnostic Power	(b + d)/n	0,237	0,243	0,235	0,212	0,226
Training accuracy		93,38	94,22	99,16	91,1	92,3
Validation accuracy		–	–	98,55	91,09	92,05

Table E2. Comparison of the performance of the 5 simple-class classification methods, after cross validation on the raw dataset. Aims is to distinguish condom from other samples Results are presented for the validation using samples present in the model. Realised on the whole dataset. SVM a linear function, SVM2 uses a second-degree polynomial one and SVM3 uses the radial basis function to compute the data.

Measure		LDA	QDA	SVM1	SVM2	SVM3
Total samples	n	831	831	831	831	831
True Pos.	a	633	633	634	633	633
False Positive	b	1	1	0	1	1
False Negative	c	1	1	1	18	1
True Negative	d	196	196	196	179	196
Correct Classification Rate	(a + d)/n	0,998	0,998	0,999	0,977	0,998
Misclassification Rate	(b + c)/n	0,002	0,002	0,001	0,023	0,002
Sensibility	a/(a + c)	0,998	0,998	0,998	0,972	0,998
Specificity	d/(b + d)	0,995	0,995	1,000	0,994	0,995
False Negative Rate	c/(a + c)	0,002	0,002	0,002	0,028	0,002
False Positive Rate	b/(b + d)	0,005	0,005	0,000	0,006	0,005
Positive predictive Power	a/(a + b)	0,998	0,998	1,000	0,998	0,998
Negative predictive Power	d/(c + d)	0,995	0,995	0,995	0,909	0,995
General diagnostic Power	(b + d)/n	0,237	0,237	0,236	0,217	0,237
Training accuracy		99.76	99.76	99,8	97.71	99.76
Validation accuracy		–	–	99.75	97.71	99.75

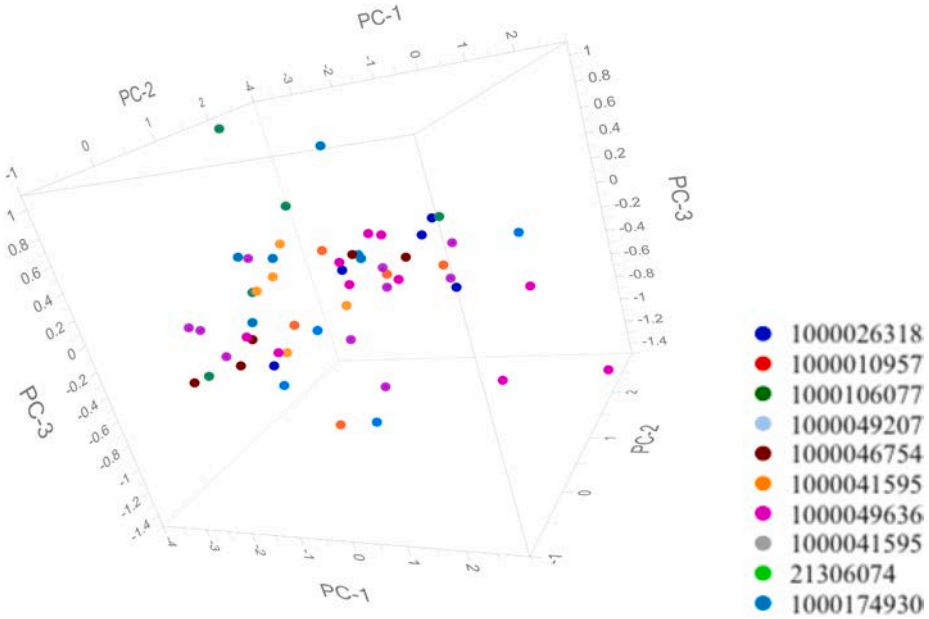

Fig. C3. 3-dimensional score's plot obtained on sample coming from a same brand, same model but presenting different lot number condom samples. Different colors and symbols stands for different lot numbers samples.

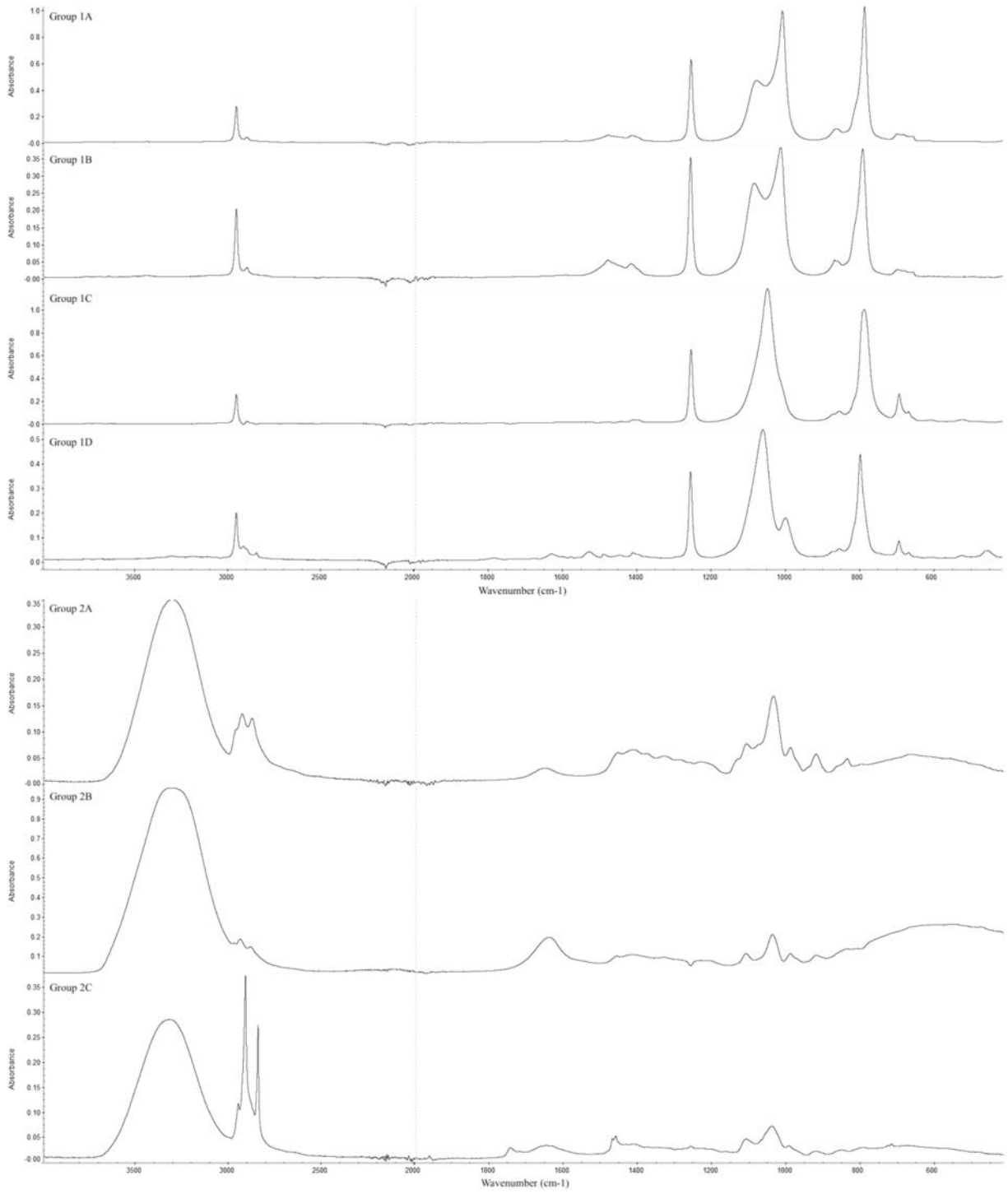
Group 1A
Group 1B
Group 1C
Group 1D
Absorbance
Wavenumber (cm-1)
Group 2A
Group 2B
Group 2C
Absorbance
Wavenumber (cm-1)

Fig. D1. .

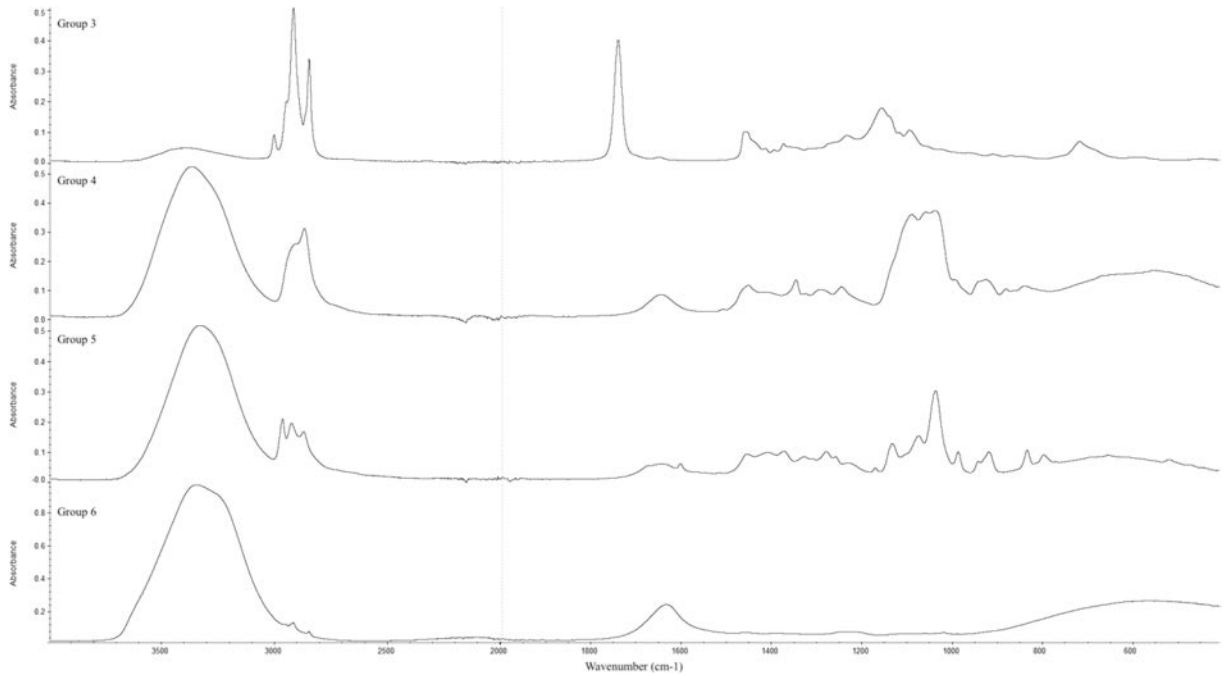

Fig. D1. (*continued*).

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Characterization and classification of water-based compounds in condoms and personal hygiene products using GC-MS

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ABSTRACT

Analysis of condom evidence commonly focusses on the detection of silicone-based lubricants, such as polydimethylsiloxane. However, water-based compounds such as propylene glycol or glycerin can also be used as condom lubricants and may, therefore, be detected as transferred traces. Evaluation of the variability amongst a large sample set from an international market is needed to determine what are the most likely compounds that may be detected in casework. In this study, 165 condoms, personal hygiene products, lubricants, creams and oils were analysed using gas-chromatography coupled to a mass spectrometer detector (GC—MS). The resulting compounds were identified using mass spectral databases, then the data were extracted and evaluated using established multivariate statistical techniques, such as principal component analysis and discriminant analysis. Qualitative visual inspection, as well as statistical analysis, revealed at least twelve different groupings within the dataset. Discrimination was based on variations in the concentration of major compounds, as well as the presence or absence of minor compounds, such as anaesthetics. For the 127 condoms examined, 2 were exclusively water-based lubricated (1.5 %) and 6 contained silicone and water-based components (4.7 %). All the others were only silicone-based (119 condoms, 93.7 %). Strong variation was observed between the different sources of products. Personal hygiene products (PHP), creams, lubricating oils, personal lubricants, and condoms were found to have different chemical compositions. Hence GC—MS can be used to assist in the differentiation of water-based residues for investigative purposes.

1. Introduction

The number of processed sexual assault kits from which no DNA is recovered are increasing. Current trends show that the analysis of condom lubricants by forensic laboratories is becoming more common, due to a serious increase in sexual assault cases. For example, 20 % of the samples collected in Lausanne and Geneva (Switzerland) from sexual assault kits did not contain any recoverable DNA but were not tested for condom lubricants.[1] The Sexual Assault Resource Centre (SARC) in Western Australia reported that requests for condom and/or lubricants testing were made at least once a week.[2] US studies revealed that condom use in sexual assaults could reach up to 16 % [1].

Condom residue evidence has been analysed over the last 30 years using various analytical procedures, targeting different components, but mainly focusing on condom lubricants. However, as previously reported [2–5], literature has focused on condom lubricants rather than bottled lubricants. Most researchers also focused only on one type of sample, such as personal bottle lubricants or condoms, and very few studies actually combined both types of samples to evaluate the variability in terms of water-based lubricant compositions.

Typical instrumentation for the analysis of water-based lubricants and/or condom lubricants was previously described by Maynard et al. in 2001 [6]. More recent studies have reported discrimination using instrumentation such as MALDI-MS [7] or

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DART-TOF-MS [2–4,8,9]. Although these techniques showed very impressive results in terms of differentiation, none of them were applied to trace samples, or to sample extracts, which are more likely to be encountered in case work, especially if only one swab is available for both DNA and lubricant evidence testing. In addition, both MALDI-MS and DART-TOF-MS are rather expensive and not readily available in most forensic laboratories.

In contrast, GC–MS is a common instrument in forensic laboratories, with various uses such as drug and fire debris analysis. GC–MS was previously reported for the analysis of condom lubricants [6,10,11] with focus on silicone-based residues and petrolatum residues, rather than water-based products, with LC–MS being recommended for any water-based traces [6]. Typical water-based components that may be found on condoms are polyethylene glycol (PEG), propylene glycol and glycerin [6,7,11–13]. It is also possible to detect anaesthetics such as lidocaine and/or benzocaine [13]. Preservatives, aromas and/or flavourings as well as colorants might also be detected [7,13–15], as long as they are present in detectable quantities. However, literature reporting the detection of these compounds is limited. From a chemistry point of view, these components are volatile and are likely to be soluble in polar solvents, such as methanol. GC–MS may thus be suitable for this type of analysis, though it has been criticized for the length of the acquisition [3]. Processed under ideal analytical conditions, GC–MS analysis should not present any major issues in terms of the separation and detection of major compounds, with each compound being expected to present a different retention time and mass spectrum. The detection of silicones is not expected, as they are not readily soluble in polar solvents and considering they are large non-volatile polymers. Therefore, a characteristic profile of the water-based composition of each sample should be obtained and statistical analysis may therefore assist in the discrimination of samples.

In this study, samples were extracted and/or diluted with methanol and analysed using GC–MS. The qualitative composition of the dataset was used to complete a first-pass visual comparison of the samples. Further objective discriminations using statistical tools were also undertaken.

2. Material and methods

2.1. Material and solutions

Methanol (Optima® LC–MS AR grade, Fisher Chemical) and diphenylmethane (99 % Sigma-Aldrich) were used as the solvent and internal standard respectively. A solution of 0.1 % diphenylmethane in methanol was prepared as the extraction solvent.

2.2. Sample preparation and analysis

One hundred and sixty-five (165) samples, consisting of 26 personal lubricants (i.e. bottled lubricants), 5 personal hygiene products, 6 creams, 1 massage oil and 127 water-based, silicone-based and non-lubricated condoms were purchased for this study from multiple shops in Australia, Switzerland and New Zealand, the vast majority of condoms being silicone-based (125 samples out of 127). A full list of the items is presented in the Supplementary Information (Table S1).

Lubricants, creams, personal hygiene products and massage oils were diluted at a concentration of approximately 1 mg/ml in the extraction solvent of methanol containing 0.1 % diphenylmethane as the internal standard (IS). Condoms were unrolled and soaked in 20 mL of extraction solvent. All extracts and dilutions were diluted 1:10 (v/v) in methanol before analysis. Each sample was analysed on the GC–MS twice from the same extract to account for sample heterogeneity.

Five (5) lubricants and one (1) personal hygiene product (see Appendix A) could not be solubilised in methanol and therefore could not be analysed using GC–MS. A total of one hundred and fifty-nine (159) products are used in this study.

2.3. Instrumental conditions

Analyses were carried out on an Agilent GC 7890B system, interfaced with an Agilent 5977 N mass spectrum detector, utilising Agilent ChemStation v. F.01.03.2357. Separation was achieved on a HP-5MS capillary column (30 m x0.25 mm x0.25 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: held at 50 °C for 2 min, then ramped at 10 °C/min to 230 °C and 20 °C/min to 300 °C, and a final hold for 5 min at 300 °C (total run time 28.5 min.). Considering mass spectral detection, the transfer line was set at 250 °C, the ion source at 230 °C and the quadrupole at 150 °C. Data was acquired in full scan mode (30−550 *m/z*), with a sampling rate of 3.

Mass spectral data coming from published literature [2,3,5,9,10,16,17] and mass spectrum computerized databases such as NIST18 (*National Institute of Standards and Technology*), TOX3 (*Wiley Drug and Pesticides*) and NBS75 K (*National Bureau of Standards*) were used to characterize the compounds detected in the samples, with identifications confirmed based on scores and comparisons of major ions observed in the mass spectra.

2.4. Data processing

2.4.1. Software

Data processing was performed using Microsoft Excel 2019 and The Unscrambler X v. 10.5 (Camo Software, Norway).

2.4.2. Data extraction and pre-processing

Areas of target ions in the chromatograms were integrated for each identified compound using Agilent ChemStation® software. Internal standard (IS) was used for quality control, to ensure there were no injection issues. IS abundance was checked in all the chromatograms by integrating the area and comparing between all the samples and with a reference solution to assess the reproducibility of the analysis. The resulting integrations were exported to Excel®. Peak areas were normalised to the sum of all the compounds detected and the square root was performed in order to reduce the influence of larger peaks and be able to compare variables on the same scale. Normalisation to internal standard was also evaluated, but did not allow to minimize intravariability and maximize intervariability.

2.4.3. Chemometric analysis

Multivariate analysis of the dataset was conducted using principal component analysis (PCA) and Support Vector Machine (SVM) algorithm for discriminant analysis. Principal components (PCs) were built from all compounds detected in the chromatograms over 30,000 abundance units (AU). This threshold was selected as to be over three times the signal to noise ratio. PCA was performed using the single value decomposition (SVD) algorithm. Three dimensional (3D) scores plots were generated using combinations of the scores from the first seven PCs to visualize structure within the dataset. Loadings corresponding to the appropriate PCs were studied to identify variables leading to the discrimination between samples.

SVM model was used for the discrimination and classification of the samples as SVM aims to establish the separation hyperplane that offers the maximum separation margin between classes, using a kernel function. This is often used when data classes do not

follow a gaussian distribution and have different variances. Radial basis function kernel (RBF) was used, with γ (kernel parameter) set at 0.01 and C (soft margin parameter) set at 0.001. This was chosen as it is a robust solution to separate non-linearly separable classes using a one-versus-one classification scheme.

3. Results and discussion

3.1. Qualitative analysis

This study focused on the overall set of compounds present at detectable amounts in the samples. In total, 26 compounds were identified in the dataset and are presented in Table 1. Internal standard was eluting at aroung 15.25 min.

Qualitative analysis allowed the creation of sub-classes amongst the various samples, based on the overall content. In terms of condoms, four populations were observed. The first population contained 93.7 % of the samples which were silicone-based condoms, without any water-based compounds. The second population represented 3.14 % of the dataset and comprised silicone-based condoms, with additional benzocaine and PEG (3 condoms, 2.3 %) or glycerine and PEG (1 condom, 0.7 %). The third population (1.5 %) comprised silicone-based condoms, which also contained propylene glycol. Finally, 1.5 % of the condoms were non-silicone-based condoms and contained propylene glycol and glycerin.

Water-based compounds were detected in 46 (28.9 %) of the 159 samples constituting the dataset. The most common compounds were glycerin, propylene glycol, phenoxyethanol and PEG. Amongst these samples, 56.25 % of the samples contained glycerin, 37.5 % propylene glycol, 6.25 % PEG, 25 % phenoxyethanol, and 21.8 % were found not to contain any of these main components. However, they were containing other compounds listed in Table 1. Glycerin and propylene glycol were often present together, this composition accounting for 31.25 % of the samples.

No compounds linked to aromas or colorants were detected in any of the samples. This suggests that if any were present, they were either not extracted by the solvent, or not separated and/or detected by the GC–MS method or were present in quantities below the detection limit. Therefore, if needed, further research into methods for the extraction and detection of these compounds is necessary using other instrumentation or extraction techniques.

Most of the analytes detected were listed as ingredients on the packaging for most of the lubricants and personal hygiene products. Interestingly, PEG was only found in 6% of the samples, mostly in condom samples. This is surprising as literature reports that besides PDMS, one common lubricant that might be found on condoms is PEG. However, the non-lubricated condoms that were analysed in this dataset had a high glycerin content and a small amount of propylene glycol. PEG was only detected on condoms that were also lubricated with silicone. Campbell et al. (2007) indicated that PEG found on condoms was often used with a spermicide [11]. However, only one of the samples in the dataset revealed traces of nonoxynol-9, the only spermicide permitted for use on condoms. This difference may relate to changes in condom regulations, in particular nonoxynol-9 is no longer permitted on condoms present on the New Zealand and European markets.

3.2. Sample discrimination

PCA performed on the entire dataset (40 samples, considering 119 condoms without any water-based compounds, and 6 samples that could not be analysed) and all 26 compounds revealed that the first five principal components accounted for 87 % of the total variance in the data. 33 % of the variance was explained along PC1, while PC2 and PC3 accounted for 26 and 13 % of the variance respectively. The total variance explained by the first seven PCs was found to be 95 %. 3-dimensional PCA scores plot were generated by plotting the sample scores, in combinations up to the fifth PC. Results of the scores plots are presented in Fig. 1 and Fig. 3. Sample groupings were assessed based on the visual clusters, independent of the sample type. As shown in Fig. 1, at least 8 groups were identified. After colour-coding by class, it was observed that the visual clusters did not always correspond to the actual class of the sample. For example, some condoms and lubricants were found to be part of the same clusters.

Examination of the loading plots for the first 3 principal components indicated that the separation of the data was mainly

Table 1
Compounds found in the samples composing the dataset, their retention time, target ions and qualifiers.

Compound	RT [min]	Target ion (*m/z*)	Qualifiers (*m/z*)
Propylene Glycol (PG)	4.167	45	61, 7629
1,2-Pentanediol	7.042	55	73
1,2-Hexanediol	8.718	69	87, 41
Glycerin	9.802	61	43, 31, 15
2-Phenoxyethanol	11.76	94	138, 77, 66
1,2-Octanediol	12.066	55	97, 115, 43
Triethylcitrate	17.75	157	115, 203, 43
Octane, 1,1′-oxybis	17.792	57	71, 112
1-Tetradecane	17.95	57	43, 91, 71
Xylitol	18.157	61	4,374,103
Cyclodecane	18.711	57	43, 168, 199
Hexaethylene glycol (PEG Oligomer)	19.415	45	89, 133
Oxalic Acid	19.918	83	139, 55, 95
Cyclododecane	20.249	83	111, 196, 224
Pentadecanoic acid, 14-methyl-methylester	20.684	74	87, 143, 55
2-Tetradecyloxyethanol	21.515	57	166, 125, 85
1-Octadecene	22.072	83	97, 111, 125
Heptaethylene glycol (PEG Oligomer)	22.147	45	89, 133, 207
811-Octadecadienoic acid methyl ester	22.192	67	81, 109, 294
Lauryl Acetate	22.748	145	168, 213, 127
Nonoxynol-9	23.930	45	89, 133, 206
Cyclotetradecane	23.978	173	168, 83, 43
Octaethylene glycol (PEG Oligomer)	24.078	45	89, 133, 283
Glycerol-1-Palmitate	24.865	239	299, 98, 134
Cyclohexadecane	25.223	145	173, 57
Glycerol Tricaprylate	28.134	127	327, 57, 201

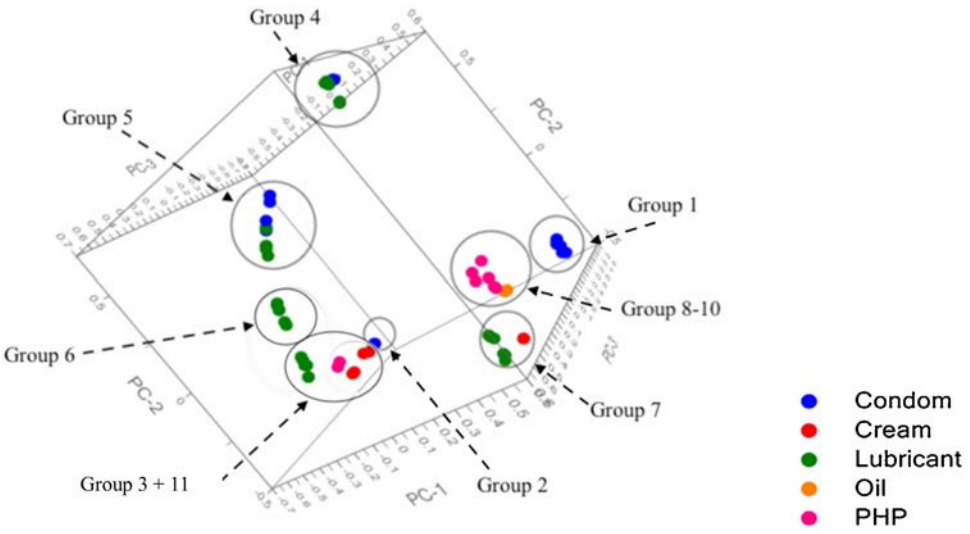

Fig. 1. 3D Scores Plot of the dataset along PC1(33 % variance explained), PC2 (26 % variance explained), and PC3 (14 % variance explained),. Black circles indicate visual clusters observed when modelling the three-dimensional space. Groupings are named after Table 2, and refer to the chemical content of the samples in the groups.

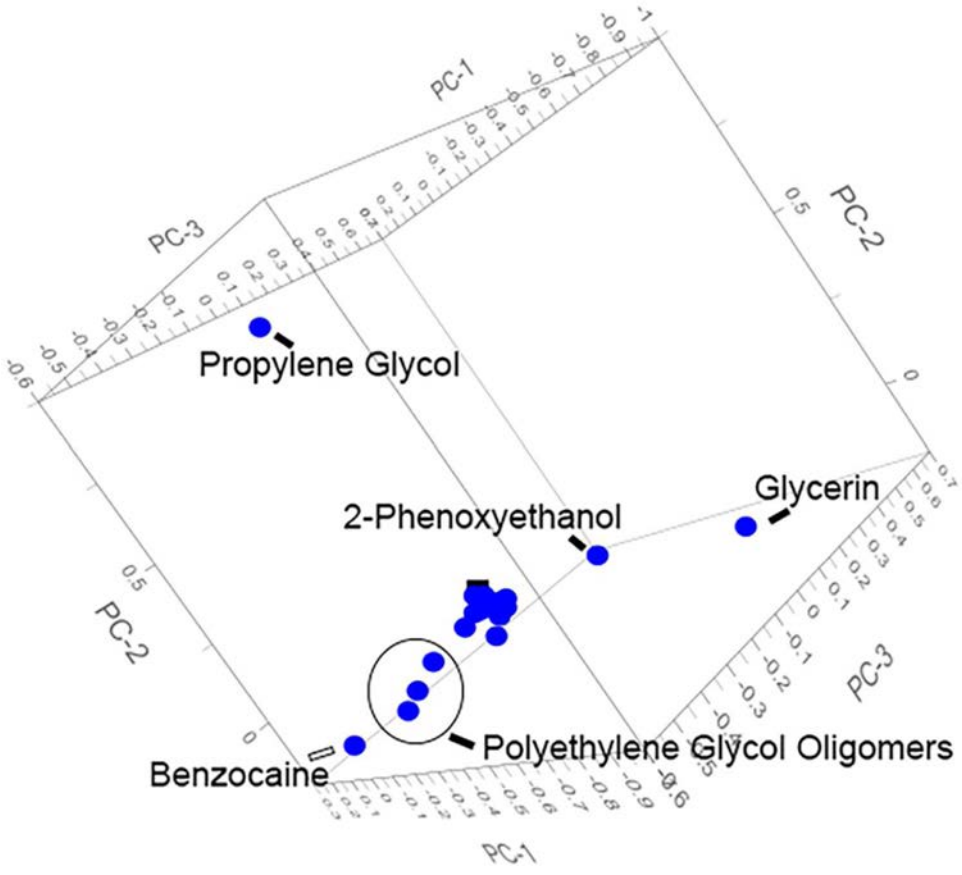

Fig. 2. Loading plots associated with 3D-Scores Plot of Fig. 1. The non-labelled cluster represents the rest of the variables used to build the discrimination plots.

dictated by the glycerin, phenoxyethanol and propylene glycol content (Fig. 2). Glycerin was found to have a strong negative impact along PC1 and PC2, and a small positive impact along PC3 was also noted. Propylene glycol had a positive impact along PC1 and PC2 but had no impact on PC3. Phenoxyethanol presented a positive impact along PC1 and PC3 and did not affect separation along PC2. Along PC3, oligomers from PEG (i.e. hexa-, hepta- and octa-ethyleneglycol) and benzocaine were found to have a negative impact, although they were close to zero along PC1 and PC2. All the other variables were found to cluster together, with a mild positive impact along PC1, and almost no impact on PC2 and PC3.

Loadings along further principal components were evaluated to see whether other variables were impacting sample discrimination (see Supplementary Information). No additional information could be drawn from this evaluation.

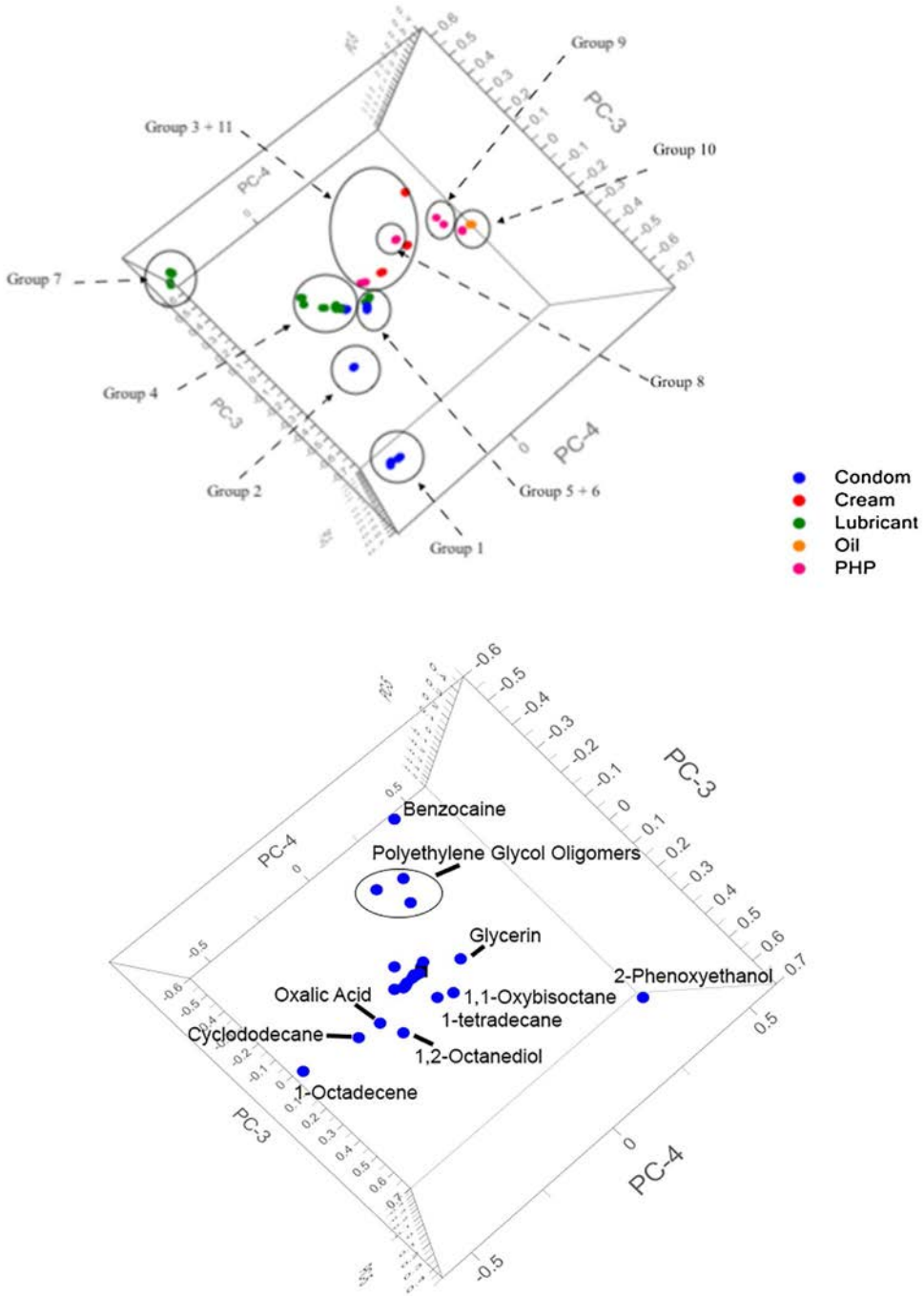

Fig. 3. 3D Scores Plot of the dataset along PC3, PC4 and PC5, and loading plots associated. Black circles indicate visual clusters observed when modelling the three-dimensional space. Groupings are named after Table 2, and refer to the chemical content of the samples in the groups.

The examination of scores and loadings for PC4 and PC5 are presented in Fig. 3. There was a positive correlation along PC4 for benzocaine and polyethylene glycol. Alkane patterns were found to generate a negative correlation along PC4. Finally, separation of samples along PC5 was found to be mainly due to differences in minor components. These separations were found to assist strongly in separating groups that were not distinguished by the first three PCs (as shown in Fig. 1) but may be limited in abundance in case work samples.

As shown in Fig. 3, one additional group was found to be highlighted when exploring further principal components. Observation of the clusters revealed that some populations that were found to be clustered together along PC1-PC3 (i.e. groups 8–10 from Table 2) were found to be separated along these further PCs. Components responsible for sample separation along PC4 are octadecene and oxalic acid, while triethylcitrate, cyclodecane and 1-tetradecane are responsible for the separation along PC5. Glycerin, propylene glycol and 2-phenoxyethanol were found to be missing in these PC. Additionally, samples that were initially clustered with group 3 were found to cluster with group 4 along PC4. In contrast, clusters that were found to be distinguished along PC1-PC3, i.e. groups 5 and 6 from Table 2, were clustered together along PC4-PC5. Group 7 illustrated in Fig. 2, shows PC1-PC3 was found to be separated into two parts, with samples moving from group 7 to group 3. This observation was confirmed by groups created in Fig. 4, based on the qualitative content of the samples.

Table 2
Summary of the sample(s) comprised in each grouping. PEG = Polyethylene glycol oligomers; GLY = glycerin; PE = phenoxyethanol; PG = propylene glycol; OCT = 1,2 octanediol; other = minor compounds in the chromatograms. 6 lubricants could not be analysed as they could not be solubilised in methanol, and 1 water-based lubricant is missing as there was no sample left for GC-MS analysis. Total number of samples is 159. Chromatograms examples for Group 1-11 are presented in Supplementary Information.

Group	Source of the sample	Content	Samples in the group
Group 1	Condom (3)	Benzocaine + PEG	Samples 14, 19, 22
Group 2	Condom (1)	GLY + PEG	Sample 124
Group 3	Lubricants (1) Creams (5) PHP (1)	GLY + PE + PG + other	Samples 140, 159, 160, 161, 162, 163, 164
Group 4	Condom (2) Lubricants (8)	GLY + PG	Samples 57, 70, 108, 135, 141, 165, 175, 176,177, 182
Group 5	Condom (2) Lubricants (2)	PG only	Samples 112, 113, 134, 136
Group 6	Lubricants (2)	PG + other	Samples 168, 170
Group 7	Lubricants (3) Creams (1)	OCT + other	Samples 142, 158 166, 167
Group 8	PHP (1)	OCT + PG	Sample 154
Group 9	PHP (1)	PEG + other	Sample 155
Group 10	Massage oil (1) PHP (1)	Other water-based	Sample 138, 157
Group 11	Lubricants (2)	GLY only	Sample 137,172
Group 12	Condoms (119)	No detectable compounds	All condoms with no observable pattern

From observation of the extracted data obtained from central objects of each group visually identified, based on the presence and/or absence of certain compounds, it was determined that 11 groups were created. It was also observed that samples that apparently contained the same major compounds (i.e. glycerin, propylene glycol) may contain various minor compounds detected in the qualitative profile, therefore creating additional discrimination of the different samples (Fig. 4). One new group was created based on the observations of Fig. 4, leading to group 11, containing only one sample. This sample was not differentiated using PCA.

Evaluating the mean of each of the 26 components in the 11 groups, a large variability amongst the samples was observed, although samples containing similar features, were differentiated by variations in components and/or concentration variations. Based on the PC scores observed from Fig. 2 and 3 and the qualitative analysis, four stand-alone groups were identified within the dataset, consisting of individual samples, which were Ceylor Gold condom (Group 2 - glycerin, PEG and nonoxynol), Multi-Gyn Active Gel (Group 7 - phenoxyethanol, octanediol), Summer's Eve Feminine Wash Sensitive Skin (Group 8 – propylene glycol, PEG, alkanes) and Astroglide Gel Personal Lubricant (Group 11 - glycerin). The remaining groupings contained multiple samples and the separation was achieved based on the various combinations available. For example, group 1 contained condoms with benzocaine and PEG. Condoms containing glycerin and PG (Group 4) were found to cluster with all the lubricants containing both components. Creams, as well as personal hygiene products, presented various compositions and some were easily clustered with lubricants.

Coupling data obtained from both PCA scores plot and Fig. 4 reveal that 12 groups were observed amongst the population studied, given their qualitative and semi-quantitative content, group 12 being samples that yielded no observable components in the chromatogram. Table 2 summarises the sub-groups and the samples contained in each group that were established based on both the qualitative and multivariate analyses.

It is now interesting to understand if the groups obtained in the PCA are significantly different from the ones obtained in Table 2. Groups 1, 2, 4, 5 and 7 from Table 2 were found to be clustered together in the PCA provided along PC 1, 2 and 3, as well as along PC 3, 4 and 5. Groups 8, 9 and 10 of Table 2 were only found to be separated in the PCA provided along PC 3, 4 and 5, suggesting that PC 4 and 5 were using other minor compounds (e.g. oxalic acid, cyclodecane, tetradecane) for the separation of the groups, assisting in creating extra sub-groups. Group 3 was found to contain all the left over samples that could not be clustered with other groups, which leads to a considerable inhomogeneity, confirmed by the spread of the data belonging to this group. However, groupings were obtained based on a certain number and amount of compounds detected, and some subclasses using additionnal accurate visual comparison in group 3 might lead to further subgroupings. Examining only this group for further discrimination using chromatogram overlays, the samples presented compounds common to all, but also additional ones, which lead to the creation of as many sub-groups as there are samples in that group. These samples are therefore grouped as one, but may be differentiated further when additional samples are added to the dataset.

3.3. Classification model

SVM classification model was created using the first five PCs, the 11 groupings derived from the content clustering, observation of the PCA of the dataset and the marketing type (i.e. condom, bottled lubricant, etc). The model was constructed using the training set containing 2/3 of the dataset, and then the validation set (1/3 of the dataset) was predicted to provide a more realistic estimate of model performance. The discriminant analysis presented an 82.1 % classification accuracy. Condoms, whose composition were limited to either propylene glycol and glycerin, or propylene glycol only, were found to misclassify (Table 3). Similarities between the chemical profiles of these samples and lubricants were observed, with these common compounds detected in both. Massage oils, creams and personal hygiene products were also found to be misclassified, as the separation was not based on glycerin, propylene glycol and/or phenoxyethanol, but rather on the alkane series detected in the chromatograms (Table 3). These variables were found to be of minimal influence in terms of model loadings and sample discrimination, thus explaining the lack of correct classification. Validation accuracy was 79 %. In both cases, misclassifications occurred between massage oils and personal hygiene products, as expected from their close PC scores. Regardless, GC—MS yielded an interesting classification potential. A 2-stage process for classification could be considered, with the first run in a model designated to determine the group a sample belongs to, and the second run through another more

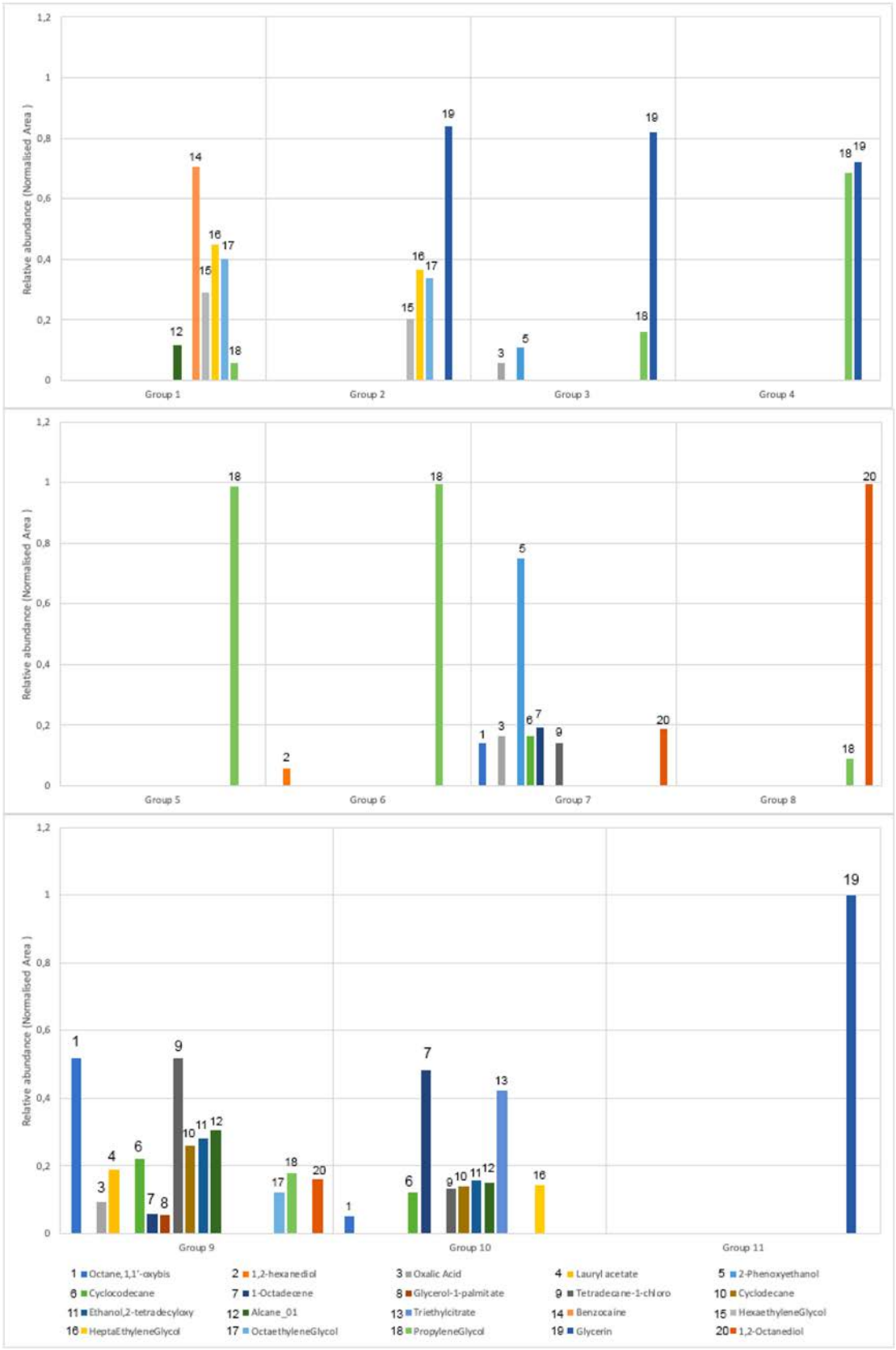

Fig. 4. Qualitative data obtained on the mean of the data present in each group, highlighting the disparity of the groups. Only compounds that were presenting a relative abundance (normalised area) over 0.05 are presented, which results in 20 out of the 26 compounds presented in Table 1.

Table 3
Confusion matrix for SVM classification model.

		Actual Class				
Predicted Class		Lubricant	Condom	PHP	Cream	Oil
	Lubricant	35	8	0	0	0
	Condom	0	8	0	0	0
	PHP	0	0	8	2	2
	Cream	0	0	0	4	0
	Oil	0	0	0	0	0

adequate model which would allow the separation of massage oils, creams and personal hygiene products. Future investigations should be led in that direction.

Comparison of this study's results with previous research outlined that GC—MS achieved different results than the ones obtained with MALDI-MS and DART-MS. In the dataset used by [7], samples were found to contain water-soluble compounds such as PEG, benzocaine and nonoxynol-9. Other additives were identified using FT-IR spectroscopy. Glycerin and propylene glycol were not reported, and discrimination of the samples was found to be lower than that achieved using GC—MS. On the other hand, results obtained by [3] were found to be more accurate, as they were able to detect more components in the samples. Their classification model presented a higher accuracy than the one obtained for the present GC—MS model. This is due to the group definition, as we used the marketing information to do our prediction, whereas they used the groups based on the chemical information.

A recent publication by [5] investigated the discrimination of sexual lubricants from personal hygiene products using DART-TOF-MS. 22 PCs were used to build the discrimination model whereas our model used 5 PCs to account for the same total explained variance. PCA scores plots of the DART-TOF-MS data also illustrated clusters which were difficult to highlight, and significant overlap of lubricants, personal hygiene products and condoms, similar to those presented in this study. This observation suggests that these products are unlikely to be clearly separated from each other, independent from the analytical instrumentation used. The most likely hypothesis to explain such results is that all the samples contain the same base formula, which is a logical step as there are heavy regulations regarding chemicals authorized for use on human skin [18–22]. In addition, LDA was used to classify the samples acquired using DART-TOF-MS, thus assuming equal variance of the groups as well as a Gaussian distribution of the data, which is not the case [5]. Using 22 PCs, their classification model reached a correct classification rate of 99.2 %. However, the methodology has not yet been used on diluted samples, nor on real world samples, and their instrument is not readily available in forensic laboratories. With regards to these results, the methodology presented here has the advantage to consider the non-Gaussian distribution of the data, and thus offers a classification scheme more representative of the dataset. The classification is certainly less powerful in terms of correct classification rate, but the presented model only uses 5 principal components, on 26 compounds, and works for diluted samples.

4. Conclusion

In this study, 159 samples consisting of condoms, lubricants, personal hygiene products, creams and massage oils, from 3 international markets, were analysed to determine distinguishing 'water-based' compounds, and hence what compounds may be useful in case work. GC—MS provided a rapid method to identify the various water-based components after extraction with methanol. Based on chemical compositions, a total of 11 groups were determined, though it was found that some samples from different classes, mainly condoms and lubricants, were misclassified due to similarities in compositions.

Chemical profiles were found to be repeatable with good resolution obtained in all chromatograms. The major compounds detected were glycerin, phenoxyethanol, propylene glycol and polyethylene glycol. Minor components such as benzocaine, octanediol or an n-alkane series were also detected and found to contribute to sample characterization. These compounds also enhanced the discrimination between samples in the dataset. No traces of aroma, flavourings or colorants were detected in the chromatograms of the samples, highlighting the need for alternative methods if these compounds are of interest in case work.

As this study highlights, the complete differentiation of all samples might be difficult using GC–MS, as only water-based components present in detectable quantities were observed. However, the methodology presented in this research is applicable in case work situations. Future research should investigate the possible interaction with the cotton swabs used for sampling, as well as interaction with the vaginal matrix, including preferential absorption of some compounds, and other persistence factors, to evaluate applicability to case work. This will provide a baseline to implement this analysis in real world cases.

Credit statement

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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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Appendix A. Supplementary data

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Condom evidence: Characterisation, discrimination and classification of pyrolysis-GC-MS profiles

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ABSTRACT

Analysis of condom evidence commonly focusses on the detection of silicone-based lubricants, such as polydimethylsiloxane (PDMS). Although various instruments are used to analyse silicone lubricants, pyrolysis-gas chromatography coupled to mass spectrometry (py-GC-MS) is one of the few instruments that presents immediate applicability to casework. However, considering that this technique detects silicone-based evidence, it is important to evaluate the discrimination potential of the method when applied to various samples. Examination of the variability within a large sample set from an international market is needed to evaluate the most distinguishing compounds likely to be detected in casework. In this study, 70 condoms, personal hygiene products, and lubricants, were analysed using py-GC-MS. Resulting pyrograms were characterised using published spectral databases. Pyrolysates data were extracted and evaluated using multivariate techniques. Qualitative visual inspection of the data, as well as statistical analysis, revealed at least five groups within the dataset. Discrimination was based on four main oligomers, as well as six minor compounds from siloxane degradation. Condom lubricants were found to present a very regular pattern, allowing for the majority of them to be differentiated from personal lubricants.

1. Introduction

Condom residues are an important evidence type in investigations of sexual assault, and the number of cases where this type of evidence is relevant has increased within the last decade [1]. Evidence recovered is usually linked to the lubricant on the condom, as it is the most abundant trace that transfers during protected intercourse [2–7]. The most common condom lubricant formulation is based on polydimethylsiloxane (PDMS), and is found on over 95% of the condoms from the international market [2,3,6,8–10]. Other common lubricants are water-based formulations containing glycerol, propylene glycol and/or polyethylene glycol (PEG) [11–13]. The candidates for inclusion within condom formulations are regulated by international norms [14–17], which significantly reduces the substances that can be used and hence present within transferred residues. However, the regulations applying to personal lubricants used as intimate products are slightly different, as their use does not imply protection against sexually transmitted diseases and/or pregnancy. It is therefore possible that personal lubricants may contain PDMS different from that used in condom formulations.

The differentiation of lubricants using Matrix Assisted Laser Desorption Ionisation – Mass Spectrometry (MALDI-MS) was investigated by several authors: Bradshaw et al. in 2011 [18] and in 2013 [19] have illustrated differences within the mass spectra obtained from different brands and models while Spencer et al. in 2011 [4] processed statistical analysis but could not clearly identify any subclasses in populations containing PDMS. Moustafa and Bridge in 2017 also proposed a discrimination model for the differentiation of condoms and personal hygiene products using Direct Analysis in Real Time- Mass Spectrometry (DART-MS) [20]. Their model differentiated samples with different chemical profiles, such as those containing PDMS, glycerol or PEG. They also demonstrated the possibility of differentiating a silicone personal lubricant from a silicone condom lubricant, but the sample size was moderate (n = 36) and the samples were all sourced from the US market. The most recent discrimination model for silicone lubricants was drawn by Baumgarten et al. [9] who used DART-MS for the discrimination of 56 silicone products purchased on the American market. The model was found to be able to distinguish 11 different classes of chemical profiles based on their mass spectral profile. These are the

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only three existing models for the discrimination of sample classes present in the literature. However, the two techniques have not been applied to diluted samples, case simulations or real cases.

In contrast to these two techniques, pyrolysis gas chromatography mass spectrometry (py-GC/MS) was applied in multiple studies for the detection of condom traces in simulated cases, as well as to study the persistence in different matrices [5,7,21]. This is a proven technique, along with Fourier transform infrared (FTIR) spectroscopy, more specifically with DRIFTS-FTIR [3,6,22], and has demonstrated potential applicability to extracted traces and real cases [7]. A recent study by Maurer et al. [23] reported which analytical and pyrolysis conditions were the most adequate for the separation and identification of PDMS, to ensure good reproducibility of the results. Given the lack of discrimination model existing for py-GC/MS, an attempt of discrimination was made [23]. However, the number of samples was too small (n = 5) to infer on their discrimination. Given the potential offered by py-GC-MS, it is relevant to investigate its discriminatory power, in order to determine if the technique is able to differentiate samples from different classes that have indistinguishable profiles by infrared spectroscopy. Indeed, as demonstrated by [3,24,25], FTIR spectroscopy is successful at identifying silicone and non-silicone based samples, which in casework would be important to know, in order to use the most relevant method for the analysis of the evidence. Therefore, in casework, the analytical sequence should be constituted of FTIR analysis prior to any chromatographic or mass spectrometry technique [3,24].

The advantage of using py-GC/MS is that large and non-volatile polysiloxanes, which cannot reasonably be analysed by GC/MS otherwise, can be analysed using this instrument. Silicone lubricants, such as PDMS, are the first target of this type of analysis, especially as more than 95% of condoms found on the international market contain PDMS [2,6,8]. It has also been established that glycerol or PEG-type water-based lubricants are easily analysed using GC/MS without requiring pyrolysis [5,26]. Moreover, the pyrolysis of these water-based lubricants does not result in characteristic profiles, probably due to the decomposition to CO_2 and H_2O of the molecules.

During the pyrolysis, PDMS is degraded into cyclic oligomers of low molecular weight (see Fig. 1), called dimethylsiloxanes (DMS) [5]. These DMS oligomers are usually labelled according to the number of silicon atoms in them: the cyclic trimer (IUPAC name: hexamethylcyclotrisiloxane) as presented in Fig. 1, is called D3 and is the smallest and most stable oligomer generated [27]. Pyrolysis generates a range of cyclic oligomers of increasing molecular weight, based on their chain lengths. Their separation through the capillary column is now possible, knowing that they are volatile molecules, which is not the case of pure PDMS. Pyrolysis is therefore often used for the analysis of polysiloxanes [6,23,28–31].

It turns out that the viscosity of the siloxanes, given by the number of repeated [Si(CH_3)-O] units, affects the size of the area of the peaks in the pyrogram: the lower the viscosity, the lower the area [30]. The effect of the pyrolysis temperature is not negligible because the number of pyrolysis products varies if the temperature increases or decreases [23,31–33]. A fairly high temperature must be applied to obtain the degradation of PDMS [5,27], but not too high otherwise the degradation of PDMS is altered [29].

The aim of this study was therefore to investigate the chemical profile obtained using py-GC-MS on lubricants extracted with hexane, the solvent recommended by Maynard et al. [3]. This has the added benefit of making the samples comparable to expected concentrations arising from the extraction of case samples. Visual examination of the composition of the dataset was undertaken first to attempt a qualitative differentiation of the samples. Chemometrics was then used to evaluate objective discrimination and classifications.

2. Material and methods

2.1. Material and solutions

Hexane (AR grade, Sigma Aldrich, USA) was used as a solvent. Pyrolysis sample holders were Eco-Cups SF and Eco-Sticks SF purchased from Frontier Laboratories. PDMS 200 centiStokes (cSt), with a molecular weight ~9430, obtained from Sigma Aldrich (USA) was diluted in hexane at a concentration of 1 mg/ml.

Samples were obtained from commercially available distributers in Australia, New Zealand and Switzerland. The samples obtained were considered representative of the markets, as they covered major condom brands and sub-brands and were available to consumers. The dataset includes 2 personal hygiene products (PHP), 7 lubricants and 61 condoms, that are all known to contain silicone lubricants. Table 1 presents the list of the samples used in this study.

2.2. Sample preparation and analysis

For py-GC-MS analysis, condoms were opened, unrolled, deposited in a 40 ml glass bottle and covered with 25 ml of hexane. The bottles were then capped and ultrasonicated for 15 min. The extracts were then diluted 10-fold prior to analysis. Liquid samples, such as personal lubricants, were weighed and diluted in hexane to the approximate concentration of the diluted, extracted condom, between 1.5 and 2.5 mg ml^{-1}.

For each sample, 10 µL of the hexane solution was spiked in the stainless-steel cups and left to evaporate prior to analysis. Three replicates were prepared from each condom extract, to account for sample variability, as well as any variation due to the instrumentation and sample preparation. Blanks were run between each analysis to avoid cross contaminations.

For GC-MS analysis, lubricants, creams, oils and personal hygiene products were diluted at a concentration of approximately 1 mg ml^{-1} in the extraction solvent of methanol containing 0.1% diphenylmethane (vol/vol) as an internal standard (IS). Condoms were unrolled and soaked in 20 ml of extraction solvent. All extracts and dilutions were diluted 1:10 (vol/vol) in methanol before analysis. Each sample was analysed on the GC-MS twice from the same extract to account for sample heterogeneity.

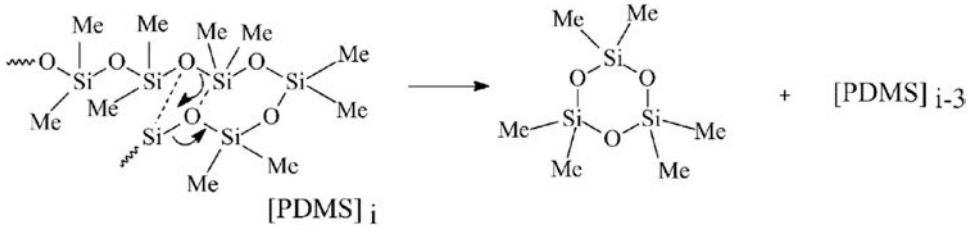

Fig. 1. Pyrolysis of PDMS to produce cyclic oligomers.
Reproduced from [29].

Table 1
List of the samples used in the study. Samples were taken from a larger database, which contained multiple samples from the same brand and type. Therefore, replicate samples were removed, thus explaining non consecutive number in the table.

Sample N°	Class	Brand	Type	Country of purchase
1	Condom	Durex	Extra Safe	NZ
10	Condom	Durex	Classic	NZ
11	Condom	Durex	Unknown	NZ
12	Condom	Durex	Pleasure Me	NZ
13	Condom	Shield	XL	NZ
14	Condom	Durex	Mutual Climax	NZ
20	Condom	Durex	Intimate Feel	NZ
23	Condom	Durex	Confidence	NZ
24	Condom	Durex	Banana	NZ
33	Condom	Durex	Apple	NZ
34	Condom	Ansell	Contempo Rough Rider	NZ
36	Condom	Durex	Orange	NZ
38	Condom	Durex	Thin Feel	NZ
40	Condom	Durex	Confidence	NZ
41	Condom	Gold Knight	Chocolate	NZ
42	Condom	Marquis	Flavoured	NZ
44	Condom	Marquis	Regular	NZ
45	Condom	Gold Knight	Strawberry	NZ
47	Condom	Ansell	SKYN-Original	NZ
48	Condom	Ansell	Lifestyles - Ultra Thin	NZ
50	Condom	Ansell	Lifestyles - Regular	NZ
53	Condom	Ansell	Lifestyles - Zero	NZ
54	Condom	Ansell	Lifestyles - Party Variety - Snake Skin Textured	NZ
55	Condom	Ansell	Lifestyles – Party Variety - O'Max	NZ
56	Condom	Ansell	Lifestyles – Party Variety - Tutti Frutti	NZ
58	Condom	Ansell	Lifestyles – Party Variety – Glow in the Dark	NZ
59	Condom	Ansell	SKYN-Elite	NZ
60	Condom	Ansell	Lifestyles – Assorted – Banana Bump Studded	NZ
61	Condom	Ansell	Lifestyles – Assorted – Sonic Strawberry Ribbed	NZ
62	Condom	Ansell	Lifestyles – Assorted – Berry Blast Smooth	NZ
63	Condom	Ansell	Lifestyles – Assorted – Vanilla Thriller Smooth	NZ
64	Condom	Ansell	Lifestyles – Assorted – Choc Ripple Ribbed	NZ
65	Condom	Ansell	Lifestyles – Assorted – Mintensity Studded	NZ
66	Condom	Ansell	Lifestyles – Assorted – Sonic Berry Ribbed	NZ
68	Condom	Ansell	Lifestyles – Party Mix – Choc Ripple Ribbed	NZ
69	Condom	Ansell	Lifestyles – Party Mix - Dynamint Studded	NZ
79	Condom	Durex	Performa	NZ
109	Condom	Ansell	LifeStyles Luxe Silicone-based lubricant	AUS
110	Condom	Ansell	Manix Contact	CH
111	Condom	Ansell	Manix Natural	CH
112	Condom	Ansell	Manix Orgazmax Plus	CH
113	Condom	Ansell	Manix Endurance	CH
114	Condom	Ansell	Manix Fraise Gourmande	CH
115	Condom	Ansell	Manix Xtra Pleasure	CH
116	Condom	FairSquared	Sensitive dry	CH
117	Condom	FairSquared	Original	CH
118	Condom	FairSquared	Max Perform	CH
119	Condom	Migros	M-Budget	CH
120	Condom	Migros	Cosano Regular	CH
121	Condom	Migros	Cosano Sensual	CH
122	Condom	Migros	Cosano Feeling 0.05 mm	CH
123	Condom	Coop	Prix Garantie	CH
124	Condom	Ceylor	Gold	CH
125	Condom	Ceylor	Thin Sensation	CH
126	Condom	Ceylor	Non-Latex UltraThin	CH
127	Condom	Ceylor	Strawberry	CH
130	Condom	Durex	Strawberry	CH
133	Lubricant	Ansell	SKYN Maximum Performance Lubricant	CH
156	PHP	Femfresh	Feminine deodorant spray	AUS
158	PHP	Vagisil	Soothing Oatmeal Cream	AUS
168	Lubricant	Ansell	Skyn Intimate Moments	AUS
169	Condom	Ansell	Skyn Intense Feel Non-Latex Condoms	AUS
171	Lubricant	Astroglide	Diamond Silicone Gel Personal Lubricant	AUS
172	Lubricant	Astroglide	Gel Personal Lubricant	AUS
174	Lubricant	Astroglide	Waterproof Silicone Liquid	AUS
177	Lubricant	Astroglide	Strawberry Liquid Personal Lubricant	AUS
178	Condom	Four Seasons	Naked Black Condom	AUS
179	Condom	Four Seasons	Stubbed & ribbed Stimulating condoms	AUS
180	Condom	Checkmate	ExtraSensitive Lubricated Condoms	AUS
181	Lubricant	Durex	Play Perfect Glide	AUS
184	Condom	Ansell	LifeStyles Ribbed Condoms	AUS

2.3. Instrumental conditions

2.3.1. Pyrolysis-GC-MS

Analyses were carried out using an isothermal oven Frontier Lab py-3030S single shot pyrolyzer device coupled to an Agilent GC 7890B system, interfaced with an Agilent 5977N mass spectrum detector. Software used were respectively Py3030S Control (v. 1.77) from Frontier Laboratories and ChemStation v. F.01.03.2357 from Agilent. Pyrolysis was undertaken at 720 °C for 20 s, under an inert atmosphere, helium as carrier gas.

Separation was achieved on a HP-5MS capillary column (30 m x 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 programme was as follows: held at 50 °C for 2 min, increased at 10 °C/min to 230 °C, then 20 °C/min to 300 °C, then held for 5 min at 300 °C, making a total acquisition time of approximately 29 min. Considering 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.

2.3.2. Attenuated total reflectance (ATR)-FTIR

Infrared spectra were collected using a Nicolet iS50 FTIR spectrometer equipped with single-bounce diamond crystal ATR accessory. Data collection was carried out using the OMNIC software. Spectra were collected over the 4000–400 cm^{-1} range with 4 cm^{-1} resolution and 32 co-added scans. ATR correction was performed on all spectra to account for variations in penetration depth based upon wavelength.

Condoms were rubbed directly on the ATR crystal and analysed with no further preparation. All other products were applied as thin films to cover the ATR crystal and analysed with no further preparation. The sampling window was thoroughly cleaned using ethanol and lint-free tissue before each sample, and a background scan of the clean crystal was obtained between each replicate acquisition. For each sample, 5 replicates were acquired, to be able to statistically consider any sample variation.

2.4. Data processing

2.4.1. Qualitative analysis

Identification of the compounds was undertaken using three different mass spectral databases; NIST18 (*National Institute of Standards and Technology*), PP (*Pyrolysis Products*, in-house pyrolysis library) [34–36] and TOX3 (*Wiley Drug and Pesticides, Wiley138*), as well as comparison with retention time and mass spectra obtained from the analysis of bulk PDMS (Fig. 2), and published literature. However, the identification of pyrolysates is a difficult task as pyrograms are complex with numerous peaks. There were some compounds that were not identified using this methodology, and therefore are referred to as "Unknown" followed by their retention time.

Using Agilent ChemStation® software, areas of the target ions within all the acquired pyrograms were integrated for each peak. Peaks were selected as to be repeatable, and distinguishable from the background, over a threshold value of 30,000 A.U. Table 2 references the peaks and their parameters. Data were exported to Microsoft Excel, the whole dataset was normalised to the area sum, and the double square root was calculated prior to multivariate statistical processing.

2.4.2. Chemometrics

Principal component analysis (PCA) was undertaken on the normalised data, using the non-iterative partial least squares (NIPALS) algorithm. Three dimensional scores plots were used to visually explore the data structure and to assess the loadings of the main components. Firstly, groupings related to class (i.e. condom, lubricant, PHP) were examined, to determine if separations were clear or if overlaps between classes existed. The loadings plots related to these sample scores were evaluated to understand the variables most important for sample discrimination and to

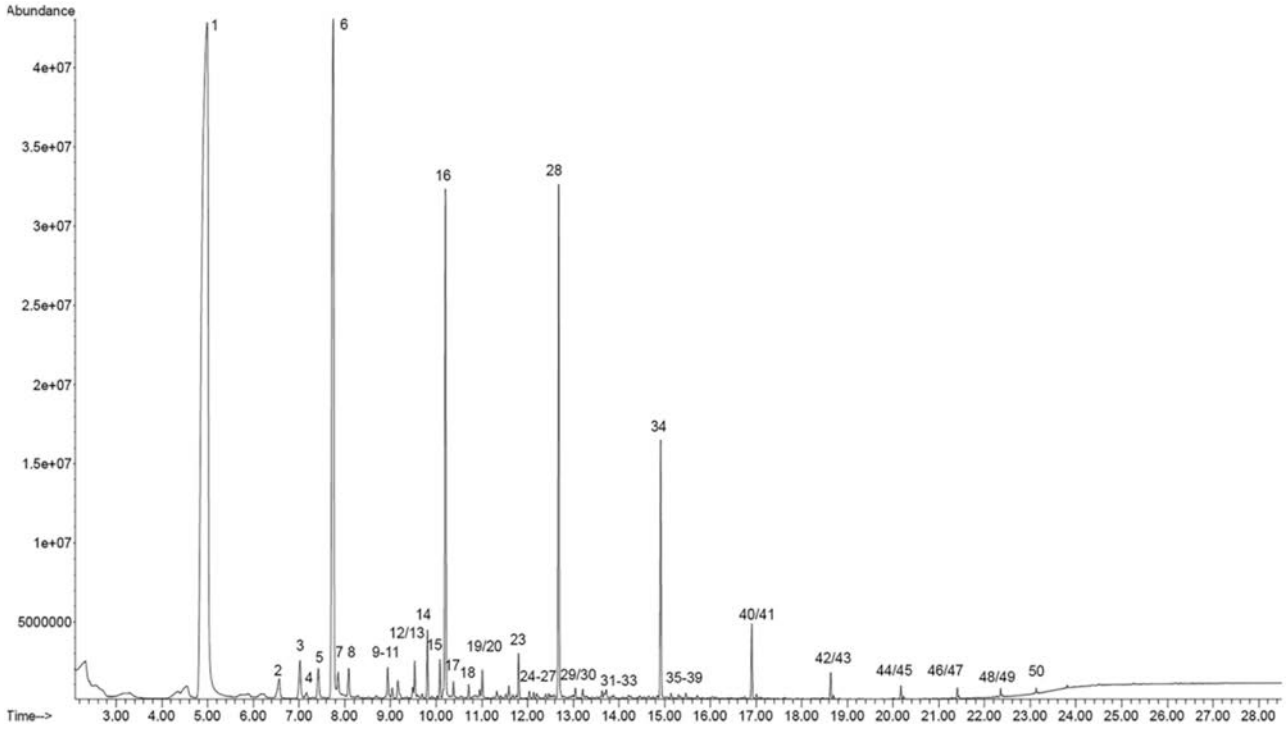

Fig. 2. Chromatographic pattern of PDMS 200 cSt reference, analysed under optimised conditions. Compounds were selected to create the extraction macro. Compound numbers relate to Table 2.

Table 2
Compounds selected for comparison. Names in brackets are suggestions for compounds that were not identified using databases or literature. Qualifiers are listed following their abundance ratio to target ion.

Nº	Name	RT (min)	Target ion (*m/z*)	Qualifiers (*m/z*)
1	D3	4.93	207	191, 133, 96
2	(linear D3)	6.56	207	193, 221, 177
3	Unknown 7.02	7.02	192	209, 97, 134
4	Unknown 7.16	7.16	267	193, 207, 281
5	Unknown 7.41	7.41	207	223, 191, 133
6	D4	7.73	281	265, 191, 249
7	Unknown 7.86	7.86	267	281, 250, 126
8	(linear D4)	8.08	281	265, 207, 133
9	Unknown 8.94	8.94	265	125, 249, 191
10	Unknown 9.03	9.03	207	193, 247, 176
11	Unknown 9.16	9.16	281	295, 233, 193
12	Unknown 9.48	9.48	267	250, 192, 126
13	Unknown 9.53	9.53	267	126, 250, 283
14	Unknown 9.80	9.80	341	325, 163, 73
15	Unknown 10.08	10.08	341	325, 163, 73
16	D5	10.21	355	267, 73, 251
17	(linear D5)	10.37	355	267, 250, 73
18	Unknown 10.71	10.71	355	267, 250, 73
19	Unknown 10.95	10.95	339	323, 162, 128
20	Unknown 11.00	11.00	339	323, 162, 154
21	Unknown 11.32	11.32	281	339, 267, 321
22	Unknown 11.59	11.59	341	324,163,73
23	Unknown 11.80	11.80	326	415, 73, 399
24	Unknown 12.04	12.04	326	415, 73, 398
25	Unknown 12.19	12.19	326	399, 415, 73
26	Unknown 12.40	12.40	326	415, 269, 253
27	Unknown 12.46	12.46	401	341, 429, 73
28	D6	12.67	341	429, 325, 147
29	(linear D6)	13.04	341	324, 429, 147
30	Unknown 13.21	13.21	413	324, 399, 73
31	Unknown 13.63	13.63	400	489, 326, 384
32	Unknown 13.67	13.67	324	413, 207, 190
33	Unknown 13.72	13.72	400	489, 326, 384
34	D7	14.90	415	281, 147, 326
35	(linear D7)	15.14	503	415, 147, 281
36	Unknown 15.29	15.29	399	487, 325, 147
37	Unknown 15.46	15.46	487	399, 147, 281
38	Unknown 15.71	15.71	475	147, 73, 400
39	Unknown 16.74	16.74	73	147, 221,281
40	D8	16.90	355	401, 281, 221
41	(linear D8)	17.00	221	147, 281, 355
42	D9	18.62	429	355, 221, 147
43	(linear D9)	18.70	221	355, 147, 429
44	D10	20.17	503	281, 355, 147
45	(linear D10)	20.23	533	281, 221, 147
46	D11	21.40	355	535, 147, 281
47	(linear D11)	21.44	429	355, 207, 281
48	D12	22.35	429	355, 207, 147
49	(linear D12)	22.38	207	281, 355, 429
50	D13	23.12	207	281, 355, 429

investigate the potential reduction in the number of variables. Finally, quadratic discriminant analysis (QDA) was undertaken on the entire dataset to build the classification model. Each variable was assumed to have equal a priori probabilities, with a variable weight of 1.0 for each variable. Both PCA and QDA were performed using the Unscrambler X v. 10.5 (Camo Software, Norway).

3. Results and discussion

3.1. Qualitative analysis

All of the samples were analysed in triplicate and the pyrograms were found to be repeatable between replicates in relation to the number of compounds detected, retention times and relative intensities. No major visual differences were noted in the pyrograms of the condom extracts. However, before the appearance of compound D3, i.e. before 4.9 min, peaks of hexan-2,5-dione were identified with a high-quality ranking in the databases. They were found not to be reproducible between replicates. Non evaporated samples were run and their chemical profile was compared to the one of evaporated samples to investigate if this compound could originate from a solvent issue. Chemical profile were found to be affected if the evaporation was incomplete (data shown in Supplementary Information). It is therefore possible to state that this is more likely that this compound is derived from the pyrolysis and recombination of hexane and hence, was likely due to the solvent not sufficiently evaporating prior to pyrolysis, than from any other compounds pyrolysis.

Peaks that were repeatable in the condom samples were characterised using the various databases available as well as published literature. Up to 50 compounds were found in the chromatographic pattern obtained from PDMS 200 cSt (Fig. 2), with 10 of them being identified as cyclic oligomers generated during the pyrolysis of PDMS, i.e. D3-D13. The remaining 40 compounds could not be conclusively identified, as was mentioned previously. However, by examining the mass spectra and the literature [37] these compounds were consistent with originating from siloxane degradations, and not from other compounds. The retention time and mass spectra were repeatable enough to be used to ensure a proper integration of the compounds. The %RSD for the integrated abundance of the chromatographic peaks in TIC mode were found to be lower than 5% for most of the compounds. Table 2 lists the 50 compounds integrated and extracted for further statistical analysis.

The study of the pyrograms obtained for the 70 samples analysed revealed at least 6 different profiles, which are illustrated in Fig. 3 respectively, amongst a population which presented undistinguished patterns when run in ATR-FTIR (data not shown). Although condom samples did not present any significant visual differences in their chemical profiles, personal lubricants were found to produce different profiles. Most condoms presented a pattern as illustrated in Fig. 3A, with the exception of two condoms, Ceylor Gold and Fair-Squared Sensitive Dry, which presented slightly different chemical profiles, including the presence of PDMS oligomers (Fig. 3B). These condoms were however already differentiated using their FTIR spectra (data not shown) but were run to confirm the observation. Further investigation showed these profiles differed due to terminated silicones, with methylterminated silicones present in Fig. 3A and hydroxyterminated silicones present in Fig. 3B. Amongst the silicone-containing personal lubricant populations, four different profiles were observed, one of them being indistinguishable to condom pyrograms (Fig. 3A) and the three others being visually discriminated (Fig. 3C–E) based on the presence or absence of peaks in the pyrograms. The sixth reported profile (Fig. 3F) was observed for the FemFresh sample, which indicates that this either did not contain a silicone lubricant, or it was present below the limit of detection.

The observation of these different profiles within a population of silicone-based products is an important point to consider. From a qualitative point of view, condom presented two types of profiles according to silicone content. Methylterminated-silicone lubricated condoms presented indistinguishable chromatographic profiles as presented in Fig. 3A. Multiple condom brands chromatograms are gathered in Supplementary Information. Some condoms presented hydroxyterminated silicone lubricant, which allowed the distinction due to a different distribution pattern. Other sources presented different profiles based on the presence or absence of given components in the pyrograms (Fig. 3C). Chromatographic patterns of major cyclic oligomers observed in Fig. 3C and E were very similar. The main difference between these two patterns was due to the minor compounds present, which differed either in terms of presence or absence of peaks, or in terms of relative abundance. Most of the samples contained the same oligomeric degradation pattern, from D3-D6, and the major variations were within the rest of the oligomers (D7-D13), as well as in the minor compounds detected. Fig. 3D also a huge

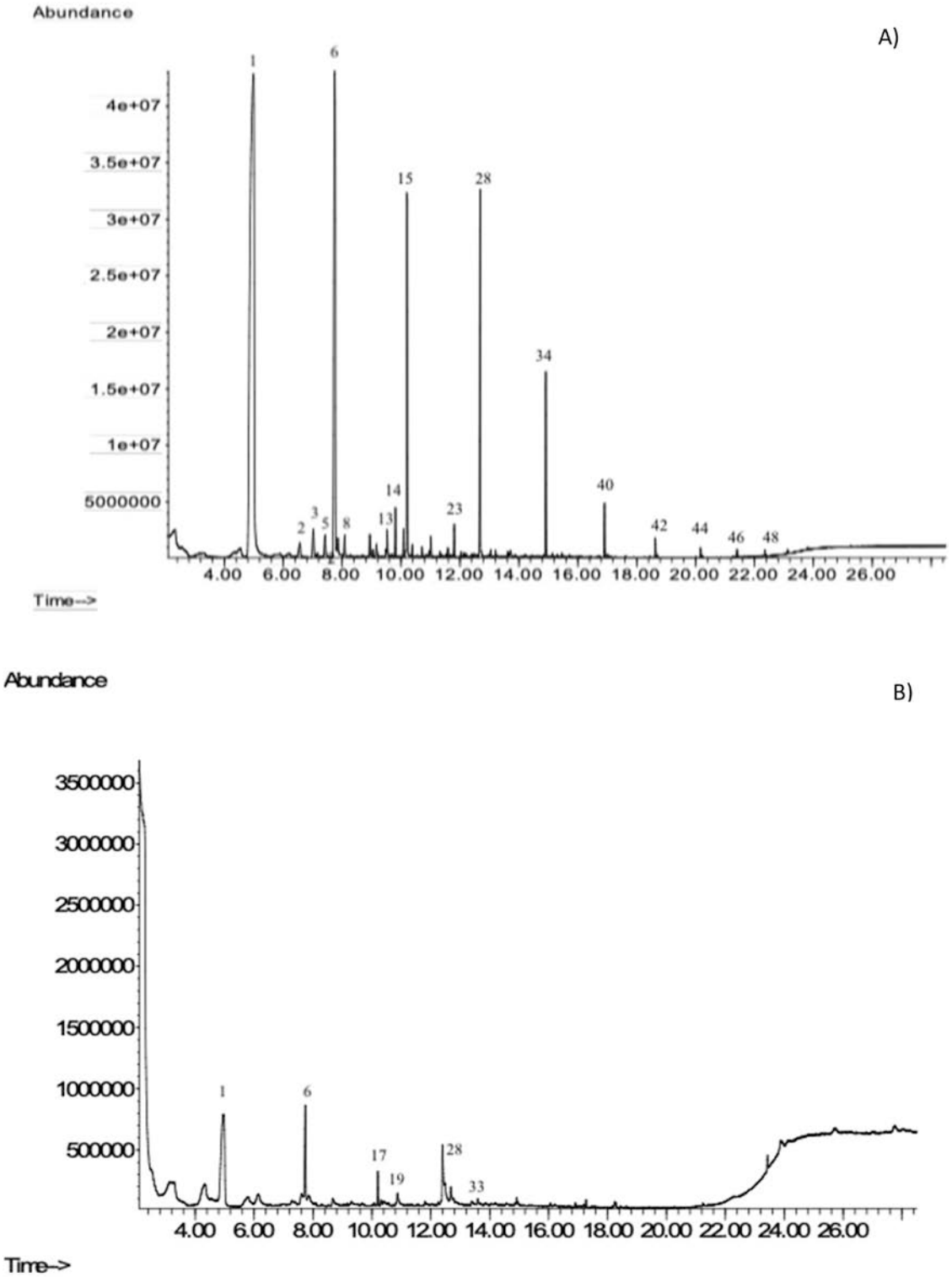

Fig. 3. Illustration of the different chemical profiles observed amongst the dataset. (A) Condom profile (Sample 01-Durex Classic)- methylterminated silicone, (B) condom profile (Sample 124-Ceylor Gold) – hydroxyterminated silicone, (C) lubricant type 1 profile (Sample 109 - Ansell LifeStyles Luxe Silicone-based lubricant), (D) lubricant type 2 profile (Sample 171 - Astroglide Diamond Silicone Gel Personal Lubricant), (E) lubricant type 3 profile (Sample 174 - Astroglide Waterproof Silicone Liquid), (F) profile obtained for Sample 156 - FemFresh Feminine deodorant. Some compounds are annotated with numbers corresponding to Table 2.

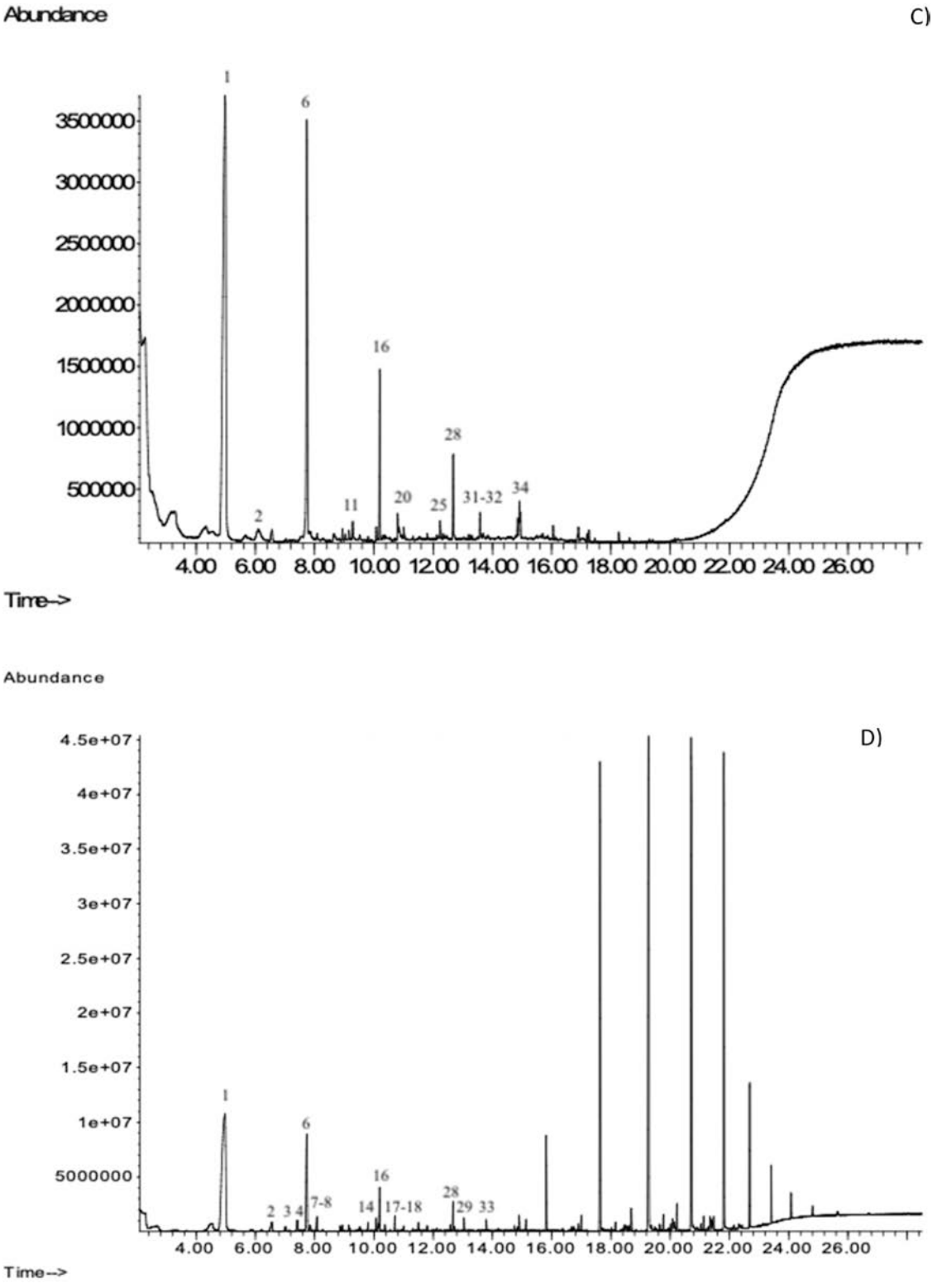

Fig. 3. (*continued*)

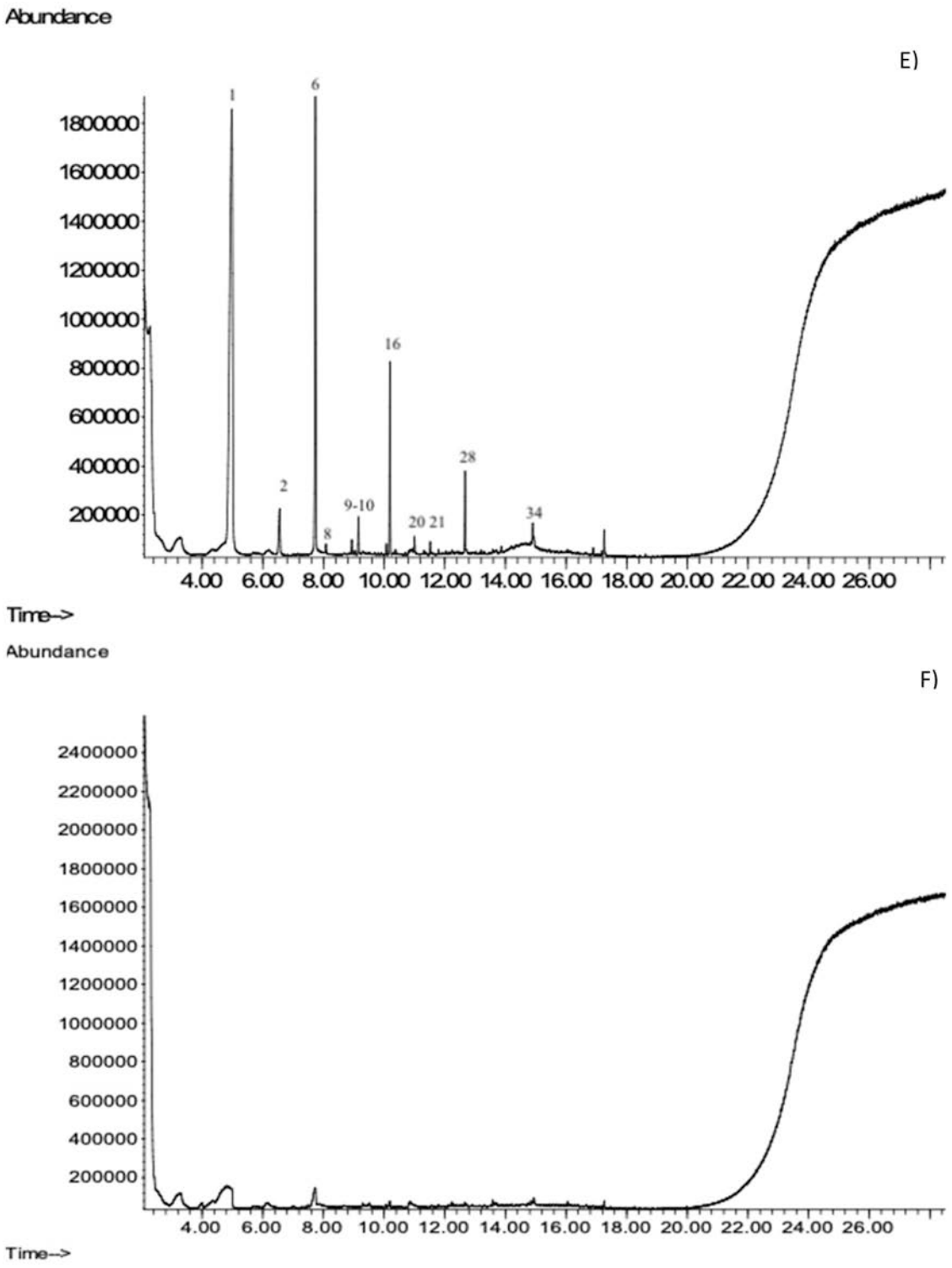

Fig. 3. (*continued*)

distribution of 9 additional peaks visually distinguished from the rest of the sample set. These peaks are not present in Table 2 as they occurred in only one sample, and hence were not included. These peaks presented similar mass spectra and different retention time. Comparison of the mass spectra with the NIST database allow to asses they were long chain silicones: respectively hexa- (15.712 min), hepta- (17.632 min), octa- (19.270 min), nona- (20.712 min), deca- (21.810 min) and undeca- (22.683) siloxanes. This suggests that silicones of different chain lengths, and therefore of different viscosities, were used for different products [30,31].

Since the silicone-containing condoms all resulted in indistinguishable pyrograms, regardless of brand or type, it may be assumed that minor compounds, such as flavours or dyes, were not extracted by hexane. This was expected, due to the polarity of these compounds, and indicates that a polar solvent extraction followed by conventional GC-MS analysis should be considered in order to

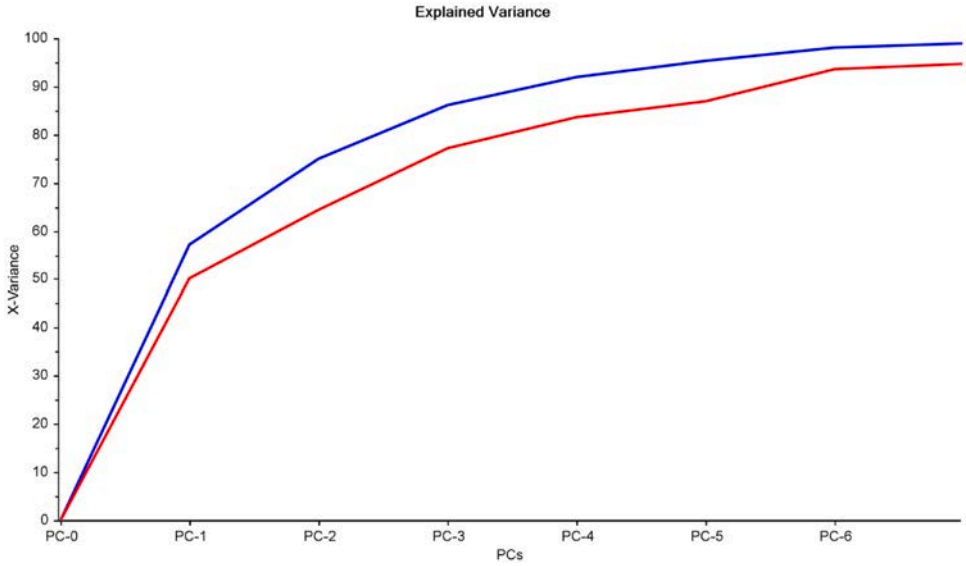

Fig. 4. Scree plot depicting the cumulative variance in the dataset retained by each PC. The red curve represents the first model with 50 PC and the blue curve the one with 10 PC. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

understand the exact composition of the sample. This may also further isolate compounds of interest for the discrimination of samples within classes. However, this must be handled with caution, as the likelihood of the transfer and persistence of these minor compounds is unknown, and they may potentially not be found in trace swabs.

Condoms are a mass-produced product in a regulated industry. Observations of visually indistinguishable profiles confirms the hypothesis that either very similar formulations are used by manufacturers or that all the PDMS used may come from one chemical manufacturer. Both hypotheses are possible as the number of PDMS suppliers around the world is unknown. Although DNA is the most common evidence to be collected from condoms, lubricants can also be used for both investigative and evaluative purposes. If no DNA is detected, the presence of a condom lubricant can infer the use of a condom and provide a possible explanation for the lack of any DNA. In these cases, the py-GC technique can answer questions of interest as the presence of diagnostic patterns from condom lubricants can infer the use of a condom and potentially the profile can be linked to one of the condom profiles in the database. The chemical profiles obtained from condoms were indistinguishable between condom manufacturers, and the chemical profiles obtained from personal lubricant were, in most cases, distinguishable from the condom ones. However, the initial target molecule was found to be the same, i.e. PDMS. Therefore, the hypothesis that the manufacturers use the same source of PDMS for condoms may be true. These observations also suggest that the PDMS used in lubricants is different to that used on condoms, in terms of viscosity and chain length, which results in the generation of different chemical profiles. Although pyrolysis is affected by the concentration of the sample [38,39], previous researches reported that higher viscosity lubricants produce more higher molecular weight cyclic oligomers (such as D9-D13) than lower viscosity lubricants [30,31]. In addition, it is known that peak area is linked to viscosity, the higher the viscosity, the higher the peak area of the cyclic oligomers, especially for the D3 oligomer [30]. This is a very interesting point to consider when it comes to a potential discrimination of the samples. The use of chemometrics and statistics was applied to evaluate the potential discrimination and classification of the samples constituting the dataset.

3.2. Sample discrimination

PCA was first performed on the entire dataset and considering all the 50 compounds listed in Table 2 after normalisation. Using 7 principal components explained up to 86% of the total variance of the dataset. After analysing the correlation of the variables using the loading plots, and considering the coefficient of variations for each compound as described by [33,40,41], a reduction of the variables set to 10 variables explained 99% of the variance of the dataset. Retained variables were D3, D4, D5, D6, linear D3, Unknown 7.02, Unknown 7.41, Unknown 7.86, linear D4 and Unknown 9.80.

Within this model, the first four principal components (PC) accounted for 94% of the total variance of the dataset, as illustrated by the scree plot (Fig. 4). Increasing the number of principal components up to 7 explained 99% of the variance of the dataset.

Fig. 5 shows the scores for the first 3 PCs (Fig. 5A) and for PC 1, 2 and 4 (Fig. 5B). Discrimination was not enhanced using supplementary principal components.

As illustrated in Fig. 5, condom samples were found to form two clusters: the first one from samples presenting the clear diagnostic pattern of siloxane degradation, and the second containing only 2 samples (FairSquared Sensitive Dry and Ceylor Gold) presenting unclear patterns of siloxane degradation, with significant variations of peak concentrations (see Fig. 3B). If FairSquared Sensitive Dry did not present any evidence of the presence of silicone compounds, Ceylor Gold presented a pattern different from the CH_3-PDMS (Fig. 6) but indistinguishable from OH-PDMS as illustrated in [42].

The pyrogram obtained from Ceylor Gold showed traces of cyclic oligomers (D3, D4 D5 and D6) in significantly lower amounts than what was previously observed for condom samples. A GC-MS analysis of polar compounds, extracted with methanol, detected a water-based lubricant (i.e. polyethylene glycol) and spermicide (i.e nonoxynol-9) in the Ceylor Gold condom. FairSquared Sensitive Dry sample was difficult to analyse, as it is a dry condom. Dry condoms are usually not lubricated with classic lubricants (i.e. PDMS, Glycerol, PEG) but contain significant amounts of solid particles (e.g. polyethylene powder, cornstarch), which are not expected to be detected with the method presented here, as they are not extracted with hexane [37]. However, traces of cyclic oligomers D3-D6 were observed, in very low concentrations. No traces of solid particles, such

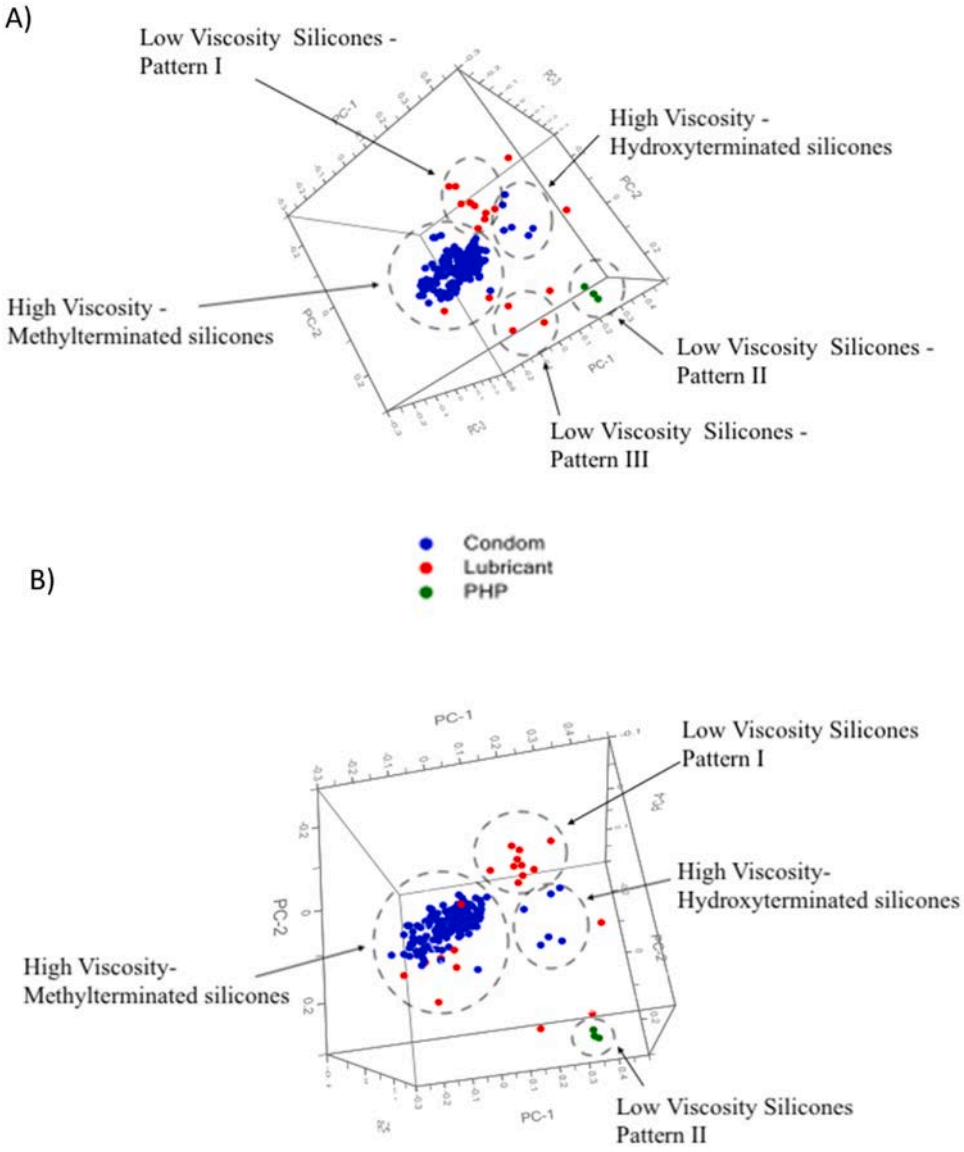

Fig. 5. 3-dimensional scores plot showing the distribution of the data collected from the 70 samples constituting the dataset. (A) Along PC1, PC2 and PC3, (B) along PC1, PC2 and PC4. In blue are the condoms, green the personal hygiene products and red the lubricants. Only one PHP sample is presented, as Sample 156-FemFresh Deodorant could not be properly analysed, due to solubilisation issues. Dotted circles indicate clusters assumer to represent a same population. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

as furfural, furaldehyde or furane derivatives, were found in the pyrolysis patterns. In the eventuality of the use of a dry condom in an alleged assault, stereomicroscopy and microscopy should be used to help detect these particles [2,24,43,44], as they are not being specifically extracted and/or detected using the hexane/Py-GC-MS protocol. These two samples are challenging, as their profiles might be misinterpreted as a "negative" profile when present in real world samples, given the low concentration of silicone lubricants. Hence, the absence of chemical residues should be carefully evaluated in the forensic context, with other techniques used to detect other types of compounds, or an evaluation of the factors affecting transfer and persistence of the samples in the matrix.

Within the cluster containing the majority of the condom samples, it was determined that one replicate from sample 40 (Durex Confidence) was slightly separated from the rest of the dataset, but not enough to be clustered with the other samples. It was found to be very close to lubricant sample 171 (Astroglide Diamond Silicone Gel Personal Lubricant). This replicate can be considered an outlier, possibly due to a cross contamination and was removed from classification models using the classification algorithm. Replicates from sample 64 (Lifestyles Assorted Choc Ripple Ribbed) were found to be slightly separated from the major condom group, as their scores along PC1, 2 and 3 were observably different. Two replicates presented a positive value along PC1, whereas the rest of the condoms were found to have negative values along this PC. Similarly, values along PC2 for most condoms were around 0.002 and was over 0.1 for sample 64. The PC3 value for sample 64 was negative, whereas all other condoms had positive values. As the pyrogram for sample 64 was visually similar to all the other condoms, flavourings were not considered as the source of the observed classification difference. A possible hypothesis is that changes in the structure of the latex (i.e. ribbed condoms) generated variations in the amount of lubricant that can be added, resulting in this condom being distinguished from the rest of the sample set. However, the other ribbed condom present (sample 184 – Ansell LifeStyles Ribbed Condoms) was not found to be distinguished from the rest of the dataset.

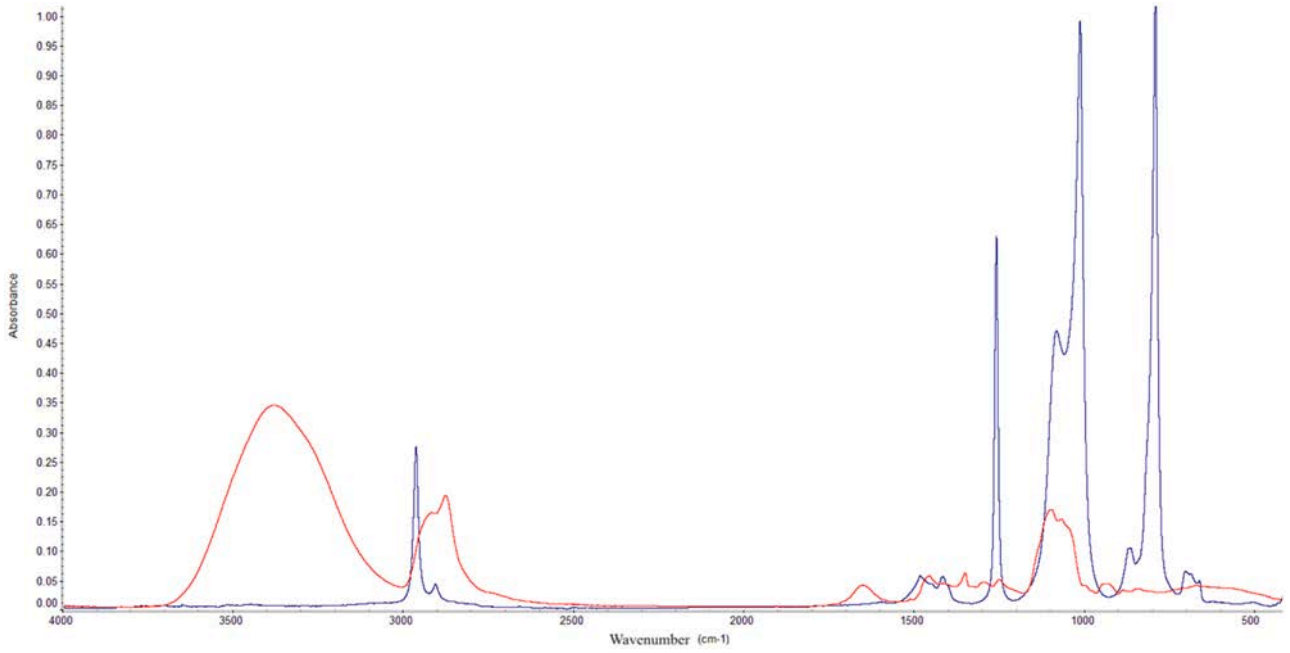

Fig. 6. ATR-FTIR spectra of Ceylor Gold sample (in red), and a silicone-based sample (in blue) for comparison. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Therefore, except for condoms presenting visual distinguishable patterns, it was not possible to differentiate silicone-based lubricated condoms.

Lubricants initially presented several visually different patterns. The statistical model confirmed that it was possible to differentiate the samples, mainly into 3 groups. One lubricant, sample 181 (Durex Perfect Play Glide), was found to cluster with condom samples, and could not be separated with subsequent PCs. When examining the chemical profile, there was no difference noted between this profile and the ones coming from the condoms. It was the only lubricant that was classified with condom samples. Sample 171 (Astroglide Diamond Silicone Gel Personal Lubricant) was found to separate to an isolated cluster, close to the condom samples. However, PC2 and PC4 helped separate this sample from the condom sample set. As the chemical profiles obtained for lubricants were differentiated, visually, statistically and semi-quantitatively, it can therefore be concluded that in general, the chemical profiles of condoms differ from those of lubricants, although in both types a silicone composition is observed. This suggests that silicones of different chain lengths, and therefore different viscosities, are used for different products.

Three outliers from the groupings were noted, which were replicate 2 from sample 172 (Astroglide Gel Personal Lubricant), replicate 3 from sample 109 (Ansell LifeStyles Luxe Silicone-based Lubricant) and replicate 2 from sample 168 (Ansell Skyn Intimate Moments). These replicates were significantly spread and plotted away from the other replicates of the same samples, thus indicating that there may have been some variation, either at the acquisition of the chromatogram or during the extraction of the data procedure. These samples were not considered in the classification steps. Regarding sample 172, the pattern of silicone peaks was close to the background and hence, may not be detected in casework. In addition, sample 172 was a water-based lubricant, thus GC-MS analysis may be more appropriate for analysis and interpretation than py-GC-MS [45]. Therefore, it was removed from the sample set when performing the classification process.

The smallest class of the dataset, personal hygiene products, was under-represented in this model. Indeed, only one of the 70 samples available in the initial dataset presented a silicone-based chemical profile when analysed with a screening method, such as FTIR [25]. The chemical profile obtained for this sample 156 (FemFresh Intimate Deodorant) was inconclusive, resulting in it being clustered significantly separately to the rest of the dataset. Replicates showed a higher variability than for the rest of the dataset, and the detection of silicones was found to be inconsistent between analyses. This might be due to its aerosol nature, which made it quite challenging to collect sufficient residues for analysis. However, from the chemical and statistical point of view, the residues from this sample were found to be distinguishable from the rest of the dataset.

Finally, all seventy samples constituting the dataset were grouped into five categories that were observed on the overall dataset using both qualitative and statistical analyses, the lists of which are presented in Table 3. Results gathered from Table 3 shows that 85.7% of the silicone profiles observed are belonging to Group 1, 7.14% to Group 2, 2.86% to Group 3, 1.43% to Group 4% and 2.86% to Group 5. 96.72% of condom present a Group 1 profile, and the left-over condoms present a Group 3 profile. Amongst the lubricant population, 14.28% presented a Group 1 profile, 71.42% a Group 2 profile, 14.28% a Group 4 profile. Group 5 was exclusively made of personal hygiene products.

The discriminating power of the method was calculated, and was found to be 0.26 for the overall groupings. This is not really high, but is not surprising either, firstly because condoms are overrepresented in the dataset, and secondly because ISO norms regulations on condom manufacturing make it harder to find different chemical profile in a condom population. The discrimination of the samples

Table 3
Summary of the sample(s) comprised in each grouping, considering that all samples were containing silicones.

Group	Source of the sample	Samples in the group
Group 1	Condom (59) Lubricants (1)	Samples 1, 10, 11, 12, 13, 14, 20, 23, 24, 33, 34, 36, 38, 40–50, 53–56, 58–66, 68, 69, 79, 110–115, 117–123, 125–127, 130, 178–184
Group 2	Lubricants (5)	Samples 109, 133, 168, 172, 174
Group 3	Condom (2)	Samples 116, 124
Group 4	Lubricants (1)	Sample 171
Group 5	PHP (2)	Sample 156, 158

Table 4
Confusion matrix for discriminant analysis applied on the entire dataset, constituted of the replicates of the 68 samples, classification based on the class using QDA algorithm.

QDA	Condom	Lubricant
Condom	169	1
Lubricant	4	16

contained in Group 1 could probably be enhanced by the use of other instrumentations, such as DART-MS as described by Baumgarten et al. [9], but such a choice should be dictated by the question targeted by the forensic scientist.

3.3. Classification model

Replicates of the seventy samples were classified according to the class to which they belonged. However, considering that the personal hygiene product class contained only 2 samples clearly distinguished from the rest of the dataset, they were removed from the classification model. Thus, the samples were grouped into two categories. The quadratic discriminant analysis was applied to the scores of the first four principal components, since these were necessary for the separation of the samples, using 2/3 of the dataset as the training set and 1/3 of the dataset as the validation set. A good classification rate of 97.37% was obtained. The confusion matrix is presented in Table 4.

The misclassified replicates were evaluated. One replicate of Sample 40 (Durex Confidence) was classified in the lubricant category. The PCA results indicated a very close proximity between the chemical profile of this replicate and that of sample 171. The replicate of sample 40 that misclassified was the closest to the replicates of sample 171.

Two replicates of sample 64 (Ansell Lifestyles - Assorted - Choc Ripple Ribbed) were classified in the lubricant category instead of the condom category. These samples were not outliers. The results of the PCA showed that the chemical profiles of these condoms were slightly separated from the condom population to which they were expected to belong, although it was not possible to assign them to another class. The centroid of the condom group was found to be located around 0.005 along PC1 and − 0.018 along PC2, whereas the lubricant group was located around 0.17 along PC1 and − 0.06 along PC2. Classification values obtained for the two replicates were found to be negative for clustering to the condom group and were found to be positive for clustering with the lubricant group, the distance being 0.05 to the condom group, and 0.11 to the lubricant group for the first replicate, and 0.04 and 0.12 for the second. The third replicate presented eigenvalues of − 0.007 and 0.03 along PC1 and PC2, making this sample closely clustered with the condom class. Given the difference in coefficient of distance to the centroid, this suggests that an additional class should be suspected. As previously stated, such a class could be due to changes in the structure of the latex (i.e. ribbed condoms), which would generate variations in the amount of lubricant that can be added to it, thus they could be distinguished from the rest of the sample set. This hypothesis is also suggested as it is known that pyrolysis is sensitive to the amount of sample deposited in the cup, as illustrated by previous publications [23,32,33,46]. Therefore, a difference in the concentration is likely to generate a difference in the amount of sample in the cup after evaporation and thus affect the chemical profile.

A replicate of Sample 60 (Ansell Lifestyles - Assorted - Banana Bump Studded) was classified into the lubricant category, instead of the condom category. Visual examination of the chemical profile did not allow it to be distinguished from the rest of the condom population. The results of the PCA showed that the chemical profile of this condom was slightly separated from the condom population to which it was expected to belong. However, the other 2 replicates were clustered appropriately within the condom population. These observations correspond to the previous ribbed sample that also had classification issues, reinforcing the hypothesis that changes in the structure of the latex (i.e. ribbed condoms), would generate variations in the amount of lubricant and therefore in the discrimination and classification patterns.

One replicate of Sample 168 (Ansell Skyn Intimate Moments) was classified in the condom category, instead of the lubricant category. The PCA results indicated proximity between the chemical profile of this replicate and that of the condom population. The classification results are thus compatible with what was observed for the PCA, and it was not surprising that the classification model was not able to correctly classify this sample.

The three replicates of sample 181 (Durex Perfect Play Glide), which presented a chemical profile different to the condom ones, were correctly classified in the lubricant category. Visual analysis and observation of the PCA scores plot indicated that the chemical profile of this silicone-based lubricant was indistinguishable to the chemical profiles obtained for condom-type samples. However, the QDA algorithm correctly clustered these samples in the lubricant classes. Evaluation of the eigenvalues showed out that the separation was led along PC4, with the condom groups presenting an average value of 0.00029 and the lubricants one of 0.023. The sample eigenvalue was 0.04 (± 0.01) which makes it cluster to the lubricants class. This is surprising, since it would have been reasonable to expect these samples to be misclassified. The algorithm was able to differentiate samples that were very close during the analysis by principal component, however this may not be the case when additional samples are added to the dataset. In addition, the distance between this sample and the centroid of the lubricant samples is smaller than the distance between the sample and the centroid of the condom samples, which is not surprising considering the variability coming out of the condom cluster. These observations reveal that QDA is reinforcing the quality of the classification procedure, as visual analysis of the data, or investigation of the eigenvalues might not be sufficient to classify the samples in the correct classes.

The results previously discussed show that the quadratic discriminant analysis provided results corresponding to what had been observed for PCA. Most false classifications were one of the replicates of a sample having slightly variable characteristics, leading to a correlation to samples within the population of another class. The study of misclassifications, supported by the results of the PCA, indicates that these samples generally differ not in terms of their visual chemical profile but in semi-quantitative terms. These differences can generally be explained by analytical and operator variations, such as spiking reproducibility or manual integration of the peaks, especially considering the great variability that occurs in pyrolysis events. Variations in the quantitative amounts present in the various samples may also explain the observed variations, although the chemical profiles do not differ significantly.

The classification model based on classes of silicone-based samples can thus be validly used to predict the class (i.e. condom or lubricant) of a trace whose origin is unknown. Two limitations to the use of this model can be encountered. The main limitation is that the detection of the fifty peaks used to build the model may not be present in real cases, since the interaction with the vaginal matrix has not, at present, been fully examined. A focus on the major cyclic oligomers is recommended, and this is the reason why the presented model was built only with 10 out of 50 compounds. The second limitation is that, the proportion of lubricants and intimate hygiene products based on silicones is relatively low and consequently, it is possible that other samples present on the market may contain different chemical profiles. On the opposite, the condom population included different brands and types, flavoured and coloured

samples, those containing specific additives, with or without latex and a range of prices to be the most representative. However, a comprehensive model may not be feasible, the number of brands and types on the market being relatively large (more than 200 products on the Swiss market, and almost as many on the Australian market). In addition, new products are frequently released on the market and hence a continuous update of the model may prove necessary, although the list of authorised lubricants is not constantly changing.

The discriminant analysis models were used to assess the possibility of statistically differentiating the samples from the dataset, based on their chemical profiles. The main conclusion from these models is that condoms of different brands and types, the lubricant of which is based on silicones, are generally not differentiable. Samples of different classes that do not differ qualitatively can be differentiated. Samples of different sources (condom, lubricants, PHP) that show slight differences in the level of minor compounds can generally be differentiated, but only if these compounds are detected. Application to casework revealed that these minor compounds were also observed when trace evidence was analysed. When considering a possible use to courtroom, the forensic expert could either use the classification scheme with reported error rates or choose to use a Bayesian approach to provide the court with a likelihood ratio on the source level. Examples of classification using Bayesian framework are presented in many different areas of forensic sciences and could easily be derived for an application to condom evidence [47–49]. However, further research are required regarding other pending questions such as background, transfer and persistence of the evidence in a vaginal matrix, so as to be able to provide the Court a more adequate information on the observed evidence.

3.4. Investigation of brand discrimination

Although the results of the qualitative analysis did not reveal any profiles specific to a type of condom or to a brand, it was of interest to investigate potential brand discriminations. The results of the PCA did not reveal any features that would allow separation by brand or type (Fig. 7). The chemical profiles of different condoms do not differ significantly between brands, nor do the chemical profiles of condoms of different types within the same brand. These findings indicate that the variation between the different condom manufacturers is very small. This is likely due to the very high level of control and international regulations for the production of condoms and limited PDMS suppliers [14–17].

Under these conditions, the classification model has 14 categories. LDA and QDA were not attempted as some classes contained too few samples for reliable modelling. The classification performed with an SVM model gave a classification rate of 54.74%, with 51.05% for cross validation. The classification rate was not satisfactory, but this was not surprising in view of the observations highlighted during the PCA. Multiple other discriminations were investigated, such as the purchasing location, whether different models coming from a same brand were different, or even if same brand, same model and different production could be distinguished, but none of these were successful.

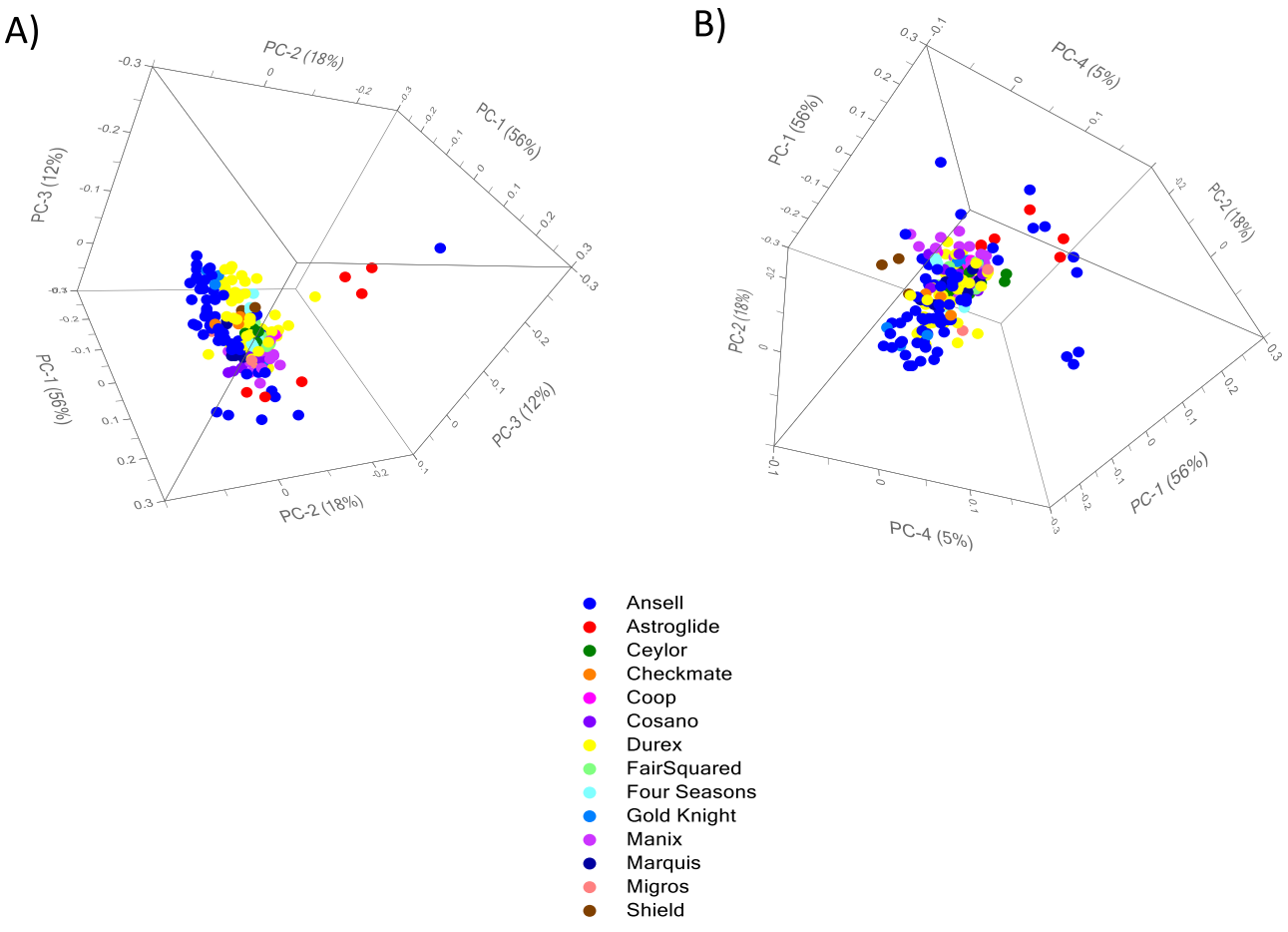

Fig. 7. 3-dimensional scores plot showing the distribution of the data collected from the 70 samples constituting the dataset. (A) Along PC1, PC2 and PC3, (B) along PC1, PC2 and PC4. Classification based on the brand.

4. Conclusion

In this study, 70 samples consisting of condoms, lubricants and personal hygiene products containing silicone, and purchased on 3 different market, were analysed to evaluate the potential discrimination of these samples. To achieve this goal, py-GC-MS was used as it is known to be able to detect siloxanes with good sensitivity. Based on chemical compositions, at least 6 different groups were observed, and it was found that some lubricants were belonging to the same group than condom, due to similarities in compositions.

Chemical profiles were found to be repeatable with good resolution obtained in all the pyrograms. Upon visual examination, pyrograms were dominated by cyclic oligomers resulting from the degradation of PDMS, but another 40 compounds were also detected, without being linked to any specific molecule. Only 10 out of 50 compounds were necessary to obtain a good discrimination, the rest of it not allowing to enhance sample separation in the dataset. No traces of aroma, flavourings, colourants or water-based residues were detected in the pyrograms of the samples, highlighting the need for complementary methods, such as GC/MS or ATR-FTIR, if these compounds are of interest.

This study confirms that pyrolysis GC-MS is one of the most suitable techniques for PDMS lubricant analysis. However, it does not solve the question of water-based lubricated condoms and non-lubricated condoms, outlining the need for further research for these sample types. The methodology was found to be applicable in casework situation. Future research is needed to investigate transfer interaction with the vaginal matrix, and other persistence factors to evaluate full applicability to casework, including relevant interpretation of the evidence. This will provide a baseline to implement this analysis in real world cases.

CRediT authorship contribution statement

Céline Burnier: Writing - original draft**. Geneviève Massonnet, Sally Coulson, Kari Pitts, David DeTata** : Writing/Reviewing. **Kari Pitts, David DeTata, Sally Coulson:** Conceptualization. **Céline Burnier, Kari Pitts, David DeTata:** Methodology. **Céline Burnier:** Validation, Investigation, Data curation, Visualization. **Kari Pitts, David DeTata, Sally Coulson:** Resources. **Kari Pitts, David DeTata:** 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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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.forsciint.2021.110793.

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Analysis of condom evidence in forensic science: Background survey of the human vaginal matrix using DRIFTS and pyrolysis-GC/MS

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ABSTRACT

Condom traces are increasingly detected from victims of sexual assault, mostly from vaginal swabs. Protocols have been developed for the analysis of silicone-based condom lubricants using DRIFTS-FTIR and py-GC/MS, but very little research is concerned with the background contribution of the vaginal matrix itself. The present contribution would be an asset for more fundamental research on condom residues in the vaginal matrix, as well as for interpretative purposes in the forensic area. This study investigated vaginal matrix residues using Diffuse Reflectance Fourier Transform Infrared Spectroscopy (DRIFTS-FTIR) and pyrolysis Gas-Chromatography coupled to Mass Spectrometry (py-GC/MS) to obtain fundamental information about the vaginal matrix's initial composition. Differences between women of a given population were investigated as well as the prevalence of silicone-based residues for natural purposes in the population. Apolar fractions of the samples were investigated after extraction with hexane, as it is the one targeted for silicone-based lubricants used in condoms. Infrared spectroscopy outlined the presence of various proteins and lipids in all the samples, and the spectral regions 1000–1850 cm^{-1} and 2700–3600 cm^{-1} were identified as the most relevant zones of the spectra. Pyrolysis-GC results confirmed the presence of lipids, more specifically the presence of cholesterol residues. Chemometrics analyses showed that it was not possible to distinguish the samples based on the qualitative nor semi-quantitative content. This suggest that the same type of compounds are extracted regardless of the donor. None of the samples were found to contain any silicones residues. These results are promising from a forensic evidence interpretation perspective. Further research is required to fully validate such models and assess their robustness and limitation in casework conditions.

1. Introduction

In recent decades, analysis of the chemical composition of condom evidence has seen increasing focus in forensic research. This in an effort to be able to successfully analyze condom lubricants, more specifically silicone-based lubricants, and overcome analytical issues facing condom evidence. These lubricants were found to be preferentially extracted with hexane and detected by Diffuse Reflectance Infrared Fourier Transform System (DRIFTS-FTIR) and pyrolysis-gas chromatography-mass spectrometry (py-GC/MS). It is recognized that there is a need to identify not only the analytical parameters that would allow evidence to be detected, but also the factors that would influence their detection in casework [1]. Previous researchers have highlighted issues regarding the lack of knowledge on the interaction between the target compounds and the vaginal matrix [1–3]. Other investigators have conducted research to answer questions concerning the transfer and persistence of condom residues [4–6], but the question of the background is still pending.

The vaginal matrix is of particular interest as this is the most likely support of condom traces that can be encountered in casework [7–10]. This matrix is made up of a stratified squamous epithelium, characteristic of the mucous membranes that are found in moist areas of the human body. The epithelial tissues have a polarity: the apical surface is exposed outside the organism, and the basal surface is attached to the underlying connective and muscle tissue. The cells of the apical surface carry for the most part microvilli, increasing the surface area and thus to improve the absorption of substances. The basal surface lies on top of the basal lamina, a support sheet composed of glycoproteins and collagen fibers. The basal lamina acts as a scaffolding allowing the migration of epithelial cells for the growth or repair of organs. The epithelia are innervated but are not

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vascularized. The cells are then nourished by substances which diffuse from the underlying connective tissue. The further the cells are from the basement membrane, the less they are nourished by the connective tissue, and the less viable they are [11]. The cells of the apical surface are constantly abraded and replaced by the process of mitosis of the cells of the basement membrane. The cells of the surface layer are rich in glycogen, to be able to survive. The glycogen produced by the epithelial cells is used to protect the vagina. The lactobacilli present naturally in the vagina anaerobically metabolize glycogen to lactic acid, giving the vagina an acidic environment, with a pH of 4.0–5.0. This acidity is toxic to sperm, but it helps protect the vagina against infections [11–13]. At the same time, the vaginal mucosa is permanently moistened despite the absence of glands, thanks to three types of secretions: cervical mucus, mucus and transudate. Cervical mucus is secreted by glands located at the level of the cervix and flows along the vaginal wall, thus implying a constant humidification of the mucous membrane. The secretions are however subject to physiological variations due in particular to the period of the ovulatory cycle, the taking of hormones (e.g. HRT, contraceptives) or sexual arousal [12,14–16]. Mucus and transudate are produced during sexual arousal. Mucus comes from the vestibular glands located on either side of the vaginal opening. Transudate is produced by the venous system and which percolates through the mucosa by osmosis. It then flows from the walls of the vagina, ensuring good lubrication [11,17]. The exact composition of the vaginal matrix is not completely known. However, some articles have listed and reported compounds that can be easily detected, and they are listed in Table 1.

There are many reported medical studies investigating changes in the vaginal matrix under certain circumstances, such as infections, diseases, pregnancy or surgery. However, none of these studies report observations of spectral or chromatographic data that would provide information regarding possible siloxane content. In 2015, Orphanou [21] analysed various human secretions, including vaginal secretions, using ATR-FTIR spectroscopy and broadly reported the variations that could be observed, which are very relevant for forensic purposes. Since then, most research regarding condom evidence has focused on the development of analytical techniques to enable the detection of condom residues, but interactions with the vaginal matrix have only recently been reported [6]. However, to assist the interpretation of evidence [1,22,23], there is a need to conduct a prevalence study as well as report possible interactions, or specific problems linked to interactions, that may be observed with condom target compounds.

We present a comprehensive study of the qualitative composition of the human vaginal matrix and variation amongst donors using Diffuse Reflectance Infrared Fourier Transform System (DRIFTS-FTIR) and pyrolysis-gas chromatography-mass spectrometry (py-GC/MS). Samples were collected from a small population of donors to explore the range of qualitative and semi-quantitative variation, and to identify the composition of the matrix, with specific species as potential targets for further studies in background investigation. Several approaches were taken to identify the composition, such as comparison with previously reported data on human skin, latent fingermarks or vaginal matrix. The prevalence of silicone-based traces in the given population will be discussed.

Table 1
Composition of the vaginal matrix.

Compound Class	Type of compounds [11,14,17–20]
Trace elements	Calcium, iron, magnesium, zinc
Electrolytes	Potassium, chlorine, sodium
Proteins	Albumin, immunoglobulins, lactoferrin, glycoproteins (mucins)
Organic acids	Lactic, citric, acetic, propionic, butyric acid
Amino acids	Histidine and other amino acids
Lipids	Triglycerides, cholesterol, phospholipids
Degradation products	Urea, amines
Carbohydrates	Glucose, fructose, maltose, glycogen
Enzymes	Vaginal peptidases, lysozyme, oxidases, peroxidases, alkaline phosphatases, lactate dehydrogenase
Cells	Macrophages, flaky epithelial cells, granulocytes
Other	Water, squalene, pyridine, immunoglobulins

2. Materials and methods

2.1. Chemicals

Hexane of analytical grade was from Sigma Aldrich (USA) and was used as received. PDMS 200cSt 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. Cotton swabs used for sample collection were COPAN150C (Copan Diagnostics Inc).

2.2. Sample collection

42 volunteers each provided 3 swabs, collected all at once, which were analyzed in triplicate, which resulted in a total 378 analyses for the FTIR technique. 8 volunteers selected based on the FTIR results were analysed in triplicates using py-GC-MS, for a total of 24 pyrograms.

The volunteers were aged between 18 and 35 years old. They were asked to self-sample 3 cotton swabs from their vaginal matrix, at any time of the day, independently of any specific activity. It was asked to sample all the swabs together, as one set, on the same day. Volunteers were asked not to collect samples during menstruation. Information was collected regarding the date of their last menstruation, the sampling date and the hour and if they had had intercourse in the week before sampling. If they had intercourse the week before the sampling, they were asked to report if the intercourse was protected, and when it last happened.

Sample collection was led in accordance with the Swiss Federal Act on Research involving Human Beings (Ordinance on Human Research with the Exception of Clinical Trials) and approved by Swissethics (2018-00690). Implicit consent was obtained from all donors, and collected data was entirely anonymized.

2.3. Sample extraction and preparation

Based on previous researches [9,24,25], cotton swabs collected from the volunteers 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 triplicate.

For DRIFTS analyses, KBr was finely ground for about 5 min using an electric mechanical grinder. The finely mixed KBr was placed in sample cups and dried for 15 min at 100 °C. Solution samples were individually deposited on the dry KBr filled cups using an eVol® syringe. Four cups filled with just KBr were kept for blanks. After spiking with 10 µl of solution, samples were put in an oven for 15 min at 100 °C to ensure solvent evaporation. Samples were left to cool down before analysis. Blanks were collected every three analyses, e.g. after 3 replicates coming from the same sample, and solvent blanks were run to account for interferences.

For the py-GC/MS analysis, 10 µl of the solution was spiked into the quartz tube on glass wool and left to evaporate before analysis. Three replicate samples were prepared from each donor to take into account homogeneity in the sample composition as well as the variation due to the instrumentation and sample preparation. Blanks

were collected before each replicate of each sample due to the type of pyrolysis device used.

2.4. Instrumental conditions

2.4.1. DRIFTS-FTIR

Spectra were collected using a Digilab FT3000 FTIR spectrometer equipped with Spectra-Tech 0030-05 Collector II Diffuse Reflectance Accessory (Spectra Tech). Data collection was carried out using the Agilent's ResolutionPro v.4 software. Spectra were collected over the 4000–400 cm^{-1} range with 4 cm^{-1} resolution and 64 co-added scans.

2.4.2. pyrolysis-GC/MS

The instrumentation used in this study was a resistively heated filament Pyroprobe 5150 from CDS Analytical Inc. The pyrolysis device was coupled to an Agilent GC 6890N 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.

2.5. Data processing

2.5.1. DRIFTS-FTIR

Visual and qualitative analysis of DRIFTS-FTIR data were performed on ThermoFisher Omnic32™ software (v. 8.2.0.387). Data pre-processing and chemometric analysis were performed using the Unscrambler® X 10.1 software (Camo Software AS, Oslo, Norway). Spectra were truncated to omit the 2340–1880 cm^{-1} region due to interference from CO_2. Then range normalization, automated using Unscrambler software, was applied to remove variation related to the amount of sample deposited on the KBr. Identification of the compounds was based on the comparison with published literature and analysis of reference material.

2.5.2. Py-GC/MS

GC/MS data were processed 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.

Identification of the compounds was undertaken using three different mass spectral databases; NIST18 (*National Institute of Standards and Technology*) and TOX3 (*Wiley Drug and Pesticides, Wiley138*), as well as comparison to published literature. Using Agilent ChemStation® software, areas of the target ions within all the acquired pyrograms were integrated for each peak. Peaks were selected as to be repeatable, and distinguishable from the chromatographic noise, over a threshold value of 30,000 A.U. Data were exported to Microsoft Excel, normalised to the area sum, and the double square root calculated prior to multivariate statistical processing, using the Unscrambler® X 10.1 software (Camo Software AS, Oslo, Norway).

2.5.3. Chemometrics

Using the Unscrambler® X 10.1, principal component analysis (PCA) was carried out on the dataset using non-linear iterative partial least square (NIPALS) algorithm, with 1000 iterations. This method was used to explore visually the structure of the data, by reducing their dimensionality and therefore evaluating the variation within the data. PCA is also used to determine which variables affect the most the principal components by studying the loadings, which are the coefficient of linear combination, associated to each principal component. Principal component analysis is necessary to reduce the data dimension and to explore visually and study the variation within the dataset. If the samples present a lower intravariability than intervariability, replicates from a same sample should be clustered together and separated from the replicates of other sample replicates on the first principal components. Principal component analysis was realized using the Unscrambler® X 10.1.

3. Results and discussion

3.1. Preliminary considerations

The method selected for sample collection asked the volunteers to self-sample. Although this is a minimally invasive method, causing minimal restriction for the volunteers, large variations can be generated during sampling. For example, a difference in pressure may cause a variation in the amount of material collected. In addition, several factors are likely to influence the matrix content as illustrated by [1–5,24]. As the vaginal matrix undergoes cyclic evolution [12,14,16,26], parameters such as the time of the menstrual cycle, hormonal intake, specific medication, pathologies, use of toiletries or genetics will influence the composition of the samples. The questions asked to the volunteers were deliberately very general and were related to the time of the menstrual cycle and previous protected intercourse, which could lead to detection of silicone-based lubricants. No personal questions regarding medications, pathologies, personal hygiene or hormonal intake were asked.

Preliminary experiments and previous research [4,5,27,28] showed that silicone-based lubricants were easily distinguished from the vaginal matrix and could be readily detected using the analytical framework presented in this study. As the present study focuses on silicone-based compounds, apolar solvent, i.e. hexane, was used as an extraction solvent. Therefore, a limited number of non-polar matrix constituent are expected to be detected. Water-based molecules are not likely to exhibit strong infrared signal and will not be detected using py-GC/MS, due to the degradation process, generating H_2O and CO_2, which are not detected by the mass spectrum. These compounds may instead be easily analysed using GC-MS. The focus of this work was constrained only to the non-polar fraction.

Finally, regarding data processing, several normalization and preprocessing methods were investigated, including normalization to major peaks of the IR spectra (i.e. cholesterol peaks) or of the pyrograms (i.e. cholesta-3,5-diene), in order to maximize the between sample variability and minimise within sample variability. The use of an internal standard, although being one of the more common ways to standardize data, is not recommended when using pyrolysis-GC/MS, as recombination could happen and chromatographic patterns might not be reproducible. The various combination of processing did not allow enhancement of the separation of between and within sample variability.

3.2. Qualitative analysis of the vaginal matrix

3.2.1. FTIR analysis

All 378 spectra acquired were found to have very good reproducibility between sample replicates within analytical replicates as well as between the different samples collected by the same volunteer. Typical spectra obtained from three different volunteers are illustrated in Fig. 1A. Fig. 1B illustrates the results of the blank swabs extracted for comparison.

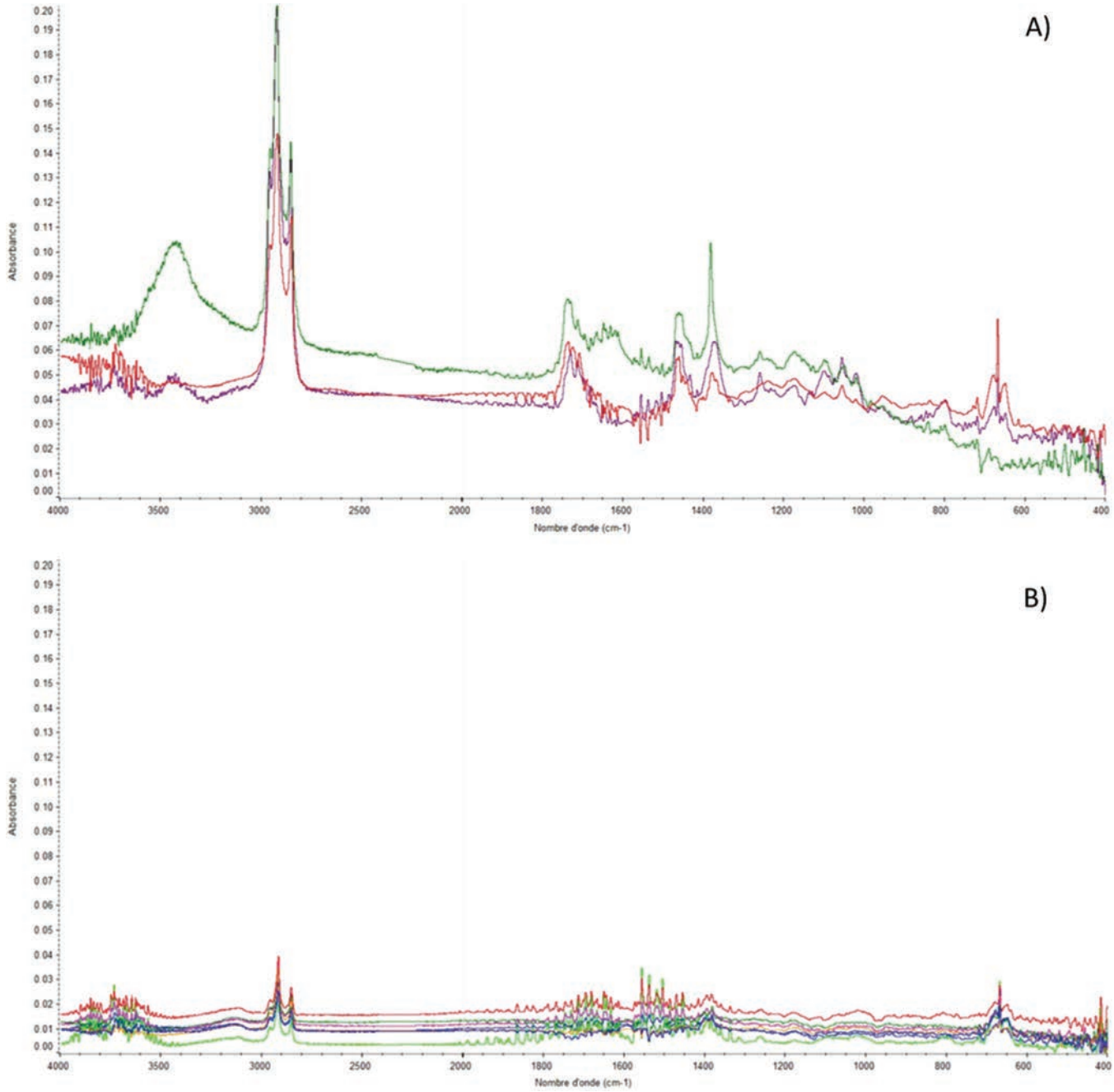

Fig. 1. Example of the variability observed on typical vaginal matrix spectra after hexane extraction and baseline correction. Spectra acquired (A) from the extracts of the vaginal matrix of three different volunteers randomly selected (volunteer A in red, volunteer B in green, volunteer C in purple), (B) from the extracts of the blank cotton swabs.

In this study, the main vibrational bands identified in all background vaginal matrices were found to be globally comparable to those identified in earlier research into vaginal secretions, from a qualitative point of view [21]. However, many differences were observed with the results from [21]. Indeed, Orphanou used ATR-FTIR and deposited the samples directly on the ATR crystal [21] whereas the present study used DRIFTS analysis, based on its relevance in the forensic analysis of condom residues [27]. As an extraction procedure was required in this study, some compounds might not be visible in the extracts, due to their polarity. Except for the bands coming from CH_2/CH_3 vibrations between 2850 and 2920 cm^{-1}, bonds coming from the cotton swabs were not dominant in the vaginal matrix swabs, thus suggesting that the vibrations observed in the collected spectra could be attributed to the vaginal matrix components.

Given the chemical structure of cellular membranes and the use of hexane as the extraction solvent, one could reasonably expect IR bands characteristic of glycoproteins, glycolipids, peripheric proteins or cholesterol to be present in the spectra. The presence of glycogen, nucleic acid and amino acids was also expected. The presence of a peak at 1741 cm^{-1} in addition to other lipidic peaks in the low region of the spectrum, below 1800 cm^{-1}, indicated the presence of esterified lipids, such as di- or triglycerides.

Proteins were observed through C=O stretching at 1649 cm^{-1} and C–N stretching at 1542 cm^{-1} which are characteristics of Amide I and Amide II bonds. Additional peaks coming from the methyl bending of proteins were observed at 1467 and 1383 cm^{-1}. However, these peaks were found to be significantly more important than the ones reported by Orphanou [21]. This suggests either a high concentration of proteins or the presence of other molecules that present

significant C=C vibrations as well as bending and stretching of C-O-H and OH bonds. However, if the protein concentration was higher, it is likely that Amide I and Amide II bands would also be significantly larger, which was not observed. In addition, vibrations at 2850, 2920 and 2960 cm^{-1} were found to be more important in terms of relative intensity compared to the ones presented in [21]. This observation is more likely to be due to the extraction step, as apolar solvent was used: therefore non-polar bonds appear to be dominating the spectra.

Lipidic material was indicated by the free hydroxyl (OH) bond around 3432 cm^{-1}, asymmetric vibrations from the C–H bond present at 2850, 2920 and 2960 cm^{-1}, 1467 and 1383 cm^{-1}, as well as C-O vibrations at 1055 cm^{-1} and a C-C backbone vibration at 938 cm^{-1}. Comparisons with reference spectra found in the literature [19,29–31] indicated that these peaks were associated with cholesterol. Traces of cholesteryl stearate were observed in the spectra. Steroids were also found to produce several various vibration bands in the domain between 600 and 1400 cm^{-1}. As well as cholesterol, steroid hormones (e.g. progesterone and estrogens) secreted during the menstrual cycle may also be detected in infrared spectra. Given the strong apolar composition of these molecules, it is not surprising to see them being extracted in hexane [32]. The concentrations of these hormones vary naturally during the menstrual cycle [11] and could possibly affect sample discrimination (see section 8.3). Nucleic acid phosphates were detected via a peak at 1232 cm^{-1}.

Carbohydrates were detected in the form of glycogen, with the main bands observed in the region 1034–1126 cm^{-1} [21]. Although these bands were present in the vaginal matrix spectra in the area where Si–O doublet vibration would appear (1020/1090 cm^{-1}), it was previously demonstrated that the peaks from silicone-based lubricants are well resolved from vaginal secretion spectra [27]. As there could be some misattribution of peaks to either glycogen or PDMS in the eventuality of the presence of traces, reference material of glycogen and PDMS were analysed and compared, as illustrated in Fig. 2. If the Si–O doublet appears to be in a similar region than peaks from glycogen, Fig. 2 also highlights that the bonds at 1263 cm^{-1} and 800 cm^{-1}, which are known to be diagnostic peaks for PDMS [27], are very important and are not overlaid with any other components coming from glycogen. Therefore, the presence in the spectra of the four diagnostic peaks of PDMS, i.e. 1263, 1090/1020 and 800 cm^{-1}, is necessary to infer on the presence of PDMS in the sample.

Based on these results, it was possible to identify the most informative regions of the IR spectra for exploring dataset variation through chemometric analysis (Fig. 3 and Table 2).

The spectra obtained from the 42 volunteers were largely similar, with no significant distinguishing peaks. In one third of the samples, Amide I peak at 1650 cm^{-1} was shifted to 1640 cm^{-1}, which was found to happen in several cases as reported in the literature [35,36]. Variations in the abundance of the peaks were observed in the region between 1850 and 600 cm^{-1}, which may be explained by natural variations as well as by the impact of the different contraception devices used. Sampling should also be considered as a source of variation.

Variations of the vaginal matrix were studied visually, with no indication of strong variation between the different volunteers from a qualitative point of view. From the information obtained from the volunteers, 66% (28/42) did not have any sexual intercourse in the week before sampling. Of those that reported sexual intercourse, 3 volunteers (21.4%) reported the use of a condom in the week before the sample collection. The spectra obtained for these volunteers are presented in Fig. 4.

In the spectra of two out of the three volunteers, traces of silicone material were suspected to be present (Fig. 4A and B), with very low concentration bands. Given that not all the bands were present, and they are not well defined, it is difficult to conclude that silicone was present in the sample. These two samples were first targeted using

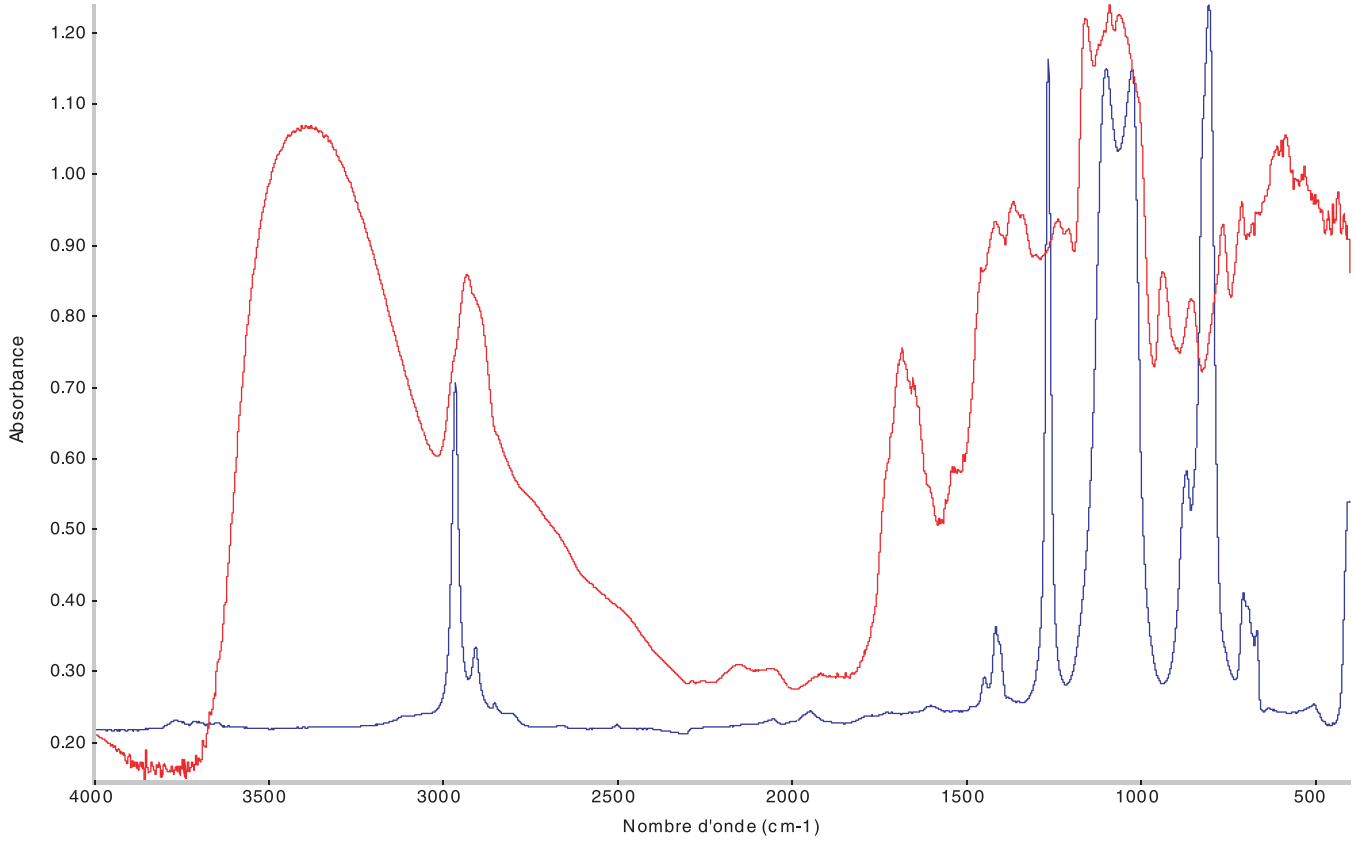

Fig. 2. Overlay of reference spectra of glycogen (in red) and of PDMS (in blue).

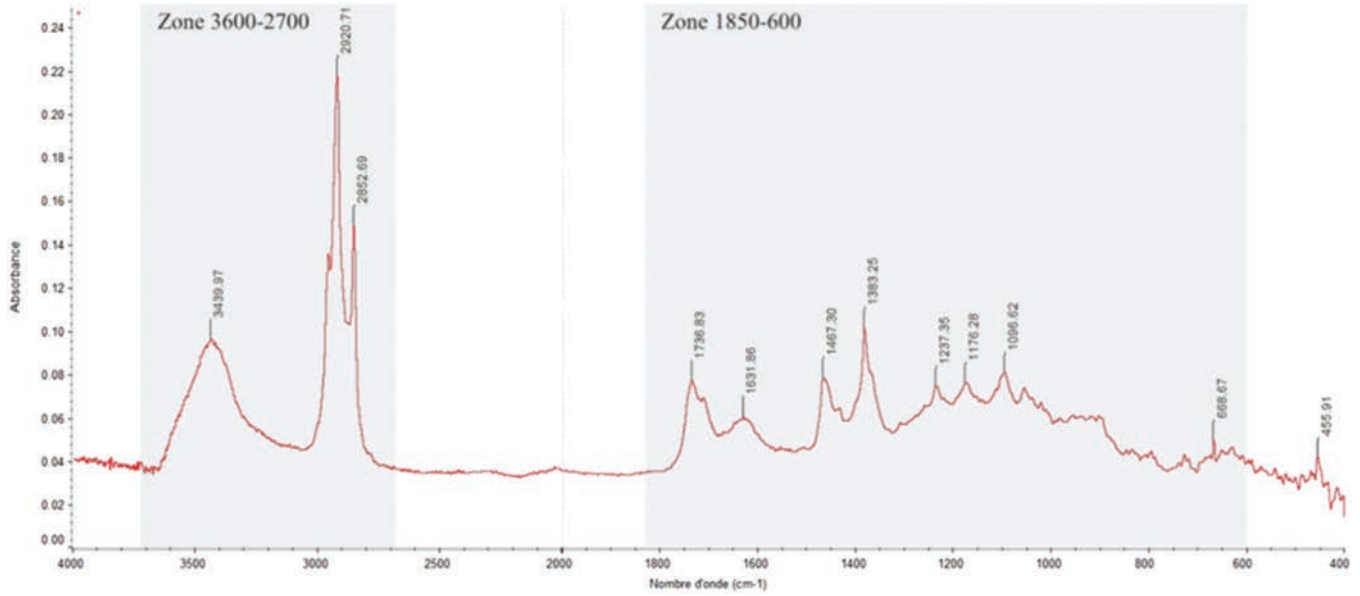

Fig. 3. Example of a vaginal matrix spectrum obtained after hexane extraction. The shaded zones were selected for further chemometric analysis.

Table 2
Major vibrational bands corresponding to proteins, lipids and various material observed in the IR spectra of vaginal matrix extracts [21,29,33,34].

Peak position [cm^{-1}]	Vibrations	Compounds	Category
3150–3600	Large OH-bond		
2960	C–H_2 stretch	Esters	Proteins, lipids
2920	C–H stretch (1st carbon)	Aliphatic chains	Proteins, lipids
2850	C–H stretch (2st carbon)	Aliphatic chains	Proteins, lipids
1730	C=O stretch	Fatty acids	Lipids
1713	C=O stretch	Carboxylic acid	Organic and amino acids
1630	C=O stretch	Amide I	Proteins
1542	N–H and C–N	Amide II	Proteins
1465	C–H_3 asymmetric bend C–H_2 symmetric bend	Aliphatic chains	Lipids
1380	C–H_3 symmetric bend	Aliphatic chains	Lipids
1200–1250	C–N stretch	Amide I/II	Proteins
1175	C–C–O stretch	Saturated esters	Lipids
1100	C–O stretch	Secondary alcohol	Lipids (cholesteryl stearate)

py-GC/MS to investigate this observation. Regarding the third volunteer (Fig. 4C), presence of silicone is excluded, as none of the diagnostic peaks from PDMS are visible. No other spectra were found to present any silicone material.

3.2.2. py-GC/MS analysis

Before processing any qualitative analysis, instrumental blanks as well as blank swabs were run and evaluated to identify compounds coming from the instrument or from the cotton swabs. Instrumental blanks were found to be completely clean. All the cotton swabs analysed contained 11 compounds, respectively toluene, ethylbenzene, styrene, 1,2,4-trimethyl-benzene, alpha-methylstyrene, 3-phenyl-1-propyne, naphthalene, 1,2-dihydro-1-phenyl-naphthalene, undecane, nonadecane, and indene (Fig. 5B). These products are characteristic of cotton pyrolysis [37]. Most of these compounds were present in very low abundance and were not detected in the vaginal matrix pyrograms. It was also found that although these compounds could originate from the swabs, the relative abundance of some of these peaks was higher in the vaginal matrix pyrograms, suggesting that pyrolysis of vaginal matrix residues also produced these compounds. The relative amount of these compounds was therefore subtracted from the ones obtained from the vaginal matrix swabs.

All the pyrograms acquired were found to have very good reproducibility between sample replicates. Some differences were noticed between the different volunteers, with certain peaks being detected only in certain chromatograms. Typical spectra obtained from three different samples are illustrated in Fig. 5A.

The major peaks seen towards the end of the pyrogram, i.e. peaks 21–27 in Fig. 5A, are found systematically in all the pyrograms. were present in all of the pyrograms. These peaks were attributed, using their mass spectra, literature [38–42] and databases, to cholesterol and stigmasterol derivatives (Table 3), which are two compounds classified as steroids. These molecules are found in cell membranes but are also the basic structure of sex hormones such as estradiol or progesterone. The presence of steroid hormones in the extracts had already been noted during the infrared analysis of the same samples.

The majority of the compounds detected in the pyrograms had a naphthalene or indene base or were small molecules such as xylene and styrene. Compounds such as phenanthrene and anthracene, as well as their derivatives were also detected. Literature [41,42] confirmed that these compounds did indeed come from the degradation of cholesterol and steroid-based compounds present in the samples. Compounds which had an abundance higher than 30,000 AU and which were sufficiently distinct from the background noise for integration are reported in Table 3.

The smallest peaks present in the pyrograms, with abundances approaching the limit of 30,000 AU, presented mass spectra characteristic of products derived from cholesterol and analogous steroids. These compounds could have been attributed to naphthalene derivatives (for example 2-ethenyl-, 1-ethyl-, 1,5-dimethyl-, 2,3-dimethyl-) or to phenanthrene derivatives. These were not

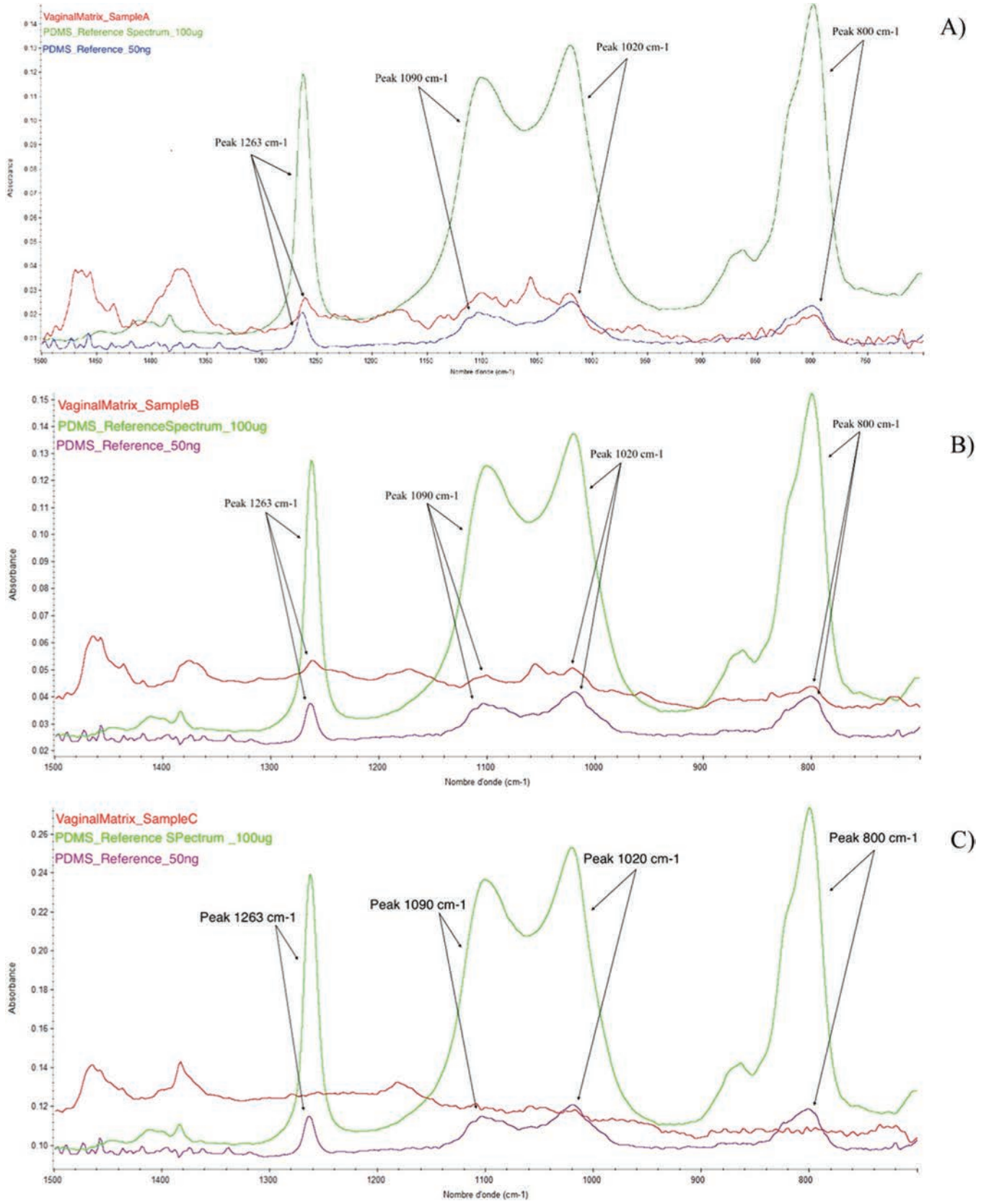

Fig. 4. Illustration of the FTIR spectra obtained from the volunteers who reported sexual intercourse in the week before sampling. A) 2 days before sampling, B) 7 days before sampling, C) unknown time interval. Spectra are displayed between 700 and 1500 cm^{-1} to enhance readability. Overlay of a 100 µg PDMS reference spectrum (green) and 50 ng PDMS reference spectrum (purple) are used for comparison.

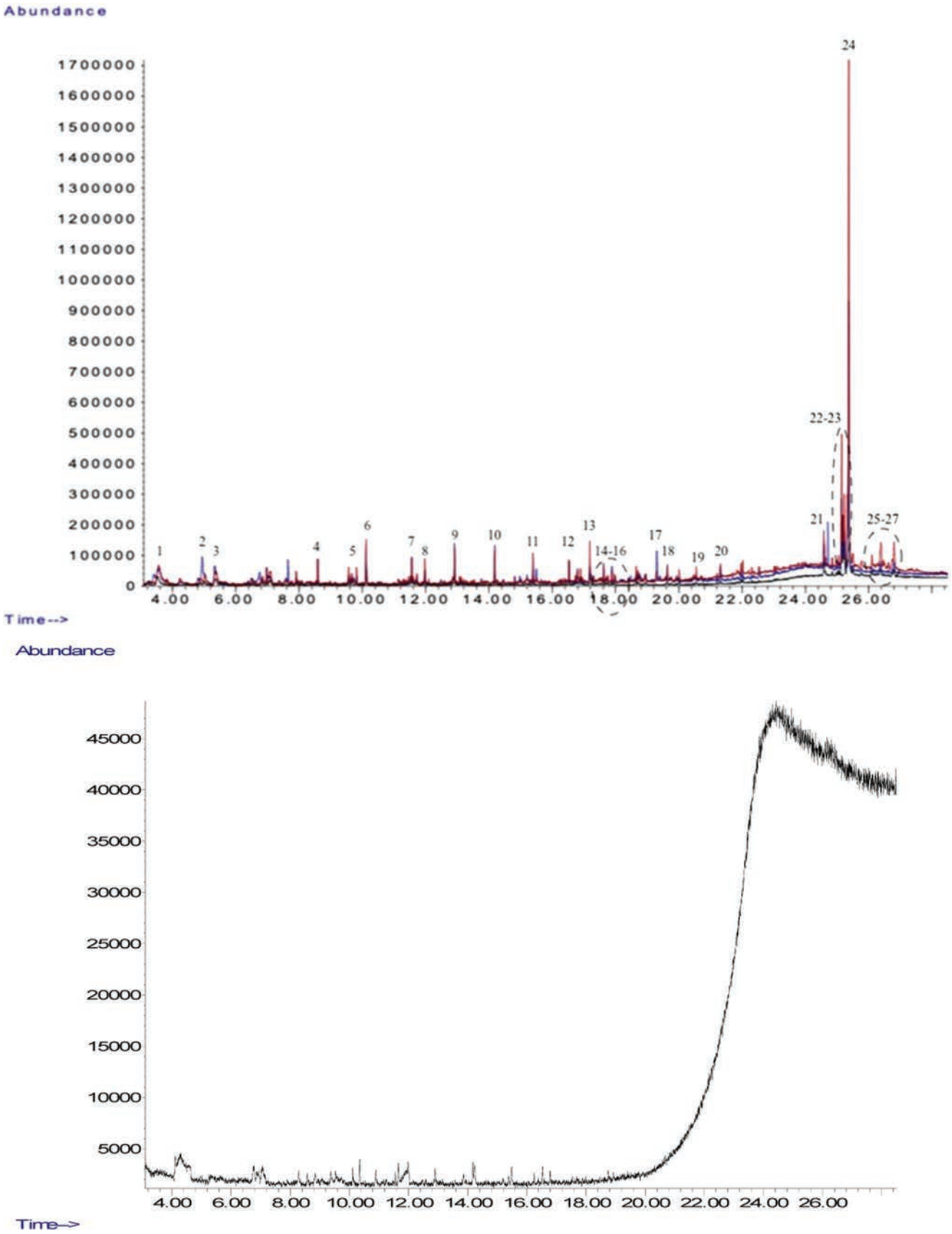

Fig. 5. A) Typical pyrogram obtained for three different volunteers. Annotated peaks are the ones that were found to be coming from the vaginal matrix, and not from the cotton swabs. Numbers refer to peak identification from Table 3. B) Blank swab for comparison.

Table 3
Identification of the compounds found in all the pyrograms obtained from the vaginal matrix samples. Peak numbers are related to Fig. 5A.

Peak	Compound	RT [min]	Target ion [m/z]	Qualifiers [m/z]
1	Toluene	3.530	91	79
2	p-xylene	4.929	91	106, 77
3	Styrene	5.354	104	78, 91
4	1-Undecene	8.589	154	117, 97, 83
5	Trimer containing:		104	
	1-Methylindene	9.562	130	115, 102, 77
	2-Methylindene	9.667	130	115, 102, 77
	Naphthalene, 1,2-dihydro	9.813	130	115, 91
6	1-Dodecene	10.116	128	97, 83, 70
7	1-Tridecene	11.550	83	97, 111, 196
8	Naphthalene, 2-methyl	11.719	142	115, 129, 158
8b	Naphthalene, 1-methyl-	11.964	142	115, 126, 89
9	1-Tetradecene	12.908	83	196, 125, 111
8c	Naphthalene, 1,3 dimethyl	13.124	141	156, 115, 127
10	1-Pentadecene	14.179	83	210, 182, 111
11	1-Hexadecene	15.385	97	224, 125, 111
12	1-Heptadecene	16.522	97	238, 125, 111
13	Naphthalene,1-(1,1-dimethylethyl)-7-methoxy	17.186	214	199, 143, 91
14	1-Octadecene	17.612	83	252, 125, 111
15a	Phenanthrene	17.699	178	152, 89, 76
15b	Anthracene	17.752	178	152, 89, 76
16	Tetradecanal	17.880	82	194, 96, 138
15c	Phenanthrene,2-methyl-	18.952	192	165, 176, 95
17	n-hexadecanoic acid	19.296	73	129, 213, 256
18	5-Eicosene, (*E*)-	19.634	97	111, 125, 280
19	1-nonadecene	20.549	97	111, 125, 266
20	1-docosene	21.307	97	111, 125, 308
21	Cholesta-2,4-diene	25.148	368	255, 353, 106
22	Unknown_25.18	25.185	197	352, 144, 117
23	Cholesta-4,6-dien-3-ol, (3 beta)-	25.236	366	247, 143, 91
24	Cholesta-3,5-diene	25.370	368	353, 147, 247
25	Cholest-7-en-3one-4, 4-dimethyl	26.098	207	193, 348, 181
26	Cholesta-7,9(11) dien-3-ol-4,4-dimethyl	26.390	207	398, 344, 281
27	Stigmasta-3,5-diene	26.804	207	147, 396, 81

detected in the blank analysis from the cotton swabs. Certain compounds, such as dienol or compounds containing hydroxy groups, may be produced from the breakdown of estradiol and estriol, which are the two most important female sex hormones produced by the human body. Their chemical structures presented below (Fig. 6), include phenolic groups as well as alcohol groups. Considering recombination happening during the pyrolysis process, the presence of these molecules might explain the hydroxy or phenolic content detected in the minor peaks in the pyrograms.

The pyrograms were further examined for other steroid hormone derivatives and pyrolysis products, based on the report by [43]. No residues characteristic of progesterone or androsterone, nor any of their derivatives or pyrolysis products were detected in the pyrograms.

The remainder of the compounds listed in Table 3 were 1-alkenes. According to Maher et al., 1-alkenes are generated from the pyrolysis of triglycerides [44]. It was previously suggested that the compounds observed in the FTIR spectra and pyrograms originate from cell membranes. Cell membranes contain, amongst other compounds, glycoproteins, cholesterol and glycolipids. The previously described cholesterol residues do not explain the presence of 1-alkenes. Triglycerides contain fatty acids and are found in both phosphoglycerides (e.g. phosphatidylcholine) and glycolipids. They contain, for example, stearyl, oleyl, linoleyl acyl groups [41,45] which can explain the presence of 1-alkenes in the pyrograms.

Although most of the peaks detected in the pyrograms were sourced from cholesterol derivatives and glycolipids, indoles were also detected in small amounts, though not abundant enough to integrate for chemometric purposes. However, indole is a typical marker from protein compounds [46]. 1,2-Cyclopentanedione, 3-methyl was also found as a marker from carbohydrates [46].

From a qualitative point of view, the pyrograms showed no signs of the presence of silicone traces. Regarding samples that were found to present possible evidence of silicone in the infrared spectroscopy, the sample presented in Fig. 4C was confirmed to be negative to silicone residues. The overlay of the samples with the blank associated illustrated that it was likely that the vaginal sample A would contain some silicone, but an extraction of the ions would be necessary. Regarding sample B, the overlay did not allow to visually distinguish any peaks from the background. The pyrolysis procedure was inspired from [4,5,47,48] and extracted ion chromatograms of the silicones patterns as illustrated by [47,48] for the vaginal matrix sample A and B, showed traces of the main D3 to D5 degradation, but in very low abundance. Given that the abundance was not over 3x the noise at the same retention time for any of the two samples, the presence of the compounds in the matrix cannot be fully assessed. These observations support the hypothesis that silicone compounds are not naturally found in the vaginal matrix.

3.3. Variation of the vaginal matrix

3.3.1. FTIR analysis

This section aims to assess whether statistical differences can be observed within a population of women, based on variations in absorbances. Based on previous studies on the classification of human samples according to population characteristics [33], spectra were pretreated using the Savitsky-Golay second derivative, which corrects for baseline variations and allows for the separation of overlapping peaks.

PCA of the overall dataset (378 infrared spectra) revealed that 75% of the variance within the dataset was accounted for by the first seven PCs. The scores plot constructed from the first 3 PCs (Fig. 7) showed no significant differences in compositions were observed between the 42 volunteers. PCA of data processed with Savitsky-Golay second derivative was compared against PCA following

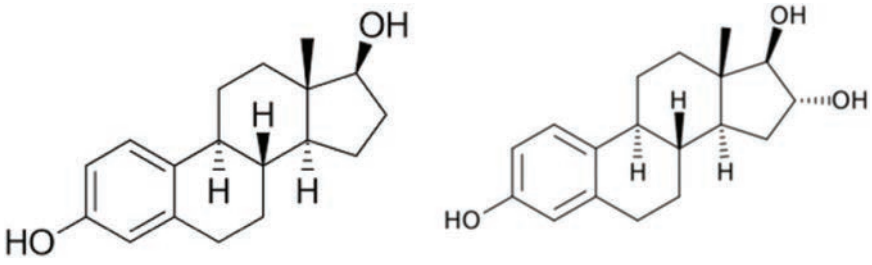

Fig. 6. Chemical structure of estradiol (left) and estriol (right).
Reproduced from https://pubchem.ncbi.nlm.nih.gov/compound.

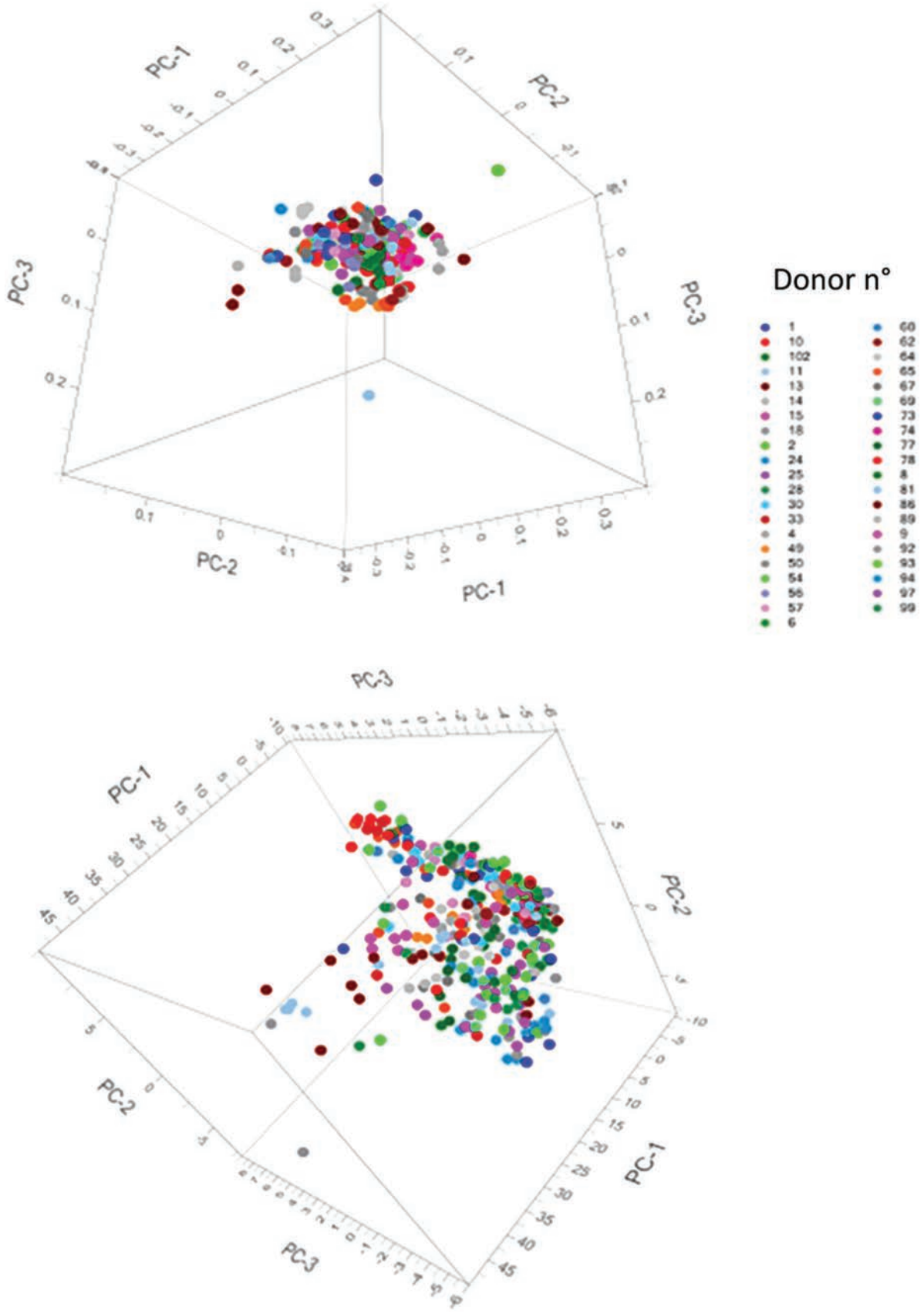

Fig. 7. 3-dimensional scores plot generated from the first 3 PCs, demonstrating the distribution of vaginal matrix compositions collected from 42 volunteers, after second derivative (top) and range normalization (bottom). Samples are colored by individual volunteer.

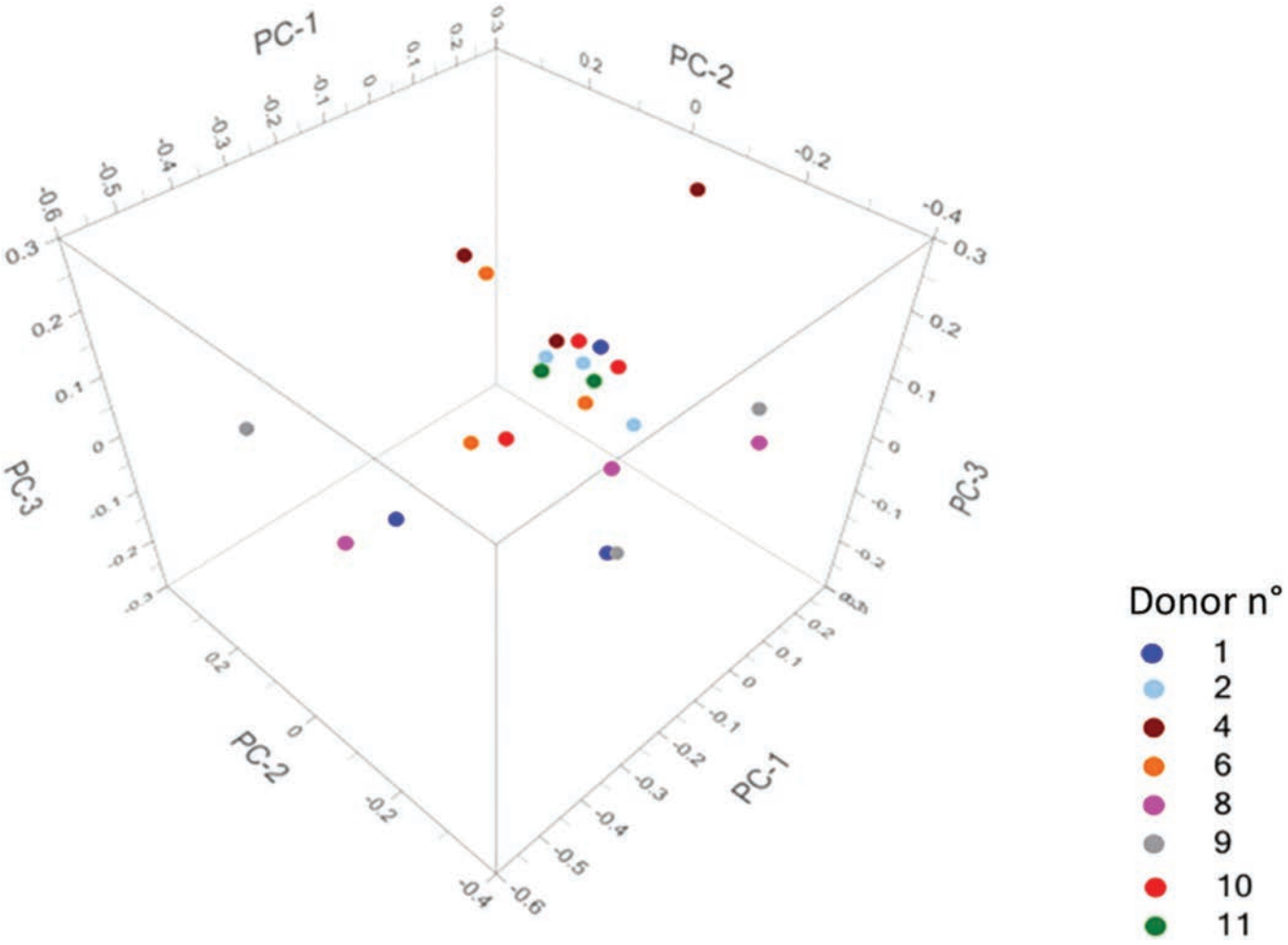

Fig. 8. 3-dimensional scores plot generated from the first 3 PCs, demonstrating the distribution of vaginal matrix compositions collected from 8 volunteers, after area sum normalization and double square root processing. Donors 8, 9 and 11 reported protected intercourse in the week prior to sampling.

baseline correction and range normalization, where 95% of the variance was explained by the first seven PCs. While the cumulative variance of the range normalized data was higher than that of the data processed with derivation, it was found that baseline correcting and normalizing the data generated a broader spread of the samples within the scores plot. However, no trends were visible in the dataset, and it was not possible to see any influences from certain region of the spectra.

As the vaginal matrix undergoes cyclic changes, the relative amounts of molecules linked to sexual hormones (such as estriol, estradiol or progesterone) that could be detected in the spectra are reflective of compositional changes. Investigation into discrimination of the samples based on the time in the menstrual cycle found no commonalities at different phases of the menstrual cycle, which is likely due to inter-donor compositional differences.

The disparate projection of samples from the 42 volunteers is caused by inter and intra-donor variation, as well as variations due to sample collection and analytical impact, which creates difficulties in interpreting the dataset in its entirety. PCA indicates that there are no sufficiently significant changes in the infrared spectra to differentiate between women nor between different stages of the menstrual cycle.

3.3.2. *Py-GC/MS analysis*

In order to assess and compare within and between sample variability, the extracted and normalized area were used. The coefficient of variation obtained within samples was not different from to that obtained between sample. In fact, both within and between samples coefficient of variations ranged from 1.4% to 37%. Classification using hierarchical cluster analysis was also investigated, to see if variation between donors could be observed, but replicates from the same donors were not found to plot together.

PCA of the overall dataset (24 pyrograms, from 8 different donors) showed that 90% of the variance within the dataset was accounted for by the first seven PCs. The scores plot constructed from the first 3 PCs (Fig. 8) showed that no significant differences in composition between the 8 volunteers. Although strong variability was observed, the same pattern was observed as with the infrared data. No trends were visible in the dataset. The loadings plots demonstrated that PC1 was influenced by styrene and xylene, and PC2 by cholesta-3,5-diene and cholesta-2,4-diene. These compounds were also found to generate separation along PC3. The rest of the compounds were not found to contribute significantly to sample separation. The rest of the variables were found to be too close to 0 to be relevant for discrimination purposes.

The disparate projection of samples from the 8 volunteers is caused by inter and intra-donor variation, as well as variation in sample collection and analytical impact. PCA of the dataset indicated that there are no statistically significant differences in the pyrograms to observe any differences between women nor between different times of the menstrual cycle.

4. Discussion

The observations described above indicate that there was no endogenous presence of silicone compounds in the vaginal matrix. However, these observations were made within the scope of a pilot study, studying a small population of women within a restricted age group (18–35 years). If certain IR spectra suggested the presence of a silicone compound, the chromatographic analysis did not reveal any trace of these compounds, even in samples from women who had protected intercourse in the week before the sample. No instances occurred where a volunteer had protected intercourse in the 24 h preceding sampling, in which case silicone traces would have been expected given previous research [27,28].

The data obtained made it possible to understand the structure of the vaginal matrix, in order to identify relevant peaks for the analysis of real samples. The application of data pretreatments (for example normalization or derivation) or multivariate statistical analyses (for example PCA) did not enable any classification linked to the volunteers or any stages of the menstrual cycle. Intravariability was found to be as important as intervariability, preventing differentiation between samples from different sources. In order to conclude if the within sample variability is really as important as the between sample variability, it is necessary to standardize the sample acquisition and the analytical methodology, as it cannot be excluded that the observed within and between sample variability is due to differences in the self-sampling of the volunteers. Validation of the entire procedure according to reliable and repeatable statistical models would be recommended, so as to be able to subsequently reduce within sample variability, while keeping between sample variability high enough to be able to discriminate samples from different sources. The presence of different populations based on the vaginal matrix content, and variations due to the menstrual cycle or the intake of certain types of contraceptive might affect the chemical profile of the matrix. Therefore, being able to distinguish these differences would be of interest so as to be able to see these when encountered in casework.

In addition, the results obtained in this study raise more fundamental questions about the necessity of acquiring more data in order to be able to draw a model and to understand the chemistry and behavior of the vaginal matrix, especially in view of a forensic application. The acquisition of a larger data set, particularly in terms of pyrolysis-GC-MS analysis, is necessary so as to obtain a model which can be considered statistically reliable. In view of the data now obtained and considerations regarding the observed variability, the acquisition of more data will result in the creation of an unreadable grouping at the center of the 3D scores plot, in a similar manner to the FTIR data.

The non-differentiation of different sources with the present protocol can be explained by different factors: on one hand by the effects of the extraction and on the other by human cellular composition. The use of a non-polar solvent, i.e. hexane, only allow to extract a non-polar fraction of the original sample, which seems to be mainly constituted of cell-membrane constituents. These constituents are not expected to show much variation between individuals. Any possible variations due to the menstrual cycle or contraceptives are masked by operator, instrumental or extraction variability. In order to conclude about such, as mentioned before, standardization of sampling to avoid variability due to self-sampling, as well as a bigger dataset is needed. As previously discussed, self-sampling is a strong source of variability, and one possibility to reduce this variation is to standardize the procedure, which could imply that a medical expert would collect the samples.

However, the analytical methodology was set up in order to extract and analyse silicone compounds, so hexane was chosen as an extraction solvent because PDMS has a particularly high affinity for it. This means that the extracted molecules are likely to be the same for each donor. The use of another extraction technique, which would include extraction of polar compounds, might allow discrimination of samples coming from different donors.

Another element to consider regarding sample variation is linked to the analytical technique. Indeed, sample preparation for DRIFTS analyses implies a significant amount of variations such as a different KBr grain size or KBr packing within the cell. An optimization of the latter is to be considered for a better study of the background noise. Indeed, in order to guarantee the quality of the results, it is necessary to minimize the variation induced by the handling of samples. According to the study by [27], micro-transmission and micro-ATR offer good reproducibility. However, these techniques do not work well on diluted samples which left only DRIFTS as a suitable FTIR technique for trace evidence analysis.

Independently of the question regarding the composition of the vaginal matrix, both methods (py-GC and FTIR) were suitable to determine the presence or the absence of silicone components in the samples [4,5,27,47]. In this study, none of the samples presented any evidence of siloxane components. The data presented suggested that it is unlikely to observe silicone compounds in the vaginal matrix if there has been no protected intercourse.

Finally, the modeling of interpretive parameters is generally carried out so as to derive from the data a model which is applicable to the entire considered population. Knowing that each woman has a different vaginal microbiome, which undergoes regular cyclical variations, the influence of the menstrual cycle on the data warrants further investigation. However, such information would be specific to each woman, and the model might never be applicable in general. These results are however promising for future practical use, since the results of this study as well as the ones published by Burnier et al. on condom traces [27] show that once the traces are extracted, there is a priori no matrix residues present in the extracts. Thus, only lubricant residues are likely to be detected, making it more relevant for case work investigations. However, matrix residues are here not to be mixed up with matrix effect. Because no matrix residues are detected does not mean there is no matrix effect on the traces, effect which will happened along the persistence, which is defined as the elapsed time between the intercourse and the sampling.

5. Conclusion

Several trace samples are likely to be investigated when looking for condom evidence in forensic casework. While the samples received by the forensic laboratories are mostly collected from the victim (vaginal, anal, endocervical or vulvar swabs), other trace media may be encountered, such as the victim's underwear [1,7,8,49]. This research focused on the vaginal matrix on the assumption that these samples would be more often encountered in forensic practice. FTIR and py-GC/MS were successfully used to understand the vaginal matrix composition.

Spectral areas between 700 and 1850 cm^{-1} and 2700–3600 cm^{-1} were identified as being the most interesting based on FTIR analyses carried out on several volunteers. These regions contained the majority of the relevant spectral information, particularly in relation to the components of the vaginal matrix (i.e. lipids, proteins). If these regions are more interesting for the vaginal matrix itself, it is not completely true for silicone, where the four major peaks appear in a smaller range, as reported by [27]. As far as silicone detection is concerned, the most interesting area ranges from 800 to 1300 cm^{-1}.

A py-GC-MS study of the composition of the vaginal matrix was carried out and characterized 27 compounds present in vaginal matrix samples which were not detected in the silicone products analysed from reference material and condoms on the market [47]. These compounds included cholesterol and its degradation products.

The human vaginal matrix exhibited a high degree of complexity and completing a full characterization of the matrix and any potential discrimination is challenging. DRIFTS and py-GC/MS were

used in a prospective approach, from a chemical and forensic point of view, to investigate the composition of the matrix that could be detected in case work samples, and to evaluate the prevalence of silicone traces amongst a given population.

None of the 126 vaginal swabs coming from 42 donors were found to contain any silicone residues. This can be used in forensic interpretation of the evidence as a background parameter, although it would be recommended to pursue further investigation on a larger population to ensure statistically representative results.

CRediT authorship contribution statement

Céline Burnier: Writing - original draft, Conceptualization, Methodology, Validation, Investigation, Resources, Data curation, Supervision, Visualization. **Anaïs Hermelin:** Writing - original draft, Validation, Investigation, Data curation, Visualization. **Julia Fischer:** Writing - original draft, Validation, Investigation, Data curation, Visualization. **Nikola Saric:** Writing - original draft, Validation, Investigation, Data curation Visualization. **Loïc Fabien:** Writing - original draft, Validation, Investigation, Data curation, Visualization. **Geneviève Massonnet:** Writing - review & editing, Resources.

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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A preliminary investigation of transfer of condom lubricants in the vaginal matrix

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ABSTRACT

Condom evidence has become in the past years a very relevant item of evidence in sexual assault or rape cases, being an objective help in the reconstruction of the activity. Traces recovered from a vaginal swab might help to identify whether a condom or other lubricants were used, and thereby possibly confirming or infirming allegations of the parties. However, the interpretation of condom traces can be challenging and requires a detailed understanding of various factors like condom lubricant chemical composition and occurrence on the market, transfer and persistence parameters and background. Herein, we aimed at improving our understanding of factors affecting the transfer variability of condom residues recovered from vaginal matrix.

This work employed Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFTS) to provide new data for a characterization of condom lubricants and their traces after a transfer in a vaginal matrix has occurred. Condom traces were recovered from volunteers and the traces characteristics were investigated and analyzed. The effects of donor (condom) and receiver (vaginal matrix) were firstly evaluated, as they are known factors, and these data that could be obtained in real caseworks. Using principal component analysis (PCA), this study highlighted that the effect of the donor was more important than the receiver effect. Vaginal matrix residues were not detected in transferred extracts. The discrimination pattern amongst the donor was found to be indistinguishable from the one obtained on reference material using ATR-FTIR (Attenuated Total Reflectance).

1. Introduction

Condom traces, more specifically silicone residues coming from condom lubricants, have recently been more investigated for forensic purposes. The issue of condom traces in forensic science has been illustrated since the 1980s, especially in cases of sexual assaults on women [1–3]. According to the information obtained during an investigation, it may become necessary to determine whether a trace of a condom is present, in order to confirm or deny the allegations of the parties: it is not the occurrence of a sexual act but the way it happened that is questioned [4]. The condom trace is also used as associative evidence to establish the *corpus delicti* and verify penetration [2,5,6].

Multiple researches have focused on different, but complementary, approaches to be able to exploit the condom evidence. Most studies focused on the composition of the condoms and the discrimination of samples present on various markets [4,7–12,12,13]. More rarely, chemical markers in the vaginal matrix were investigated to identify which compounds were the most easily detected and what could be found in traces [14]. Another important aspect seems to have been neglected by researchers: the question of interpretative parameters. This approach generally takes into account parameters such as occurrence, background, transfer and persistence parameters. Despite the recurrence of such questions, there's is no existing model to assist the forensic scientist to understand what happens during the transfer as well as after a sexual intercourse, and thus to allow adequate interpretation of the evidence. This is likely due to barriers related to this type of study. Indeed, the variations observed in a profile are influenced by various factors which are difficult or even impossible to control [15,16], and whose effects are for the most part unknown (e.g. the effects of the contact itself (duration, intensity.), or the influence of the vaginal matrix) [15]. Added to this are ethical considerations, which make these studies long and tedious but demonstrate the need to increase research on the subject.

As described by Burnier and Massonnet [15], several factors are likely to influence the trace and its recovery by influencing the transfer and

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persistence of the evidence. Both the initial composition and recovered composition of the evidence are significantly affected by different influence factors, classified in five groups: the donor, the receiver, the contact, the elapsed time between intercourse and sample and the activity of the receiver between the elapsed time [15].

Donor characteristics can easily be assessed using market studies. In a recently published ATR-FTIR market study [17], silicone lubricants were found to be the more common lubricant on condoms, whereas most of the other types of lubricants were containing water- based compounds. The classification model built in this study, as in many other publications, was based on pure lubricants, and the classification of real traces has so far not been investigated, to see whether they classify in the same way than pure lubricant samples do. Indeed, chemical profiles coming from traces are likely to be affected by the vaginal matrix, and in a forensic context, it is important to understand how the target compounds react when in contact with the matrix, and the effects of the donors (condoms) and receivers (human vaginal matrix). Receiver characteristics and contact have yet never been reported, although these are more than likely to affect the evidence as illustrated in multiple caseworks [5,18].

As such, this study aims to provide data to investigate the transfer of silicone lubricants in the vaginal matrix using DRIFTS [14] as a tool to detect condom residues. This study sought to contribute to an empirical evidence base to establish the nature of the transfer of condom lubricants and develop a scientific basis to develop further interpretation model when considering the detection of condom evidence.

2. Material and methods

2.1. Chemicals

PDMS 200 cSt was purchased from Sigma Aldrich (USA) and was diluted in hexane (analytical grade; Sigma Aldrich (USA)) at concentrations of 0.1, 1.0, 2.0 and 3.0 mg/mL. KBr used for DRIFTS analysis was purchased from Acros Organics. Human sample collection was led by self-sampling procedure, using COPAN 150C cotton swabs (Copan Inc., USA). All the sample preparation and spiking were realized using a 5 µl syringe eVol XR ® from SGE Analytical Science (Australia).

2.2. Samples

To investigate the donor (i.e. condom) effect, 2 volunteers had sexual intercourse using 11 different condoms, listed in Table 1. Volunteers self-sampled 3 blank swabs prior to intercourse, and 3 samples right after intercourse. To avoid any cross-contamination, the volunteers were asked to wait one week between each protected intercourse. Each sample was analyzed 3 times, resulting in 9 replicates for a same donor. As each volunteer provides 3 swabs and each swab is analyzed in triplicates, a total of 180 analyses (blank included) were obtained for volunteer 1, whereas 90 were obtained for volunteer 2. This leads to a total of 270 analyses run for the aim of this paper.

To investigate the receiver (i.e., volunteer) and therefore matrix effect, the donor was fixed as the Ceylor Blue condom (D1-latex silicone lubricated condom). In the eventuality the volunteer asked for a latex free condom, the selected condom was Manix Skyn (D9). These choices were made as they are common condoms found on the market, and they are not statistically distinguishable based on their chemical composition. 9 volunteers were asked to have sexual intercourse using the provided condom, and to self-sample using the cotton swabs right after the intercourse. Volunteers R2, R3 and R8 asked for a latex-free condom (D9-Manix Skyn), and all the other volunteers used the latex silicone lubricated condom (D1-Ceylor Blue). Here again, blanks were collected prior to intercourse to ensure there are no silicones in the vaginal matrix, and therefore that the traces detected come from the given intercourse and not from previous use.

Sample collection was led in accordance with the Swiss Federal Act on Research involving Human Beings (Ordinance on Human Research with the Exception of Clinical Trials) and approved by Swissethics (2018-00690). Informed consent was obtained from all donors, and collected data were entirely anonymized.

2.3. Extraction procedure and sample preparation

Sample extraction procedure was taken from Refs. [3,5,10], with cotton swabs separated from the wooden stick and put in a glass vial. 1 mL of hexane was added prior to vortexing for 1 min and sonicating for 15 min. Extracts were analyzed in triplicate.

KBr pellets were prepared by filling sample cups with finely manually ground KBr powder. Cups were then dried for 15 min at 100 °C. 10 µl of extract solution were spiked on the pellets, and one pellet was spiked with hexane only. Three cups were left unspiked for blank analysis. Samples were then put back in the oven for 15 min at 100 °C to ensure solvent evaporation. Samples were left to cool down in a Pyrex® dish, filled silica beads to absorb exceeding humidity.

2.4. Data acquisition

Data were acquired using a Digilab FT3000 FTIR spectrometer equipped with Spectra-Tech 0030-05 Collector II Diffuse Reflectance Accessory. Agilent's Resolution Pro v.4 software was used for data collection. Spectra were collected over the 4000–400 cm^{-1} resolution, using 64 co-added scans and a 4 cm^{-1} resolution. KBr background was collected every 3 analysis and solvent blanks were run to account for interferences.

2.5. Data analysis

Qualitative analysis and visualization of the spectra was led on ThermoFisher Omnic32™ software (v. 8.2.0.387). The Unscrambler®

Table 1
List of the donors used for the donor effect investigation and their features, with the number attributed and which receiver used which samples. Composition is known based on observations from Ref. [17].

No	Donor	Type	Lubricant	Other components	Receiver 1 (R1)	Receiver 2 (R2)
D1	Ceylor Blue	latex	silicone	no	X	X
D2	Ceylor Gold	latex	Water based + silicone	Glycerin, PEG, nonoxynol-9	X	X
D3	Ceylor Ultrathin	non latex	silicone	no	X	X
D4	Ceylor Green	latex	none	no	X	
D5	Manix Contact	latex	silicone	no	X	X
D6	Durex Natural	latex	silicone	no	X	
D7	Durex Gefühlsecht	latex	silicone	no	X	
D8	Manix Orgazmax Plus	latex	silicone	Propylene Glycol	X	
D9	Manix Skyn	non latex	silicone	no	X	
D10	Prix Garantie	latex	silicone	no	X	
D11	Manix Fraise	latex	silicone	no		X

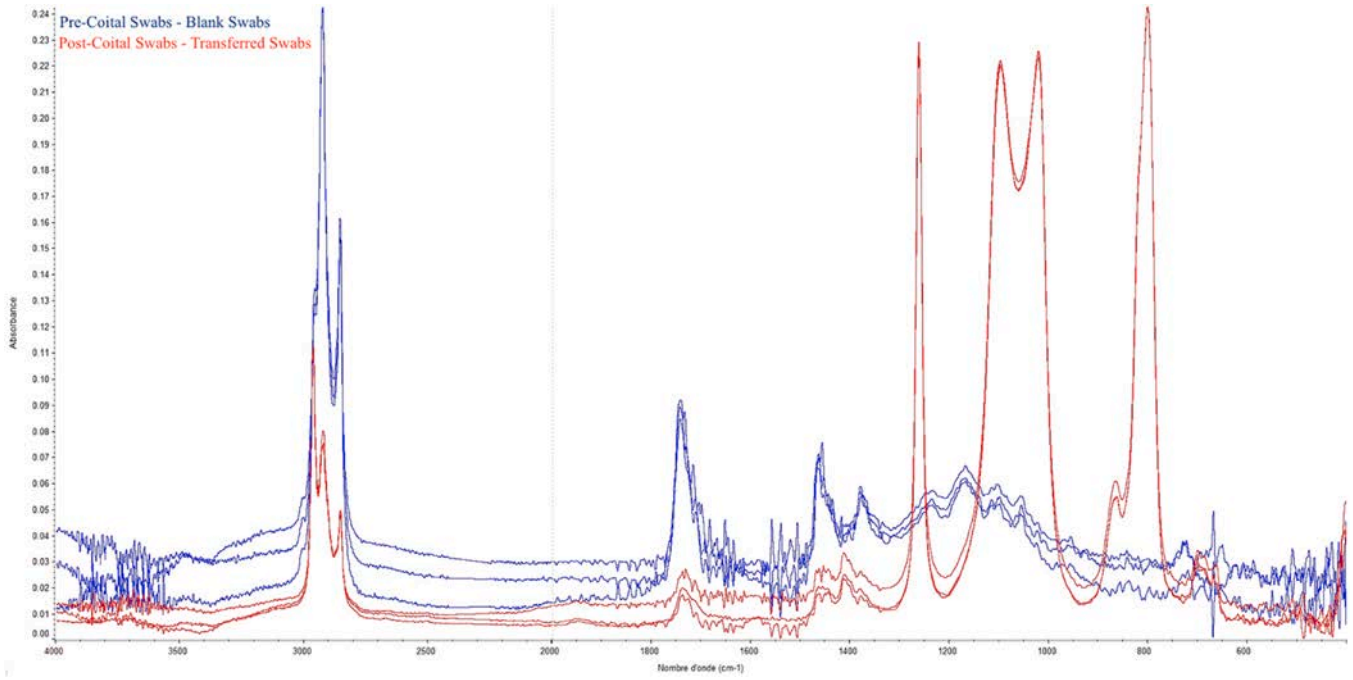

Fig. 1. Illustration of the vaginal secretions (blank swabs – Blue spectra) and an overlay of the transferred spectra (PDMS containing – Red spectra). There is no interference noted between the blanks and the transferred residues. Blanks do not present any signs of a presence of PDMS.

X 10.1. software from Camo Software AS (Oslo, Norway) was used to perform all the automated data preprocessing and chemometric analysis. Spectra were truncated to omit the 2340–1880 cm^{-1} region and range normalized before processing principal component analysis (PCA).

PCA was run using non-linear iterative partial least square (NIPALS) algorithm, with a total of 1000 iterations. This allows to visually explore the structure of the data and evaluate the variation within the latest. Loadings, known as linear combination coefficients, are used to help understanding which variables affect the most the sample separation. Sample replicates presenting a lower within-sample variability (intra) than between-sample variability (inter) should be clustered together and separated from other sample replicates on the first principal components. PCA was run using the Unscrambler® X 10.1.

3. Results and discussion

3.1. Preliminary considerations

As illustrated in Ref. [15], transfer is affected by three main categories of factors, the donor, the receiver and the contact, which lead to an initial post-coïtal transferred composition of the trace. The donor is here assessed as the condom, and its characteristics can easily be known based on the different published market studies. Similarly, the receiver characteristics, in this study the vaginal matrix, can be investigated, although the cyclic evolution of the menstrual cycle [19–22], specific medication, the use of toiletries or genetics are likely to influence the composition. Contact parameters includes the pressure and the duration of the contact between the donor and the receiver, and more broadly various parameters linked to the sexual intercourse itself [15]. If both donor and receiver parameters can be monitored within such a study, the contact parameter cannot be monitored without causing ethical issues. Therefore, the contact parameter will not be discussed further in this study.

All the volunteers were asked to collect 3 blank swabs prior to sexual intercourse. All the blanks were run before the rest of the samples to ensure that there were no silicone residues within the blanks. This allows to ensure that the detected compounds come from the questioned sexual intercourse and not from previous use of silicone containing products. All the blanks were found to be blank for all the volunteers. Fig. 1 illustrates the results of the blank swabs and swabs with silicone collected from one volunteer.

These observations confirm the observations of [14] who indicated that silicone bands were well resolved from the vaginal secretions and were indicative of the presence of PDMS in the vaginal matrix when some is present.

Prior to investigating the donor and receiver parameters, it is necessary to evaluate the vaginal matrix effect on the collected PDMS traces, the influence of different concentrations of pure lubricant as well as the influence of diluted lubricant extracts from the cotton swab. Three different types of samples were used: a standard reference PDMS diluted in hexane at a concentration of 1 mg/mL, with no substrate, cotton swabs spiked with various concentrations of PDMS between 1.0 and 3.0 mg/mL, introducing the cotton support, and cotton swabs with residues from self-sampled volunteers, thus including the vaginal matrix.

This allows evaluation of whether there are any specific patterns linked to dilution, to cotton swabs and/or to the human matrix. No visual differences were observed as illustrated in Fig. 2.

Principal component analysis was performed to see if the samples were statistically distinguishable. Results are presented on Fig. 3.

Three clusters can be observed from Fig. 3:

- Cluster A contains 3 replicates from the PDMS traces in the vaginal matrix separated from the rest of the dataset
- Cluster C contains 7 replicates from the PDMS standards
- Cluster B contains the rest of the samples analyzed.

These data highlight the significant variability that can be encountered in casework. Indeed, samples from Cluster A were found to originate from a single volunteer, whereas all the rest of the receivers were found to be clustered together (Cluster B). Therefore, the separation of Cluster A from the rest of these replicates might be

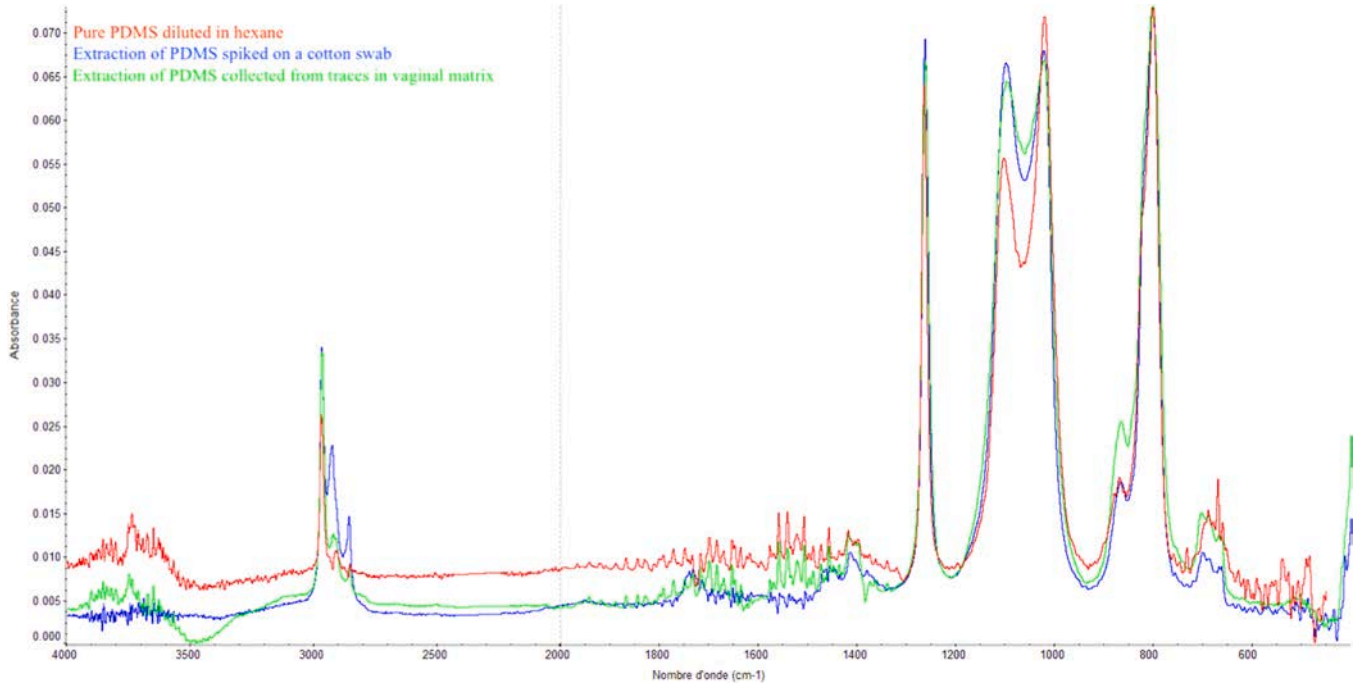

Fig. 2. Illustration of the chemical profile obtained from pure PDMS diluted in hexane at a concentration 1.0 mg/mL (red spectrum), extract of PDMS spiked on a cotton swab at concentration 3.0 mg/mL (blue spectrum) and extract of PDMS collected as trace evidence in a vaginal matrix after intercourse (green spectrum).

due to the receiver rather than to the matrix effect. Investigation of the loading's plots indicated that the separation of this cluster from the rest of the data was due to the CH_2/CH_3 content, which was previously assigned to cell membrane components and sex hormones. Variation in the concentrations of these compounds in this sample type may contribute to this clustering. These variations could originate from a specific contraceptive use as well as from the hormonal cycle variation. No information on the type of contraceptive used by the volunteers was asked, therefore this hypothesis cannot be confirmed. Regarding the hormonal cycle variation, there is, to the authors' knowledge, no publication monitoring these variations of the vaginal matrix over multiple cycle period, but such studies would definitely help assisting further interpretation of transfer and persistence results of other type of evidence in the forensic area.

Samples constituting Cluster C were found to all come from the reference PDMS samples. Loadings plots highlighted that this cluster was separated from the rest of the samples due to its silicone content. Variations in concentration would explain such clustering. However, as previously outlined in regarding DRIFTS analysis, significant variability can be observed for this type of analysis due to the sample preparation [23,24]. The particle size of the ground KBr used for the analysis might generated variable adsorption of the silicone molecules and variable coating of the KBr while depositing the extract on the cell, as previously described by Ref. [14]. If grain size varies, the distribution of PDMS around KBr grains will differ and so will the resultant coating. The contact area is maximized when the KBr particles are small, which increases the infrared signal. Moreover, it is impossible to control how the particles percolate through the KBr, and whether they remain on the surface or not. If the KBr particles are too small, adsorption may not occur, whereas if they are too large, the results will not be repeatable. These phenomena were reported to significantly affect the variability and thus are more likely to be the reason of the separate cluster.

Multiple concerns can be raised regarding the variability implied by the sample preparation and the implication on the overall feasibility of the results when applied to casework samples, where concentrations would vary. DRIFTS-FTIR was reported as a valuable screening method in several papers [10,25], as FTIR is available in most forensic laboratories

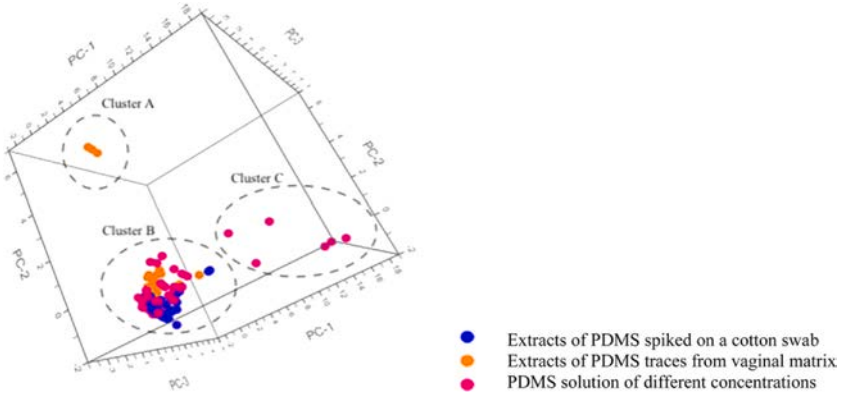

Fig. 3. 3D-Scores plot obtained from the PCA on the data used to investigate matrix effect. Several clusters are outlined, but more importantly is outlined the variability of the samples. PC 1–3 are presented. Separation is made according to the type of sample: extracts of PDMS on a cotton swab (blue), PDMS traces from vaginal matrix (orange) and PDMS solution (pink).

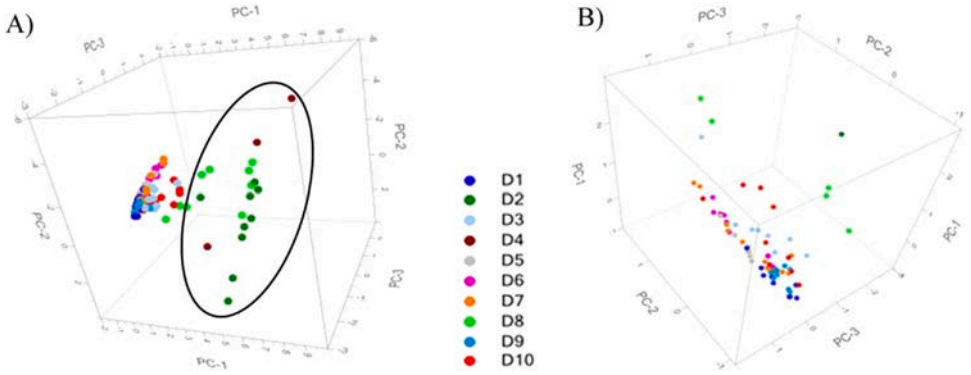

Fig. 4. 3D-Scores plot obtained from the PCA on the data used to investigate donor effect with **receiver 1**. PC 1–3 are presented. Samples are colorized according to donor (i.e. condom). For each donor, replicates are n = 9, except for D4 which has n = 3 replicates due to complications in sampling. A) considering the whole dataset B) after removing samples circled on Fig. 4A.

and offers the possibility of a rapid detection. When used in casework, a confirmation method such as for example py-GC/MS [16,26] or mass spectrometric techniques [4,5] should be used to infirm or confirm the results. FTIR is also very valuable in order to know which technique to use for further investigations. For example, the use of py-GC/MS is only relevant on silicone-containing samples, whereas GC/MS is more relevant on water-based samples [27]. However, more studies on the application to casework are needed for most of the confirmation techniques.

Regarding Cluster B, it was not found to be possible to differentiate the samples prepared from different substrates. These observations show that hexane extraction might not be affected to the same extent as methanol extraction in terms of matrix residues, as most of the matrix is expected to be aqueous/hydrophilic, and thus not soluble in hexane [15,21,22,25]. This might also reinforce that the chemical model presented in Ref. [17], could be directly applied to trace evidence without any major concerns. The preprocessing methods previously used to discriminate samples [17] were found to be unable to account for variability due to sampling types, as samples were not projected according to their sampling procedure in PCA scores plots. Investigation of further principal components (up to 7) did not enable further separation of the samples.

3.2. Influence factor I: donor

To investigate the effect of the donor, 11 donors from the market dataset published in Ref. [17] were used (Table 1). Two volunteers (receivers) had protected intercourse, the volunteer 1 doing the experiments with 10 out of 11 condoms while volunteer 2 only used 5 out of the 11 different condoms (donors). The volunteers self-sampled immediately after transfer (T = T0).

Given that the receiver might be affecting the results, PCA was performed separately on the data acquired for receiver 1 and receiver 2. Results are presented in Fig. 4 for Receiver 1, and in Fig. 5 for Receiver 2. As this section aims to observe the difference between the donors, and its consistency, data acquired for both volunteers will not be computed together.

Fig. 4A shows the PCA plots obtained when discriminating donors according to their chemical profiles. Donors D2, D4 and D8 were found to be statistically differentiated from other donors. Although reported as non-lubricated, D4 was found to present a very light silicone composition, which suggest that manufacturers need to put silicone to avoid the latex to stick to itself, no matter the type of condom [15,17]. Visually, sample D2 and D8 were found to present a different chemical profile than the other PDMS lubricated samples

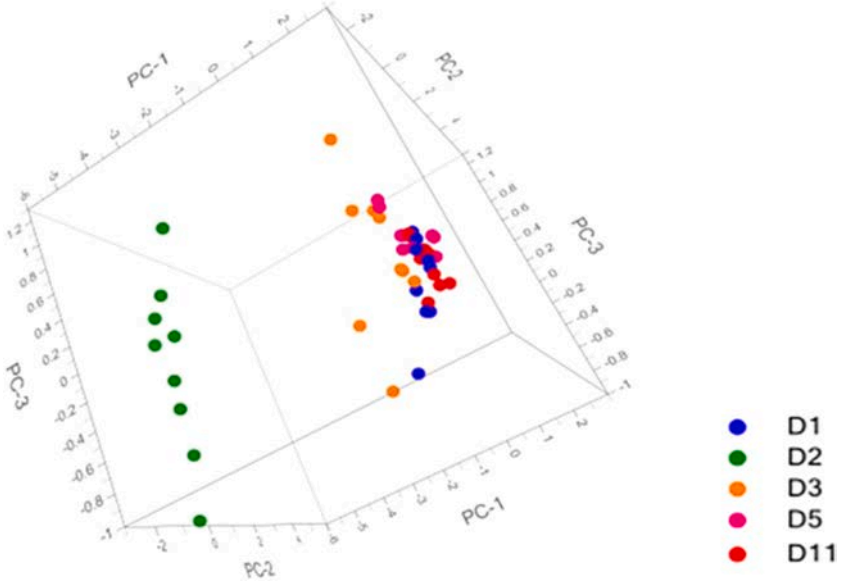

Fig. 5. 3D-Scores plot obtained from the Principal component analysis on the data used to investigate donor effect on **receiver 2**. PC 1–3 are presented. Separation is made according to the donor. For each donor, replicates are n = 9.

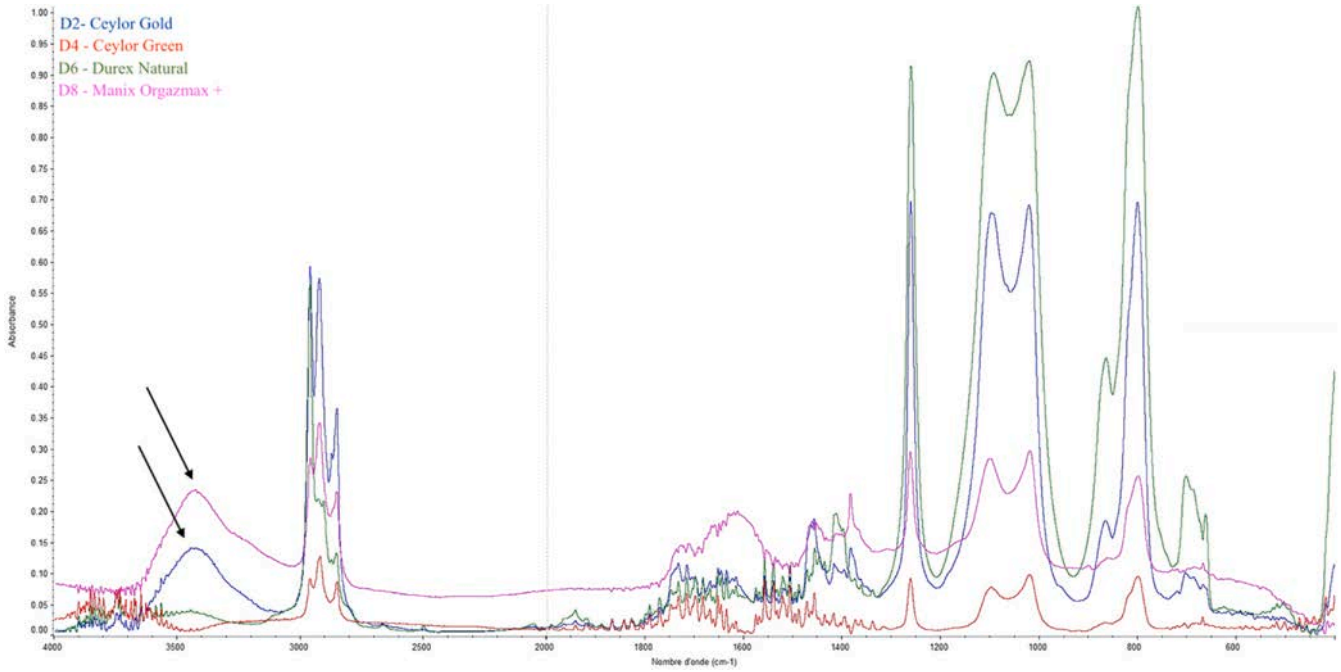

Fig. 6. Illustration of the different DRIFTS-FTIR chemical profile obtained for Durex Natural condom (D6 in green), Ceylor Gold condom (D2 in dark blue), Ceylor Green condom (D4 in red) and Manix Orgazmax Plus condom (D8 in pink). The black arrows indicate the OH bond, sign of the presence of water-based components. These chemical profiles perfectly match the ones previously observed when leading the market study using ATR-FTIR.

like D6, as illustrated on Fig. 6, due to the presence of OH bonds. This is due, for D2- Ceylor Gold sample to the presence of OH-siliconated lubricants and presence of PEG and nonoxynol-9 in the composition of the sample and for D8-Manix Orgazmax + to the presence of propylene glycol [17].

These observations are consistent with the market survey [17] as donors D2 and D8 were already found cluster separately from the rest of the condom samples. Regarding donor D4, such a classification is not surprising as it is a dry condom type, which means it is not lubricated. It looks like D4 is clustered with samples D2 and D8 and such an observation was previously observed with FairSquared Sensitive Dry sample, which was clustered with non-silicone-based samples [17].

Further observations on Fig. 4A and 5 also indicate that the composition of the body of the condom does not affect the chemical profile of the trace. This observation was already noted by Ref. [17] in their paper but is now also confirmed to be the case for trace evidence.

As donors D2, D8 and D4 significantly impacted discrimination, these samples were removed from the dataset and PCA was rerun on the remaining samples. Fig. 4B illustrates the scores plot generated from the reduced dataset. Samples from donors D10 and D3 were found to present significant variability. However, this variability can be considered normal and more likely to be linked to the analytical instrumentation or the self-sampling procedure than to other factors. The variability observed in the data is in accordance with the variability observed in the ATR dataset from the market study.

In both cases, further principal components up to PC7 were used to project the data, but none of them enabled improved separation of the samples in the dataset.

The same procedure was applied to samples collected from receiver 2. As illustrated on Fig. 5, donor D2 was again found to be clearly separated from the rest of the dataset, which confirms previous observations in the market study and receiver 1. The pattern observed on Fig. 4 strongly resembles the pattern observed in Ref. [17] which illustrated the various types of chemical profiles observed within the condom population. According to Ref. [17], Donor D2 is known to belong to another category than the rest of the samples. Therefore, the clustering of donor D2 in Fig. 4 and 5 is due to the variability within the condom samples.

Discrimination patterns are in agreement with previous observations on the discrimination of condom profiles conducted with another instrument (i.e. ATR) and applied on raw samples (i.e. non extracted condom rubbed on ATR crystal) [17].

Observations of donor variation were found not to be distinguishable from those made in the market study presented in Ref. [17], although two different instruments were used. This suggests that the model created on the condom dataset in presented in Ref. [17], can be considered as relevant and representative as the trace samples presented in this study exhibit exactly the same discrimination patterns.

3.3. *Influence factor II: receiver*

To investigate the effect of the receiver, the donor was fixed (here, D1-Ceylor Blue or D9-Manix Skyn, both silicone-lubricated condoms), and 9 volunteers (here after receiver) were asked to self-sample post-coïtal residues.

Typical spectra obtained from real samples were already presented in Ref. [14]: no interaction with the matrix was noted, and spectra presented clear and well-defined Si-O-Si and Si-C stretching bands, thus confirming the efficacy of DRIFTS analysis for case work. PDMS was detected in all the swabs that were collected right after coitus. From a qualitative point of view, abundances were found to vary between different volunteers but also within the same volunteer (Fig. 7). Such variation is likely to happen due to self-sampling or parameters linked to the contact itself and cannot be reasonably monitored.

The PCA scores plot shown in Fig. 8 contains two main clusters, one consisting of replicates from receiver R7, the second containing the rest of the samples. Investigation of PCs 4–7 did not produce any further enhancement of sample clustering.

Such a strong separation of the samples can originate from variation in self-sampling procedure or from the analytical instrumentation. Samples coming from receivers R7, R8 and R9 were run on the same

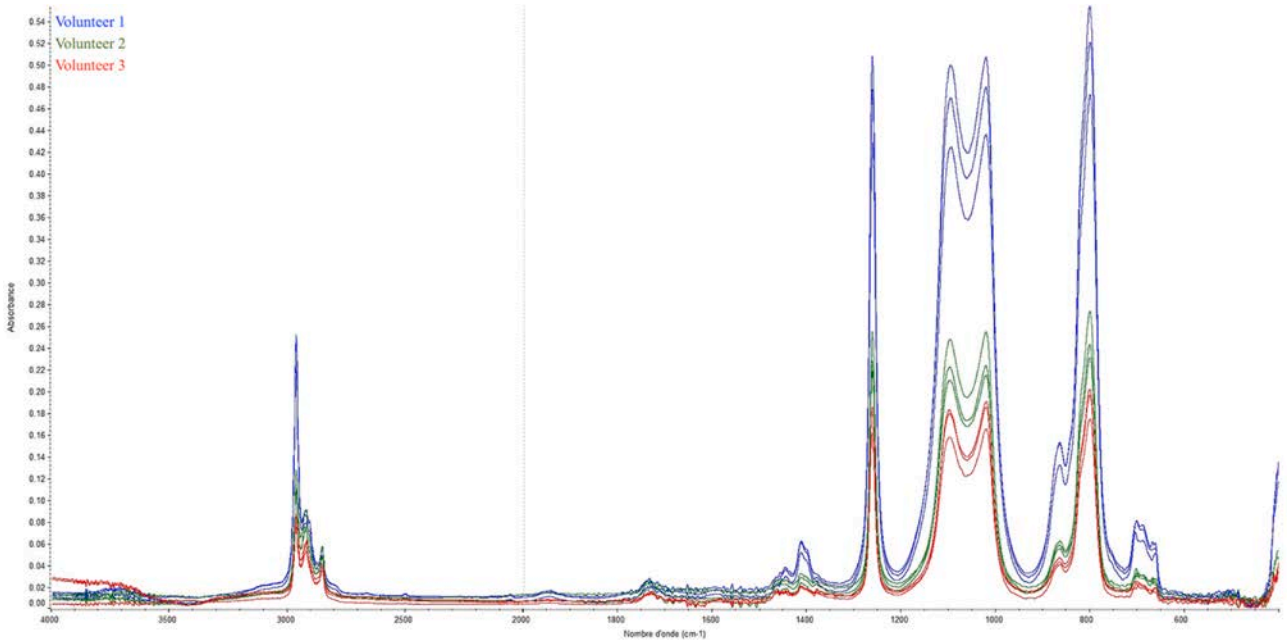

Fig. 7. Infrared spectra of post-coïtal transferred residues from 3 different volunteers. For each volunteer, the 3 replicates acquired are presented.

analysis day, which means it is unlikely that the variability of the sample preparation would be the main reason for R7 to be separated from the rest of the samples. Observation of the spectra obtained from R7 compared to the rest of the dataset coupled to the analysis of the loading plots obtained from the PCA computation allowed to note that the difference of abundance between the two bands coming from the Si-O doublet (Fig. 9) was the source of the variation.

Although Fig. 8 shows no significant differences between the volunteers when it comes to the relative abundance of the Si-O doublet band, similar difference of abundance was observed on Fig. 2, with pure PDMS samples analyzed after dilution in hexane. The first hypothesis considered for such difference was a variation of concentration. However, as illustrated on Fig. 9, not all the peaks are affected, only the silicone doublet, which does not make this hypothesis valid. The second hypothesis is that the variability induced in the DRIFTS results is partly due to the sample geometry: KBr particle size, influence of a manual KBr grinding or influence of a manual spiking on the pellet. A possible contribution from specular component, which differs depending on the KBr surface, can not be neglected. All these can induce a relative intensity changes.

Receiver R7 was clustered significantly separately from the rest of the samples. The corresponding samples were removed from the dataset and PCA was rerun on the remaining samples in order to investigate the variability between the other receivers. Fig. 10 illustrates the results of the new PCA. Receiver 3 was found to be clustered separately from the rest of the samples. Other samples were found to cluster together. Spectra were found to be visually similar, with no distinguishable features that could be used for sample separation according to the receiver. As the algorithm is able to detect a significative difference between spectra coming from Receiver 3

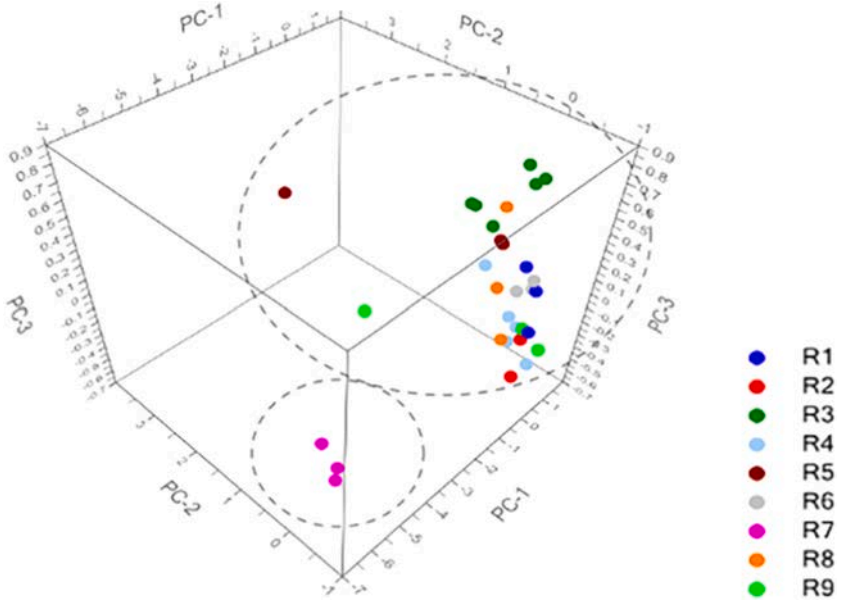

Fig. 8. 3D-Scores plot obtained from the Principal component analysis on the data used to investigate receiver effect. PC 1–3 are presented. Separation is made as a function of the receiver. Transfer was generated with silicone-lubricated condom D9-Manix Skyn for R2, R3 and R8, and with D1-Ceylor Blue condom for the other receivers.

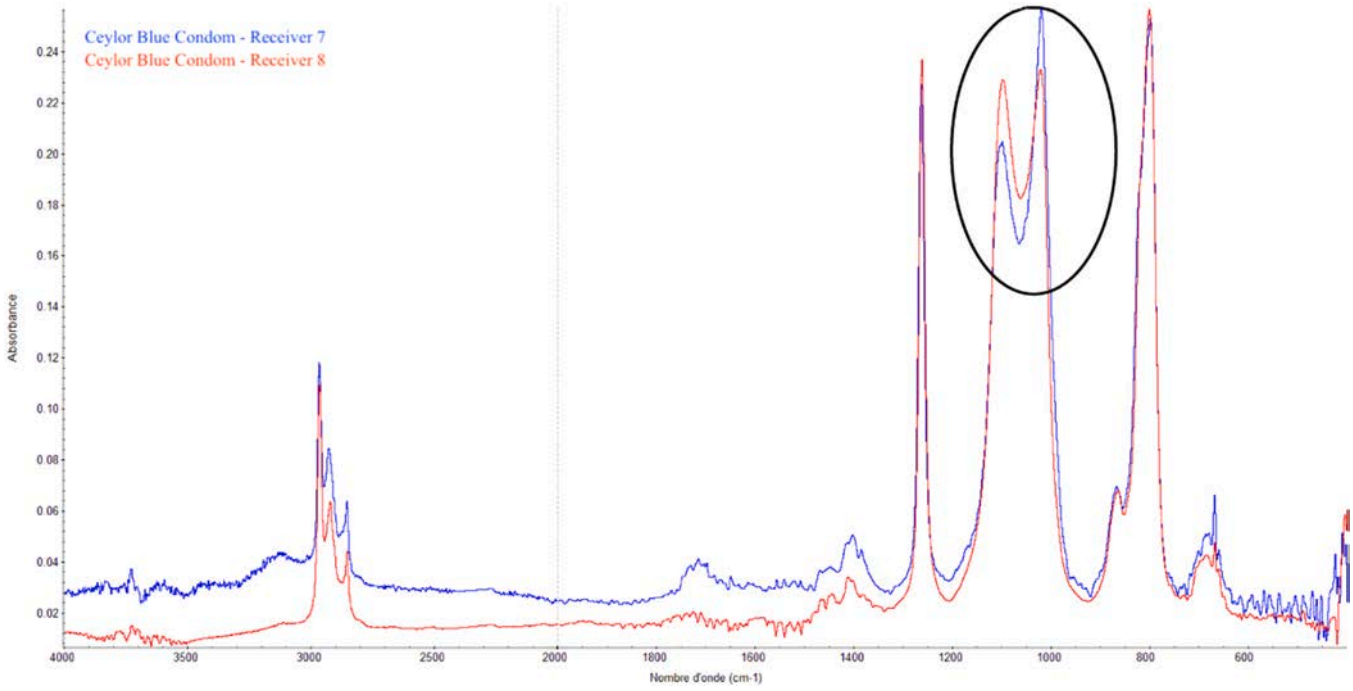

Fig. 9. Illustration of the difference of the abundance of the Si-O doublet peaks, highlighted in the black circle, between Receiver 7 and 8, samples collected at T = T0.

and the rest of the receivers, multiple hypothesis can be drawn, the main ones being:

1. A different initial concentration. This can result from variations during contact or during self-sampling.
2. Interactions between the matrix and the lubricants have generated chemical changes. Although these interactions have not really been reported and investigated and knowing that PDMS is highly nonpolar and not supposed to be reactive to most of the microbiota, this hypothesis is not very likely.

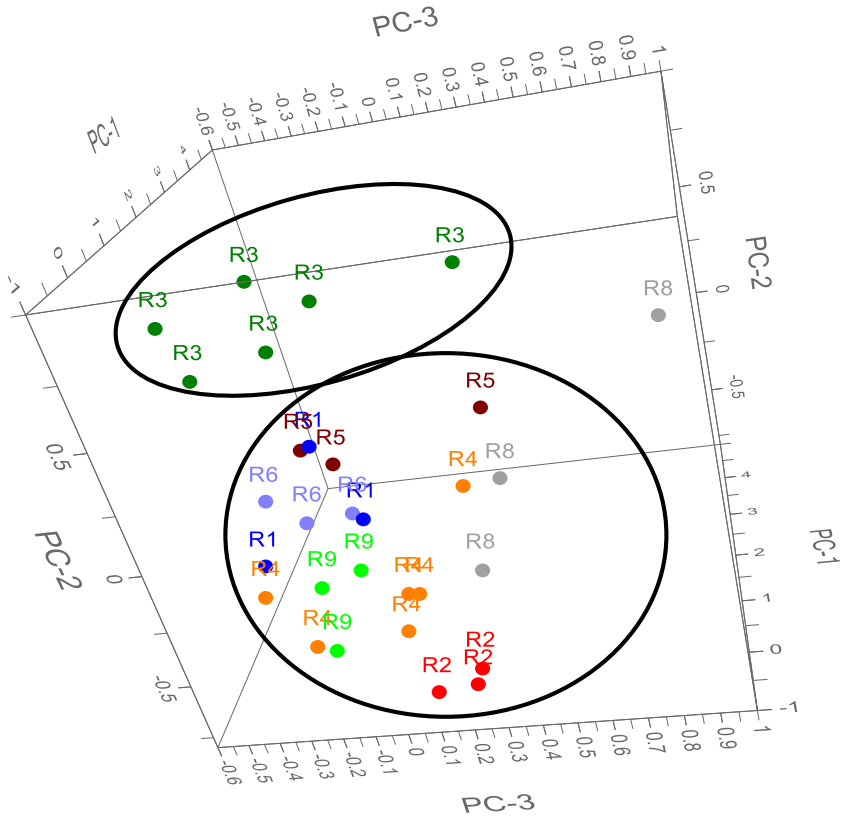

Fig. 10. 3D-Scores plot obtained from the Principal component analysis on the data used to investigate receiver effect. Receiver 7 removed from dataset. PC 1–3 are presented. Transfer was generated with silicone-lubricated condom (D1 -Ceylor Blue and D9- Manix Skyn).

3. The matrix has overcome some important changes at the time of the experiments, which explains the separation. Given the information obtained from the volunteer, there is no way to confirm that statement.

The clustering of the data is consistent with the results obtained in the background publication of Hermelin et al. [28] who showed that all the donors were not presenting different chemical profile nor were statistically differentiated. The present data illustrate a similar dispersion than the one observed in Ref. [28] and most of the data were found to cluster together independently of the receiver. Based on the indication discussed in Burnier and Massonnet [15], this strongly illustrate that the discrimination of the transferred residues does not seem to be less affected by the receiver than by the contact. The impact of the contact itself cannot be investigated and will remain an "uncontrolled" factor, as it implies to get information on the length of the penis, the intensity of the contact, or the duration of the sexual intercourse. Self-sampling also adds some variability, but such variations will be observed even though sampling is realized by a health care professional /SANE nurse, regardless of standardized training. Finally, some variations within the sample preparation, especially due to the manual preparation of the samples, which adds undesired additional variation to the data.

The great variability observed amongst the DRIFTS-FTIR results does not make it the gold method for an application in casework. Indeed, reproductibility is one the important parameters to control when considering a validation procedure in the eventuality of an accreditation for use in court. However, DRIFTS is a very interesting and successful screening method, fast and easily available. However as described in Refs. [10,25], FTIR is a screening method that should be coupled to an adequate confirmation method, such as GC/MS or py-GC/MS for example.

4. Conclusion

This study investigated the classification of condom traces after their transfer in a vaginal matrix as well as factors impacting transfer, such as the donor and the receiver, using human collected samples.

Visually, none of the condom extracts containing silicones were found to be significantly distinguishable, whereas non lubricated or water-based containing samples were found to be distinguishable. Clustering patterns observed using unsupervised statistical analysis were found to be similar to the ones obtained using ATR-FTIR in previous studies [17]. Traces were found to be not visually distinguishable from the reference material they originated from. Statistically, the algorithm created 4 clusters, which were found to be consistent with groupings created in previous publications. The results indicate that reference condom material and transferred traces do not present distinct chemical profiles. The experiments described in this study illustrate the potential to distinguish condoms in a similar way to what was observed during the construction of the infrared profile database.

Transfer was found to be affected mainly by the donor (i.e. the condom) rather than by the receiver (i.e. the vaginal matrix), which is not surprising when considering an apolar extraction of the cotton swabs. The contribution of the support after extraction of the traces from the cotton support proved to be practically nonexistent: the diluted silicone standards and spiked cotton having statistically indistinguishable profiles from the trace samples from a living vaginal matrix.

The experiments also highlighted the current difficulties in reducing the variability of the parameters due to different volunteers. If the procedure were to be limited to the use of known factors, the use of comparison material from the identified victim would be required, but this practice would be ethically questionable. These ethical questions were already highlighted in fingermark ageing works, where authors showed out that a model would need to be built using the suspect (if you can find them) and replicating the exact environment of the evidence [29–32].

The results obtained are no less interesting, but it is clear that additional studies must be carried out in order to assess the impact of the various influence factors and whether it is possible to construct a more relevant statistical model. Investigation of persistence of the traces as well as the ability to visually and statistically distinguish background from transferred and persisted evidence are still needed so as to allow a proper interpretation of the evidence in a Bayesian framework.

CRediT authorship contribution statement

Nikola Saric: Writing - original draft, Methodology, Validation, Data curation, Visualization. **Loïc Fabien:** Writing - original draft, Methodology, Validation, Data curation, Visualization. **Julia Fischer:** Writing - original draft, Methodology, Validation, Data curation, Visualization. **Anaïs Hermelin:** Writing - original draft, Methodology, Validation, Data curation, Visualization. **Geneviève Massonnet:** Writing - review & editing, Resources. **Céline Burnier:** Writing - review & editing, Conceptualization, Methodology, 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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The use of an optimized DRIFTS-FTIR method for the forensic analysis and classification of silicone condom lubricants

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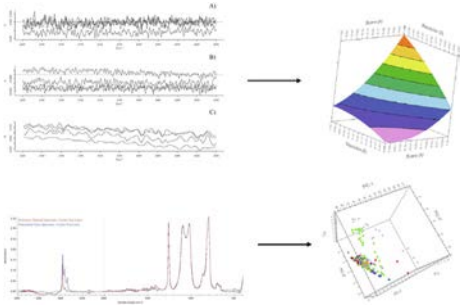
HIGHLIGHTS

- Optimal analytical conditions for DRIFTS-FTIR analysis are 64 scans and 4 cm^{-1}.
- Condom discrimination is not enhanced with DRIFTS compared to ATR-FTIR.
- Traces were found to cluster with the rest of the condom pattern.
- It was not possible to attribute a source to the trace that was recovered.

GRAPHICAL ABSTRACT



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ABSTRACT

Condom residues may be encountered in forensic investigations as traces left in sexual assault or rape cases. Considering casework samples analysis, where material from swabs will need to be extracted, Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFTS) was reported as the most relevant method for trace evidence analysis. However, there has been no study to identify which specific 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 resolution and number of scans 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 and extended to a face centered central composite design (FCCD) for the optimisation. Repeatability of the results was also investigated using principal component analysis (PCA) and hierarchical cluster analysis (HCA) in order to select the most relevant analytical parameters. The optimized DRIFTS parameters were then used to collect data from reference material and from traces after a transfer in a vaginal matrix has occurred. Discrimination models were built with DRIFTS data and compared to pre-existing models built with Attenuated Total Reflectance (ATR)-FTIR data. Condom traces were recovered from volunteers who had sexual intercourse using different types of silicone-lubricated condoms. The corresponding traces characteristics were investigated and analysed.

1. Introduction

Condom trace evidence; including particulates, lubricants and spermicides; has been reported in the forensic investigation of sexual assaults and rape cases for the past 40 years [1]. These traces can be used by forensic investigators to determine whether or not a condom was used during intercourse, potentially supporting the statements of one of the parties.

The majority of condoms consist of latex coated with solid particles and lubricant. The latter is usually polydimethylsiloxane (PDMS), found in more than 85% of condoms on the international

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market [2–10], or polyethylene glycol (PEG). Solid particles are added to the latex during production to avoid the latex to stick to itself. They are likely to be used as forensic evidence in casework [10,11]. Other additional components such as anaesthetics, colourants or flavourings, can also be found in very small quantities [10]. Condom residues recovered in the investigation of a sexual assault will contain a combination of these compounds along with components of the vaginal matrix. This complexity poses a challenge for detection, which is affected by the initial transfer, including the influence of the source of the trace (i.e. the condom) but also by the vaginal matrix itself and the type of contact (i.e. duration, intensity). Finally, the time elapse between the alleged activity and the sampling as well as the activity of the victim during this period will influence the persistence of the target compounds [8–10,12].

Condom traces are typically sampled using cotton swabs, then extracted into a solvent for analysis [1,12]. Dichloromethane is the most common extraction solvent, although hexane and isooctane have also been reported as dichloromethane may not provide for complete extraction of the condom traces [12]. A screening method is usually performed to determine the classes of compounds present, followed by a confirmatory technique to identify specific components [8–10,12]. Spectroscopy is frequently used for preliminary analysis of condom residues [12–15] due to its rapidity, simplicity of use and availability in forensic laboratories. In particular, Fourier Transform infrared (FTIR) spectroscopy is frequently employed in both research and casework as a screening method, with a preference for diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) when handling caseworks [1,12,15]. DRIFTS is ideal for this purpose as it allows the analysis of polymers such as PDMS, and has been confirmed by several authors as the most relevant method for the analysis of condom residue in solution, based on the spectral quality, the reproducibility, the analysis and preparation time as well as the applicability to trace evidence [1,16].

Although DRIFTS has been established to be suited to the analysis of condom traces [1,12,15], the analytical parameters are multiple and various in the literature. Although this works is for qualitative analysis, analytical conditions should be statistically reproducible and repeatable for an application to casework. Factors influencing the repeatability of DRIFTS analysis have never been reported to the best of our knowledge and there is a need to obtain more reproducible and repeatable results, with a good signal-to-noise ratio (SNR), to be able to work with traces. Development and optimization of the SNR using statistical algorithm, such as design of experiments, is still necessary. Enhancing SNR will ensure that that vibrational bands of the target compounds are distinguishable from the background for qualitative examination. Minimising noise is also mandatory to ensure that detected information can be attributed to the evidence rather than instrumental artefacts, and to improve reproducibility. Forensic sciences attach a strong importance to the identification of the source of the recovered evidence. In the case of condom evidence, inferring on the source means identifying the brand or type of condoms. The most common way to answer such questions is to conduct a statistical exploratory study on a large population set. Such studies have been widely reported with different analytical techniques, and amongst all ATR-FTIR was one of the most important. However, there's no such published available study for DRIFTS analysis, and this can be a serious issue as this is the recommended method for the analysis of condom trace evidence in a forensic context. Therefore, it is necessary to create such a model and compare it with the previously published ATR-FTIR model. In addition, it is of prime importance to see whether real traces are classify the same way pure lubricant samples do.

The present paper aims at first to determine which factors, between the number of scans and the resolution, most significantly affect the SNR, in order to obtain a more adequate understanding of how to analyse silicone-based condom residues. Designs of experiments were used to explore all the possible combinations of parameters variations within the experimental plan [17], with the advantage to allow data collection and analysis with proper statistics to obtain maximal information, such as important factors affecting the targeted response (here, the SNR). In a second part, the optimised parameters were then applied to silicone-lubricated condom extracts and real trace samples, with the aim to build a discrimination model and investigate the classification of real samples in the given model, to observe the differences with pre-existing models and provide relevant information for the forensic scientist, when it comes to casework application.

2. Material and methods

2.1. Material

Hexane (Sigma-Aldrich, Damstadt, Germany) and Methanol (Sigma-Aldrich, Damstadt, Germany) of analytical grade were used as received. Dimethylpolysiloxane 200 cSt was purchased from Sigma-Aldrich (Damstadt, Germany). Cotton swabs (COPAN150C) were purchased from COPAN (Brescia, Italy). Potassium bromide (analytical grade) was purchased from Fluka Chemika (Honeywell International) and was manually grinded before use. eVol XR® syringe from SGE Analytical (Trajan Scientific Australia) was used for spiking the samples on KBr pellets.

2.2. Instrumentation and sample analysis

2.2.1. DRIFTS-FTIR

Infrared **DRIFTS** spectra were acquired with a Digilab FTS 3000 Excalibur FTIR spectrometer (Portmann Instruments AG, Biel, Switzerland), equipped with a Spectra-Tech 0030-05 Collector II diffuse reflectance accessory (Portmann Instruments AG, Biel, Switzerland) and DTGS detector. Spectra were collected from 4000 and 400 cm^{-1}. 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 in a 100 °C oven. For analysis, 10 µl 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. Blanks were prepared in the same manner using 10 µl of hexane and analysed every 3 measures to account for background interference. Extraction blanks were also prepared in the same manner using extraction of a clean swab.

2.2.2. ATR-FTIR

Infrared **ATR** spectra were collected using a Nicolet iS50 FTIR spectrometer equipped with single-bounce diamond crystal ATR accessory (ThermoFisher Scientific). Data collection was carried out using the OMNIC software v. 8.2.0.387 (ThermoFisher Scientific). Spectra were collected over the 4000 to 400 cm^{-1} range with 4 cm^{-1} resolution and 32 co-added scans. Samples were deposited or rubbed directly on the crystal as described in [18].

2.3. Optimization of the analytical DRIFTS conditions

2.3.1. Identification of factors influencing the Signal-to-Noise ratio (SNR)

Several experimental designs were conducted in this study, as an iterative process in order to obtain the highest and less variable

SNR. All the designs were realized using a single standard solution of bulk PDMS diluted in hexane (1 mg/ml) and spiked on a KBr pellet. 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 four replicates of each point including the central point. This resulted in a total of 20 randomized program experiments. The central point was defined at 64 scans and 4 cm^{-1} because it is the closest point to regular practice in forensic laboratories [27].

The second experimental cycle was led to estimate the effects of each factor. A new FFD was designed with new scan number, chosen within a factor 2 from the central point, i.e. 32 and 128 scans. That was decided to reduce the time of analysis as some of the highest level of scan and resolution might significantly increase the latest. The resolution variable was modified, to correspond to a variation of a factor 2 around the value of the central point.

Finally, a third experimental cycle was an extension of the FFD into a Central Composite Design (CCD), more specifically here, a face centered composite design (FCCD). The remaining points were added to the initial FFD to capture the true relation between the factors and the SNR. For each cycle, effect significance, lack of fit, regression significance and curvature were evaluated. A total of 80 experiments were led for these designs.

Data analysis for experimental designs were performed in Unscrambler X v.10.1 (Camo Software, Oslo, Norway) 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 [19], and the curvature of the plan according to a Student's test [17]. Several regression models of different complexity (from linear to quadratic) were fitted on the data. The model describing the relation between the factors was then selected based on the highest lack-of-fit p-value and the lowest regression significance p-value.

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

Factor	Level −1	Level 0	Level 1
Scan number	16	64	256
Resolution [cm^{-1}]	8	4	1

Table 2
Factor and levels used for the calculation of the effects, using a FFD design.

Factor	Level −1	Level 0	Level 1
Scan number	32	64	128
Resolution [cm^{-1}]	8	4	2

2.3.2. Repeatability evaluation and parameters selection

Repeatability of the instrument is important, especially when it comes to trace evidence analysis. To evaluate the repeatability, two different statistical methods are usually used: principal component analysis and hierarchical cluster analysis, with distance or correlation measurements. All these techniques will be used to investigate which number of scans provides, for a given resolution, the highest repeatability, with spectra clustered the closest to each other. This will allow the identification of the number of scans which provides the highest repeatability of the data.

2.4. Application to condom samples

In order to evaluate the applicability of the optimized method to real samples, 16 condoms and 2 lubricants from major distributors and manufacturers on the Swiss market were purchased from Swiss supermarkets and pharmacies (Table 3). All the samples were previously categorized as containing a silicone-based lubricant [18] except for Fair Squared Sensitive Dry (P11), which is a non-lubricated condom.

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. Before analysis, samples were aliquoted and diluted 10 times. 10 µl of the solution were spiked in the quartz tube on the glass wool and the analysis was processed. Three 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.

2.4.1. Data pattern recognition – ATR vs DRIFTS analysis

The results of the 16 samples acquired with the 2 different IR methods were analysed using Principal Component Analysis to identify the potential clustering and classification in the data. The hypothesis to corroborate is the following: samples are not clustered according to their analysis type but according to their

Table 3
Samples used for DRIFTS discrimination model. Composition is known based on observations from [18].

N0	Brand	Model	Type	Lubricant	Other Component
P0	Durex	Performa	Latex	silicone	no
P1	Durex	Invisible	Latex	silicone	no
P2	Durex	Natural Feeling	Polyisoprene	silicone	no
P3	Migros	M-Budget	Latex	silicone	no
P4	Migros	Cosano Regular	Latex	silicone	no
P5	RFSU	Profil	Latex	silicone	no
P6	Manix	Contact	Latex	silicone	no
P7	Manix	Skyn Original	Polyisoprene	Silicone	no
P8	Ceylor	Blauband	Latex	Silicone	no
P9	Ceylor	Non-Latex	Polyurethane	Silicone	no
P10	Coop	PrixGarantie	Latex	Silicone	no
P11	Fair Squared	Sensitive Dry	Latex	Non lubricated	Solid Particles
P12	Vitalis	Natural	Latex	Silicone	no
P13	Amor	Nature	Latex	Silicone	no
P14	ESP	Skin	Latex	Silicone	no
L1	Durex	Play play eternal - Perfect glide	Personal lubricant	Silicone	no
L2	Ceylor	Silk sensation	Personal lubricant	silicone	no

sample category. This hypothesis has, to the author's knowledge, never been reported before, these two techniques usually being tested one against the other, and not as the potential of complementary techniques. This investigation aims to verify if samples obtained with DRIFTS analysis can be projected in the market survey discrimination and classification models built with ATR results [18], and if not, to evaluate if the discrimination patterns obtained on the two techniques are similar. This would allow to transpose the discrimination pattern observed on the entire dataset from one technique to the other, although variability could affect the pattern.

2.4.2. Trace vs reference classification

To investigate the classification of real samples, 2 volunteers had sexual intercourse using 10 different condoms, coming from the list presented in Table 1 as well as from other condom types presented in [18]. 3 blank swabs were collected prior to intercourse, and 3 samples swabs were collected right after intercourse. To avoid any cross-contamination, the volunteers were asked to wait one week between each protected intercourse. Each sample was analysed 3 times, as described for the condom samples, resulting in 9 replicates. A total of 132 analysis were run for this purpose. Sample collection was led in accordance with the Swiss Federal Act on Research involving Human Beings (Ordinance on Human Research with the Exception of Clinical Trials) and approved by Swissethics (2018-00690). Informed consent was obtained from all donors, and collected data was entirely anonymized. Based on previous researches [1,12,14], cotton swabs collected from the volunteers 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 triplicate.

3. Results and discussion

3.1. Identification of factors influencing SNR

3.1.1. Response surface screening

Analyses carried out on the Full Factorial Design were first visually analysed to evaluate the variability among all the replicates, by looking at the variability around the baseline, and the noise variation. The results showed that both number of scan and resolution affect the SNR, as well as their interaction. The quadratic effects were found to be non-significant. The non-significance of the lack of fit (p-value = 0.805) allowed to assume that all main effects are linear in this model. If the number of scans is increased, SNR increases as well, but so does the analysis time. In the same manner, the lower the resolution, the higher the SNR, but the lower the quality of the spectral information will be. Choosing the adequate analytical parameters is a compromise between the amount of spectral information and the SNR.

The visual comparison of the noise observed in the spectra obtained at 16 scans for the resolution at respectively 1 and 8 cm^{-1} present a good repeatability, in terms of intensity fluctuation. The noise is very important when the resolution increases and reaches 1 cm^{-1}. Similarly, at 256 scans, a very good repeatability is observed, with a noise higher if the resolution is higher. Repeatability is globally higher at 256 scans than at 16 scans for a same resolution (cf. Fig. 1A and B). The scans at 64 scans and 4 cm^{-1} have a rather good repeatability (Fig. 1C). The impact of the resolution is clearly visible compared to the other sets of analysis.

To investigate the variation of the SNR as a function of the number of scans and of the resolution, the noise (RMS) was integrated on the 2200–2000 cm^{-1} region as it is the range where the beam

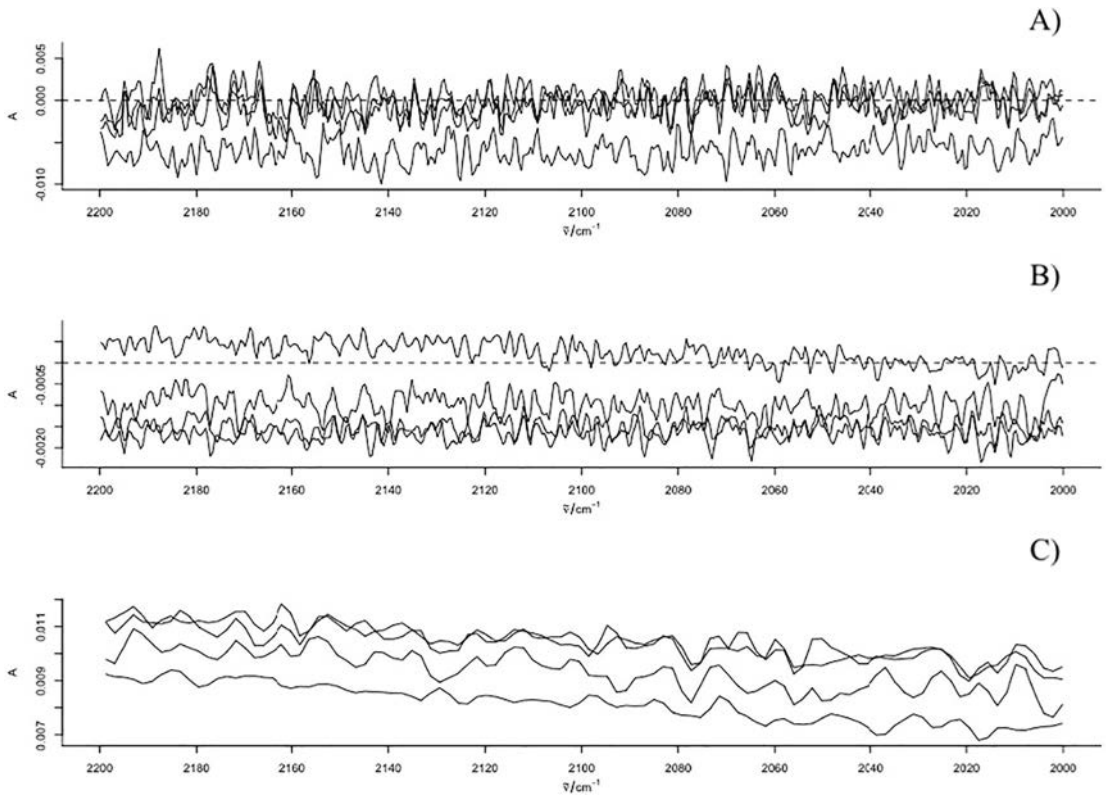

Fig. 1. Illustration of the noise of the spectra acquired under different number of scans and resolution over the 2200–2000 cm^{-1} region, four replicates per design point are presented, A) 16 scans/1cm^{-1}, B) 256 scans/1cm^{-1}, C) 64 scans/4cm^{-1}.

Table 4
Observed coefficient of variation (%) of the SNR as function of the number scans and of the resolution.

Scans	Resolution	SNR	CV %
16	1	283 ± 15	5.60
16	8	883 ± 65	7.43
64	4	1424 ± 91	6.42
256	1	1440 ± 204	14.16
256	8	3662 ± 443	12.09

intensity is the greatest [20]. SNR was calculated as follows: $SNR = \frac{Baseline\ \%\ Transmittance\ Value}{RMS}$. The baseline transmittance value was usually 100% but was sometimes slightly lower (i.e around 99.5%) and therefore was adapted as a function of the value obtained for each spectrum.

Table 4 highlighted the lower variability of the central point (64 scans, 4 cm^{-1}). As illustrated in Table 4, the SNR is lower when a high resolution set up is used than when a lower resolution is used. To this effect, a high resolution at 1 cm^{-1} is not recommended if planning to work on real cases: when dealing with trace evidence, there's a need to have the highest SNR. Similarly, for the number of scans, a too low number of scans provides a lower SNR and therefore is not recommended.

The variability of the SNR was also investigated as it was assumed that the variability detected on the SNR would be observed as well on the data collected from real samples. Variability was found to increase as the number of scans increases (cf. Table 4). Thus, it seems inappropriate to expose the sample to too many scans, since not only the analysis time increases, but so does the variability. The coefficients of variation obtained for the low number of scans are less than 10%, which is rather good considering the mode of analysis and the problems related to diffusion that can be observed. These observations confirm that both number of scan and resolutions are important parameter influencing the reproducibility of the data.

Higher number of scans induce higher variability of the SNR, and the reproducibility is affected as well. Therefore, analyses at 256 scans were discontinued for further investigations. The surface screening showed more repeatable results for a number of scans closer to 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.

3.1.2. Calculation of the main effects

The knowledge acquired in the first cycle of experiment allowed reducing the factors closer to the center points. A new two-level factorial design of experiment was run to estimate the effects of each of the factors. Each point was analysed four times to get replicates. Calculation of the main effect of each parameter were realized as described in [17] and respective effects of ~3196 (p-value = 0.0001) for the number of scans and ~−2096 for the resolution (p-value = 0.0008) were obtained. These results show that both effects almost equally influence the response of the SNR. Both factors have positive effects. This means that decreasing the resolution (i.e. 8 cm^{-1}) and increasing the number of scans cause an increase in the SNR. The goal is to increase this ratio, since the signal must be maximized with respect to noise. To achieve the goals set, the number of scans must be maximised, and the resolution minimized. The effect of the interaction has also been calculated and is ~1496 (p-value = 0.0016). The effect here is half as important as the one of the numbers of scan, and almost as important as the one of the resolution, while positively affecting the SNR. This interaction is therefore important for the model because its effect is as important as any of the main effects.

This design was still not sufficient to have a complete coverage and understanding of all the interactions. Thus, an extension to a FCCD design which allows computing more complex interactions and create a final response surface modeling with the best understanding of the impact of each parameter was achieved.

3.1.3. Response surface modeling

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 number of scans and the resolution, then considering their interaction

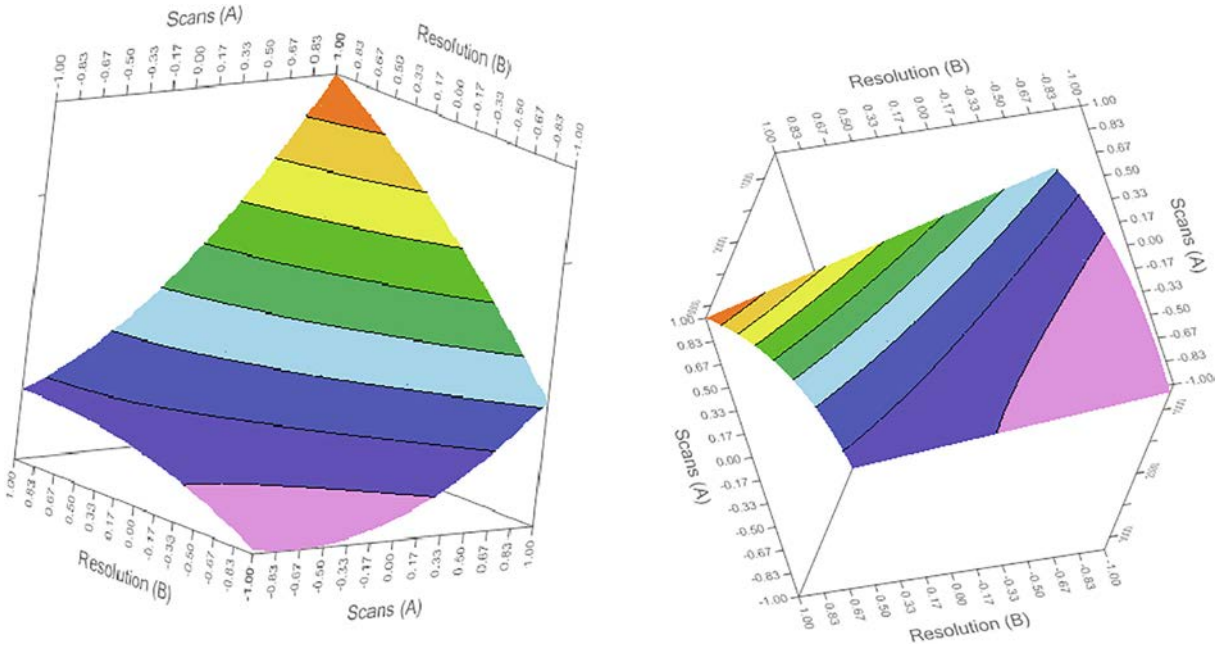

Fig. 2. Surface response obtained for the quadratic model with interaction (left) and for the optimization using only the main effects (right). Factors level are the ones described in Table 2.

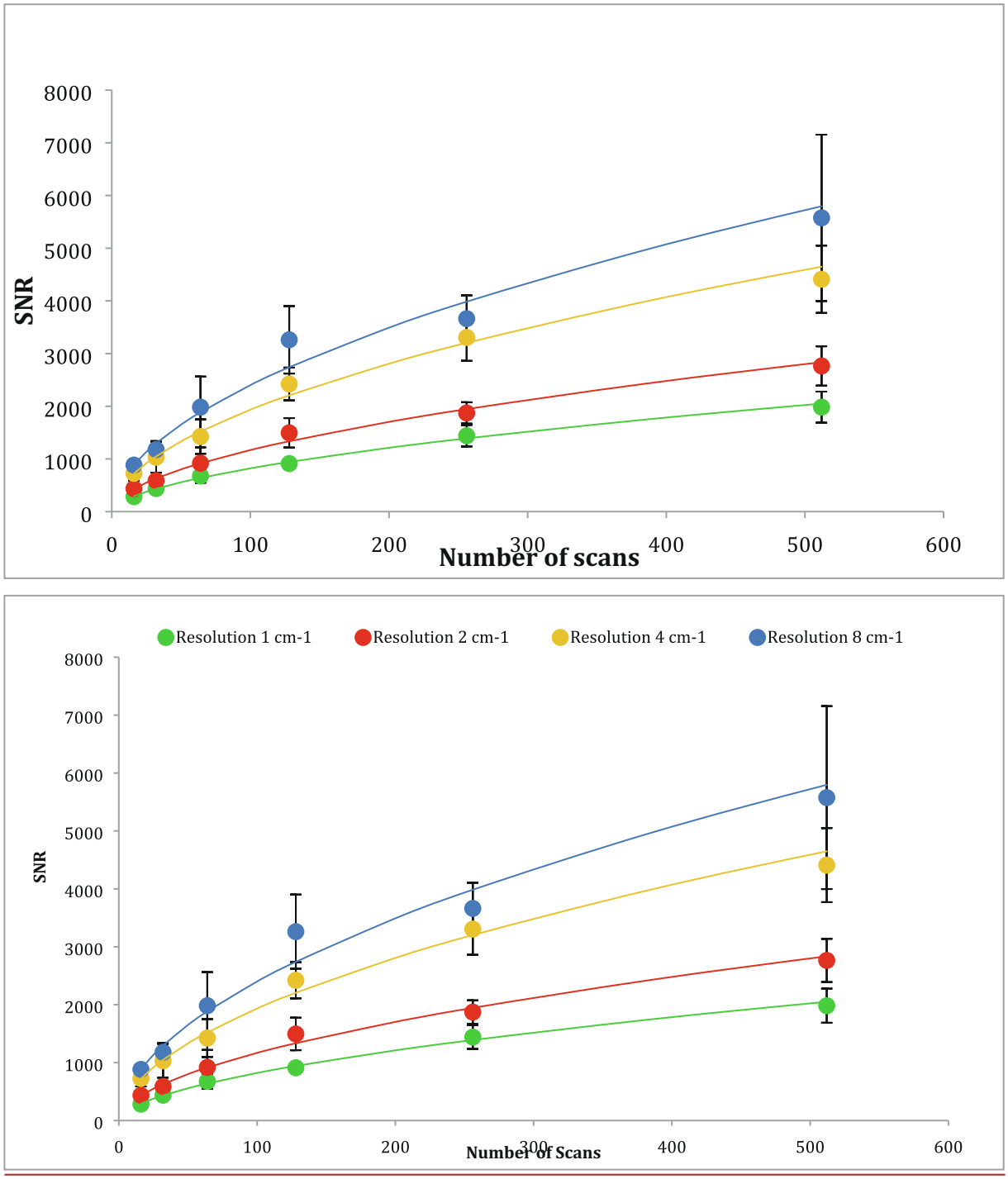

Fig. 3. . Illustration of the relationship between scan number and resolution.

and finally considering second order terms. First order effect were found to be statistically significant, with p-values < 0.001. Within this model, the AB interaction was found to be non-significant, with a p-value of 0.47. Quadratic effects were also investigated but were not found to be significant, with p-values respectively 0.646 for the Scan × Scan (AA) parameter, and 0.388 for the resolution × resolution (BB) parameter. The curvature was not found to be significant, indicating that only a linear model would fit on the data. In addition, the lack of fit was non-significant with a p-value of 0.8051 which indicates the model is not adequate for such a model. The different models were all compared using the adjusted R^2 with a partial Fisher-test. None of the models were found to fit properly. Lack-of-fit values were found to be 0.085 for the first-degree model with interaction, 0.805 for the second-degree model with all the factors and 0.8725 for the second order models without the squared resolution. This indicate that none of these models are likely to fit the data. Surface response obtained for the quadratic interactions and the linear modelling are gathered in Fig. 2.

Multiple regression models were tested and showed out that only the number of scans and the resolution were significant for the surface response. In addition, when removing the quadratic

interactions, the obtained surface response was completely linear, suggesting an increase of the SNR with the increase of the number of scans and the decrease of the resolution. To be able to select adequate analysis parameters, focus was set up on literature reporting that the SNR increases according to an exponential curve of the type $y = ax^b$, the exponential of which is close to a theoretical value of 0.5 [20]. Therefore, optimal analysis parameters will be selected based 1) on the exponential curve for the optimal resolution number, and 2) on the shorter distance or correlation between spectra acquired for a same resolution and different number of scans.

It also needs to be considered that these second and third design cover a very limited area of the global S/N curves. Therefore, it might appear that, on this small area, the global trends are not always respected. However, one of the important parameters to consider is the variability on the sample, and to this extent, there is a need to evaluate the repeatability of the analysis in order to select the most relevant parameters for the forensic analysis of condom evidence.

3.2. Repeatability evaluation and parameters selection

3.2.1. Resolution

As illustrated in [20], optimal resolution can be selected when plotting the resolution as a function of the number of scans, fitting a power function of the type $y = ax^b$, the exponent of which is close to a theoretical value of 0.5 [20]. The optimal analysis parameters are therefore those whose exponential is closest to 0.5, with the regression coefficient the closest to 1.

Relation between resolution and number of scans will be plotted and the parameters of the curves were calculated, as well as the regression coefficients of the latest. The relation between resolution and number of scans is illustrated on Fig. 3. However, it has to be considered that a lower resolution won't allow the optimized separation of the infrared signal. In the practice, a resolution of 4 cm^{-1} usually offers the best compromise between the S/N value and spectral separation.

Parameters of the curves were calculated, as well as the regression coefficients of the latest (Table 5). Although 8 cm^{-1} resolution is the one which offers the highest SNR, both the regression coefficient and the power function parameter *b* are more fitting to the power function when using a resolution of 4 cm^{-1}. As it is also a very common parameter in most forensic laboratories, the resolution of 4 was selected as a final parameter for further analyses.

3.2.2. Number of scans

To select the adequate number of scans, the repeatability of the analysis should be assessed. Therefore, the data were first plotted into a principal component analysis to evaluate the variability of the different measurements. In addition, hierarchical cluster analyses were used with Ward's Linkage, Euclidean Distance measurement and Pearson's correlation measurements, and different linkage were tested, to see whether the results were consistent.

As illustrated on the PCA results on Fig. 4, the spectral data are rather spread out and are not really clustered together. The only ones clustered together are the ones acquired at 64 scans, as represented with a black circle on Fig. 4.

Table 5
Resolution impact.

Resolution cm^{-1}	a	b	R^2
1	88.951	0.4692	0.9334
2	141.65	0.4503	0.899
4	246.51	0.4897	0.9958
8	319.4	0.4253	0.8194

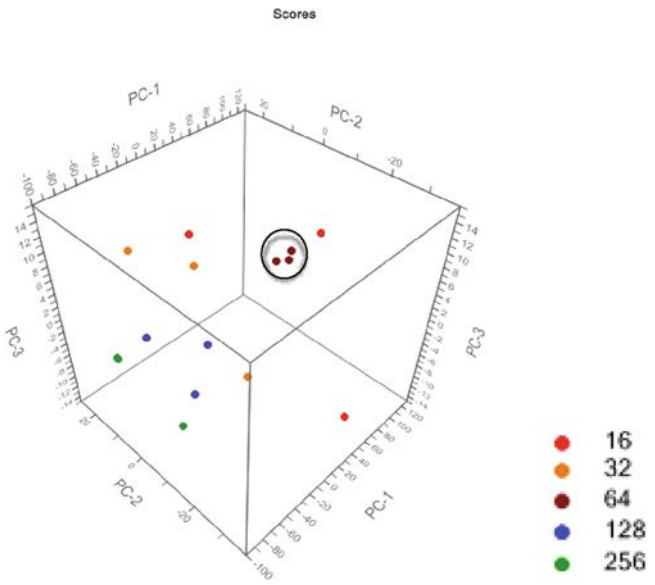

Fig. 4. 3-dimensional scores plot of the data acquired on different number of scans but a same resolution. Samples are coloured as a function of the number of scans.

All the hierarchical clusters provided the same results, with the smallest distance or correlation between the spectra being observed at 64 scans independently of the measurement methods or linkage method, thus suggesting a more appropriate repeatability of the data. An example of the results obtained with a Pearson's correlation and a complete linkage analysis is presented on Fig. 5. The black circle highlights the measurements obtained for 64 scans. The grey circle outline measurements obtained for 256 scans: interestingly, 2 replicates acquired at 256 scans are very close to each other, as close as 64 scans ones together, but the third replicate systematically is more distant to the rest of the samples. 256 scans would be a very interesting option of analysis as some authors have used a larger number of scans (256 or 512) to deconvolute the spectra and classify samples of different types. Increasing the number of scans to 512 would not significantly improve the SNR but increase analysis time by a factor of 10: analysis were found to last around 3 min for 64 scans and up to 15 min for 256 scans. Hence, this is not a cost-effective compromise based on spectral variability, but it might be requested for specific aims (i.e., spectral deconvolution).

The results presented above show that the more relevant conditions regarding the number of scans and the resolution are **64** and **4 cm^{-1}**, respectively. These are the parameters that will be used for further analysis.

3.3. Application to condom samples

3.3.1. Comparison of DRIFTS and ATR discrimination models

Most of the previous classification and discrimination models build for condom evidence analysis were constructed using ATR-FTIR. However, DRIFTS has been reported as the most adequate analytical method for the analysis of condom residues when it comes to casework, as described by [15]. This may be an issue for a forensic scientist facing condom evidence in case work, especially if the main concern is the proper identification of a condom, its brand or a specific model, as previous classification and discrimination models were constructed using ATR-FTIR, which is the reason why a model dedicated to DRIFTS analysis should be considered.

It is therefore of great interest to observe if the clustering identified in ATR data is also highlighted in DRIFTS data. Considering

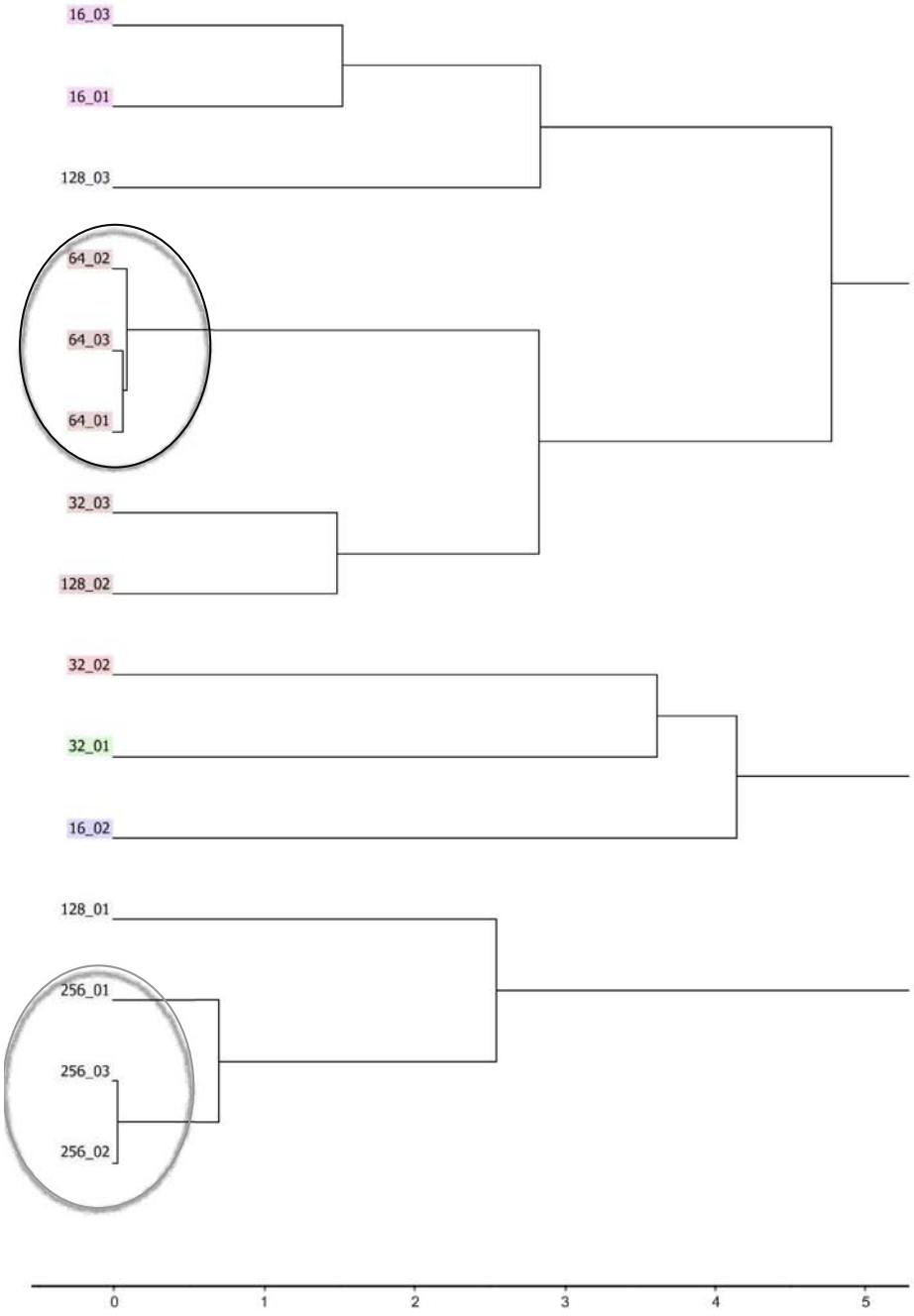

Fig. 5. Hierarchical Cluster Analysis obtained with a Pearson's correlation measurement, and a complete linkage clustering.

the chemical profiles obtained on a batch of different samples using DRIFTS, the main difference between ATR and DRIFTS is based on the silicone-based samples and only on the 1100–1000 cm^{-1} region of the spectra. Indeed, it is where the silicon-oxygen double bond presents its symmetric and asymmetric stretching vibrations, which are better resolved and show higher intensity in DRIFTS than in ATR, as previously illustrated in [15]. There seem to be an opportunity here to possibly differentiate using DRIFTS samples that could not have been distinguished with ATR-FTIR.

At first, PCA was computed on the acquired data sets separately. For the DRIFTS analysis data, the variance explained by PC1 to PC3 was respectively 64%, 15%, and 7%. The first three principal components explain 86% of the variance whereas for the ATR data, the variance explained along PC1 to PC3 were respectively 84%, 8%, and 6%, leading to 98% of variance explained. Fig. 6 shows the scores of the first three principal components (PC1-PC3) plotted against each other for all data. In this figure, replicates of condom of same *model* are represented by the same color. Fig. 6 illustrates the comparison of the PCA scores plot obtained for the ATR (left) and DRIFTS (right) data. Previous research [18] has highlighted specific data patterns using ATR-FTIR analysis. A similar structure in the data can be observed, with samples L2 and sample P11 being separated from the rest of the sample set. Similarly to previous

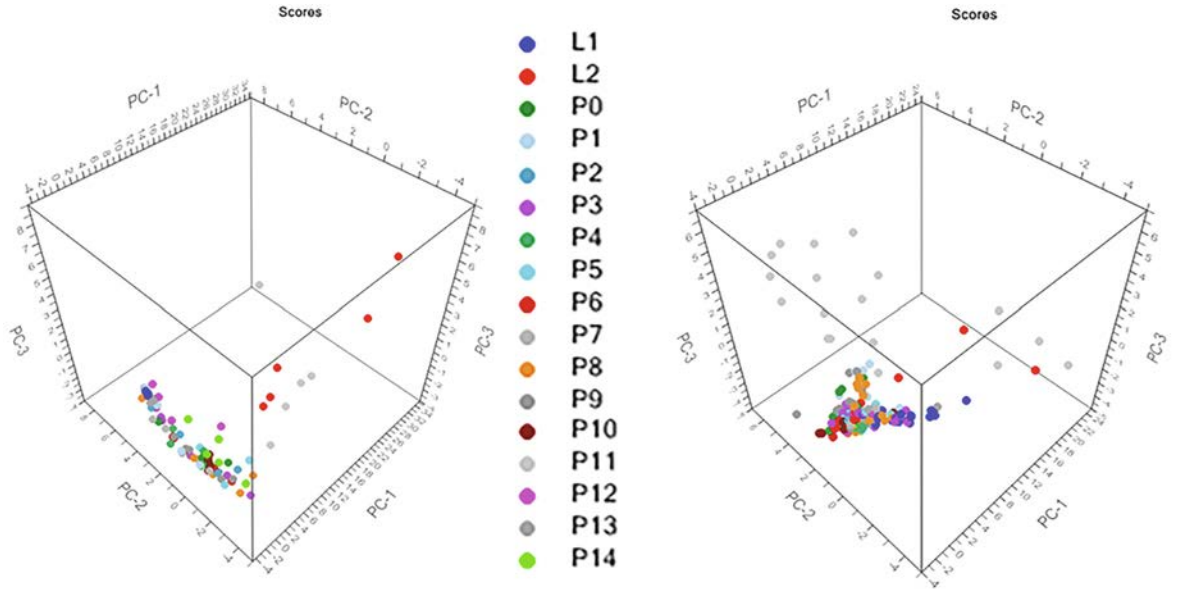

Fig. 6. 3-dimensional PCA scores plots showing the distribution of the samples acquired with ATR-FTIR (on the left) and DRIFTS-FTIR (on the right) for comparison. Legend numbers refers to Table 1.

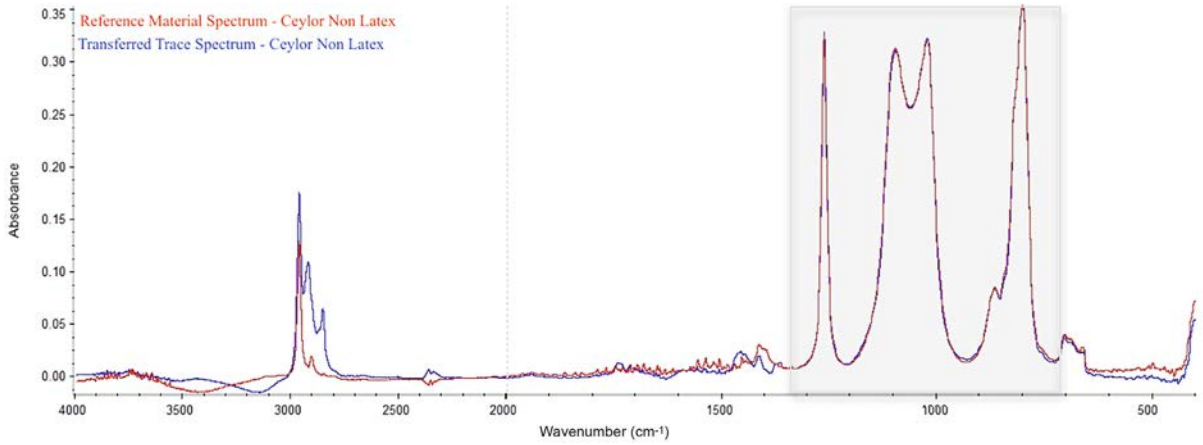

Fig. 7. Illustration of the overlay between a condom reference material spectrum (in red) and a trace evidence spectrum (in blue). The diagnostic peaks of PDMS are highlighted in grey, and are 1263, 1090, 1020 and 900 cm^{-1}. The overlay does not illustrate any significant variations in the area of PDMS, the four bands being present (grey zone), and a similar abundance being observed.

results published on ATR-FTIR results [18], the rest of the silicone containing samples cannot be differentiated of each other, and it is not possible to differentiate samples coming from different brands or models. Further PC did not help enhancing the discrimination of the samples. This suggests that although DRIFTS offer better visualisation of the siloxanes chemical patterns, it does not enable any improved discrimination of samples.

A very important difference that can be noted when comparing the two models presented on Fig. 6 is the dispersion of the data: DRIFTS model presents a far more important dispersion of the data than ATR model. This can easily be explained by two main reasons: the sample preparation and the extraction procedure. Regarding the sample preparation, DRIFTS analysis required the manual preparation of KBr pellets followed by spiking the liquid sample. Not only the KBr grinding might not always be reproducible between all the pellets, but an important modification of the KBr surface is generated at the moment of spiking, which is a source of variation.

Secondly, the samples analysed using DRIFTS were samples extracted from the condom whereas the ATR data were acquired by rubbing the sample on the crystal. Not only will there be variation resulting from the transfer, but also the extraction efficiency has not been evaluated, as it is known from [12] only that the use of a solid-liquid extraction using hexane allows adequate extraction of the target compounds. ATR was not found to offer significant better results for the analysis of condom evidence on swabs. *In situ* analysis was shown to be applicable on reference material (i.e. when condom was rubbed on a cotton before squeezing it on the ATR crystal) but produced no results from real samples, when an external matrix (i.e. vaginal matrix) was present. *In situ* analyses present considerable risk of contamination if DNA extraction has not been previously processed. Previous researchers reported that DNA extraction did not affect silicone-based residues analysis using hexane extraction on the same cotton swab [8]. The use of extracted samples is not adequate for ATR analysis, as at the moment of the deposition, the solvent evaporates, generating ran-

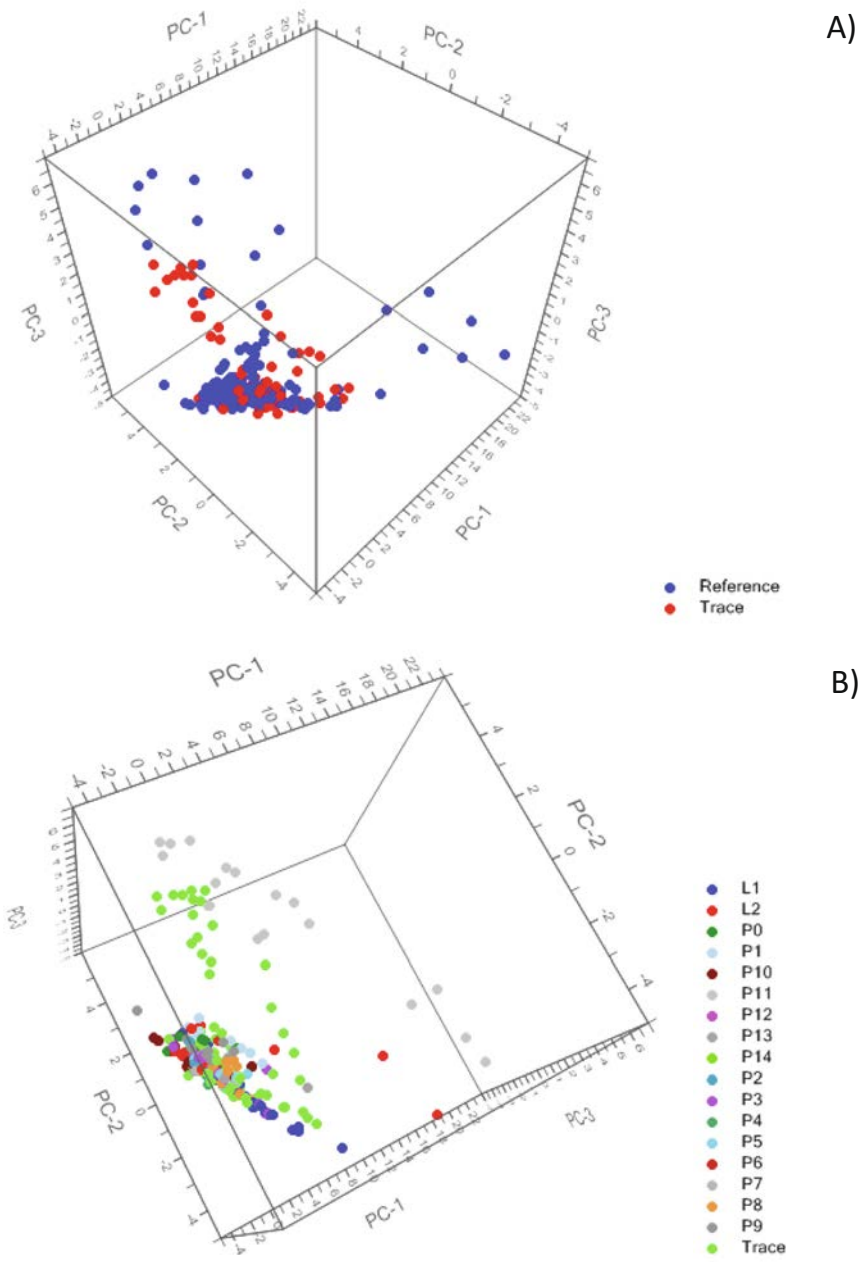

Fig. 8. . 3D-scores plot of the PCA on 16 reference samples (n = 5) and 11 samples collected from human volunteer (n = 8) analysed with DRIFTS, A) distinguishing traces and references, B) distinguishing all the different types of condom used as reference material.

domly deposited PDMS aggregates on the ATR crystal, and thus causing reproducibility issues. On the other hand, DRIFTS was reported as the most adequate technique for the analysis of real samples in case works [1] and a very sensitive technique. In this case, an extraction step is mandatory.

Although the number of samples is limited in this study, the analysis of such a big dataset as presented in [18] would unlikely provide more accurate results, considering that condom production is regulated by international ISO norms [21–24]. The conclusions of a discrimination and clustering investigation of a bigger dataset would be unlikely to provide different results on the discrimination of condoms than the ones observed in [18]. Should further discrimination be required, other analytical instrumentations such as DART-TOF-MS would be recommended given their discrimination ability [5,25].

3.3.2. Trace vs reference classification

Another recurrent and important question is to know whether real traces classify the same way pure lubricant samples do [18]. To this aim, samples collected from real samples were analysed. The visual analysis did not allow to differentiate samples coming from traces from the reference, except for the ones coming from sample P11, which is not a silicone-based condom. An illustration of a reference spectrum obtained from the condom Ceylor Non-Latex (P9), compared to the trace spectrum obtained after a sexual intercourse is presented in Fig. 7. The four diagnostic bands of PDMS are present in the spectra (grey zone in the spectra). The overlay do not highlight any significant visual differences, except in the area around 3000 cm^{-1} where more CH_2-CH_3 vibrations can be observed, due to the presence of the vaginal matrix [26].

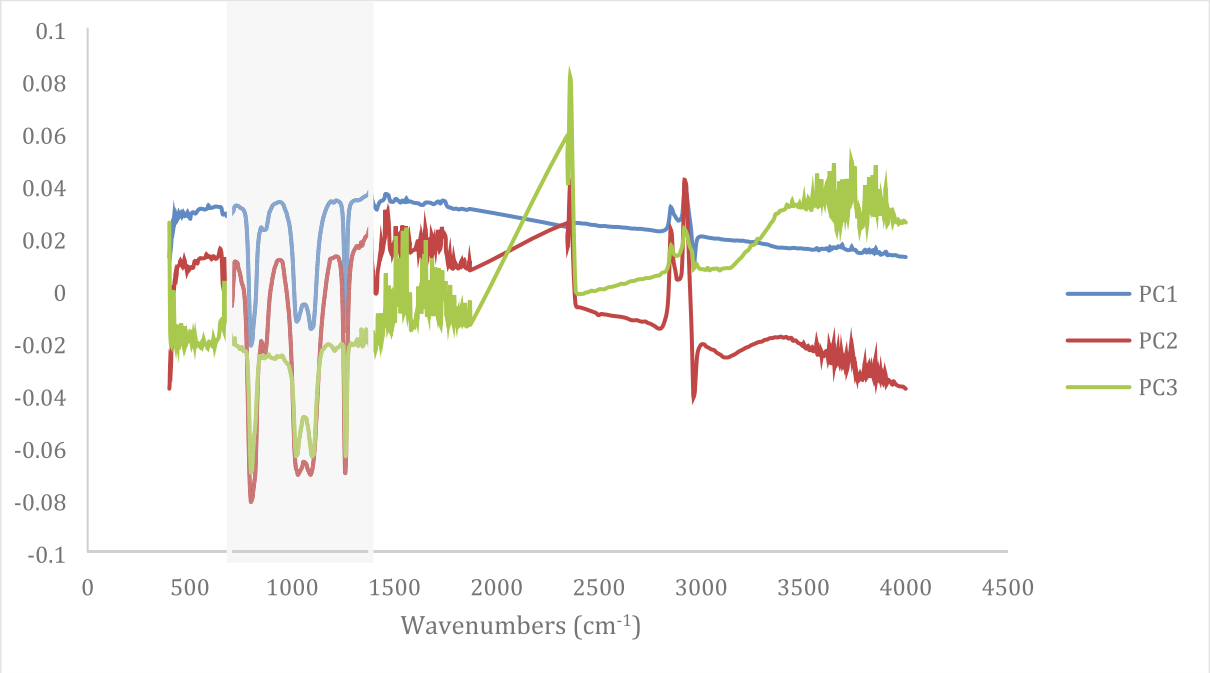

Fig. 9. Factor loadings of PCs 1–3 for the PCA conducted in Fig. 8, based on their DRIFTS-FTIR spectra.

The real samples were then processed and added to the PCA plots to investigate their possible discrimination pattern within the reference material (Fig. 8). As illustrated in Fig. 8A, trace samples are clustered within the same pattern as the one obtained with reference material. Traces present a slightly higher variability than the reference material when it comes to the silicone content (Fig. 8B). Replicates of the same samples were clustered together. Most of the traces were clustered very close to the reference material, thus suggesting that the model would be appropriate to classify traces at the moment of their transfer and that the chemical profiles are not affected by matrix residues which is not surprising considering the type of extraction processed on the samples. Indeed, non-lubricated condom traces were found to be clustered in the same zone (in terms of PC eigenvalues) than the non-lubricated reference P11. In a similar manner, silicone-containing traces were found to cluster within the silicone-containing reference cluster. These observations are very interesting, as they suggest that the chemical profile gathered from condom lubricants after intercourse is not affected at T0 by the receiver or the contact to generate a different pattern or a new specific cluster in the PCA plots.

Samples were clustered together along all the PC, and no clusters were observed differently than from the ones previously observed from the visual inspection of the data. The factor loadings for PC1-3 (Fig. 9) were used to identify the spectral region generating the sample discrimination. Along all the principal components, a strong negative correlation was observed with the peaks at 790, 1020, 1090 and 1260 cm^{-1}, which are linked to the PDMS silicone backbone [15]. A positive correlation was observed with peaks at 2912 and 2925 cm^{-1} consistent with the bonds coming from the vaginal matrix [26], and this discrimination was found to be stronger along PC2, although all PC offer this discrimination. Consequently, the discrimination between the samples is mainly due to the silicone content, most likely based on the concentration content.

In addition to PCA, HCA was also performed, as an unsupervised method to see the similarity between the data, without taking into account any other information. All the traces and the references were used, and 16 clusters were asked. All of the different distance measurements and linkage measurement revealed the same pattern: P11, L2 and the traces coming from these references were clustered together and presented a higher distance to the rest of the samples. Considering the silicone samples, the cluster analysis failed to correctly group the traces with their corresponding condom, independently of the type of linkage or distance/correlation measurements. These results also illustrate that whilst traces can be clustered with the reference material, it is not possible to link a trace to a specific material. This informs that when a trace is recovered, inferring on its exact source might not be possible, although in most casework it might not even be relevant, as the questions usually target the presence or the absence of traces. Whether it is a condom, or another type of sample can also be answered, but the exact source of the trace cannot be inferred, as the chemical profiles are all undistinguishable. This is interesting from the interpretative point of view, as the method is not able to discriminate condoms coming from different brands and models. Interpreting the evidence on a source level would be more relevant in the discrimination from condom vs lubricants rather than between condoms. When considering the use of a given condom vs the use of another one, the likelihood ratio (LR) obtained would be of 1, and therefore uninformative to the Court.

4. Conclusion

The choice of analytical parameters for infrared analysis was assessed using experimental design, as literature was not providing any consistent parameters. Full Factorial design was used and extended to Central Composite Design, using SNR as the target response factor. Distance and correlation measurement were also used to select the more adequate conditions in relation to the repeatability of the data. Most adequate conditions parameters in terms of variability and statistical representation of the data were found to be 64 scans and a resolution of 4 cm^{-1}.

The discrimination pattern of the reference material obtained using DRIFTS was compared to the ones previously published using ATR-FTIR. Similar patterns were observed, with a slightly higher dispersion for DRIFTS analysis, which is attributed to the extraction

and sample preparation step. Data acquired with both models could not be computed together, as the chemical profiles obtained were too different. The discrimination of different brands or model types of condom was similar to the one observed with ATR-FTIR.

Reference and traces were found to be undifferentiated, independently from the condom at the source of the trace. The results indicate that reference condom material and transferred traces do not present distinct chemical profiles. As expected, no differentiation was observed according to the transfer effect itself. The experiments described in this study illustrate the potential to distinguish condoms in a similar way to what was observed during the construction of the infrared profile database.

CRediT authorship contribution statement

Céline Burnier: Writing - original draft, Conceptualization, Methodology, Validation, Investigation, Data curation, Visualization. **Virginie Favre:** Methodology, Investigation, Resources, Data curation. **Geneviève Massonnet:** Writing - reviewing & editing, Resources.

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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