Analysis of transferred fragrance and its forensic implications

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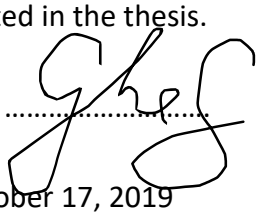
Declaration

I, Simona Gherghel, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signed ……



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Abstract

At present, the analysis of volatile organic compounds (VOCs) from fragrances is not employed in forensic science despite its potential as a form of trace evidence. Perfumes are used by many men and women on a daily basis, contain a large and diverse number of fragrances, and are invisible to the naked eye. Moreover, research on VOCs from human scent has shown that solid phase microextraction (SPME) with gas chromatography-mass spectrometry (GC-MS) is a robust method for the analysis of these odorous compounds. This enables insights into the transfer and persistence of the VOCs, which is a prerequisite for the use of a trace in forensic reconstructions.

This thesis presents the development, optimisation, and validation of a SPME GC-MS method for quantification of VOC traces from clothing. The method created was robust and sensitive, allowing quantification of VOCs from clothing even when the fragrance mixture was diluted up to 1500 times.

Experiments that addressed the transfer characteristics of fragrance VOCs demonstrated that fragrances can transfer from one fabric to another even when the contact times between fabrics is as short as 10 s, and even when the perfume was aged on the primary fabric for as long as 48 h before transfer took place. The nature of the fragrance transfer also depended on the fabric type, so that a clear discrimination was observed between the transfer that occurred from a cotton fabric swatch onto a natural (cotton) fabric and onto a synthetic (polyester) fabric.

Further experiments considered the persistence of fragrances. The data generated indicate that the highest VOC amounts are generally obtained from recipient fabrics after shorter persistence times of up to 1 d, however VOCs were successfully quantified for persistence times of up to 4 weeks. Lower environmental temperatures resulted in higher recoveries for most VOCs, especially for short persistence times. These findings demonstrate that the optimal recovery of VOCs from clothing occurs when the fabric is kept at cooler temperatures and analysed soon after the fragrance transfer occurred.

Therefore, given the ability to recover VOCs from fragrances from clothing, and the transfer and persistence characteristics of these VOCs, there is potential for fragrance to be used as a form of trace in forensic reconstruction approaches to address both source and activity level propositions.

Keywords: experimental studies, trace evidence, volatile organic compounds (VOCs), transfer, persistence, forensic reconstruction

Impact statement

Currently, analysis of fragrance from clothing is not investigated in forensic laboratories despite its potential applicability for reconstructing events in sexual assault cases that involve close contact between people. This thesis presents the first experimental studies investigating the viability of fragrances as a form of trace evidence, and thus represents a novel line of research investigation. As such, this work has created, optimised, and validated a gas chromatography-mass spectrometry method for the analysis of trace level amounts of fragrances from clothing. This new research method has implications not only for forensic research, but also for the textile industry regarding the persistence on fabrics of fragrances, such as the ones from laundry detergents and fabric softeners.

Forensic science faces clear challenges that address not only the validity of techniques, but also the state of the evidence base to underpin the interpretation of trace evidence. It is therefore more important than ever to undertake empirical studies to explore the dynamics of traces with a particular focus on transfer and persistence. Fragrance is an underexplored form of trace, so establishing whether it is possible or not to recover it in different scenarios, demonstrates the feasibility of being able to reconstruct events using fragrance as a trace indicator. One of the findings of this thesis highlighted that fragrance transfers onto a secondary piece of clothing in sufficient and consistent amounts when the contact between fabrics was between 10 sec and 5 min. This shows the applicability of fragrance traces even for sexual assault cases where the contact between individuals was brief.

The research in this dissertation has also had impact within the academic sphere with three presentations at national and international conferences and the publication of key findings in internationally peer reviewed journals that relate to the development of fragrances as a form of trace evidence in forensic science (eg Gherghel et al. (2018) and Gherghel et al. (2019)). Aspects of the MRes thesis which formed the foundation of this present work have been published (Gherghel et al. (2016)), and this paper won the PW Allen Award for the most meritorious paper published in the journal ‘Science & Justice’ (2016). This research was distributed to a broad audience being covered by news articles “Perfume traces could help to solve crimes” (BBC News) and two radio programmes: BBC Radio 4’s “PM” programme, BBC World Service’s “Newshour” programme.

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Presentations and Publications:

Aspects of the MRes thesis which formed the foundation of this work have been presented at the following:

S. Gherghel, R.M. Morgan, C.S. Blackman, K. Karu, I.P. Parkin (2015) "Determining the potential of transferred fragrance as a viable form of trace evidence", *Spectrometry for Security Applications workshop,* Birmingham University, UK (poster)

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S. Gherghel, R.M. Morgan, C.S. Blackman, J. Arrebola-Liébanas, I.P. Parkin (2019) "Fragrance transfer between fabrics for forensic reconstruction applications". *Sci. Justice*. 59 (2019) 256-267. doi:10.1016/j.scijus.2019.02.002

S. Gherghel, R.M. Morgan, C.S. Blackman, J. Arrebola-Liébanas, A. Garrido Frenich, I.P. Parkin (2019) "Persistence of transferred fagrance traces on fabrics for forensic reconstruction applications". *Sci. Justice*. In Press. doi:10.1016/j.scijus.2019.09.002

Additional publications undertaken during the time of the PhD research:

J.R. Belmonte-Sánchez, **S. Gherghel**, J. Arrebola-Liébanas, R. Romero González, J.L. Martínez Vidal, I. Parkin, A. Garrido Frenich (2018) "Rum classification using fingerprinting analysis of volatile fraction by headspace solid phase microextraction coupled to gas chromatography-mass spectrometry", *Talanta.* 187 (2018) 348–356. doi:10.1016/j.talanta.2018.05.025.

Abbreviations:

ANOVA (analysis of variance)

CAI (case assessment and interpretation)

CAR (carboxen)

CCD (central composite design)

DVB (divinylbenzene)

FM (fragrance mix)

HCA (hierarchical cluster analysis)

HS (headspace)

LOD (limit of detection)

LOQ (limit of quantification)

PA (polyacrylate)

PAT (perfume ageing time)

PCA (principal component analysis)

PDMS (polydimethylsiloxane)

PLS (partial least square)

RT (retention time)

RSD (relative standard deviation)

SEM (scanning electron microscope)

SPME GC-MS (solid phase micro-extraction gas chromatography-mass spectrometry)

VOC (volatile organic compound)

1. Introduction

1.1. Current forensic science landscape and the need for research

In the last decade, concerns have been articulated in the forensic science literature regarding issues on the reliability of the forensic science culture, methods, practices, and standards. At the forefront of this scrutiny was a paper by Saks and Kohler (2005), who challenged the underlying assumptions of uniqueness in traditional forensic identification methods. Traditional forensic identification sciences generally aim to compare a pair of marks and traces, such as fingerprints, bite marks, handwriting, hair, with the assumption that two indistinguishable marks must have been produced by a single object. As a result, crime scene evidence is often individualised, by linking it to a single object or person, "to the exclusion of all others in the world" (Saks and Koehler 2005, p. 892). The authors argue that at the basis of these traditional forensic identification sciences should be empirical and probabilistic data, rather than "untested assumptions and semi-informed guesswork" (Saks and Koehler 2005, p. 895). Their criticism did not apply to DNA analysis, which they presented as a model to guide scientific forensic identification. However, the underlying principles of DNA are foundationally different from those of any other forensic traces, so that while DNA evidence can lead to a single individual with a high degree of probability (due to the existence of allele frequency population data), other forms of identification evidence cannot (Broeders, 2006; Morgan, 2017).

DNA analysis has been developed on a theoretical foundation, supported by the work of scientists from various disciplines, such as biochemistry, population genetics, molecular biology, and statistics. DNA evidence is often presented as a "random match probability", i.e. the probability that a randomly selected person from the population, other than and unrelated to the suspect, has the same DNA profile as the one found at the crime scene. Due to its theoretical basis and its probabilistic conclusions, DNA profiling has been considered "gold standard" in forensic science (Lynch, 2003), despite rising issues surrounding the interpretation of "trace DNA" (Gill et al., 2000; Meakin and Jamieson, 2013; David et al., 2016; Meakin and Jamieson, 2016) and the interpretation

of mixed and degraded profiles (Gill, Guiness and Iveson, 2012; Dror and Hampikian, 2011; Butler, Kline and Coble, 2018).

The issue of the lack of reliability of some forensic methods and practices were strongly presented in 2009, when an independent committee formed by the National Academy of Sciences (NAS) in the US published its "Strengthening forensic science in the United States: A path forward" landmark report (National Academy of Science, 2009). The NAS report revealed that with the exception of DNA analysis, none of the other investigated forensic analyses held up to rigorous examination. It highlighted that many of the techniques employed in forensic laboratories whose aim is to individualise had never been subject to scientific scrutiny. However, it was highlighted that analysis methods drawn from well-characterised methods of chemistry and biology (such as toxicology, DNA analysis, fibre analysis, paint analysis) are more robust than analysis methods drawn from law enforcement (such as ballistics, fingerprints), as in theory it is possible to obtain an understanding of the uncertainties associated with these analyses. The report mentions that, with the exception of DNA, few of these methods have had their limits and uncertainties examined, or their assumptions scrutinised. As a result, one of the emerging recommendations from the NAS report was the development of rigorous research to determine the capabilities and the limits of forensic science. The report was a significant catalyst to calls within the forensic science community for a paradigm shift towards a more research-focused culture.

The NAS report led to a number of independent reports in the UK, such as those from the Law Commission (2011), the annual reports from the current Forensic Regulator (Forensic Science Regulator, 2015, 2016, 2017, 2018), and the Government Chief Scientific Adviser annual report (Government Office for Science, 2015). One of the recurrent themes in all these reports has been the need for research in forensic science. For example, the four annual reports from the UK Forensic Science Regulator highlighted the importance of carrying out experimental studies on the transfer and persistence of trace evidence, and the factors affecting the transfer, in order to enable a more robust interpretation and evaluation of evidential significance. This advice resonates with that of Mnookin et al. (2011, p. 2), who argue for the "use of sound research rather than experience, training and longstanding use". This comes after a number of court cases

such as R v Reed (Court of Appeal of England and Wales (Criminal Division), 2009) and Reed and R v Weller (Court of Appeal of England and Wales (Criminal Division), 2010), where the judge favoured claimed experience from an expert over and above the findings of experimental data. However, quoting 'x years of casework experience' does not have a scientific basis (Meakin and Jamieson, 2016) as it is not possible to test the validity of someone's experience.

For the development of a research culture able to answer questions such "What do we know?" "How do we know that?" and "How sure we are about that?" (Mnookin et al. 2011, p. 17), there is a prerequisite need for experimental studies investigating the behaviour of traces under various situations, blind studies evaluating the uncertainties, and population studies looking at background levels and occurrence. This thesis, framed around the potential of fragrance traces to be used as a form of evidence in forensic investigations that involve contact, resonates well with the call for a more research-focused culture. Using a well-developed and mature analytical technique, gas chromatography-mass spectrometry (GC-MS), a method for the analysis of fragrances from clothing was devised, optimised, and validated. This method was then applied to further experimental studies to develop an understanding of the evidence dynamics of fragrance traces between fabrics, in a casework informed manner so as to establish the potential for this form of trace for forensic reconstructions, in addition to the benefits and the limitations of this approach.

1.2. Thesis aim

The ultimate aim of this PhD work was to determine the potential of volatile organic molecules from fragrances to be used as a form of trace evidence by carrying out empirical experiments to recover fragrance VOCs, and to assess the transfer and persistence of fragrances between and onto items of clothing. The first objective of the thesis was to carry out experimental studies in order to devise an optimal method for the extraction of fragrance traces from clothing materials. After a robust analysis method was devised, transfer and persistence experiments were carried out to gain insights into the different factors, such as time, fabric type and environmental conditions that affect how fragrances transfer and persist onto/on a piece of fabric. The

resulting empirical data provided the capability to assess the viability of fragrance traces for forensic reconstruction.

1.3. Thesis outline

This thesis contains a literature review (Chapter 2), a material and methods chapter (Chapter 3), three experimental chapters (Chapters 4-6), and a final summary and conclusion chapter (Chapter 7).

Chapter 1 has provided an overview of the forensic science domain and has offered insights into the current forensic science landscape. The call for a move towards a more research-based framework grounded in empirical studies looking at how traces behave in terms of transfer and persistence has been highlighted.

Chapter 2 presents the concept of trace evidence and the importance of understanding how such evidence transfers and persists. A discussion about volatile organic compounds, analysis methods and their study in human scent forensic literature is provided, followed by an examination of the literature gap in the use of fragrances as a form of trace evidence. The chapter concludes with the research questions put forward in this thesis.

Chapter 3 presents the methods and materials used for the experimental studies in the following three experimental chapters.

Chapter 4 presents a new analytical method for the extraction of fragrance traces from fabrics for forensic applications. The solid phase microextaction gas chromatography-mass spectrometry parameters are optimised using a response surface methodology, more specifically a central composite design. The optimised method is validated and successfully used for the analysis from fabrics of commercial perfumes diluted up to 1500 times. Based on these results, guidelines and recommendations for the extractions of fragrances from garments in forensic casework are then outlined.

Chapter 5 presents the results of three experimental studies designed to offer insights into the transfer of fragrance traces on clothing, and thus aid in determining the potential of fragrances in forensic casework. The findings showed that the fragrances

transfer even for contact times between fabrics as short as 10 s, and even when the perfume was aged on the primary fabric for as long as 48 h. Also fabric type played a role in the transfer of fragrance, so that a clear discrimination was observed between the transfer that occurred onto a natural fabric and the transfer onto a synthetic fabric.

Chapter 6 presents the results of an empirical study looking at the persistence of transferred fragrances for two different fabrics and at different environmental conditions for persistence times as long as 4 weeks. The findings showed that shorter persistence times of up to 1 d result in the highest amounts of VOCs recovered. Nevertheless, VOCs were successfully quantified for persistence times of up to 4 weeks. Lower environmental temperatures resulted in higher recoveries for most VOCs, especially for short persistence times.

Chapter 7 provides a conclusion to the thesis, summarising the main findings of the experimental studies within the thesis, discussing their implications, before making suggestions for future research. This body of work has demonstrated the potential use of fragrance traces as form of trace evidence by showing through empirical experiments that there is value in using the developed and validated method even for assault cases where contact between clothing may have been brief, and even when the crime is reported several weeks after it has been committed.

2. Literature review

2.1. Outline

Due to the multidisciplinary nature of this research, this review of the published literature starts with an examination of trace evidence, followed by a consideration of volatile organic compounds (VOCs), their analysis and applications documented in the forensic science literature, and then addresses the potential use of fragrance VOCs as a form of trace evidence in forensic reconstruction.

The first section addresses the forensic value of trace materials and their interpretation. The importance of understanding trace evidence dynamics for forensic reconstructions is addressed as a foundation for creating experimental studies to investigate the potential of fragrance VOCs to be used as a form of trace evidence. The second section outlines the general applications and importance of VOCs, followed by methods commonly used in analytical laboratories to analyse VOCs, followed by a review of the published literature that addresses empirical studies of the use of VOCs from a human body in forensic reconstruction applications. The third section discusses the current gap that exists in the body of knowledge and the published literature, and the potential use of fragrance VOCs as a reconstruction tool in the investigation of sexual assault cases. The importance of carrying out transfer and persistence studies for traces, especially for under-researched traces, such as VOCs, for robust and transparent interpretation of trace materials in forensic reconstruction is also addressed, followed by the presentation of the research questions for this thesis.

2.2. Trace evidence

2.2.1. Introduction

In forensic science, trace evidence refers to any type of evidence that occurs in sizes so small, generally microscopic, that can be transferred between objects/people/locations and persists in time (Houck, 2001). Traditionally, trace evidence refers to physical traces, such fibres, hair, glass, soil, paint, and gunshot residue. The technological advances from the last two decades have enabled forensic scientists to identify and recover a broad

range of traces valuable for casework (De Forest, 1982), including biological and chemical trace evidence that are not readily seen under a microscope, for example DNA traces (where the biological source in unknown) (Meakin and Jamieson, 2013) and human decomposition scent traces (where the cadaver was scattered by animals or moved by individuals) (Alexander et al., 2015).

Trace evidence is often presented in the published literature under various names, such as trace material, trace evidence, and particulate evidence. A "trace" only becomes "evidence" when it is brought forward into the court (Houck, 2001; Jasanoff, 2006), and it is important to acknowledge the value of trace materials as intelligence in a case. The value of trace materials is predicated on Locard's exchange principle, often summarised as "every contact leaves a trace". This generation and transfer of physical evidence onto a secondary surface can allow a comparison with the original material, and thus a record of association between objects, people, environments, and events.

In an investigation, there are a series of stages from the generation of a trace material until presentation of that material as evidence in court (Morgan and Bull, 2007), as shown in Figure 2-1. Once the material has transferred to a surface, it needs to persist sufficiently to be available for collection. After collection, the forensic scientist can analyse that evidence and interpret the resulting data within the context of the forensic case, followed ultimately by the presentation of the material as intelligence and/or as evidence in court.

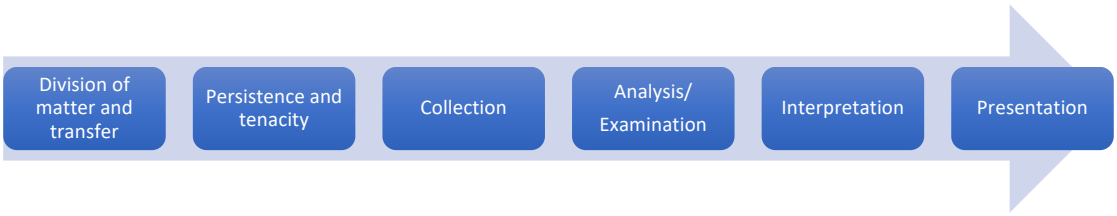

Figure 2-1 The process of a forensic science enquiry from the division of the trace material to the presentation of that trace evidence in court (Produced by the author after Morgan and Bull 2007)

2.2.2. *Trace materials: interpretation*

The interpretation of a trace should take into account each step of the forensic science process, as outlined in Figure 2-1. For a more complete and accurate understanding of the evidential weight of a trace, the forensic scientist should not limit their interpretation of evidence to just the analysis step, but he/she needs to consider the evidence dynamics of that trace within all stages of the forensic process, from the creation of the trace material up to the presentation of that material as evidence in court (Morgan and Bull, 2007). The ultimate value of a trace depends not only on the trace material being collected and analysed appropriately, but also on a robust interpretation that provides an understanding of what that evidence means in a given scenario (Morgan et al., 2009).

The Case Assessment and Interpretation (CAI) framework was designed to assist with the interpretation of evidence process (Cook et al., 1998a; b, 1999; Evett, Jackson and Lambert, 2000). CAI was designed to deliver more transparent and robust expert opinion and is based on the underlying logic of Bayes Theorem and the use of likelihood ratios. As such, the framework introduced the notion of the hierarchy of propositions, which encourages a balanced view of the evidence as it requires that the interpretation of evidence takes into consideration two alternative propositions, that of the prosecution (Hp) and of the defence (Hd). Propositions can be classified into three main categories: “offence level” (level III, referring to the commission of a criminal offence), “activity level” (level II, referring to human activities) and “source level” (level I, referring to the source of the trace material). An example of propositions at the three different levels for a sexual assault case is provided in Table 2-1 and discussed next.

In a sexual assault case, an example of a pair of offence level propositions can be “The accused sexually assaulted the victim” and “The accused did not sexually assault the victim”. In most cases, this simplistic alternative proposition formed by a negation might need to be replaced by a more appropriate proposition such as “The accused had consensual sex with the victim” (Taylor, Kokshoorn and Biedermann, 2018). In a sexual assault case where semen was found on a victim’s clothing, activity level propositions can be “The accused had sex with the victim” and “The accused’s semen transferred to victim’s clothing through touch”. Source level propositions in the previous scenario can

be “The semen from the victim’s clothing originated from the accused” versus “The semen from the victim’s clothing originated from someone other than the accused”.

Table 2-1 Examples of hierarchy of propositions for a sexual assault case

Level	Prosecution (Hp)	Basis for interpretation	Defence (Hd)	Basis for interpretation
Offence (III)	The accused sexually assaulted the victim	Court’s decision based on information from activity and source propositions	The accused did not sexually assault the victim The accused had consensual sex with the victim Some other man sexually assaulted the victim	Court’s decision based on information from activity and source propositions
Activity (II)	The accused had sex with the victim	Transfer and persistence experimental studies Background surveys Circumstantial evidence about the assault	The victim wore clothes belonging to the accused The accused’s semen transferred to victim’s clothing through touch	Transfer and persistence experimental studies that answer questions such as “what is the probability of finding this quantity of semen on victim’s underwear if the victim wore clothes belong to the accused?” Background surveys Circumstantial evidence about the assault
Source (I)	The semen from the victim’s clothing originated from the accused	Comparison between the DNA profile obtained from the suspect ‘semen and the DNA profile from the semen found on the victim’s clothing	The semen from the victim’s clothing originated from someone other than the accused	Comparison between the DNA profiled from semen found on the victim’s clothing and a DNA database

The offence level, the highest level in the hierarchy, is the ultimate issue on which the court must decide (and is not the realm of the forensic scientist). Nonetheless, the offence level is directly affected by the forensic interpretation from the two lower levels. The activity level propositions require consideration on the transfer and persistence of evidence, for example on how the semen was deposited on the victim's clothing and how it is related to the alleged event. As such, activity level propositions often require circumstantial information. The lowest level propositions, the source level, require empirical comparison between the recovered trace material and the alleged source, often involving some form of analysis and comparison with population studies.

In cases of sexual assault where biological material has been detected and identified, it is often not the source level consideration of that material that becomes important (unless the complainant is a minor), but the activity and offence level. Issues of consent, the means of transfer and the timing of a transfer (persistence) of the biological material can become key issues for establishing activity level propositions, which inform offence level conclusions which can contribute to a robust and transparent reconstruction of events.

There is a strong focus in forensic science on establishing the source level of recovered trace materials (Biedermann et al., 2016) and developing technologies and advances in analytical capabilities to identify, quantify, and classify smaller amounts of trace more accurately and in shorter timescales. Recent studies have identified that 66% of misinterpreted evidence in a sample of upheld cases heard at the Court of Appeal of England and Wales was due to the interpretation of activity level propositions (Smit, Morgan and Lagnado, 2018). There is therefore, a need to address the gaps in the evidence base in order to tackle activity level propositions. The UK Forensic Scienrce Regulator has stated consistently in all four of her annual reports that empirical research on the transfer and persistence of trace evidence and the factors that affect these processes is one of the highest priorities for forensic science (Forensic Science Regulator, 2015, 2016, 2017, 2018).

2.2.3. Trace evidence dynamics: transfer and persistence

To help with a more robust interpretation, it can be important to carry out both primary and secondary level experimentation (Morgan et al., 2009). Primary level studies provide the foundation of evidence-based knowledge about the behaviour of trace materials, and thus, help with source propositions. Secondary level experimentation recreates the conditions specific to each forensic enquiry, which can provide an evidence base to underpin the collection, examination, and interpretation of trace physical evidence. Secondary level experimentations can provide crucial information when assessing activity propositions specific to a crime case, as it allows the incorporation of particular circumstantial information.

For source and activity propositions, the forensic scientist needs to make inferences about the transfer and persistence of the trace materials. In order to provide a full and accurate interpretation of trace evidence, experimental studies that aim to reconstruct the crime scene by mimicking the behaviour of trace materials are crucial.

The first experimental studies undertaken to understand the evidence dynamics of forensic traces evidence were carried out by Pounds and Smalldon in 1975 (1975a, 1975b, 1975c), who investigated the transfer, persistence, and the mechanisms involved during the transfer of fibres between items of clothing. In their initial study involving the transfer of textile fibres to wool and acrylic clothing (Pounds and Smalldon 1975a), they determined that both the fabric type of the recipient garment and the force of contact affect the amount of fibres transferred, so that high pressures and coarse recipient garments lead to the transfer of shorter fibres. In a further study exploring the persistence of transferred fibres (Pounds and Smalldon 1975b), the authors determined that the loss of transferred fibres from the surface follows an exponential decay, with a rapid loss initially followed by a subsequent slower loss. An initial loss of approximately 80% was observed after 4 h, which increased to around 95% after 24 h.

Pounds and Smalldon were the first to show empirically that the transfer of trace evidence is a dynamic process. They highlighted the importance of considering any potential changes to the trace evidence since its formation and transfer when interpreting the presence of a specific form of trace. Following their work, studies have

been carried out to investigate the evidence dynamics of a number of different forms of trace materials, such as glass (Brewster et al. 1984; Allen et al. 1998; Curran et al. 1998), paint (Pearson, May and Dabbs, 1971; Buzzini et al., 2005; Moore et al., 2012), hair (Gaudette and Tessarolo, 1987; Mann, 1990; Exline, Smith and Drexler, 1998), and pollen (Mildenhall, 2006; Bull et al., 2006; Morgan et al., 2013). Similar patterns of loss with a two-stage model as outlined by Pounds and Smalldon (1975b) have been shown in these studies; for example Bull et al. (2006) determined a significant loss followed by a slower decay for pollen grains. Their study also showed that the decay was more influenced by the recipient fabric (secondary fabric) than the particle type when conducting experiments using pollen grains, powder and metal particulates.

It is clear that an understanding of how trace materials transfer and persist is important for forensic reconstruction purposes, as it has an impact on all stages of a forensic enquiry (Figure 2-1). Therefore, the availability of empirical data that address the dynamics of trace materials can aid the forensic scientist at a crime scene, in the laboratory, and during the interpretation process.

At a crime scene, the forensic expert needs to identify any potential valuable specimens and prioritise their proper collection. Depending on the type of crime, different physical trace materials have different places where there are more likely to be present, and appropriate recovery methods need to be used. For example, it has been shown that although forensic palynology analysis is not a common analysis type for questioned documents, paper has the ability to retain a great number of pollen grains, which could be useful in determining a timeframe, and the provenance for that document (Morgan et al., 2014). This demonstrates the value of experimental studies that can offer insights to the investigator about the retention ability of substrates for specific trace materials.

In the laboratory, the forensic scientist needs to prioritise evidence analysis, depending on the potential evidential value, the sensitivity of the material, and the ability/limitations of the techniques. The evidential value of a trace material depends on various factors such as the type of material, its location, abundance, and condition (Aitken and Taroni, 2004). For example, Curran et al. (1998) used a graphical model to assess transfer possibilities and the number of glass fragments expected to be recovered from a suspect's clothing. They identified that different variables have an effect, such as

the number of fragments initially transferred from the crime scene to the suspect, the retention properties of the suspect's clothing, the distance of the suspect from the smashed window, as well as the time from the smashing of the window to the arrest of the suspect. Although not a simple task, consideration of such variables can provide a better use of resources and a more accurate value of that piece of evidence.

During the interpretation process, it is crucial to have information regarding the behaviour of trace evidence in order to determine whether its presence at a crime scene is significant. For example, Roux et al. (1999) reconstructed a crime scene, where the only physical evidence found were some dark fibres on the soles of the boots of a deceased young woman. Laboratory experiments showed that after just 5 min of walking over various surfaces very few of the fibres transferred from the car carpet to the shoe soles persisted and were recovered. Such experimental data concerning the influence of different activities on the persistence of trace materials can provide useful intelligence for determining the importance of that trace, and whether it should be included or excluded from the crime scene.

Therefore, it is important to understand how trace materials transfer and persist and how that information offers insights into the effective collection, analysis, and interpretation of that evidence. The creation of experimental studies that are casework informed, implementable, and can offer the evidence base for interpretation of trace evidence can enable a robust interpretation of what a particular form of trace evidence means in a specific case scenario and can offer valuable intelligence and evidence (Morgan, 2017). This is notably true for understudied forms of trace materials such as volatile organic compounds from fragrances.

2.3. Volatile organic compounds (VOCs)

2.3.1. Introduction

Volatile organic compounds are a diverse and extensive class of compounds ubiquitous in everyday life, with origins in various natural and anthropogenic processes (Alwis et al., 2012). They are organic compounds with low molecular weight that most often are

defined by their ability to readily evaporate in the surrounding environment at room temperature. However, there are currently many definitions of VOCs in use.

In Europe, the VOCs Solvents Directive, whose aim is to limit the industrial emission of VOCs, stated in the 1999/13/EC Directive that VOCs are any organic compounds with a vapour pressure higher than 0.01 kPa at 20 °C (Council of the European Union, 1999). Five years later, the amended Paints Directive (2004/42) redefined VOCs as any organic compounds with a boiling point of 250 °C or lower. In the UK, the Solvent Emissions Directive (Environmental Agency, 2004) was introduced to echo the 1999 European Directive. In the US, the Environmental Protection Agency (EPA), for regulatory reason, broadly defines VOCs as any volatile compound of carbon that participates in atmospheric photochemical reactions that has not been specifically exempted (Environmetal Protection Agency, 2009). The EPA first established the list of compounds exempted as VOCs in 1977, and since then several compounds have been added.

Despite the various definitions available, the main characteristics of VOCS are their low molecular weight and high vapour pressure, which in turn give them a low boiling point that allows these compounds to evaporate at room temperature. The ability to enter the surrounding air as gases from solids or liquids is known as volatility, and therefore all odours and scents qualify as VOCs.

The interest in the detection and quantification of both natural and synthetic volatile organic compounds has been increasing in the last few decades across various economic and scientific sectors (Biniecka and Caroli, 2011). Analysis of VOCs is routinely carried out in a wide range of fields, including the perfume and cosmetic industry (fragrances and allergens: myrcene, coumarin) (Bartsch, Uhde and Salthammer, 2016; Desmedt et al., 2014; Wang, 2013), the food industry (natural and synthetic flavourings: 2-methyl-3-furanthiol, acetoin) (Gasperi et al., 2001; Pacioni et al., 2014), the environmental sector (pollutants: 1,3-butadiene, methylglyoxal) (Louie et al., 2013; Tovalin-Ahumada and Whitehead, 2007), the chemical sector (paints, cleaning products and fabric softeners: formaldehyde, terpinolene, isopropylbenzene) (Kim et al., 2011; Singer et al., 2006; Anderson and Anderson, 2000), and forensic science (human decomposition odour, living human scent, drugs and explosives: carbon tetrachloride, piperonal, diphenylamine) (Vass et al. 2008; Guerra-Diaz et al. 2010).

Fragrances and flavours have become an integral part of various products used throughout daily activities, such as food and beverages, toiletries, detergents and household products. It has been reported that the average person comes in contact with 10 to 12 different fragranced items during a routine day (Su, 2014). According to the Research and Markets report (2016), the global fragrance and flavour market was estimated to be worth $24.9 billion in 2015 and forecasted to be worth $31.4 billion by 2021. The market of fragrances and flavours is split equally, with the former taking 51% of the total market (Tully & Holland, 2014). In the UK, it is estimated that 89% of women wear fragrance regularly (Amy-Chinn, 2001), and that 63% of men wear it periodically and 23% use it daily (NPD Group, 2013).

The fragrance industry has more than 3000 different chemicals available to create distinctive perfumes, but often perfumes are a blend of up to several hundred ingredients (Harder, 1998). Different chemical and physical properties of the perfume ingredients, such as volatility and molecular interactions, allow them to evaporate at different rates. Thus, the most volatile ingredients with low molecular masses, i.e. the top notes, are the first ones to evaporate, and so the most short-lived on the skin or on a piece of fabric.

2.3.2. Instrumental analysis and sampling of VOCs

In forensic science, analysis of VOCs from various samples has been carried out for different applications. For example, VOCs from decomposing human remains have been analysed with the aim to determine the post-mortem interval (Vass et al. 2008; Statheropoulos et al. 2005), or to identify the specific VOCs from human remains that result in an appropriate response from victim recovery canines (Hoffman et al., 2009). Analysis of VOCs from biological specimens, such as hand odour and saliva, has been carried out mainly to investigate the ability of the VOCs to differentiate between individuals (Kusano et al. 2013; Brown et al. 2013). Other samples analysed for the identification of VOCs include drugs (Inoue, Iwata and Kuwayama, 2008; Rice and Koziel, 2015), explosives (Brown et al. 2004; Harper et al. 2005), and more recently fragranced garments (Gherghel et al., 2016). These studies have used a variety of sampling and analysis techniques, so a review of these techniques is provided next.

Analysis of VOCs has advanced in the last couple decades due to the increasing use of gas chromatography-mass spectrometry (GC-MS), whereas other techniques such as differential optical spectroscopy and membrane induction mass spectrometry have had a very limited use (Dewulf, Van Langenhove and Wittmann, 2002). In forensic science, GC-MS with its different sampling techniques has been the analytical technique of choice for the analysis of VOCs.

Gas chromatography (GC) is the most suitable analytical technique to separate complex mixtures, such as perfumes, into their individual components. In GC, each individual component from a sample partitions between a gaseous mobile phase (such as helium, argon or nitrogen) and a liquid stationary phase, which is generally coated onto inner wall of a long thin capillary tube, called a column. The components travel through a column at different rates depending on their affinity to the stationary phase and their vapour pressure, which is influenced by the column temperature. As components elute from the column, they enter the mass spectrometer (MS) where they are ionised most often using a stream of electrons, causing fragmentation. The ions are separated according to their mass-to-charge ratio using electric and magnetic fields, before reaching the detector. GC-MS allows not only the separation of complex mixtures, but also the identification and quantification of the individual compounds. The mass spectrometer detector has very low detection limits (approximately 10^{-12}g of analyte) (Biniecka and Caroli, 2011), and it provides the fragmentation patterns for all compounds eluted. Thus, the structure and the chemical properties of the compounds can be elucidated.

GC-MS is the instrument of choice for the analysis of VOCs not only in the forensic laboratories, but also in the cosmetic industry. However, whilst in the latter the analysis of VOCs is generally carried out straight from liquid solutions, in forensic work, VOCs often need to be extracted and analysed from a wide range of matrices, such as from drugs, human remains, explosives and so on. The sample preparation process is a crucial part of any chromatographic analyses, as it can affect the analyte concentration, as well as the purity of the sample.

In general, various techniques have been applied for the extraction of VOCs from a diverse range of matrices, such as from plants (Ormeño, Goldstein and Niinemets, 2011;

Fojtová, Lojková and Kubáň, 2008), foods (Aceña et al., 2010; Merkle, Kleeberg and Fritsche, 2015), and soils (Hewitt, 1998; Brasseur et al., 2012; Stefanuto et al., 2015). However, there is limited published research investigating the extraction of VOCs from fabrics, despite its applicability.

A notable application of VOCs is in the textile industry, particularly for processes that involve microencapsulation, a technique where tiny particles or droplets (of lasting fragrances, skin softeners, disinfectants, insect repellents, and many more) are enclosed within a capsule that allows the substance to be readily released (Jyothi Sri et al., 2012). For example, to determine the fragrance durability on clothing of two different types of microcapsules containing lemon essential oils, Miro Specos et al. (2010) extracted the VOCs from treated and laundered fabrics in ethanol at various intervals after the wash cycle. For forensic applications, Gherghel et al. (2016) investigated the transfer dynamics of fragrance between fabrics, by analysing methanol extracts of fabrics that have been in contact with fragranced fabrics using GC-MS. Although the study showed great potential for fragrance analysis to be used in forensic investigations, one of the limitations of the study was poor sensitivity resulting from the dilution of the samples during the solvent extraction step.

Solvent extraction is traditionally one of the most routinely used extraction methods, particularly for organic analytes (Ridgway, Lalljie and Smith, 2007). This simple method uses the solubility properties of VOCs for selective extraction. The structure of the analytes determines their solubility into solvents of various polarities, thus solvent polarity can influence the extraction profile (Złotek et al., 2016). In addition to diluting the sample, one of the main disadvantages of solvent extraction is that it is generally a very laborious technique, especially compared to the solid phase micro-extraction (SPME) sampling technique that can be automated.

SPME, a technique developed by Pawliszyn and his co-workers in 1989 (Belardi and Pawliszyn, 1989; Arthur and Pawliszyn, 1990), is a solvent free method, that integrates sampling, extraction, and concentration of analytes. SPME is a modified syringe-like apparatus that consists of a fibre holder and a fibre assembly that has a short retractable fibre (Figure 2-2). The fibre is made of thin-fused silica fibre coated with a polymeric phase, such as polydimethylsiloxane (PDMS). Depending on the chemistry of the

analytes, polarity and volatility, appropriate phases can be selected. The fibre can be exposed to the headspace above the sample (HS-SPME), or it can be directly immersed (DI-SPME) in the solution (György and Károly, 2004). After equilibrium is reached, the extracted analytes are desorbed from the fibre into a gas chromatograph or a high-performance liquid chromatograph. Compared to solvent extraction, SPME is a non-exhaustive extraction technique, where only a small proportion of the target analyte is extracted from the sample matrix (Ouyang and Pawliszyn, 2008). This technique has the benefit of portability, the possibility of automation, and increased sensitivity, in addition to a passive sampling approach that does not interfere with the sample when used in headspace mode (Pawliszyn, 1999). Such benefits are highly valuable in forensic sciences, as mirrored by the increase in popularity of this extraction technique among forensic scientists in the last decade, especially for human scent analysis (Prada et al. 2012).

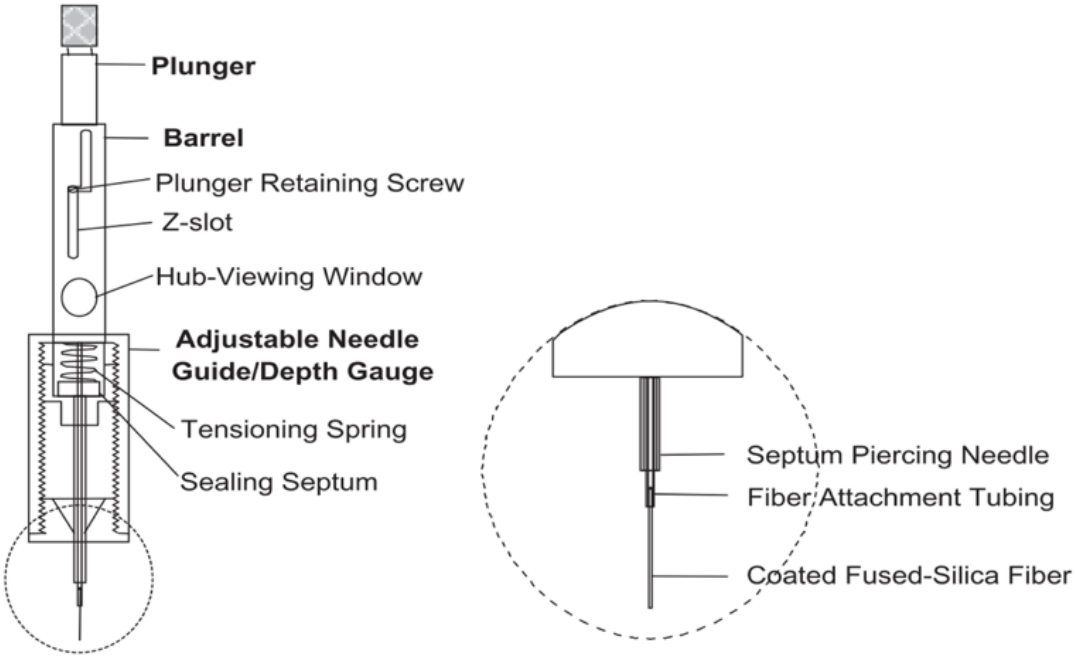

Figure 2-2 Design of the first commercial SPME device made available by SUPELCO (taken from Risticevic et al. 2010)

An alternative to SPME is stir bar sorptive extraction (SBSE), introduced by Baltussen et al. (1999). SBSE is a sampling technique theoretically similar to SPME, but with greater capacity for quantitative extraction than SPME. This technique is based on sorptive extraction, whereby the analytes are extracted into a polymeric phase, such as PDMS,

that is coated onto a magnetic stirring rod (Figure 2-3). For SBSE, the volume of polymeric phase coated onto the rod can range from 20 to 125 µL, while for SPME the volume of polymeric phase coating is usually 0.6 µL (Bicchi et al., 2002). As a result, SBSE tends to offer greater recoveries than SPME. After an exposure time, the stir bar, also called a Twister, is removed and then the absorbed analytes are either thermally desorbed and then on-line transferred to a GC-MS system for analysis, or are back-extracted using a small amount of desorption solvent followed by GC-MS analysis. SBSE is more sensitive than SPME due to the increased availability of stationary phase, however while SPME can be desorbed straight into the GC injection port, the Twister bar requires a thermal desorption unit (additional instrumental requirements), or alternatively the use of a solvent (limiting online analysis and leading to the dilution of the sample).

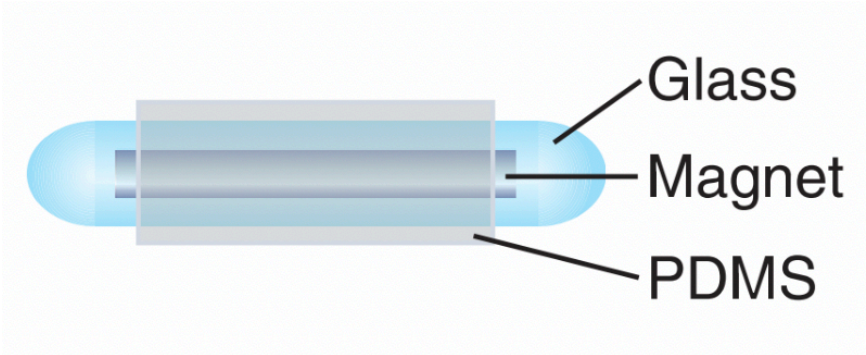

Figure 2-3 Design of commercially available SBSE devices sold as Twister by Gerstel (taken from Hoffman et al. 2002)

2.3.3. Forensic studies on VOC analysis with focus on human scent

Since 2004, there has been a growing body of published literature that addresses the analysis of VOCs using gas chromatography-mass spectrometry for forensic applications. This body of research has been mostly driven by a number of research groups investigating the VOCs associated with human decomposition (Vass et al. 2004; Statheropoulos et al. 2006; Hoffman et al. 2009), and the VOCs emitted by living human scent (Prada et al. 2008).

Because human scent is the most likely additional source of VOCs on the clothing of an individual, it is important to consider the published studies that have addressed the

analysis of VOCs from human specimens. The increased interest in the analytical investigation of human scent was fuelled by the 2005 US court case People of the State of California versus Benigo Salcido (Los Angeles Superior Court, 2005), where the dog scent discrimination evidence was challenged on issues of uniqueness and persistence of human scent and the ability of dogs to discriminate between scents.

Although dogs have been successfully used for many years in finding victims of disasters, there are still major drawbacks when it comes to their use. Drawbacks include the long period of dog training, the short period of time dogs can work for, as well as the emotional stress of the dog while searching for remains (Cablk, Szelagowski and Sagebiel, 2012). Dogs rely on a range of volatile organic compounds to detect the odours of human remains (Hoffman et al., 2009). However, it is still not known exactly whether the dogs use several chemical components, or a rather full VOC profile to match smells (Curran et al. 2007; Hoffman et al. 2009).

In the quest to understand how to produce and collect reliable and reproducible scent evidence, a number of research papers on the analysis of VOCs from humans and human biological specimens have been published (Curran et al. 2005a; Hudson 2009; Prada et al. 2011; Degreeff and Furton 2011; Brown et al. 2013a). Whilst the ultimate goal of this body of research is to distinguish between individuals (Kusano, Mendez and Furton, 2013) and to understand the detection mechanism that produces an alert from scent discrimination canines (Hudson-Holness and Furton, 2010), the research has also increased understanding in terms of how to best analyse human scent (Kusano, Mendez and Furton, 2011), how the VOCs transfer onto a collection material (Prada et al. 2011) and how it persists once transferred (Hudson et al. 2009).

2.3.3.a Method development

As previously mentioned in section 2.3.2, SPME with GC-MS has been the main analytical method for the analysis of human scent VOCs for forensic applications. In one of the early papers on this topic, the hand odour of 8 individuals was collected on 100% cotton Dukal gauze pads prior to SPME GC-MS analysis (Curran et al. 2005b) (Table 2-2). This sampling method where an intermediate collection material is used to collect human scent combined with SPME GC-MS analysis has since then become popular in this research area (Curran et al. 2007; Hudson et al. 2009; Prada et al. 2011; Kusano et al.

2013). Further method development work has been carried out to address the pre-cleaning and selection of the collection material, and also to assess the optimal extraction time for SPME.

To ensure that collection materials are “analytically clean” and free of any background compounds prior to contact with human skin, a pre-treatment step has been developed by Prada et al. (2010) (Table 2-2). Dukal pads were submitted to various processes designed to eliminate existing VOCs, and supercritical fluid extraction was shown to lead to the complete removal of human scent compounds. To avoid direct contact of collection materials with samples, and thus possible disruption or contamination, the use of a STU-100 scent transfer unit has been examined (Prada et al. 2011) (Table 2-2). The STU-100 can be placed in proximity to the human body or article of evidence and VOCs are trapped onto a collection material. However, in a study comparing direct contact and noncontact sampling, the latter method using the STU-100 provided a lower number of volatiles, as well as lower scent mass by more than an order of magnitude.

In terms of the optimisation of the analytical method for the extraction of human scent from gauze pads, published literature is limited, with focus given mainly to the optimal SPME extraction time. In a comparison study between different extraction times (from 12 to 24 h), Curran et al. (2007) (Table 2-2) showed that the most human scent VOCs in terms of abundance and number were recorded from collection materials for an extraction time of 21 h, at room temperature. The extraction temperature was not investigated.

However, work looking at optimum SPME extraction time for direct headspace analysis from human biological samples (Kusano, Mendez and Furton, 2011) (Table 2-2) showed that for blood samples, the recovery of VOCs was not achieved for any of the extraction times tested at room temperature. As such, the authors developed a further experiment where samples were heated at 37 °C using a sand bath. This allowed the successful recovery of VOCs. Although this study provides insight into using higher temperatures for better recoveries of VOCs from blood samples, this method has not been tested for the optimisation of VOCs from gauze pads with human scent.

As an alternative to intermediate collection materials combined with SPME, Soini et al. (2006) (Table 2-2) used a Twister stir bar inserted into a special roller device, designed for the direct sampling of VOCs from human skin. By rolling the stir bar over the arm of individuals, the need for a collection medium was eliminated. They determined a large number of compounds in the human scent profiles, with limited variation between samples from the same individual. Although this technique showed potential, the need for a thermal desorption unit represents an issue, and so this sampling method has not been adopted so far in the human scent forensic research community.

2.3.3.b Transfer studies

The majority of forensic studies on human scent have looked at what classes of VOCs are transferred from the human body to different intermediate collection materials. For example, Curran et al. (2005b) (Table 2-2) used SPME GC-MS to analyse the VOCs from Dukal gauze pads with human scent of eight individuals. The results showed the presence of a range of chemical classes, including organic fatty acids, ketones, aldehydes, esters, and alcohols. It has been suggested that by combining relative ratios of the odour compounds with the presence of differing compounds it is possible to distinguish between individuals (Curran et al. 2005b).

To assess the collection material that allows the best recoveries of human scent, Hudson-Holness and Furton (2010) (Table 2-2) investigated the human scent VOCs collected onto two 100% cotton materials (Dukal, Kings Cotton) and onto a blend material made of cotton, rayon, and polyester (Johnson and Johnson). Hand odour samples from the same person collected on the three different materials showed differences in the chemical classes of the compounds detected, with higher amounts of polar alcohol VOCs from the cotton blend material. Additionally, spiking the three materials with a VOC mixture showed that the cotton blend material led to the recovery of considerably higher amounts of VOCs. The cotton blend material and one of the cotton materials had similar surface morphology, so it was suggested that the chemical composition of the intermediate collection materials plays a more important role in the transfer and release of human scent VOCs.

A further study into the transfer of human scent into various materials compared four textile materials (cotton, polyester, wool, and viscose rayon) (Prada et al. 2011) (Table

2-2). After individuals clasped the palms of the hands with a material, the highest number of VOCs and highest scent mass amounts were transferred onto cotton. Different odour profiles were obtained between the different textile materials, so that cotton and rayon led to a greater variety of functional groups to be collected, whilst polyester collected predominantly high amount of acids. This differentiation was also reflected in a principal component analysis which showed that samples collected on the same textile material clustered together.

Similarly, DeGreef et al. (2011) (Table 2-2) analysed the human scent VOCs from various textiles, but transferred through the use of the noncontact STU-100 scent transfer unit. The textile materials were 100% cotton Dukal brand gauze pads, cotton material, polyester material, viscose rayon material, and Johnson and Johnson brand gauze pads (made of cotton, rayon and polyster). The study showed that both the molecular structure, as well as the weave of the material had an effect on the amount of VOCs recovered, with materials with a tighter weave collecting higher amounts, whilst the least amounts of VOCs were recovered from the polyester material.

2.3.3.c Persistence studies

Persistence in the forensic human scent research area is mostly defined as the consistency over time of the scent profile from the same person or biological material, rather than the general meaning in forensic science, referring to the persistence of a trace on a surface. It has been identified that there is a need to look into the persistence of VOCs from biological specimens (such as hair, saliva, fingernails) over time (Brown et al. 2013) (Table 2-2). However, there have been two studies that have addressed how human scent VOCs persists on a collection material over time.

Curran et al. (2005a) (Table 2-2) carried out an experiment where two individuals rolled Dukal gauze pads, that were previously weighted, between their hands for 5 min. The difference in weight prior and after scent collection was considered the initial scent weights. The pads were placed in plastic containers placed in an open cardboard box and weighed periodically for 84 d. The results demonstrated that the scent loss from the material decreased exponentially over time, with scent still being present after 84 d for two out of three individuals.

To test the optimal storage conditions for human scent evidence, the effect of the collection material (Dukal, Kings Cotton, and Johnson and Johnson) and of the environmental conditions (room temperature, -80 °C, dark room, UVA/UVB) on the persistence of human scent an additional study was carried out using a similarity index (Hudson et al. 2009) (Table 2-2). A similarity index is the result of a three-dimensional covariance mapping where the human scent collect from one individual is checked as to whether it changes over the storage period. A similarity index of 1 shows similarity, and a value of 0 shows dissimilarity. Over a 7-week period, the scent profiles from all three materials changed for all four environmental conditions, with an average similarity value of 0.65 for Dukal, 0.54 for Kings Cotton and 0.47 for Johnson and Johnson. The two 100% cotton materials resulted in the least variation over the 7-week storage period, whilst the Johnson and Johnson cotton, polyester and rayon blend resulted in the highest variation. The higher stability for the cotton materials is attributed to the more polar backbone that led to enhanced retention of polar molecular, which constituted the majority of the compounds observed. In terms of the different environmental conditions, for a storage time of 7 weeks, the averaged similarity index for the three fabrics was 0.63 for -80°C, 0.56 for room temperature, 0.52 for UVA/UVB and 0.51 for dark room. Therefore, the freezing temperature and the room temperature produced the smallest difference in the VOCs profiles over 7 weeks. Interestingly, when looking at variation between storing weeks, the greatest variation was between week 1 and 3, after which the variation decreased.

Overall, forensic studies on human scent have helped improve the extraction time for specific biological specimens, have showed what types of VOCs transfer and how they transfer onto different fabrics and how different storage conditions affect the persistence on fabrics. However, for a more complete picture research is still required on further method optimisation such as optimal extraction temperature, as well as on additional factors that affect the transfer and persistence of human scent, such as time and environmental conditions.

Table 2-2 Review of forensic studies on analysis of human scent: sample matrix, sampling size, sampling and analysis method, and the outcome of the study

Research study	Sample matrix	Sampling size	Sampling and analysis method	Outcome
Prada et al. (2010)	Headspace of various sterile gauze pads	N/A	SPME (50/30 µm DVB/CAR/PDMS, extracted at room temperature for 15 h) and STU-100, GC-MS	For obtaining gauze pads free of any human scent compounds, the supercritical fluid extraction was the most succesfull pre-treatmant method.
Curran et al. (2007)	Headspace of Dukal gauze pads with axillary (armpit) sweat	60 individuals aged 17-28 (30 males and 30 females)	SPME (50/30 µm DVB/CAR/PDMS, sample left to equilibrate for ~24 h, extracted at room temperature for 21 h), GC-MS	Determined that 21 h SPME extraction was optimal for collection of hand odour.
Kusano et al. (2011)	Headspace of buccal swabs, breath, blood and urine	N/A	SPME (50/30 µm DVB/CAR/PDMS, extracted at room temperature for different h), GC-MS	Different biological specimens have different optimal SPME extraction times. For buccal swabs and breath samples that was 21 h, for blood 18 h, and for urine 30 min.
Soini (2006)	Direct contact with inner arm	5 individuals (males and females)	SBSE (10 mm long, 0.5 mm film thickness, 24 µL PDMS volume) bars held by a rolling device, GC-MS with thermal desorption	New sampling method that does not require a collection medium. More variation between people than between repeated samples from each person.
Curran et al. (2005b)	Headspace of Dukal gauze pads with axillary sweat	8 individuals aged 17-24 (4 male and 4 females)	SPME (50/30 µm DVB/CAR/PDMS, sample left to equilibrate for ~24 h, extracted at room temperature for 15 h), GC-MS	54 VOCs identified, 7 VOCs present in all samples. Qualitative differences and similarities could be used to differentiate between individuals.
Hudson-Holness and Furton (2010)	Headspace of materials spiked with VOCs and materials that came in contact with hands	N/A	SPME (50/30 µm DVB/CAR/PDMS, extracted at room temperature for 21 h), GC-MS	Chemical composition of the material rather than the surface morphology had a higher impact on the trapping and release of VOCs.

				Cotton blends released more VOCs than 100% cotton materials.
Prada et al. (2011)	Headspace of four textile materials (cotton, polyester, rayon, wool) held between hands Vacuum of hand VOCs trapped on four textile materials	6 individuals (3 males and 3 females)	SPME (50/30 µm DVB/CAR/PDMS, extracted at room temperature for 21 h) and STU-100, GC-MS	Collection of human scent onto cotton materials through direct contact provided the most abundant and diversified profile of VOCs, that led to the highest degree of discrimination.
DeGreeff et al. (2011)	Vacuum of hands using a gauze pad	4 individuals (2 males and 2 females)	SPME (50/30 µm DVB/CAR/PDMS, extracted at room temperature for 21 h) and STU-100, GC-MS	The amount and number of VOCs trapped/released by textile fibres depended on the weave of the material and the molecular structure. Tighter weave collected greater amounts than open weave. Cellulose-based cotton, due to the differences in the molecular interactions between its molecular backbone and VOCs, collected higher amounts of VOCs than polyester.
Brown et al. (2013)	Headspace of head hair, fingernail clippings, saliva, hand swab	20 individuals. .	SPME (50/30 µm DVB/CAR/PDMS, extracted at room temperature for 21 h), GC-MS	Identified 42 VOCs from the samples that were previously reported to be of human origin. The VOCs identified included 7 functional groups: acids, alcohols, aliphatic and aromatic hydrocarbons, aldehydes, esters, heterocyclics and ketones.
Curran et al. (2005a)	Headspace of Dukal gauze pads with axillary sweat	2 males aged 24	SPME (50/30 µm DVB/CAR/PDMS, sample left to equilibrate for ~24 h, extracted at room temperature for 15 h), GC-MS	Weighing of gauze pads indicated measureable amounts of VOCs still present nearly three months after deposition.
Hudson et al. (2009)	Headspace of fabric materials held between hands	6 individuals	SPME (50/30 µm DVB/CAR/PDMS, extracted at room temperature for 21 h), GC-MS	Human scent evidence had the smallest loss of odours when kept in glass containers subjected to minimal UV light exposure

2.4. Potential of fragrance VOCs as a form of trace evidence

In the last decade, the number of sexual offences reported to police in England and Wales has increased (Office for National Statistics, 2017). For the year ending March 2017, the Crime Survey for England and Wales (Office for National Statistics, 2018) estimated that 2% of adults aged 16 to 59 had experienced sexual assault, or attempted assault, corresponding to approximately 646,000 victims, and that 0.5% experienced rape or attempted rape, corresponding to approximately 151,000 victims. Many of the sexual assaults are not reported to the police, so that for the same period 121,187 sexual offences (approximately 1 in 6 of the total estimate) and 41,186 rapes (approximately 1 in 4 of the total estimate) were recorded by the police (Office for National Statistics, 2018). These incidents are at the highest level recorded since record tracking started in 2002. However, many of these cases do not go to court. For example, in 2016, 12,572 defendants were prosecuted for sexual offences, and 7,511 were sentenced (Office for National Statistics, 2017). The c. 60% conviction rate was the lowest amongst all offence types. The disparity between cases recorded by the police and those leading to a prosecution has been explained by reference to various factors, including the vulnerability of victims, the relationship between victim and suspect, and the lack of the requisite evidence for a crime to have occurred (Hester and Lilley, 2017). The issue of insufficient evidence can be addressed by addressing the potential for incorporating relevant additional trace materials into reconstruction approaches. Trace materials can be valuable for establishing contact and obtain insights into the timing of the transfer, and so they can be particularly useful for cases where for example the offender is a stranger, the victim is a minor, or there is a dispute about the time of the sexual assault.

Clothing is often collected in the course of an investigation into sexual assault and it is generally tested for trace materials such as fibres (Bull et al., 2006). Trace material that has transferred can offer valuable information in forensic reconstruction to help identify contacts between objects, people, or locations (Chisum and Turvey, 2011).

Although not currently in practice, clothing recovered from a sexual assault victim can be also analysed for traces of VOCs from fragrances. Fragrances are traces that are invisible to the eye, and are widely used by both men and women. It is possible that

during a contact between individuals, components of those fragrances can transfer. The recovery of relevant fragrance components from clothing has the potential to be a form of trace evidence that can be used to assess the likelihood of a contact between individuals. Cosmetic products, such as perfumes, deodorants, shampoos, and make up, are well known to contain a high number of VOCs, and despite the wide popularity of these cosmetics, analysis of fragrance VOCs from clothing is not currently used in forensic analysis. Nevertheless, as seen in *section 2.3.3*, forensic analysis of materials with VOCs emanated by body odour has seen an increased interest in the in the last two decades (Prada et al. 2008).

In a more recent proof of concept study (Gherghel et al., 2016), the potential use of fragrance VOCs from fabrics as a form of trace evidence has been explored. The transfer dynamics of fragrance between fabrics was investigated by analysing methanol extracts of fabrics that have been in contact with fragranced fabrics under two different time variables using GC-MS. This study showed that both the period of time that two cotton swatches are in contact for, and the period of time that a perfume is left to dry for prior to transfer affect the transfer or fragrance between the swatches. This was the first study to demonstrate the transferability of fragrances between fabrics and the dynamics of this process. It was asserted that fragrance analysis could be potentially a very important tool in forensic investigations of cases that involve close contact between individuals, such as sexual assault cases. However, the study also highlighted the need for further investigation into methods for extracting fragrances from fabrics to overcome instrumental sensitivity.

Besides a reliable and sensitive instrumental method able to analyse a trace material identified and collected in the course of a forensic investigation, there is a need for empirical studies investigating the transfer and persistence of that form of trace material and the incorporation of these data into the interpretation process. Research studies have identified that the material type plays a major role in evidence dynamics of physical traces (Pounds and Smalldon, 1975a; Bull et al., 2006), and similar findings have been identified for chemical traces such as human scent (Hudson-Holness et al. 2010; Prada et al. 2011; DeGreeff et al. 2011).

It should be noted that VOCs differentiate in their physical state from most forms of trace material, such as fibres, pollen, hair, because of their high vapour pressure at ordinary room temperature. For example, Hudson et al. (2009) investigated the persistence of human scent VOCs collected on cotton under various environmental conditions over a 7-week period and identified a change in the human scent profile over time. Thus, it is paramount when designing experimental studies to test the ability of VOCs to be a form of trace evidence, to investigate the effect of time and environmental conditions on the amount of VOCs present at different stages in the transfer and persistence process.

For forensic reconstruction purposes, there is a need to fully understand how traces transfer and persist and the effect of factors such as material type and time (Morgan and Bull, 2007). Establishing an empirical evidence base upon which to draw conclusions about the activities of a particular form of trace is critical for a transparent and robust interpretation of what the evidence means in a specific case (Morgan, 2017). Availability of empirical data on the dynamics of trace materials can aid the forensic scientist at a crime scene, in the laboratory, and during the interpretation process.

2.5. Research questions

Therefore, this research aims to answer three main questions:

Research question 1: Can an analytical method be developed and validated for the recovery of traces of fragrance VOCs from fabrics?

Research question 2: Does fragrance VOCs spiked onto a fabric transfer onto a secondary fabric (to include a consideration of how perfume ageing time, contact time, and fabric type affect the transfer)?

Research question 3: Does transferred fragrance VOCs onto a fabric persist over time (to include a consideration of how time, fabric type, and environmental conditions affect the persistence)?

3. Experiments: materials and methods

3.1. Outline

This chapter will provide the materials and methods used to carry out the experiments from the following three chapters, namely the method development, the transfer, and the persistence experiments.

3.2. Chemicals and materials

Reference standards of seven VOCs, including (+)-α-pinene (98.5% purity), (R)-(+) limonene (97%), linalool (97%), geraniol (98%), eugenol (99%), coumarin (99%), and ethylene brassylate (97%) were purchased from Sigma Aldrich, Gillingham, UK. Their chemical structure is presented in Figure 3-1 and their CAS number, boiling point and vapour pressure values are provided in Table 3-1. These reference standards were chosen based on their popularity in the fragrance industry, assuring the standards chosen covered a wide range of volatility (see Table 3-1 for vapour pressure values) . Two internal standards of analytical standard grade, 1,4-dibromobenzene and methyl nonanoate, as well as methanol (HPLC grade, 99.9% purity) were obtained from Sigma Aldrich, Gillingham, UK. The internal stardands were chosen as they were available in high purity, they have characteristic molecular ion patterns, and because they do not occur in perfumes. Methyl nonanoate was employed for the quantification of the linear compounds, whilst 1,4-dibromobenzene was employed for the quantification of cyclic compounds. Commercially available perfumes were purchased from a mainstream retailer, with a focus given to perfumes marketed for men as 99% of sexual assaults from U.K. in 2014 were committed by men according to the Office for National Statistics (Office for National Statistics, 2015). The reference standards, internal standards, stock solutions of each compound, and the perfumes were kept refrigerated ($T < 5$ °C). In order to ensure the stability of the stock solutions, they were prepared in methanol on a monthly basis at concentrations between 10 – 25 mM.

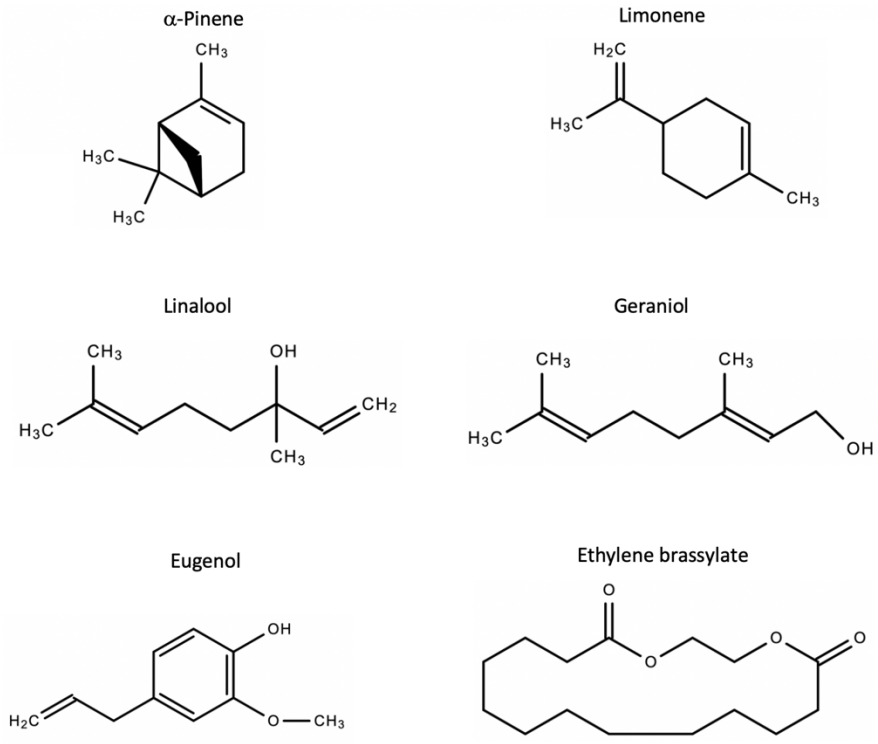

Figure 3-1 The chemical structure of the VOCs of interest

Table 3-1 The CAS number, boiling point, and vapour pressure of the VOCs of interest

Compound	CAS number	Molecular weight (g/mol)	Boiling point (°C)*	Vapour pressure (mmHg)
α-Pinene	80-56-8	136.24	156	5.098 at 23.5 °C[a]
Limonene	5989-27-5	136.24	176	2.129 at 23.5 °C[b]
Linalool	78-70-6	154.25	198	0.159 at 23.5 °C[a]
Geraniol	106-24-1	154.25	230	0.042 at 23.5 °C[a]
Eugenol	97-53-0	164.20	254	0.026 at 23.5 °C[a]
Ethylene brassylate	105-95-3	270.37	332	4.38E-07 at 25 °C[c]

* Boiling point values at 760 mmHg

a Based on calculation using Antoine Coefficients from Yaws' Handbook of Antoine Coefficients for Vapor Pressure (Yaws, Narasimhan and Gabbula, 2009)

b Based on Li et al. (1998)

c Based on Belsito et al. (2011)

For the HS-SPME procedure, the 1 cm long fibres were purchased from Supelco (Bellefonte, Pa, USA). The five different fibres investigated were polydimethylsiloxane (PDMS), polyacrylate (PA), polydimethylsiloxane/divinylbenzene (PDMS/DVB), carboxen/polydimethylsiloxane (CAR/PDMS), and divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) (Figure 3-2). Table 3-2 shows the characteristics of the SPME fibres investigated. Prior to first use and daily, the fibres were conditioned by heating them in the injection port according to the manufacturer's recommendations (time and temperature described in Table 3-2). This step was followed by a fibre blank analysis to confirm the effectiveness of conditioning. For all SPME analyses, 20 mL screw cap vials sealed with 18 mm pre-fitted PTFE-silicon septum were used.

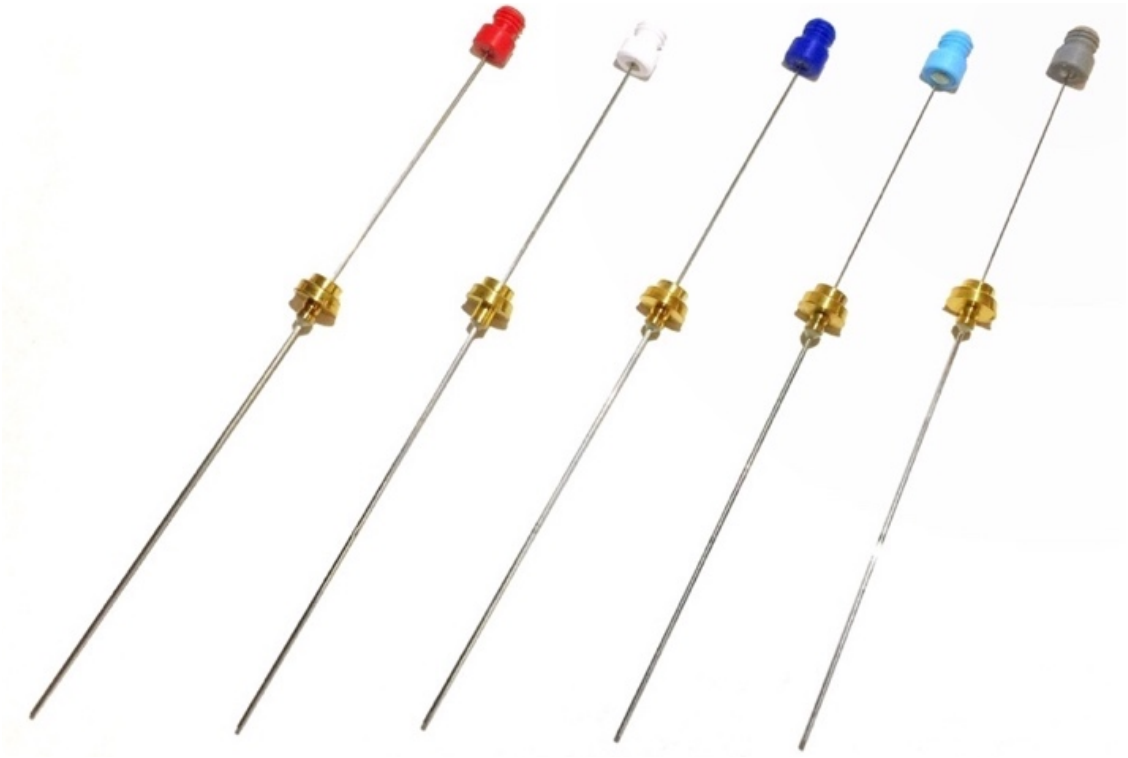

Figure 3-2 The five different fibres investigated. From left to right: PDMS (red), PA (white), PDMD/DVB (dark blue), CAR/PDMS (light blue), and DVB/CAR/PDMS (grey)

Table 3-2 List and properties of the five fibres investigated

Fibre acronym	**Fibre core/ Assembly type**	**Extraction mechanism**	**Film thickness (µm)**	**Polarity**	**Volume of coating (µL)** (Pawliszyn, 2011)	**Conditioning parameters (temperature/ time)**
PDMS	Fused Silica/SS	Absorbent	100	Non-polar	0.612	250 ℃/ 0.5 h
PA	Fused Silica/SS	Absorbent	85	Polar	0.543	300 ℃/ 2 h
PDMS/ DVB	Stableflex/SS	Adsorbent	65	Bipolar	0.418	250 ℃/ 0.5 h
CAR/ PDMS	Stableflex/SS	Adsorbent	85	Bipolar	0.528	300 ℃/ 1-2 h
DVB/ CAR/ PDMS	Stableflex/SS	Adsorbent	50/30	Bipolar	0.500	270 ℃/ 1 h

The garments used in this study were purchased from a popular UK male clothing retailer, and they were a white long sleeve T-shirt made of 100% cotton, a white T-shirt made of 100% cotton, a long sleeve denim jacket made of 100% cotton, a black jumper made of 100% wool, a beige jumper made of 100% acrylic, a blue jacket made of 100% nylon, a blue tank top made of 100% polyester, and a purple T-shirt made of polyester:viscose 50:50. Prior to the analyses, the garments were washed together in a conventional washing machine without adding any detergent. The dried garments were stored in a closed plastic bag at ambient temperature.

3.3. Instrumentation

A Jeol JSM-6700F field emission scanning electron microscope (SEM) was used for the visual characterisation of the fabrics. The fabrics were coated with gold and placed on carbon tape to minimise electrical charging.

For the VOCs analysis, the samples were analysed on a Scion Gas Chromatography system coupled to a Scion QqQ-MS/MS instrument (Bruker Corporation, Freemont, CA, USA) and equipped with a Combi Pal autosampler (CTC Analytics, Switzerland) (Figure

3-3). For confirmation of unknown compounds, a TraceTM 1310 Gas Chromatography system coupled to a Q-ExactiveTM hybrid quadrupole-Orbitrap mass spectrometer system (Thermo Fisher Scientific, Bremen, Germany) with Xcalibur 4.1 software (Thermo Fisher Scientific, Les Ulis, France) was employed. Chromatographic separation was achieved on a VF-5ms capillary column (30 m × 0.25 mm, 0.25 µm film thickness) from Varian (Palo Alto, California, USA). The carrier gas was helium (99.9999% purity) and the column flow was maintained at 1 mL/min using an electronic flow controller.

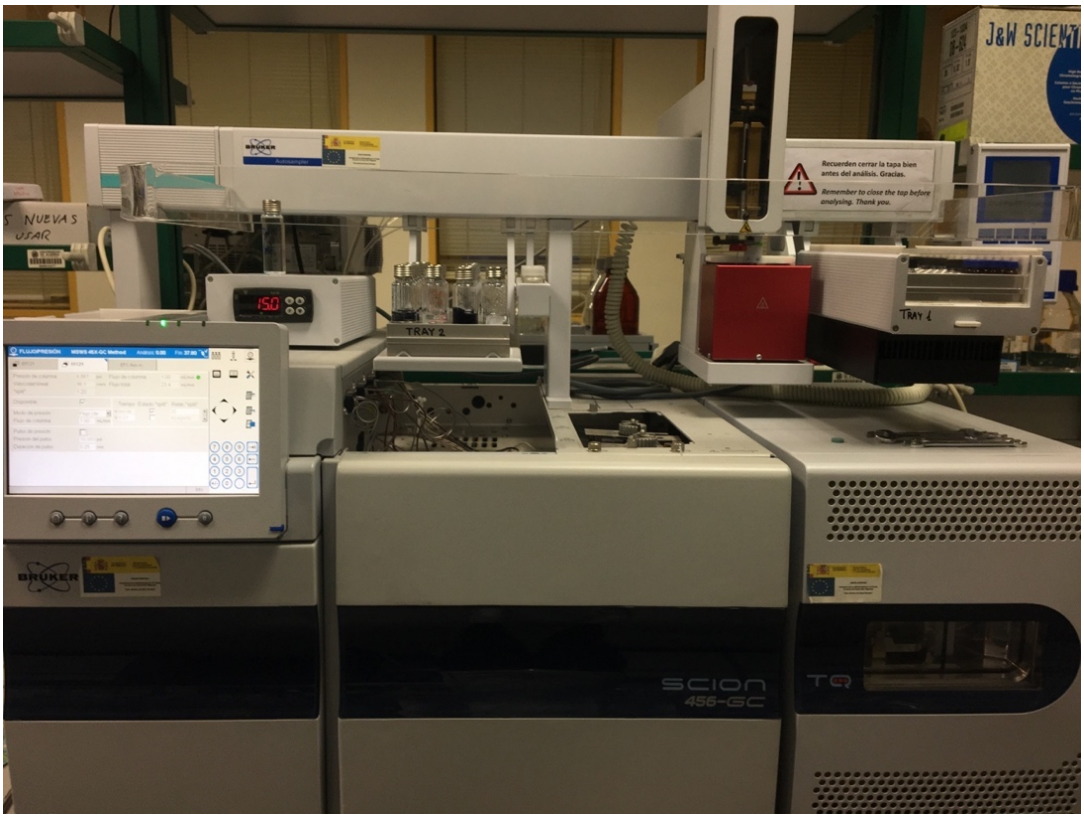

Figure 3-3 The Scion QqQ-MS/MS instrument used for analysis of the VOCs of interest

A portable digital thermometer-hygrometer (model 82021, VWR Scientific) was employed for measuring the laboratory temperature and humidity. Throughout this study, the laboratory ambient temperature ranged between 21 and 23 °C and the humidity ranged between 25 and 48%.

Work carried out by Gherghel et al. (2016) looked at the transfer of fragrances between fabrics using two different transfer methods, one that involved hand rubbing, and one that involved the two fabrics being left in contact under the pressure of a heavy weight.

The authors identified that the two procedures led to similar number of perfume ingredients being transferred. However, lower reproducibility was observed for the hand rubbing method. To overcome this issue, and to simulate closer to reality a sexual assault case where friction between fabrics occurs, a motorised Stainingtester crockmeter (Komputeskst, Hungary) (Figure 3-4) was used to simulate the transfer between fabrics for chapters 5 and 6. The crockmeter, also called a fabric abrasion tester, is generally used in the textile industry to determine the amount of colour transferred from fabrics to other surfaces by rubbing, however it has also been used in the published forensic science literature to simulate fabric contact in a reproducible way (Robertson, Kidd and Parkinson, 1982; Bennett, Roux and Robertson, 2010). The crockmeter finger covered with a piece of the primary fabric spiked with the VOCs was rubbed against a blank recipient sample fabric under a fixed load of 9 N.

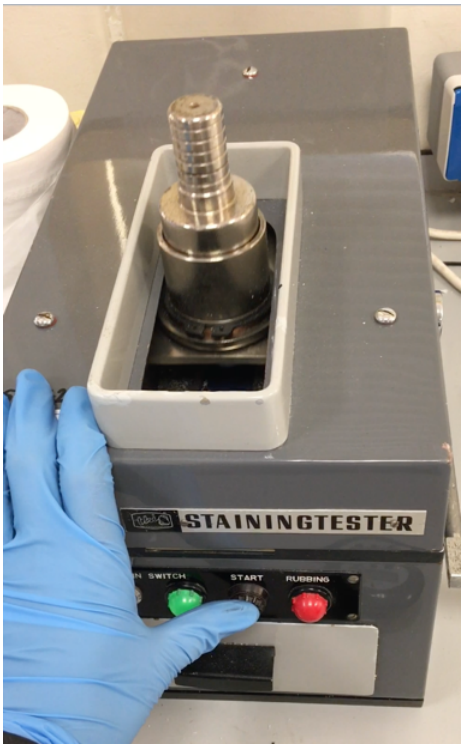

Figure 3-4 Crockmeter employed to carry out the fragrance transfer between fabrics

3.4. GC-MS conditions

The GC oven temperature was programmed from 35 °C (held for 1 min) to 180 °C (held for 1 min) at 5 °C/min rate, and then to 300 °C (held for 2 min) at 25 °C/min rate. The total chromatographic run was 37.8 min. The transfer line and the ion source

temperatures of the mass spectrometer were set to 280 °C. The thermal desorption of the SPME fibre was carried out using an injector temperature of 250 °C in splitless mode for 3 min, and a further 6 min of purging in split mode was used to help prevent carryover effects. For SPME, a specific inlet liner with a diameter of 0.8 mm was purchased from Agilent Technologies to improve GC resolution and to minimise peak broadening. The mass spectrometer was operated in Electron Ionisation (EI) mode at 70 eV for comparison with commercial library spectra. Data acquisition rate was of 5 spectra/s and the mass range was scanned from *m/z* 40 to 250. EI mass spectrum of compounds eluted from the GC column was compared to EI mass spectra from the National Institute of Standards and Technology (NIST) v 2.2 g (2014) database for identification. The conditions for the GC-Orbitratp used for the confirmation of unknown compounds were the same as the GC-MS conditions.

3.5. Procedures

As highlighted in Figure 3-5, there are three major stages in the overall fragrance transfer process. The first one (Figure 3-5, green section) involves the spiking of fabric with fragrance and the transfer of fragrance from the donor piece of fabric to the recipient piece of fabric. Various variables are in play at this point, such as the length of time the fragrances have been on the donor materials prior to transfer (the perfume ageing time), the length of time the two fabrics are in contact for (the contact time), and the fabric types. The second stage (Figure 3-5, yellow section) covers the persistence of the transferred fragrance on the recipient fabric, whilst the last stage (Figure 3-5, mauve section) is represented by the analysis of the fabric with the transferred fragrance. From an analytical point of view, the analysis method needs to be investigated first, prior to the studies regarding evidence dynamics. Thus, this section will explain the laboratory procedures carried out for the development of SPME GC-MS method, followed by the those for the transfer and persistence experiments.

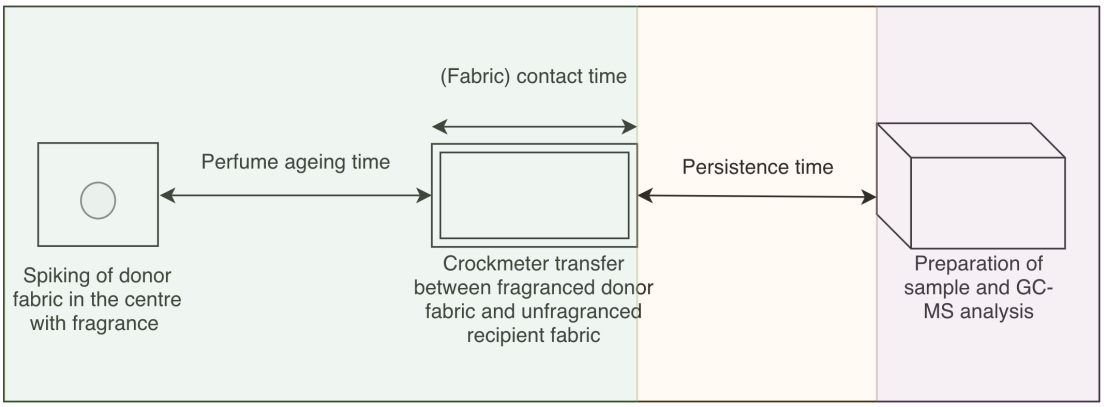

Figure 3-5 Schematic of three stages of the transfer process: the transfer itself (in green), the persistence (in yellow), and the analysis (in mauve)

3.5.1. SPME GC-MS method development

3.5.1.a Selection of the SPME fibre

For the selection of the optimal fibre towards five VOCs of interest, and also towards VOCs from a commercial perfume, five different SPME fibres, described in Section 3.2, were investigated. Firstly, the recovery ability towards the five VOCs was studied by analysing in triplicate 10 µL of a 200 µM fragrance mixture (FM) made of limonene, linalool, geraniol, eugenol, and coumarin in methanol. Secondly, 10 µL of a men's perfume was analysed in duplicate.

3.5.1.b Optimisation of the SPME extraction of fragrance solutions

After determining the optimal SPME fibre, the main extraction factors (incubation time, extraction time, and extraction temperature) were optimised using a face-centred cube central design ($\alpha = 1.682$), based on a 2^3 full factorial design plus six axial points and six replicates in the centre of the design. Therefore, 20 experiments were carried out, including six replicates, where 10 µL of a 50 µM of an FM made of α-pinene, limonene, linalool, geraniol, eugenol, coumarin, and ethylene brassylate in methanol was pipetted straight into a vial. Additionally, four empty vials used as blanks were analysed throughout the analysis sequence. The values for the incubation time varied from 1.6 to 18.4 min, for extraction time from 4.8 to 55.2 min, and for extraction temperature from 33.2 to 66.8 ˚C. The results were plotted using the Minitab 18 statistical software (Minitab, State College, USA).

3.5.1.c Optimisation of the SPME extraction of fragrance solutions from garments

After the optimisation of an extraction method for fragrance solutions, the next step was the optimisation of a method for the extraction of fragrances solutions from garments. For this purpose, pieces of 100% cotton swatches of approximately 2 cm x 2 cm and 0.2 g were employed. Each swatch was placed on a Petri dish (diameter 88 mm) and spiked with 10 µL of the 50 µM fragrance mixture used in Section 3.5.1.b. Similar to the optimization of the extraction from fragrance solutions (Section 3.5.1.b), a 2^3 full factorial CCD was created, where 20 samples were analysed in random order. However, the values varied from 4.9 to 30.1 min for incubation time, from 9.8 to 60.2 min for extraction time, and from 39.9 to 70.1 ˚C for extraction temperature.

3.5.1.d Validation of the SPME extraction of fragrance solutions from garments

For the validation of the optimised method using garments, the parameters studied were linearity, working ranges, LOD, LOQ, sensitivity, trueness (intraday and interday relative recoveries), repeatability (intraday precision), and reproducibility (interday precision). The linearity was assessed by studying the determination coefficient (R^2) of the calibration curves. Calibration curves were constructed in cotton and in polyester. Seven different concentrations of the studied VOCs were prepared in triplicate in methanol, ranging from 0.5 to 100 µM, whilst the concentration of the internal standards in the calibrants was 20 µM. Sensitivity was determined as the slope of the calibration straight-line. The LOD and LOQ for each analyte were calculated as the concentration for which the signal-to-noise ratio was 3 and 10, respectively, when the most intense characteristic ion was monitored. The working ranges were determined by the lowest and the highest concentrations tested with good linearity. Trueness of the method was evaluated by studying the relative recoveries of analytes from the gas phase when 10 µL of the FM was spiked on fabrics at two different concentrations (2.5 and 25 µM) within the same day (n = 3) and on different days (n = 4). Precision was based on repeatability (evaluated as intraday precision) and reproducibility (evaluated as interday precision), and expressed as relative standard deviation (RSD).

3.5.1.e Analysis of commercial perfumes from garments using the validated SPME method

Using the optimised and validated method, cotton and polyester swatches were spiked with 10 µL of a number of different commercially available perfumes diluted between 500 and 1500 times in methanol. The diluted perfume solution also contained 20 µM of 1,4-dibromobenzene and methyl nonanoate as internal standards. When present in the perfume, the concentration in the headspace of the analytes with available calibration curves was calculated.

3.5.1.f Analysis of commercial perfumes from garments using liquid extraction

For the comparison of SPME extraction with liquid extraction, similar to Section 3.5.1.e, a cotton swatch was spiked with 10 µL of a commercially available men's perfume diluted in methanol 500 and 100 times, and also undiluted. Following the sample preparation from Gherghel et al. (2016), the swatch was added to a 2 mL GC vial, to which 1 mL of methanol was added for the liquid extraction. The vial was shaken using a Vortex for 2 min, after which 50 µL of the liquid were transferred to a 50 µL GC vial insert. For each sample, 1 µL was injected to the GC, a five-fold increase compared to Gherghel et al. (2016).

3.5.2. Transfer studies

The series of three transfer experiments (on perfume ageing time, fabric contact time, and fabric type) were carried out as presented in Figure 3-5. A swatch of primary material was spiked with VOCs, left to dry for a period of time called perfume ageing time, followed by transfer of fragrances onto a secondary piece of fabric for a period of time (referred to as contact time) using a crockmeter. The time from the transfer until analysis (the persistence time) was kept at 0 s for the purpose of this transfer study.

A quality control process was applied by the analysis of blank samples after every three replicate samples in order to verify that no contamination of the fabrics, lab materials or instrument was observed.

3.5.2.a Perfume ageing time and contact time

For the perfume ageing time and fabric contact time experiments, a fragrance mix (FM1) containing 500 μM of each of the six VOCs, as well as 200 μM of the two internal standards was prepared in methanol. The fragrance levels in a typical personal-care perfume are in the range 0.5-2 wt% (Van Asten, 2002). The fragrance levels for FM1 were between 0.008 and 0.009 wt%, 50 times lower than the regular lower limit of 0.5 wt%.

A cotton swatch of approximately 3 x 3 cm (primary material) placed in a clean Petri dish (diameter 88 mm) was spiked in the centre with 10 μL of the FM1 using a Hamilton syringe. For the ageing time experiments, the fabrics, in triplicates, were left on the Petri dish from 10 min up to 24 h, with a leftover sample being used for 48 h. For the contact time experiments, the fabrics, in triplicates, were left on the dish for a constant ageing time of 10 min.

After the appropriate ageing time, the spiked primary fabric was placed onto the finger of the crockmeter for the fragrance transfer onto a secondary piece of cotton swatch (the recipient material). For the perfume ageing time experiment, the contact time was kept constant at 1 min, while for the contact time experiment the time varied from 10 s to 10 min.

After the appropriate contact time, the recipient fabric was removed from the crockmeter, weighed, then added to a 20 mL vial, to which 10 mL of water were added, prior to agitating the vial using a vortex for 30 s.

3.5.2.b Fabric type

The fabric type experiment was carried out in a similar manner to the first two experiments with a few modifications. The spiking fragrance mixture (FM2) contained 2mM of each of the six VOCs in methanol. The internal standard mixture was prepared separately at a concentration of 20μM of the two internal standards in methanol. The FM2 was spiked onto the primary piece of fabric as above, however the internal standard mix was added to the secondary piece of fabric (the recipient) immediately prior to adding it to the vial and adding the 10 mL water. In this way, the internal standards were not submitted to the transfer process and they were only used for the

GC-MS calibration step. Quantitative curves were verified when standards were submitted to the SPME GC-MS analysis checking the consistency of the extraction efficiency and instrumental response along the calibration range.

The ageing time was kept at 0 min, and the contact time at 1 min. Whilst the two time-related experiments above used cotton for both the donor and the recipient material, for the fabric type experiment, all seven fabrics available were used as donor materials, and cotton and polyester were used as recipient materials. However, when the nylon fabric was used as a donor material it kept slipping off the crockmeter finger, even for contact times as short as 1 min, and therefore it could not be evaluated.

The inclusion of an internal standard just prior to extraction allowed for quantification using calibration curves. Six different calibrant concentrations of the six studied VOCs were prepared in methanol, ranging from 0.2 to 200 μM. The same internal standard mixture of 20μM in methanol used for the samples was used for the calibration. The calibrants were spiked directly onto cotton or polyester swatches, followed by spiking with the internal standards mixture. The fabrics were then added to a 20 mL vial, to which 10 mL of water were added, prior to agitating the vial using a vortex for 30 s.

3.5.3. Persistence studies

For all persistence studies (presented in chapter 6), a donor cotton swatch was spiked with FM2 followed by an immediate transfer of the VOCs onto the secondary piece of fabric using a crockmeter for 1 min. Therefore, for all experiments, an ageing time of 0 s and a contact time of 1 min was employed.

The secondary piece of fabric onto which the VOCs were transferred to and left to persist on was cotton, with the exception of the room temperature experiment, where cotton and polyester were investigated. This secondary piece of fabric with transferred perfume was placed on a time-stamped clean Petri dish and left to dry in specific environmental conditions until the appropriate analysis time. Three different temperature regimes (room temperature: between 20 and 24 °C, cool temperature (fridge): between 2 and 4 °C, and very cold (freezer): between -28.3 and -27.5 °C) were chosen to replicate the environments of indoor crime event locations and subsequent storage environments of seized items of clothing in either a fridge or freezer. All samples

were prepared in triplicate. A breakdown of the number of samples and the variables evaluated is given in Table 3-3.

Table 3-3 The number of samples analysed and the values for the variables examined for the persistence studies

Donor fabric		Cotton			
Perfume ageing time (min)		0			
Contact time (min)		1			
Environmental temperature		Room temperature		Fridge	Freezer
Recipient fabric		Cotton	Polyester	Cotton	Cotton
Persistence time (d)	0.04	3	3	3	3
	0.33	3	3	-	-
	1	-	-	3	3
	3	3	3	-	-
	4	-	-	3	-
	5	3	3	-	-
	7	3	3	-	-
	10	3	3	3	3
	14	3	3	-	-
	15	-	-	3	-
	17	3	3	-	-
	21	3	3	-	-
	24	3	3	-	-
	28	3	3	-	-

3.5.3.a Room temperature

For the room temperature experiment, two different fabrics, cotton and polyester, were used as recipient fabric. The time-stamped samples were left to dry on a shelf in a laboratory storage room (Figure 3-6) at temperatures between 20 and 24 °C, and the humidity between 27 and 51% to replicate temperate climate conditions. The shelf was covered with paper on the sides to prevent external contamination. The persistence times ranged from 1 h (0.04 d) up to 28 d (Table 3-3).

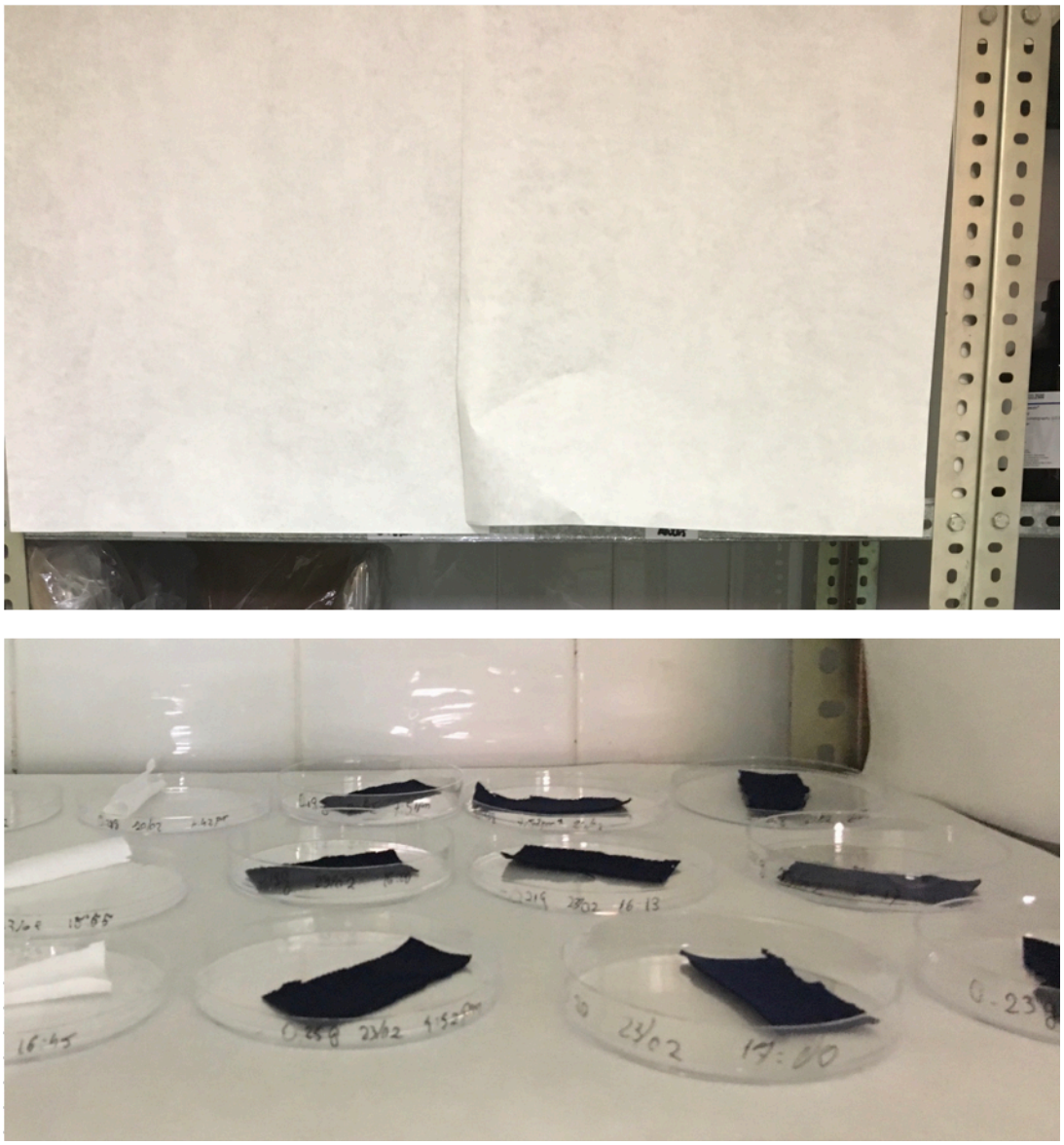

Figure 3-6 Storage conditions for the room temperature persistence experiments (each fabric rests on an 88 mm diameter Petri dish)

3.5.3.b Fridge temperature

For the fridge experiment, the samples were left in a designated shelf in laboratory fridge at temperatures between 2-4 °C. The samples used were cotton fabrics, and the persistence time ranged from 1 h to 15 d (Table 3-3).

3.5.3.c Freezer temperature

For the freezer experiment, the samples were kept in laboratory freezer at temperatures between -28.3 and -27.5 °C. The samples used were cotton fabrics, and the persistence time ranged from 1 h to 10 d (Table 3-3).

3.6. Data processing and analysis

For each of the VOCs of interest and the two internal standards, three characterising ions were chosen for calculation of the peak areas (see Table 4-6). The resulting chromatographic peak area raw data was exported to an Excel (Microsoft Inc.) file for further processing. Within Excel relative peak area ratios of analytes were calculated against the internal standards, which were further used to determine the concentration of analytes in fabric by using the calibration curves.

Minitab 18 statistical software (Minitab, State College, USA) was used in the method development study for the creation and analysis of central composite designs.

For the transfer experiment on fabric type, the quantitative composition of the VOCs recovered from the fabrics was evaluated by hierarchical cluster analysis (HCA), principal component analysis (PCA), and partial least square (PLS) analysis using the software programme SIMCA-P 12.0.1. (Umetrics AB, Umeå, Sweden).

4. SPME GC-MS method development for analysis of fragrance traces from clothing

4.1. Outline

This chapter presents the optimisation, validation, and application of a HS-SPME GC-MS method for the analysis of trace amounts of fragrances from fabrics. In addition to fibre type, three different SPME extraction conditions were investigated simultaneously, namely incubation time, extraction time, and extraction temperature, using a central composite design in order to determine the optimal conditions for the extraction of VOCs of interest. The main figures of merit of the proposed method (calibration range, limits of detection and quantification, trueness, and precision) were evaluated for six different VOCs in both natural and synthetic fibres in order to validate it and verify its capability for the proposed application. The validated method was compared to the liquid extraction employed in Gherghel et al. (2016). The benefit of the optimised and validated method for the extraction of perfume compounds from fabrics presented here is a more sensitive and robust analytical method for obtaining insights into how these fragrance VOCs have been transferred to and persisted on garments.

4.2. Results

4.2.1. Selection of the SPME fibre

Five fibres with different coating materials (Table 3-2) were investigated to check their extraction ability specifically towards a fragrance mixture (FM) made of popular perfume ingredients, but also towards a commercially available men's perfume.

For the analysis of an FM, 10 µL of a 200 µM solution of limonene, linalool, geraniol, eugenol, and coumarin in methanol were analysed in triplicate as representative compounds. For each fibre type, the mean peak area and relative standard deviations of each of the five compounds were determined, allowing in turn to calculate the sum of the mean peak areas and the average of the RSD, respectively. Whilst the sum allows

an evaluation of the amounts of compounds extracted, the average of the RSD provides information about the repeatability performance.

These two parameters enabled the determination that for the fragrance mixture, the DVB/CAR/PDMS fibre performed the best over the entire range of compounds analysed, producing the highest peak areas with the best repeatability (average RSD = 4.6%) (Table 4-1). Similar extraction rates were also obtained for the PDMS/DVB fibre, especially for lower volatility compounds, such as eugenol and coumarin (Figure 4-1). However, the PDMS/DVB fibre produced on average approximately three times poorer repeatability compared to the DVB/CAR/PDMS fibre (Table 4-1).

Table 4-1 Evaluation of the extraction abilities of five SPME fibres towards five VOCs and towards a commercial perfume marketed for men. The fibres studied were polydimethylsiloxane (PDMS), polyacrylate (PA), polydimethylsiloxane/divinylbenzene (PDMS/DVB), carboxen/polydimethylsiloxane (CAR/PDMS), and divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS)

SPME Fibre	Fragrance mixture (n = 3)		Commercial perfume (n = 2)	
	Sum of the compound mean peak areas (E+10)	Average RSD %	Sum of the chromatographic peak areas (E+14)	Average RSD %
100 µm PDMS	0.98	16.9	2.33	0.9
85 µm PA	0.77	24.5	1.64	4.3
65 µm PDMS/ DVB	6.26	12.2	2.45	1.2
85 µm CAR/ PDMS	2.99	16.1	3.71	9.5
50/30 µm DVB/ CAR/ PDMS	6.44	4.6	3.18	1.5

For the analysis of a real perfume, 10 µL of a commercially available men's perfume was analysed in duplicate for all fibres examined. The CAR/PDMS fibre extracted the highest amounts of compounds from the perfume; however, it also produced the highest variation between the duplicates (average RSD = 9.5%) (Table 4-1). The DVB/CAR/PDMS fibre produced the second highest recovery, whilst providing a low average RSD of 1.5%. As the DVB/CAR/PDMS had the best overall performance for the fragrance mixture and the commercial perfume, all further experimental SPME studies were carried out using this fibre.

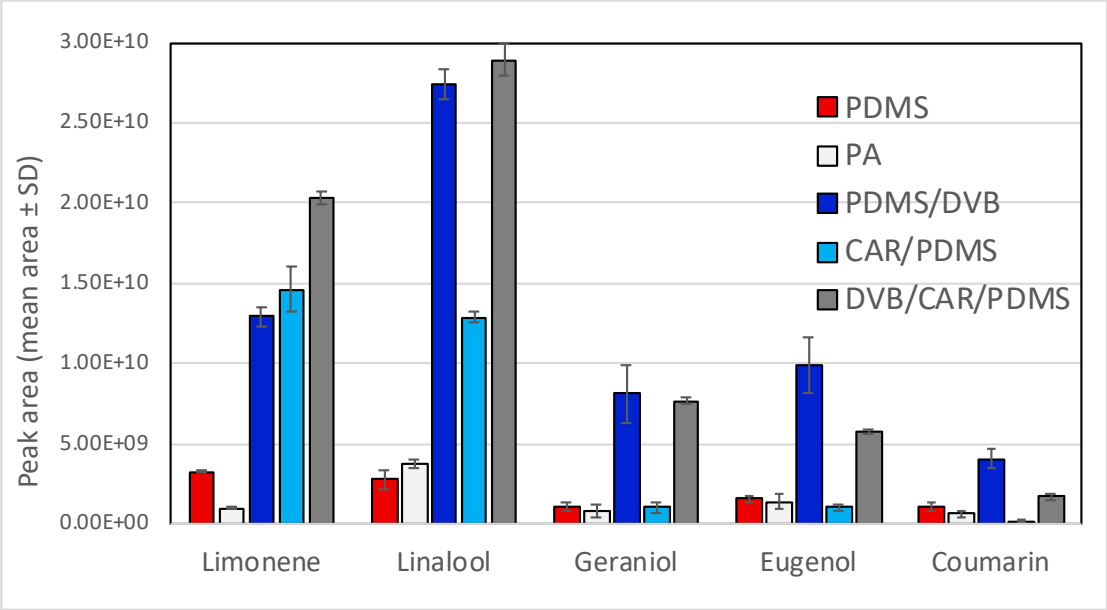

Figure 4-1 Average peak area (n=3) for five fragrance VOCs by five different SPME fibre types

4.2.2. Optimisation of the SPME extraction of fragrance solutions

After the selection of the SPME fibre, the following step was the determination of the optimal SPME variables for the extraction of VOCs of interest. To investigate the effect of different extraction parameters and their optimal values, a central composite design was used where the effect of incubation time, extraction time, and extraction temperature was tested at five different levels for seven different VOCs.

It was observed that throughout the analysis sequence, a compound related to ethylene brassylate appeared in the chromatographic runs, with its intensity generally increasing

with analysis number. The chemical structure of this compound, identified as dimethyl brassylate by the NIST library, as well as the chemical structure of ethylene brassylate are given in Figure 4-2. It should be noted that the dimethyl brassylate was observed only for this experiment, but not for any of the further experiments analysing VOCs from fabrics.

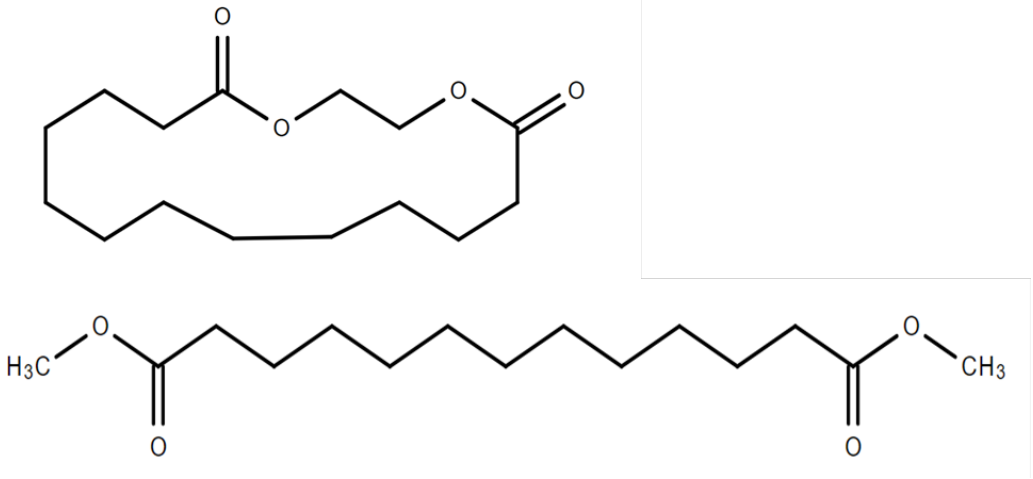

Figure 4-2 Chemical structure of ethylene brassylate ($C_{15}H_{26}O_4$) (top) and dimethyl brassylate ($C_{15}H_{28}O_4$) (bottom)

Generally, the sum of the chromatographic peak areas is used as a response in CCD for SPME optimisation (Carasek and Pawliszyn, 2006; Moreira et al., 2013). However, because of the large differences in the intensity of the analytes extracted, the sum of the normalised peaks was considered a better CCD response. The peak area of each analyte was normalised by using the maximum peak area for that analyte from the analysis sequence. Hence, the maximum possible CCD response in this case was seven, one for each analyte investigated. The conditions for the twenty experiments and the experimental responses, that is the sum of the normalised peak areas, are provided in Table 4-2.

Table 4-2 The sample number, levels of the three factors investigated, and the instrumental responses obtained from a 2^3 face centred cube central composite design (CCD) for the extraction of VOCs by SPME from a fragrance mixture

Run order	Incubation time (min)	Extraction time (min)	Extraction temperature (˚C)	Summed area of the VOCs (E+10)	Summed normalised areas of VOCs
1	5	45	40	1.48	3.67
2	10	30	33.2	1.10	2.48
3	10	30	66.8	2.00	4.68
4	15	15	60	2.31	5.24
5	15	15	40	1.95	4.14
6	10	30	50	2.40	5.31
7	10	30	50	2.60	5.96
8	5	45	60	1.95	4.79
9	10	30	50	2.48	5.64
10	10	30	50	2.45	6.13
11	5	15	40	1.68	3.75
12	15	45	40	1.72	3.94
13	15	45	60	2.25	5.4
14	18.4	30	50	2.58	6.25
15	10	30	50	1.87	4.38
16	10	55.2	50	2.62	6.19
17	10	30	50	1.68	3.91
18	10	4.8	50	1.86	4.63
19	1.6	30	50	1.38	3.79
20	5	15	60	2.12	4.95

Analysis of variance (ANOVA) provides the significance of each factor and of the interactions between factors. Based on ANOVA's analysis of the CCD results, the model's linear coefficient extraction temperature (X_3) and the quadratic term coefficient of extraction temperature (X_3^2) had a significant impact on the extraction of the VOCs investigated from the fragrances mixture by SPME ($p < 0.05$) (Table 4-3). Thus, the equation of the model can be rewritten to: $y_= -13.91 + 0.662X_3 - 0.00612X_3^2$, where y= summed normalised areas of VOCs.

The value of the R^2 indicates the percentage of the total variations in the experimentation that is not explained by the model. A R^2 of 70.08% was obtained. The lack-of-fit test is not significant (p-value = 0.784), where generally a p-value lower than 0.05 shows that the model does not accurately fit the data.

Table 4-3 Analysis of variance (ANOVA) of the central composite design experiment for the extraction of VOCs by SPME from fragrance mixture. The results indicate that the X_3 and X_3^2 terms are the only significant factor affecting the extraction of the analytes

Term	Coefficient	Degree of Freedom	Sum of Squares	F-value	p-value
Model		9	13.753	2.6	0.076
Intercept	-13.91	1			
X_1	0.126	1	2.375	4.05	0.072
X_2	-0.013	1	0.396	0.68	0.430
X_3	0.662	1	5.388	9.18	0.013*
X_1^2	-0.00419	1	0.158	0.27	0.615
X_2^2	0.00016	1	0.019	0.03	0.862
X_3^2	-0.00612	1	5.393	9.19	0.013*
X_1X_2	0.00033	1	0.005	0.01	0.928
X_1X_3	0.00062	1	0.008	0.01	0.912
X_2X_3	0.0023	1	0.009	0.02	0.901
Residual		10	5.872		
Lack of fit		5	1.889	0.47	0.784
Pure error		5	3.983		
Total		19	19.625		
R^2	0.7008				
Adj R^2	0.4315				

X_1: incubation time (min); X_2: extraction time (min); X_3: extraction temperature (˚C);

* indicate a significant difference at the 0.05 probability level

The contour plots (Figure 4-3) are bi-dimensional representations of each two factors studied at a time. If the interaction between the factors is significant, then an elliptical contour plot is obtained, where the maximum predicted value is the smallest eclipse in the diagram. In the current study, only the contour plot of extraction temperature and incubation time at a constant extraction time of 30 min (Figure 4-3 top right) has a partial ellipse shape.

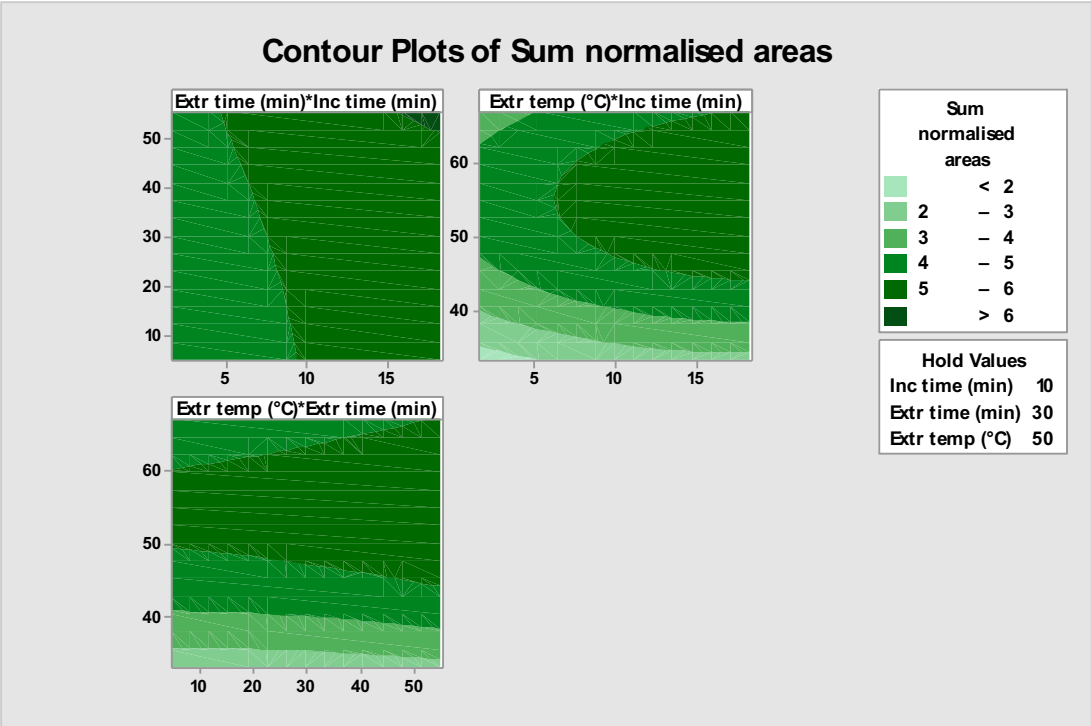

Figure 4-3 Contour plots showing the interaction effect between pair of variables: Extraction time and Incubation time (top left), Extraction temperature and Incubation time (top right), and Extraction temperature and Extraction time (bottom) for the extraction of VOCs from a fragrance mixture

Figure 4-4 represents the three-dimensional representation surface plots with the response (normalised peak area) on the Z-axis against two independent variables at a time. It can be observed how with an increase in extraction temperature there is an increase in the peak areas of the target analytes (Figure 4-4 top right and bottom).

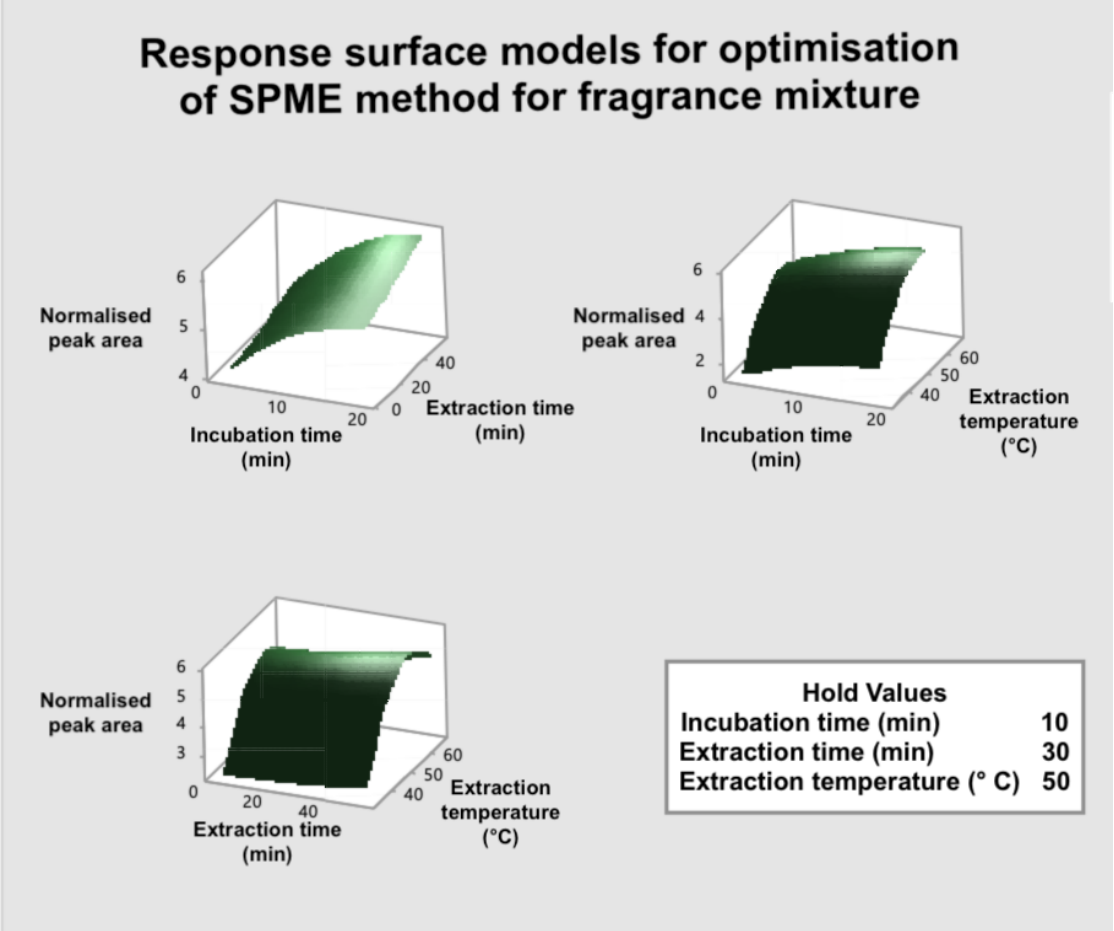

Figure 4-4 3D response surface model for total area of volatile compound for pair of variables: Extraction time and Incubation time (top left), Extraction temperature and Incubation time (top right), and Extraction temperature and Extraction time (bottom) for the extraction of VOCs from a fragrance mixture

The experimental study concluded that the optimal conditions predicted for the SPME extraction of all fragrance VOCs investigated were 18.4 min of incubation and 55.2 min of extraction time at 56 °C, where the extraction temperature was the only significant factor ($p = 0.013$).

4.2.3. Optimisation of the SPME extraction of fragrance solutions from garments

Having optimised the SPME extraction parameters for analysis of fragrance solutions, the next step was to ensure optimal relative recoveries of VOCs when the perfume was extracted from garments. Using the optimised extraction variables, a sample of the FM solution and samples of the FM spiked on various garments were analysed. The peak

area results for each VOC in the FM are plotted individually in Figure 4-5 due to the scale variation. It should be noted that the dimethyl brassylate compound was not observed in the analysis of fragrances from clothing, but solely in the previous experiment on the analysis of fragrances from solution.

Especially for high volatility compounds, polyester and acrylic provided a similar VOCs profile compared to the one in the fragrance mix itself, showing only a limited matrix interaction (Figure 4-5). On the other hand, for cotton, the high volatility compounds such as α-pinene and limonene were largely extracted, whilst a decrease (of linalool, geraniol) or an absence (of eugenol, coumarin, ethylene brassylate) in the extraction of lower volatility compounds was observed. These results were consistent with those obtained for a second different 100% cotton material. It was generally observed that the extraction of the polar VOCs was highly affected by the cotton swatches compared to the polyester and acrylic swatches.

A further experiment was designed where 10 mL of water were added to a vial containing a cotton swatch spiked with the FM and a SPME measurement made (Figure 4-5 last bar). Moreover, the addition of water enabled the extraction of eugenol and ethylene brassylate, compounds that were not previously extracted. This is probably due to the ability of water to draw out chemicals, especially polar compounds, from cotton. It should be noted that coumarin was not recovered from any of the cotton samples or from the polyester sample.

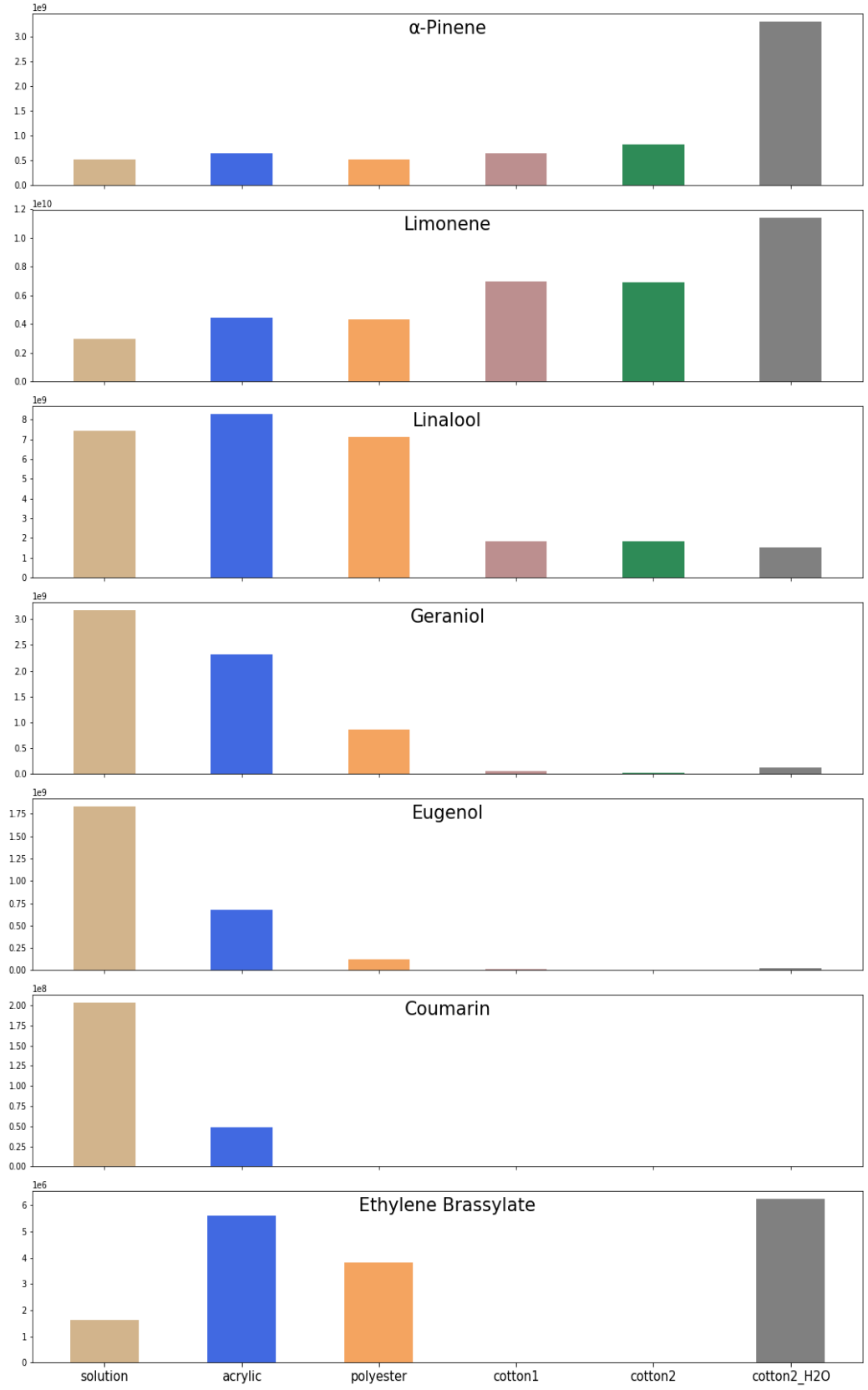

Figure 4-5 Extraction (chromatographic peak areas) of the seven individual VOCs from a fragrance solution (solution) and from a fragrance solution recovered from acrylic, polyester, cotton #1, cotton #2, and lastly from cotton #2 to which 10 mL of water were added (cotton2_H2O)

Knowing that the addition of water improves the extraction of the fragrance VOCs from fabric (see Figure 4-5), a further CCD was designed. The incubation time varied from 5 to 30 min, the extraction time from 10 to 60 min, and the extraction temperature from 40 to 70 °C. The levels of the variables studied and the experimental responses are given in Table 4-4. Once again, the peak area of each analyte was normalised and the sum of the normalised peak areas was used as the CCD response.

Table 4-4 The sample number, levels of the three factors investigated, and the instrumental responses obtained from a 2^3 face centred cube central composite design (CCD) for the extraction of VOCs by SPME from cotton

Run Order	Incubation time (min)	Extraction time (min)	Extraction temp (° C)	Summed normalised areas of the VOCs
1	17.5	35	55	3.02
2	25	20	64	2.88
3	25	20	46	1.95
4	10	50	46	3.29
5	10	50	64	4.00
6	17.5	60.2	55	4.69
7	17.5	35	55	3.26
8	30.1	35	55	3.40
9	17.5	35	55	3.20
10	17.5	35	39.9	3.03
11	17.5	35	55.0	3.26
12	25	50	64	3.13
13	10	20	64	2.09
14	10	20	46	1.54
15	17.5	35	70.1	2.64

16	17.5	35	55	2.44
17	17.5	9.8	55	1.37
18	17.5	35	55	2.62
19	25	50	46	2.36
20	4.9	35	55	2.23

Based on ANOVA's analysis of this second CCD experiment (Table 4-5), the model's linear coefficient extraction time (X_2) was the only coefficient that had a significant impact on the extraction of the targeted VOCs from cotton by SPME ($p < 0.05$). Thus, the model equation can be rewritten to: $y = -5.6 + 0.1156X_2$, where y= summed normalised areas of VOCs.

Table 4-5 Analysis of variance (ANOVA) of the central composite design experiment for the extraction of VOCs by SPME from a fragrance mixture applied on a cotton swatch.

Term	**Coefficient**	**Degree of Freedom**	**Sum of Squares**	**F-value**	**p-value**
Model		9	9.112	3.55	0.031
Intercept	-5.6	1			
X_1	0.149	1	0.136	0.48	0.506
X_2	0.1156	1	7.169	25.13	0.001*
X_3	0.137	1	0.386	1.35	0.272
X_1^2	-0.00182	1	0.121	0.54	0.484
X_2^2	-0.000126	1	0.005	0.04	0.844
X_3^2	-0.00121	1	0.138	0.48	0.503
X_1X_2	-0.00335	1	1.133	3.95	0.074

X_1X_3	0.0082	1	0.024	0.09	0.776
X_2X_3	0	1	0	0	0.999
Residual		10	2.852		
Lack of fit		5	2.222	3.53	0.096
Pure error		5	0.630		
Total		19	11.964		
R2	0.762				
Adj R2	0.547				

X_1: incubation time (min); X_2: extraction time (min); X_3: extraction temperature (˚C);

* indicate a significant difference at the 0.05 probability level

The contour plots (Figure 4-6) indicate that none of the three interactions are significant. The 3D response surface models (Figure 4-7) show an increase in the extraction of the analytes with an increase in the extraction time. The optimal values for the SPME extraction of the six VOCs from cotton were an incubation time of 5 min, an extraction time of 60 min, and an extraction temperature of 58 ˚C, where the extraction time was the only significant factor ($p = 0.001$).

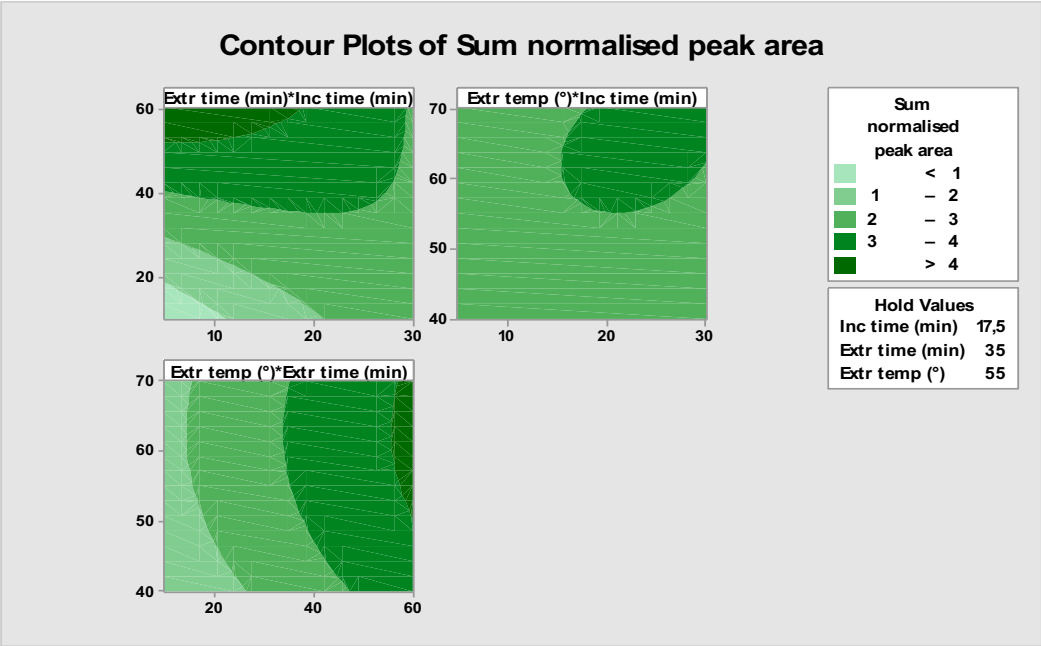

Figure 4-6 Contour plots showing the interaction effect between pair of variables: Extraction time and Incubation time (top left), Extraction temperature and Incubation time (top right), and Extraction temperature and Extraction time (bottom) for the extraction of VOCs from a fragrance mixture applied to a cotton swatch

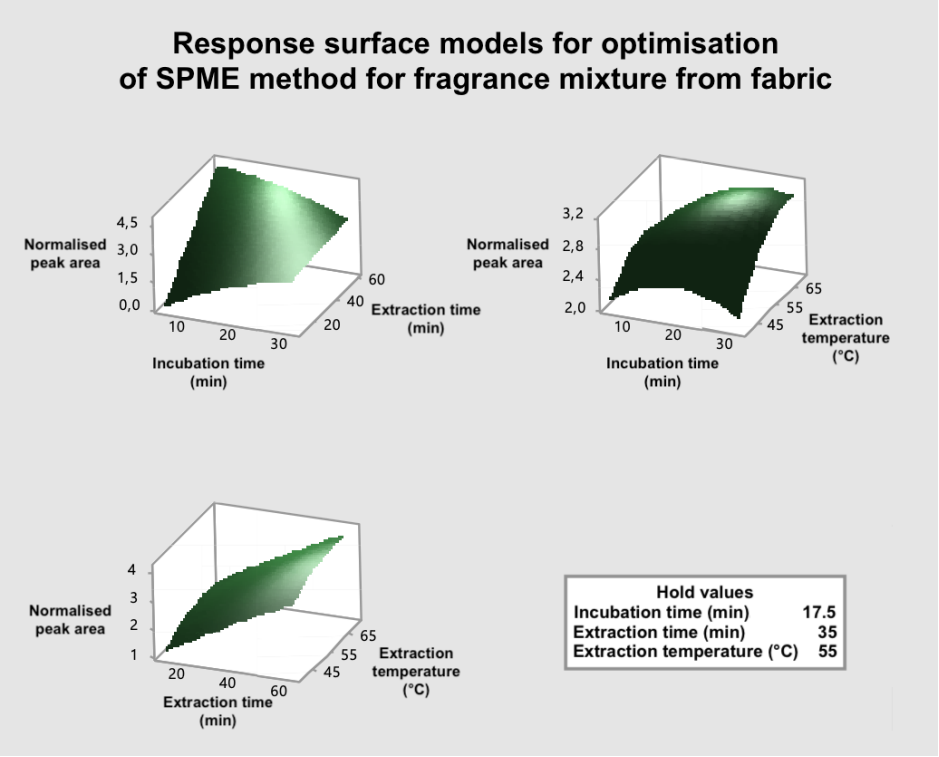

Figure 4-7 3D response surface model for total area of volatile compound for pair of variables: Extraction time and Incubation time (top left), Extraction temperature and Incubation time (top right), and Extraction temperature and Extraction time (bottom) for the extraction of VOCs from a fragrance mixture applied to a cotton swatch

Once the extraction variables were optimised, two additional trials were carried out. The first trial involved the addition of organic modifier to the water solvent, and the second trial involved an investigation in the desorption parameters, namely desorption temperature and the splitless desorption time.

In the first trial, cotton swatches spiked with 10 µL of 50 µM FM were analysed in 10 mL of either water on its own, water with 2.5% or 5% of acetone or methanol (Figure 4-8). Overall, the addition of acetone to water decreased the extraction of the VOCs. The addition of methanol at 5% lead to slightly lower or similar extraction rates compared to water on its own, therefore further SPME experiments were continued by adding 10 mL of water to the vial containing the fragranced sample.

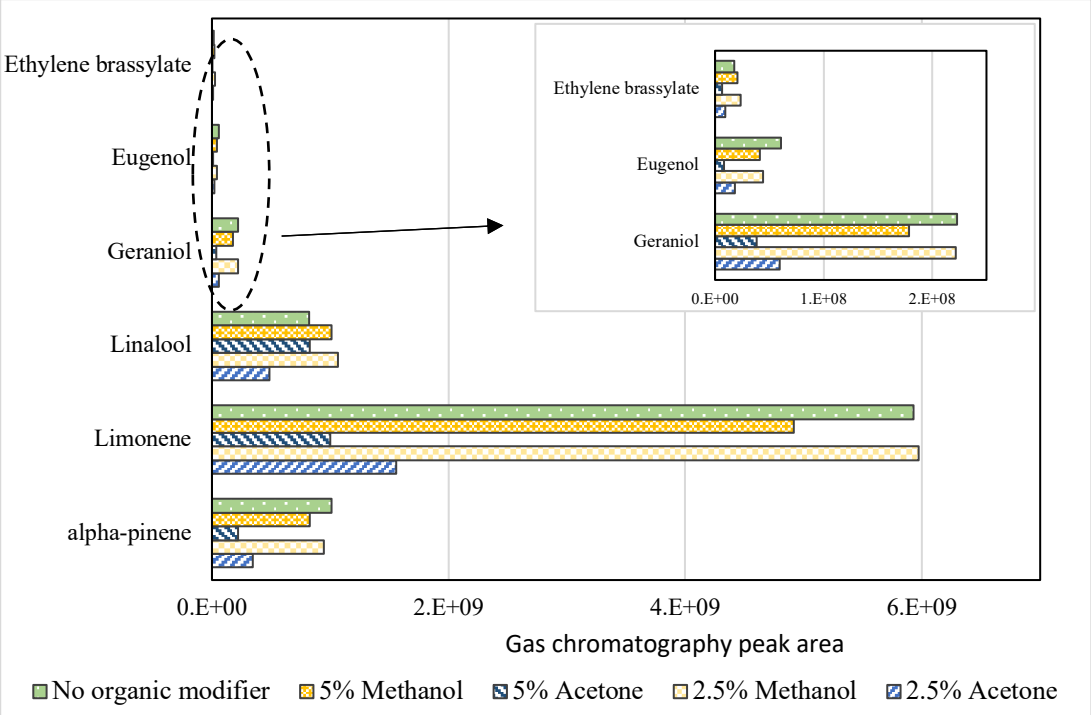

Figure 4-8 The effect of adding acetone or methanol to the water solvent for the extraction of VOCs from cotton. No major improvements were observed when organic modifiers were added to water, so water on its own was used as the SPME solvent

In the second trial, desorption temperature and splitless time, i.e. the time when the analytes are desorbed from the SPME fibre to the injector, were investigated. When increasing the desorption time from 250 to 270 °C using a constant desorption time of 0.5 min, a decrease in the recovery of most compounds was observed (Figure 4-9). When

increasing the splitless time from 0.5 to 1.5, and to 3 min, an increase in the extraction rates for most compounds was observed. Therefore, further studies were conducted using a splitless time of 3 min and an injector temperature of 250 °C.

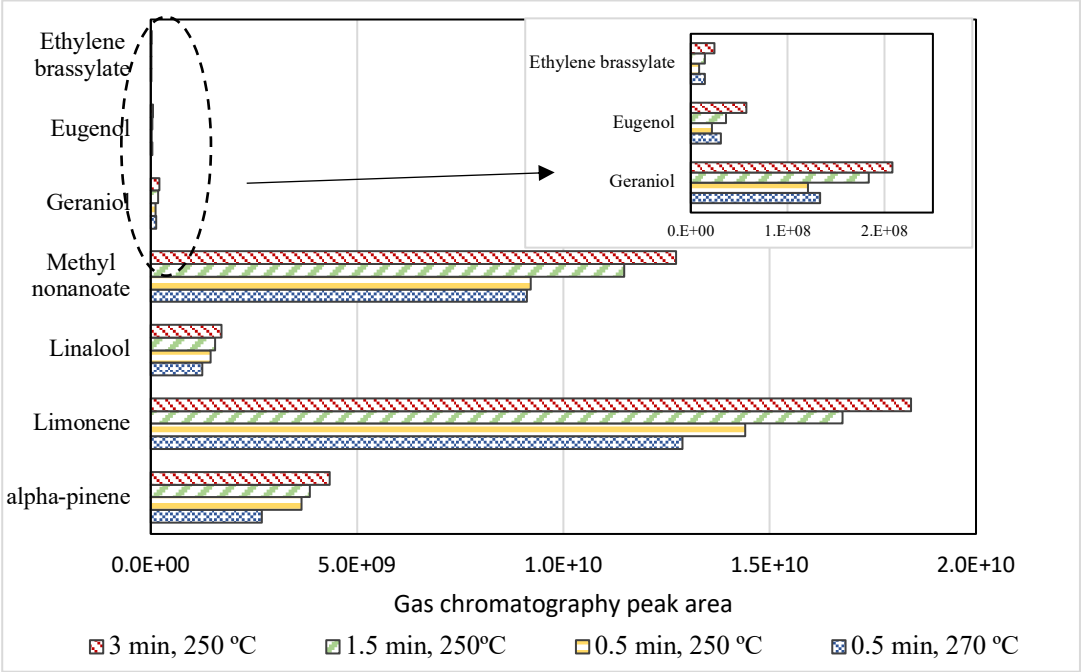

Figure 4-9 The effect of desorption splitless time and desorption temperature on the recovery of VOCs from cotton. The conditions selected were 3 min splitless time at 250 ˚C

4.2.4. Validation of the SPME extraction of fragrance solutions from garments

The optimised SPME extraction method developed for the extraction of VOCs from fabric swatches was validated for two different fabrics, cotton and polyester. To ensure adequate quantification of the analytes of interest, an evaluation of the linearity, working ranges, LOD and LOQ, relative recoveries (intraday and interday), repeatability, reproducibility, and sensitivity of the method was carried out.

Due to the diversity in the chemical structure of the VOCs analysed, two different internal standards were added at a concentration of 20 µM to all calibration standards and spiked samples. Within the concentration range investigated, coumarin showed a

non-linear response, for both fabrics studied. At this point, coumarin was eliminated from the study because of the low affinity of SPME fibre and experimental conditions tested.

The validation results for the extraction of VOCs in cotton and polyester are displayed in Table 4-6. With the exception of α-pinene and limonene which showed linearity in cotton in the range of concentrations tested, for the rest of the analytes two different concentration ranges were required. Although it was possible to fit the calibration curves to a polynomial equation, it was more desirable to split the calibration range into two different sections, as fragrance traces are expected to be found at very low concentrations when they have transferred onto a secondary piece of fabric.

Linearity was evaluated based on coefficients of determination (R^2). Most analytes provided a R^2 value above 0.99 and a number of analytes provided a R^2 between 0.98 and 0.99 (see Table 4-6). The LOD varied between 0.44 and 45 nM for cotton, and between 0.06 and 7 nM for polyester. The LOQ varied between 1.47 and 151 nM for cotton, and between 0.21 and 20 nM for polyester. Big differences for LOD and LOQ were observed for ethylene brassylate between cotton and polyester, so that in cotton the LOD was approximately 6 times higher and the LOQ approximately 11 times higher than in polyester. Generally, the sensitivity, in terms of slope, varied from 0.0005 to 0.017 Cps/μM for cotton, and from 0.002 to 0.064 Cps/μM for polyester, where Cps is the peak area expressed as counts per second.

The intraday relative recovery values varied from 85 to 114% at a concentration of 2.5 μM, and between 89 and 102% at a concentration of 25 μM. The interday relative recoveries ranged from 83 to 123% at a concentration of 2.5 μM, and between 75 and 133% at a concentration of 25 μM.

All repeatability values (evaluated as intraday precision) were below 15% at 2.5 μM, and below 9% at 25 μM. Good reproducibility values (evaluated as interday precision) were obtained for most compounds, with no RSD value higher than 21%, with the exception of α-pinene at 25 μM in polyester (RSD = 26%).

Table 4-6 Calibration curves and validation parameters of the validated SPME GC-MS method in cotton and polyester sample

Compound	m/z [a]	RT [b]	Matrix	R^2 [c]	Calibration curve	Wr [d]	LOD [e]	LOQ [f]	Intraday relative recovery % [g]		Interday relative recovery % [h]	
									2.5 µM	25 µM	2.5 µM	25 µM
α-Pinene	77.1, 91.1, 93.1	9.48	Cotton	0.995	Y=0.00396*X+0.00004	0.5-50	0.95	3.16	105 (6)	97 (5)	123 (12)	105 (7)
			Polyester	0.990	Y=0.01259*X-0.00086	0.5-2.5	2.07	6.90	100 (7)	102 (4)	113 (12)	133 (26)
				0.989	Y=0.00753*X+0.01143	2.5-50						
Limonene	67.1, 68.2, 93.1	12.47	Cotton	0.997	Y=0.01748*X+0.01214	0.5-50	0.44	1.47	103 (3)	94 (5)	117 (11)	96 (7)
			Polyester	0.992	Y=0.06395*X+0.16199	0.5-10	0.06	0.21	101 (7)	91 (1)	115 (21)	95 (12)
				0.981	Y=0.02803*X+0.48115	10-50						
Linalool	55.2, 71.1, 93.1	14.63	Cotton	0.998	Y=0.00259*X+0.00063	0.5-10	3.42	11.39	95 (8)	89 (2)	118 (20)	91 (8)
				0.995	Y=0.00191*X+0.00222	10-100						
			Polyester	0.997	Y=0.00422*X-0.00085	0.5-10	2.77	9.24	88 (7)	94 (3)	100 (13)	92 (5)
				0.993	Y=0.00331*X-0.00292	10-100						
Geraniol	41.2, 67.1, 69.1	19.04	Cotton	0.994	Y=0.00320*X-0.00112	0.5-10	12.10	40.32	90 (8)	90 (3)	95 (9)	90 (3)
				0.987	Y=0.00290*X-0.01590	10-100						

			Polyester	0.998	Y=0.00402*X-0.00022	0.5-10	6.28	20.92	92 (4)	92 (2)	93 (10)	91 (12)
				0.985	Y=0.00225*X+0.01285	10-50						
Eugenol	77.1, 103.1, 164.1	21.87	Cotton	0.991	Y=0.00108*X+0.00018	0.75-10	1.68	5.61	88 (12)	90 (1)	111 (21)	91 (7)
				0.990	Y=0.00096*X-0.00419	10-100						
			Polyester	0.987	Y=0.00205*X+0.00187	0.5-10	1.96	6.53	114 (1)	101 (5)	112 (11)	86 (17)
				0.997	Y=0.00177*X+0.00417	10-50						
Ethylene brassylate	55.1, 86.1, 98.1	34.10	Cotton	0.992	Y=0.00088*X-0.00018	0.5-10	45.45	151.52	85 (8)	94 (1)	93 (4)	75 (21)
				0.982	Y=0.00055*X-0.00012	10-100						
			Polyester	0.991	Y=0.00209*X-0.00068	0.75-10	7.11	13.70	98 (15)	99 (9)	83 (12)	82 (16)
				0.982	Y=0.00197*X-0.00813	10-100						

a Quantification ions

b Retention time (min)

c Regression coefficient

d Working range (μM)

e Limit of detection (nM)

f Limit of quantification (nM)

g Repeatability expressed as RSD is given in brackets (n = 3)

h Reproducibility expressed as RSD is given in brackets (n = 4)

4.2.5. Analysis of commercial perfumes from garments using the validated SPME method

Four different commercial perfumes, one marketed for women and three for men, were analysed using the optimised and validated method in both cotton and polyester. As the method was validated for determination of perfume traces, the samples were diluted between 500 and 1500 times in methanol prior to spiking. Additionally, blank samples of cotton and polyester were tested.

Figure 4-10 shows the chromatograms of the cotton blank sample and of a 500 times diluted men's perfume recovered from cotton by SPME. Various chromatographic peaks originating from the cotton, such as those detected at 14.8, 17.8, 24.9, 25.7 and 27.6 min RT (numbered from 1 to 5 in Figure 4-10 top) were observed in all spiked cotton samples. However, their retention times do not interfere with those of the analytes of interest, highlighted in the chromatogram of the diluted men's perfume (Figure 4-10 bottom). These cotton originating analytes were tentatively identified by high resolution mass spectrometry (Q-Exactive Orbitrap) as nonanal, decanal, 1-undecanol, 2,4-di-tert-butyl-phenol, and 2,2,4-trimethyl-1,3-pentanediol diisobutyrate.

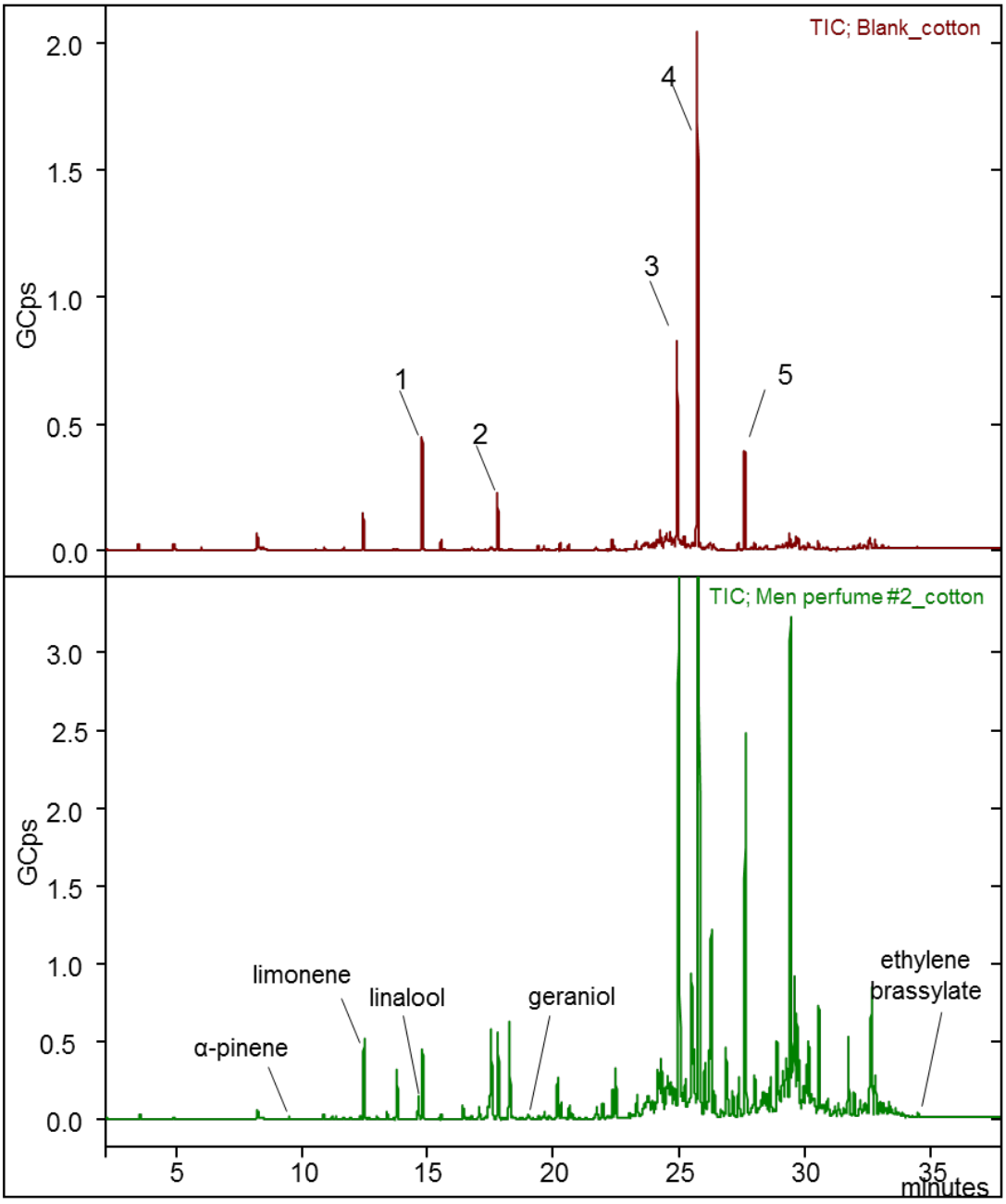

Figure 4-10 Top: gas chromatogram of a blank cotton sample, where the peaks of some cotton originating compounds were tentatively indentified as: (1) nonanal; (2) decanal; (3) 1-undecanol; (4) 2,4-di-tert-butyl-phenol; (5) 2,2,4-trimethyl-1,3-pentanediol diisobutyrate. Bottom: gas chromatogram of a cotton sample spiked with men's perfume #2 diluted 500 times, where the peaks of the target compounds found in the perfume are highlighted

Moreover, Figure 4-11 with a narrow RT window from 11.1 to 11.5 min, illustrates how certain VOCs were found in all four fragranced samples (11.2 min RT), albeit at various concentrations, whilst some VOCs (11.37 min RT) are present in only certain perfumes.

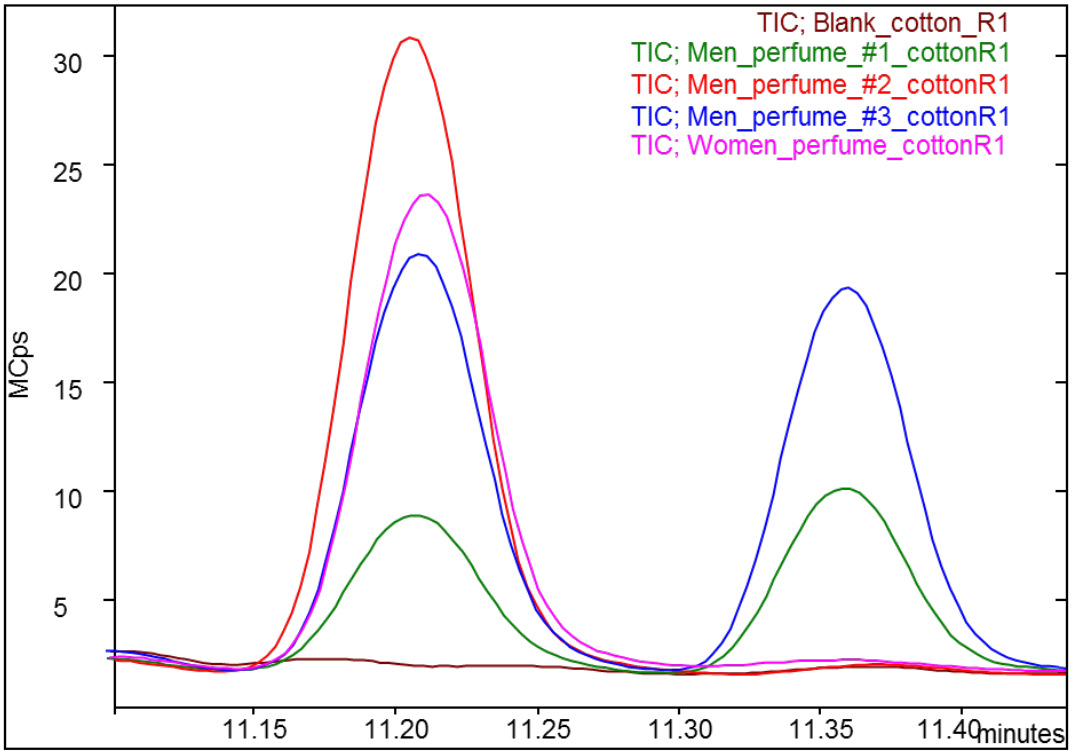

Figure 4-11 Gas chromatograms (RT 11.1-15.min) of a blank cotton sample (brown), of three cotton samples spiked with men's perfume (red, blue, and green), and of a cotton sample spiked with women's perfume (pink)

Similarly, Figure 4-12 represents the chromatograms of the polyester blank sample and of the diluted perfumes recovered from polyester. As observed with cotton, some of the compounds present in the perfume traces samples were originating from the fabric itself.

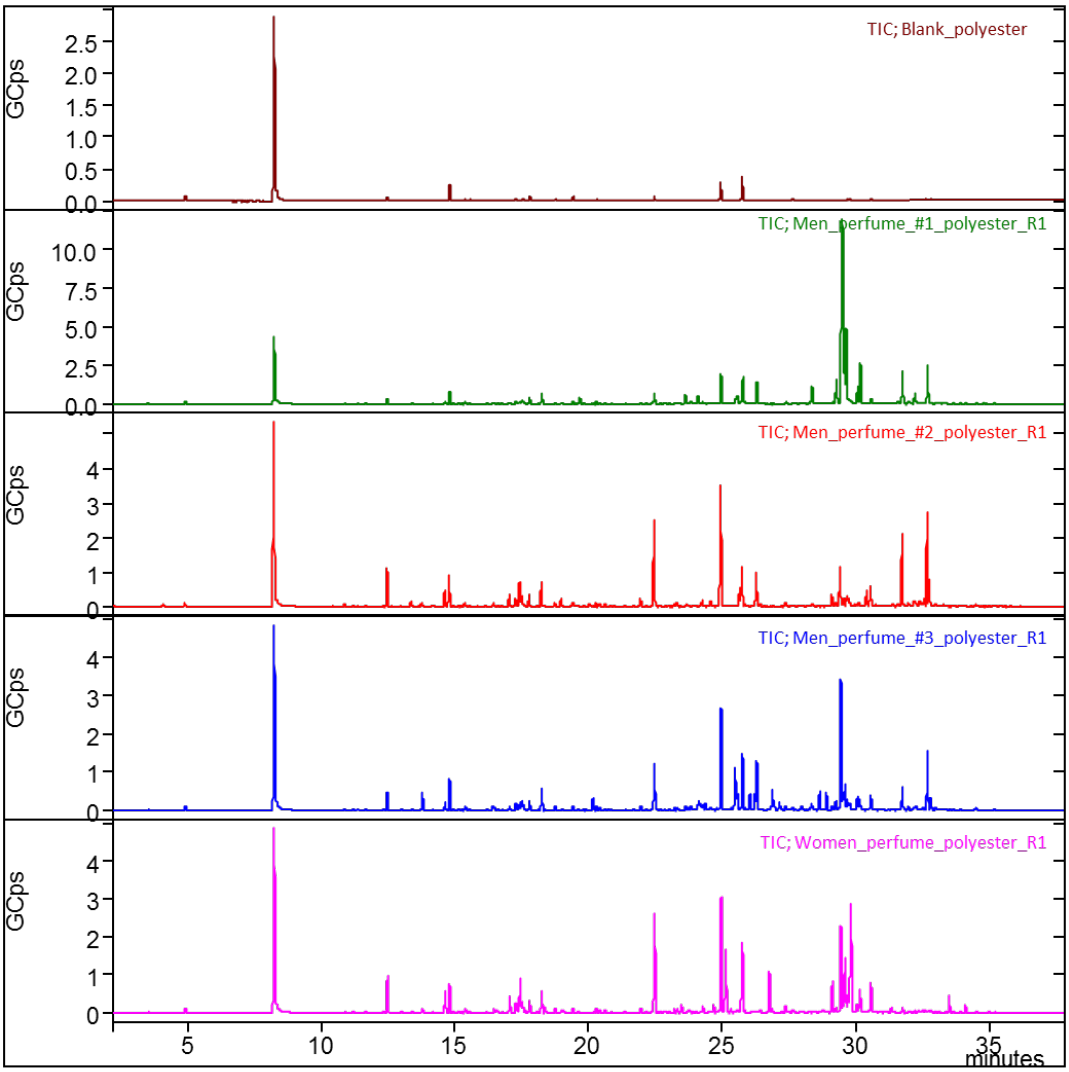

Figure 4-12 Gas chromatograms of a polyester sample spiked with no perfume (top), three polyester samples spiked with different men's perfume (middle), and a polyester sample spiked with women's perfume (bottom)

Using the calibration curves constructed during validation, the concentration of each analyte in both cotton and polyester, when available, was determined in the diluted perfumes analysed (n = 2) (Table 4-7). All RSD values were below 22%. Eugenol was not found in the four perfumes analysed.

Table 4-7 Found concentration of target VOCs in the headspace of fabric samples spiked with commercial available perfumes after appropriate dilution

Compound	Matrix	Found concentration (mM) (n = 2)[a]			
		Men perfume #1	Men perfume #2	Men's perfume #3	Women's perfume
α-pinene	Cotton	0.47 (18)	1.10 (2)	1.43 (16)	1.97 (7)
	Polyester	0.34 (8)	0.81 (3)	0.83 (12)	1.55 (11)
Limonene	Cotton	11.47 (20)	45.30 (1)	22.02 (10)	35.55 (8)
	Polyester	8.00 (13)	52.59 (6)	19.88 (7)	45.03 (9)
Linalool	Cotton	21.98 (6)	75.13 (7)	40.57 (2)	119.88 (3)
	Polyester	17.48 (5)	68.18 (2)	34.15 (2)	98.84 (1)
Geraniol	Cotton	0.71 (7)	7.52 (3)	5.44 (5)	16.43 (3)
	Polyester	0.59 (19)	5.10 (1)	2.36 (5)	19.24 (3)
Eugenol	Cotton	ND[b]	ND	ND	ND
	Polyester	ND	ND	ND	ND
Ethylene brassylate	Cotton	20.65 (6)	4.76 (9)	1.94 (20)	84.21 (22)
	Polyester	16.74 (1)	4.85 (10)	0.42 (9)	97.55 (20)

[a] RSD is given in brackets

[b] Not detected

4.2.6. Analysis of commercial perfume from garments using liquid extraction

Following the sampling procedure developed by Gherghel et al. (2016), one of the perfumes marketed for men was additionally analysed using liquid extraction. In a similar fashion to the HS-SPME analyses of real perfumes, the sample was diluted 500 times prior to extraction. The resulting chromatogram of the cotton swatch spiked with

the diluted men's perfume and extracted using methanol (Figure 4-13a) reveals very poor sensitivity of the liquid extraction method. A further test with this perfume diluted 100 times showed no major improvements (Figure 4-13b). Next, the perfume was spiked undiluted and the results obtained (Figure 4-13c) were comparable to the SPME extraction where the perfume was diluted 500 times (Figure 4-13d).

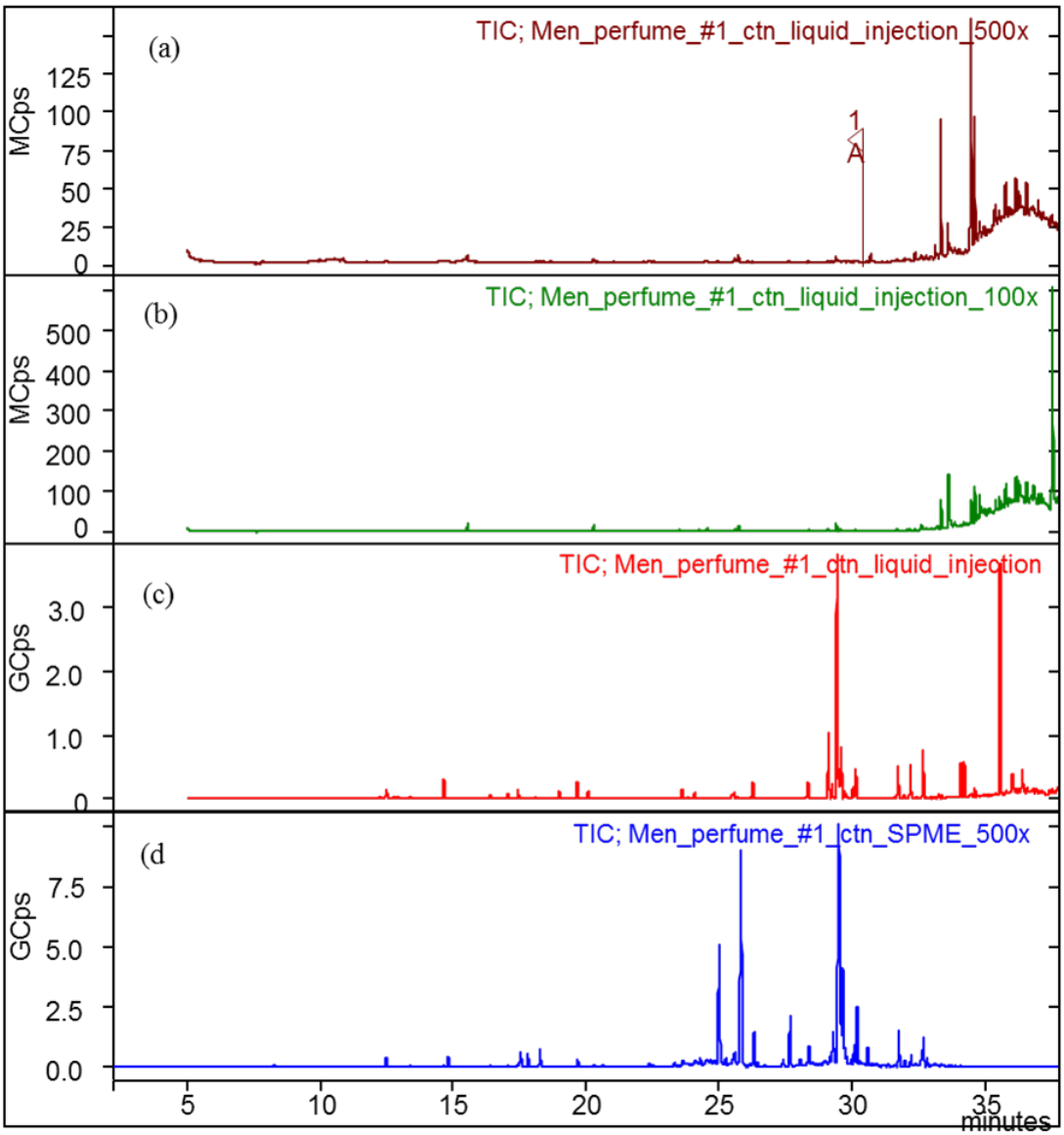

Figure 4-13 Gas chromatograms of a men's perfume recovered from cotton using liquid extraction and a dilution factor of 500 (a), 100 (b), 1 (c), and using SPME and a dilution factor of 500 (d)

A number of the validated fragrance VOCs, such as α-pinene, limonene and linalool, were recovered from this perfume using the liquid extraction method. Even with a 500-dilution factor, SPME showed greater affinity towards limonene (Figure 4-14a). For α-pinene (Figure 4-14b) and linalool (Figure 4-14c) greater amounts were recovered using liquid extraction only when the perfume was undiluted.

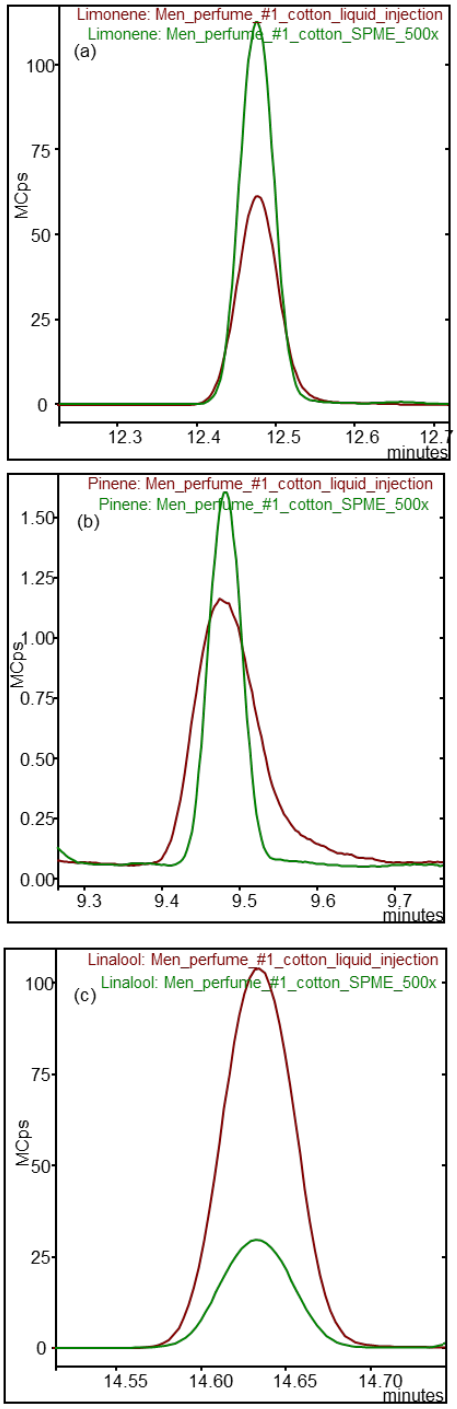

Figure 4-14 Gas chromatograms showing the recovery of: a) limonene, b) α-pinene, and c) linalool from an undiluted men's perfume recovered from cotton using liquid extraction (red), and of the same men's perfume diluted 500 times and recovered from cotton using SPME

On the other hand, whilst coumarin was not recovered from cotton using SPME, by liquid extraction it was successfully identified, albeit at a very low signal (Figure 4-15a). The mass spectrum confirming the coumarin EI fragmentation pattern is shown in Figure 4-15b.

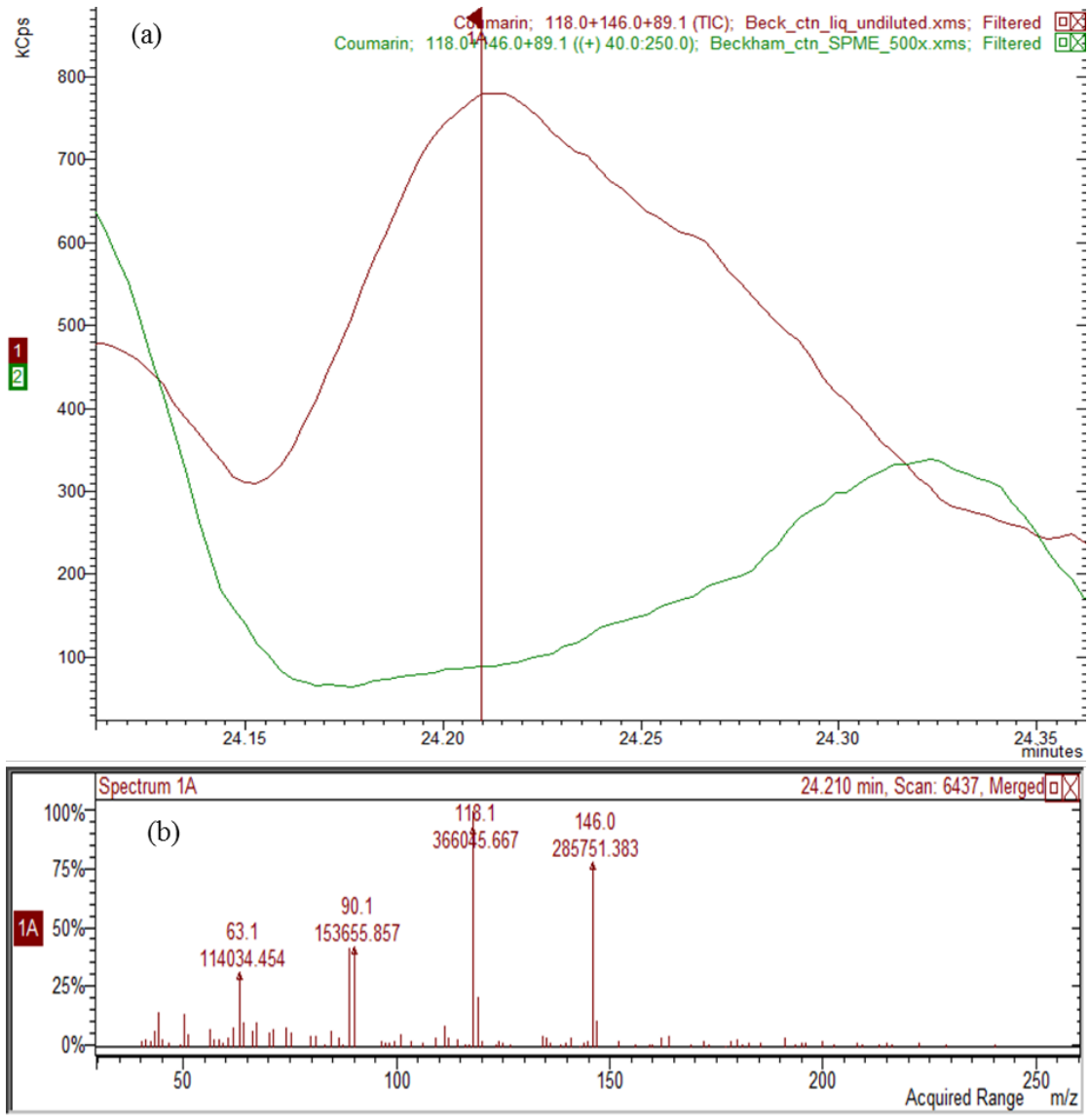

Figure 4-15 a) Gas chromatogram (RT window 24.1-24.4) showing the presence of coumarin in the liquid extract of a cotton sample spiked with male perfume (red) and the lack in the SPME extract (green), b) EI mass spectrum at 24.2 min RT corresponding to coumarin

4.3. Discussion

4.3.1. Selection of the SPME fibre

There are various factors known to affect the extraction of analytes by SPME fibres. One of the most important factors is the coating material on the fibre itself. Successful application of SPME analysis relies on the selection of a suitable fibre type for particular analytes. The five SPME fibres investigated in this study cover a wide range of analyte chemistries.

PDMS is the most typically employed coating phase in analytical applications. PDMS and PA coatings extract analytes via absorbtion, so that the compounds are dissolved and diffused into the phase, basically migrating in and out of the coating without any competition effects taking place. On the other hand, the mixed coatings such as PDMS/DVB, CAR/PDMS, and DVB/CAR/PDMS extract analytes via adsorption, and thus they physically interact with the analytes. Divinylbenzene particles are considerably larger than the carboxen particles, ideal for C6-C15 analytes, while carboxen particles are ideal for C2-C12 range (Pawliszyn 1999).

It is important that the properties of the coating materials, such as porosity and size of the particles, film thickness, and phase polarity are matched to those of the analytes. With a focus on forensic applications, Weyermann et al. (2009) obtained better results for the extraction of organic volatiles from spent cartridges with PA fibre compared to 75 µm CAR/PDMS and 100 µm PDMS, and in a later study by Dalby and Birkett (2010) involving seven fibres it was shown that 65 µm DVB/CAR had the highest extraction rates. Curran et al. (2005) determined that the 50/30 µm DVB/CAR/PDMS was the optimal fibre for the collection of human odour samples, and so this fibre has become in the last couple of years the main SPME fibre used for analysis of human scent for forensic applications (Dormont et al. 2013; Brown et al. 2013; Kusano et al. 2013). For cosmetics formulations, Ortiz and Tena (2006) determined 75 µm CAR/PDMS fibre to extract more VOCs, being more effective than 100 µm PDMS.

To overcome a general lack of literature and lack of agreement between those studies that exist, this study aimed to compare five different SPME fibres for the analysis of five

VOCs specific to cosmetic industry, but also for the analysis of a commercial perfume. In terms of both recovery and repeatability, the DVB/CAR/PDMS, which covers both polarity ranges, performed the best overall for the fragrance mixture and for the commercial perfume. The repeatability of the DVB/CAR/PDMS was the highest amongst all fibres tested for the extraction of the seven VOCs of interest with an average RSD of 4.6%, followed by the PDMS/DVB fibre with an average RSD of 12.2%. The PDMS/DVB was also the second-best fibre for amount of the five VOCs extracted. Thus, the second-best results were obtained with a similar fibre coating containing two of three coating phases of the best fibre, the DVB/CAR/PDMS. This confirms that the three-phase fibre is the most suitable for extracting the selected VOCs in this study. It is important to note that the DVB/CAR/PDMS fibre is also the most commonly used fibre in forensic literature for analysis of human scent (Dormont et al. 2013; Brown et al. 2013; Kusano et al. 2013).

4.3.2. Optimisation of the SPME extraction of fragrance solutions

Besides coating material, the amount of analytes extracted by a SPME fibre can also be influenced by extraction temperature and time, incubation time, desorption time, etc. Incubation time, extraction time, and temperature were chosen as the factors to be tested in a central composite design. The values for the three parameters were based on literature reviews for the VOCs from various matrices, such as alcoholic drinks and food samples (Januszkiewicz et al., 2008; Moreira et al., 2013).

When the SPME methodology was applied to the analysis of fragrance solutions, the only significant variable determined by the first CCD was extraction temperature. With an increase in extraction temperature to the maximum value studied of 56 °C, more of the VOCs from fabrics were extracted. The incubation time was determined not be significant, and therefore a short incubation time of 5 min was selected for further analyses. As incubation time resulted not to be a significant factor, there is an indication that headspace equilibrium happens in a very short period of time promoted by the high volatility of the studied compounds.

4.3.3. Optimisation of the SPME extraction of fragrance solutions from garments

A preliminary study showed that when the fragrance mixture VOCs were extracted from cotton rather than from the solution itself, the recovery of polar compounds was lower. This could be explained by the formation of strong hydrogen bonds with the cellulose backbone of cotton. As a result, a second CCD was designed for the VOCs extraction from a cotton swatch.

The results from the CCD using cotton determined the extraction time to be the only significant factor that influenced the extraction of the VOCs of interest, so that the maximum recoveries were obtained when an extraction time of 60 min was used. Despite the method being automated, with a chromatographic run of 38 min, an incubation time of 5 min, and a maximum tested extraction time of 60 min, each sample was taking just under 2 h. Therefore, a further increase in the extraction time was considered not a viable option in terms of time and analytical output given the constraints of forensic analysis in casework.

Whilst the CCD results for the extraction of VOCs from fragrance solutions showed that extraction temperature was a significant factor, the CCD results for the extraction of VOCs from fragrance solution from cotton swatches showed that the extraction time was the only significant factor.

With the optimal values determined for the extraction of VOCs from cotton, two further studies were carried out to optimise the extraction solvent and the desorption conditions. No improvements were observed when two different organic modifiers, acetone and methanol, were added to the water solvent or when the injector temperature was increased from 250 to 270 °C. A longer splitless time from 0.5 to 3 min in the injector allowed higher recoveries of the VOCs of interest.

4.3.4. Validation of the SPME extraction of fragrance solutions from garments

The validation was carried out with cotton and polyester fabrics. These natural and synthetic fibres are popular in the fashion industry, and therefore, in forensic science

research (Roux, Chable and Margot, 1996; Dachs, McNaught and Robertson, 2003; Bull et al., 2006). For example, cotton is one of the most popular fabric materials available commercially; so that in the US in 2010, 64% of male clothing was made of 100% cotton, while 68% of female clothing contained cotton (Cotton Incorporated, 2010). Polyester is by far the most popular synthetic fibre with a reported 82% share of the synthetic fibre market in 2014 (Krifa and Stewart Stevens, 2016). Moreover, whilst the global cotton consumption went from 38% of the global fibre mill consumption in 2000 to 27% in 2015, the polyester consumption grew from 37 to 55% in the same period (Carmichael, 2016).

For successful analyte quantification, proper calibration needs to be carried out. As the analytes studied varied in their chemical structure, two internal standards with different chemical structures were used. Methyl nonanoate was employed for the quantification of the two linear compounds, linalool and geraniol, whilst 1,4-dibromobenzene was employed for all cyclic compounds, including α-pinene, limonene, eugenol, and ethylene brassylate.

Given that this analytical method is intended for the extraction of traces of VOCs from fabrics originating from sexual assault cases, a focus on the extraction of lower concentration was given. As a result, for most analytes it was necessary to split the working range in two ranges rather than fitting a polynomial equation in order to determine more accurately the concentration of lower concentration analytes. Good linearity, with a R^2 value above 0.98 was observed for most analytes in both fabrics. Generally, polyester produced lower LOD and LOQ than cotton, especially for ethylene brassylate where the LOD and LOQ were approximately 6 and 11 times, respectively, lower. Additionally, the sensitivity of ethylene brassylate in polyester (slope for lower range = 0.0021 Cps/μM) was more than two times higher than in cotton (slope for lower range = 0.0009 Cps/μM). This could be caused by an absorption of this compound onto cotton, in turn leading to lower method sensitivity.

4.3.5. Analysis of commercial perfumes from garments using the validated SPME method

Various perfumes marketed for both men and women were successfully recovered from cotton and polyester fabrics using the validated SPME method, even when they were spiked at concentrations 1500 times lower than in the original perfumes. This highlights the suitability of the method for the analysis of fragrance traces for forensic applications.

It was observed that the blank cotton fabric contained various analytes, but they did not interfere to the chromatographic peaks of the target analytes. These compounds were identified using the high resolution Orbitrap mass spectrometer and they included aldehydes and alcohols, such as nonanal, decanal, and 1-undecanol. Aldehydes have been previously shown to be extracted by SPME from various textile materials used in forensic sciences for the collection of human scent samples, including when these materials were sterilised (Prada and Furton 2008). It is therefore important to have an understanding of the compounds originating from the fabric itself.

4.3.6. Analysis of commercial perfumes from garments using liquid extraction

The sample procedure developed by Gherghel et al. (2016) for the extraction of VOCs from fabrics using methanol was followed to analyse a perfume diluted 500 times as carried out with SPME. However, for the methanol extracts of the perfume diluted 500 and 100 times, no perfume compounds were recovered. Only when the perfume was used undiluted were similar results obtained to those found for SPME analysis of perfume diluted 500 times.

The proposed SPME extraction method is longer (approximately 2 h) than the liquid extraction option (approximately 1 h when it was carried out by a trained analyst), however the properties of SPME such as high sensitivity, lack of use of organic solvents, simplicity, ability for full automation and coupling to GC-MS, and minimisation of analyte losses or external contaminations make the SPME methodology clearly more suitable for the analysis of traces of VOCs in garments.

4.4. Conclusions

A method for the determination of VOCs specific to the cosmetic industry using HS-SPME coupled to GC-MS was developed, optimised, and validated. It was determined that a three phase SPME fibre, the DVB/CAR/PDMS fibre, was the most suitable for the extractions of VOCs from a commercial perfume and from an in-house perfume. High extraction rates were also obtained using a two-phase fibre, such as the PDMD/DVB and CAR/PDMS fibres, although with poorer repeatability. This highlights the importance of selecting a multiple phase fibre that covers a wider range of molecular weights and volatilities.

For the optimisation of the SPME extraction, a multivariate approach was used, where three different variables were studied simultaneously using a CCD. This approach allowed the identification of the most significant variables and the determination of the optimal experimental conditions, whilst taking into account the interactions between the variables. Two optimisation designs, where incubation time, extraction time and temperature were studied at various levels, were carried out for the extraction of VOCs from an in-house perfume, and from cotton matrix impregnated with the same in-house perfume. For the former, the incubator extraction temperature and the quadratic term coefficient of extraction temperature were determined as the only significant variable, so that highest extraction rates were obtained with an increase in the extraction temperature. This methodology can be a useful tool for establishing VOCs chromatographic profiles and obtaining quantitative results about commercial perfumes. For the SPME extraction of VOCs from fabric, the extraction time was the only significant variable, so that better results were obtained with a longer extraction time. This second methodology improves the ability to acquire indications of potential transfer of VOCs between the fabrics of individuals where the donor wears perfume.

The optimised SPME method was successfully validated for the extraction of six different VOCs from cotton and from polyester. This validated SPME method was applied for the analysis of cotton and polyester samples spiked with commercial perfumes diluted between 500 and 1500 times. The chromatographic results allowed the successful quantification of the analytes of interest when present. Using a liquid extraction method similar results to the SPME results were obtained only when the fabrics were spiked with

undiluted perfumes. The results presented in this chapter demonstrate that the developed SPME method represents a robust and sensitive method for the analysis of fragrance traces from fabrics that can be used to develop an understanding of the evidence dynamics of these traces for application to forensic reconstructions.

5. Transfer of fragrances between fabrics

5.1. Outline

This chapter focuses on the transfer process of fragrances onto a secondary piece of fabric and presents three different experiments where the dynamics of fragrance transfer were investigated. The three variables studied were the ageing time of the fragrances on the first fabric prior to transfer, the contact time between the two fabrics, and lastly the fabric type (of the donor material and the recipient material).

5.2. Results

5.2.1. Perfume ageing time

For this experiment, the internal standards were added to the FM1 used to spike the primary piece of fabric. It was observed that by varying the perfume ageing time (PAT), besides the expected change in the amounts of VOCs recovered, there was also a considerable change in the amounts of internal standards recovered. Figure 5-1 presents the scatter plots with error bars for the internal standards, with each point representing the mean peak area. This figure shows how for both dibromobenzene and methyl nonanoate, very large chromatographic peak areas were obtained using an ageing time of 1 h, compared to all perfume ageing times investigated. This behaviour might be due to time needed to reach equilibrium and therefore, it was deemed that the internal standards as used here are not appropriate for quantification.

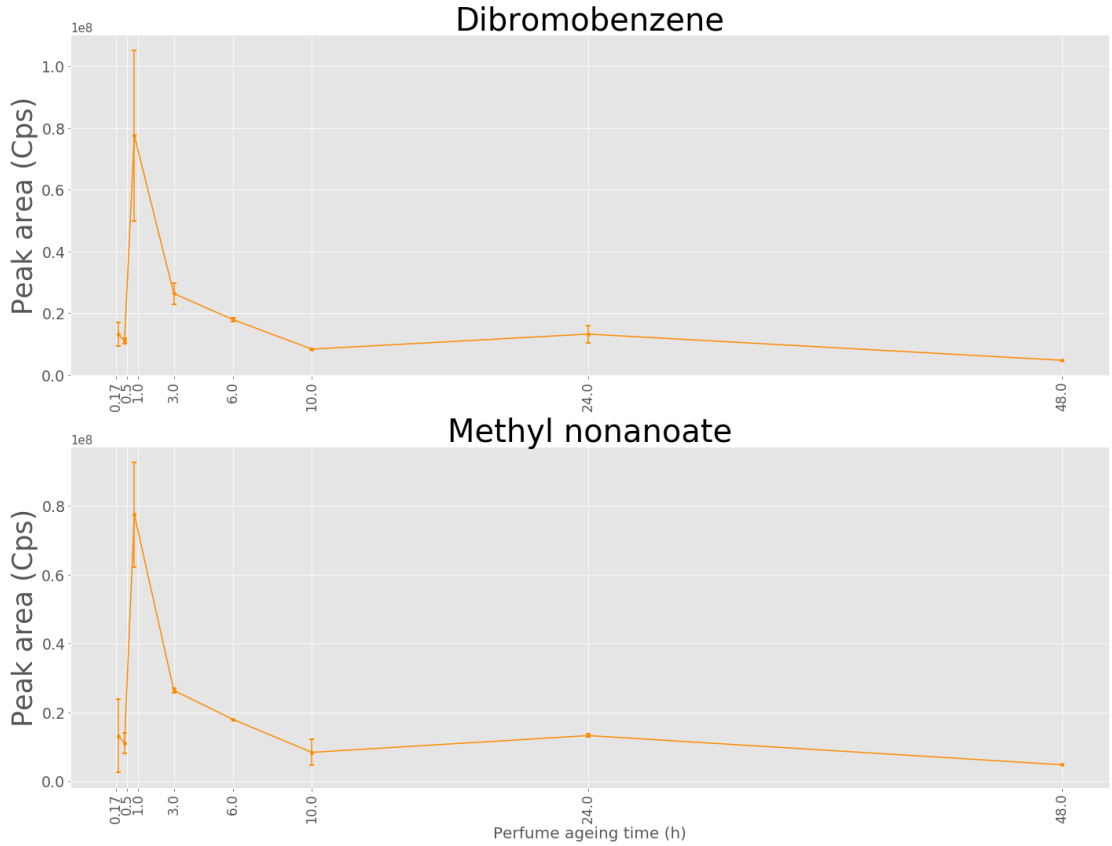

Figure 5-1 The chromatographic peak area of the two internal standards from the secondary piece of fabrics at various perfume ageing times (10 min (0.17 h) to 48 h). The error bars are standard deviations (n=3). (Cps: counts per second)

Instead, the recoveries of the VOCs were calculated using as control two reference samples. These reference samples were fabric swatches spiked with the FM1 and analysed at the beginning and at the end of the batch of the sampling sequence. Average peak area values were considered for comparison with those obtained experimentally. Additionally, the fabric weight was taken into consideration for a more accurate calculation. And so, the amounts of VOC recovered from the recipient fabrics are reported in % as sample analyte peak area per fabric weight divided by the reference analyte peak area per fabric weight.

By varying the period of time that a fragrance mix has been on a primary piece of fabric, prior to transfer, considerable differences in the amount of VOCs recovered from the secondary piece of fabric were observed (Figure 5-2). Generally, and especially for lower volatility molecules, it was noted that a short PAT lead to higher amounts recovered from the secondary fabric. Linalool, geraniol, and eugenol follow a typical decay curve,

where high amounts were recovered for short times followed by a steady decrease until a plateau was reached. For example, for linalool approximately 3% of the total 100% reference peak area was recovered from the secondary fabric using a 10 min PAT, and then the recovery slowly decreased with the time increasing, so that at 1 h PAT around 1% was recovered, at 3 h around 0.4%, after which it levelled off until 48 h PAT. Limonene and ethylene brassylate display an initial spike prior to the decay curve, at 3 and 1 h PAT, respectively. For pinene the trendline is not very clear.

Moreover, it was noted that different VOCs were recovered at different rates as it can be noted on the y scale in Figure 5-2 . For example, for pinene, very small amounts were recovered from the recipient fabric for all ageing times, between 0.005 and 0.05% of the original spiked amount. In comparison, the lower volatility compounds were recovered in higher levels (2-5% of the amount spiked on the original fabric), especially for shorter PAT.

Additionally, when looking at the error values for the triplicate samples, it can be observed that good RSD values (n=3) were obtained for most samples. Pinene displays higher RSD, with values between 9.8 and 76% for PAT lower than 6 h. The pinene recoveries are generally around 100 times lower compared to low volatility compounds, which can explain the higher RSD. It should be noted that only one sample was available for the 48 h time, and so no RSD values are available for this ageing time.

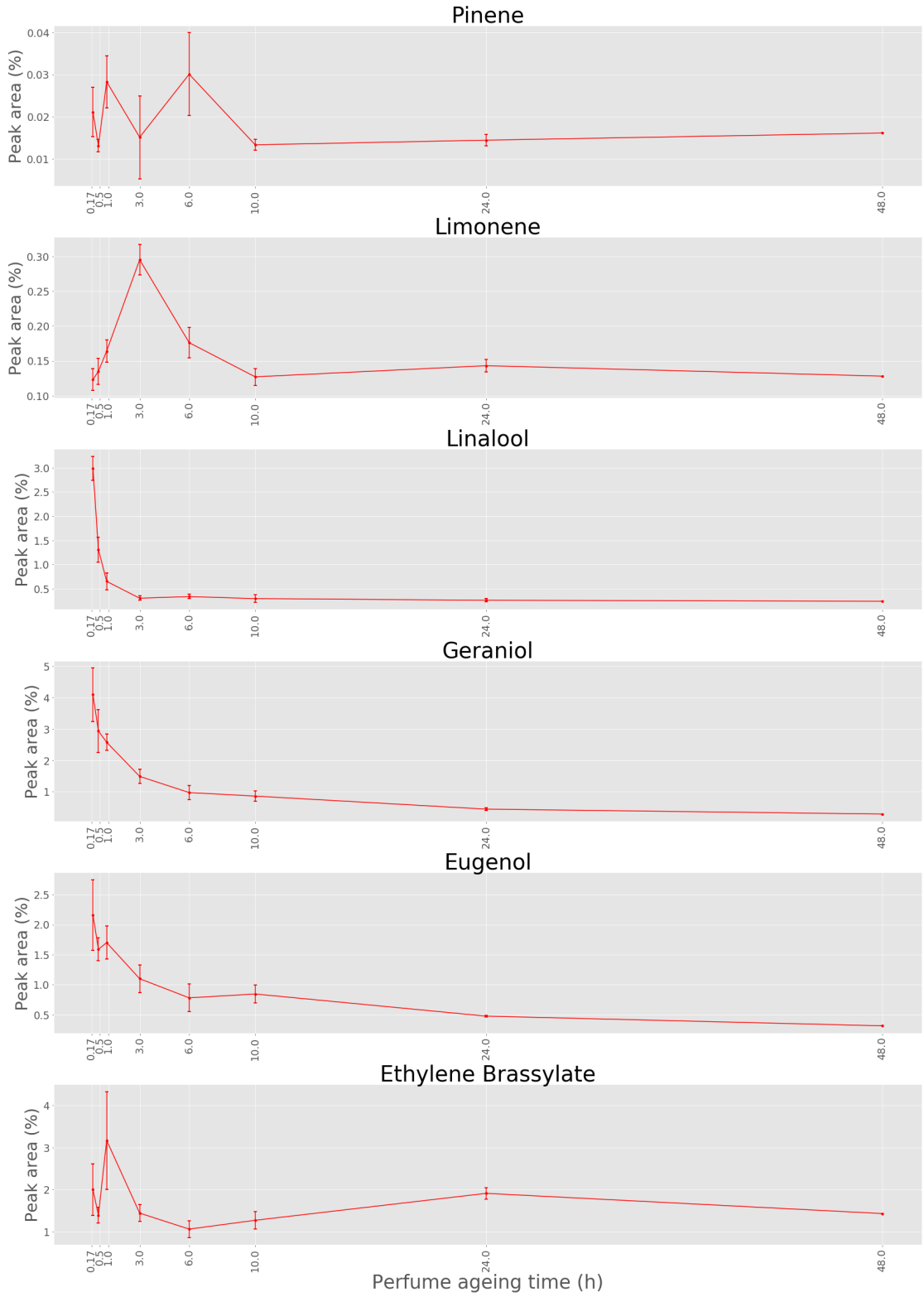

Figure 5-2 Recovery of the six analytes from the secondary piece of fabric when the perfume on the primary piece of fabric was aged for different periods (10 min (0.17 h) to 48 h) prior to contact. The error bars are standard deviations (n=3). The difference in scaling between analytes should also be noted

5.2.2. *Contact time*

For the contact time study, the donor and recipient fabrics were rubbed against each other using the crockmeter for a series of different lengths of time ranging from 10 s (0.17 min) to 10 min. It was noticed that with the longest contact time (10 min), there was a higher risk of the primary piece of fabric becoming detached from the crockmeter finger, in which case a new sample was prepared.

Similar to the PAT experiment, the internal standards added to the fragrance mix behaved differently depending on the contact time (Figure 5-3), thus the quantification of VOCs from the samples was again carried out without the use of internal standards, but rather using reference samples spiked with FM1, and thus the amounts of VOCs recovered are expressed as percentages.

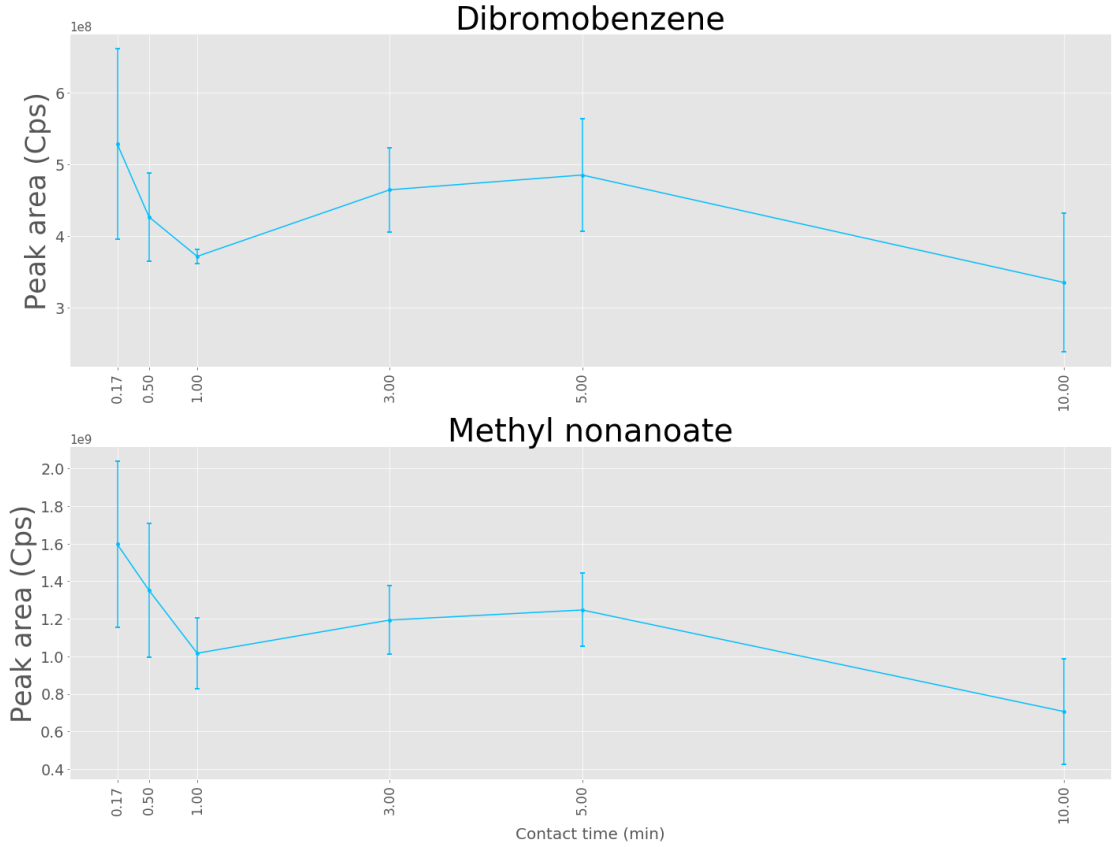

Figure 5-3 The chromatographic peak area of the two internal standards from the secondary piece of fabrics at various contact times (10 s (0.17 min) to 10 min). The error bars are standard deviations (n=3). (Cps: counts per second)

As can be seen in Figure 5-4, for all six VOCs, with an increase in the contact time between the fragranced fabric and the fragrance-free fabric, a consistent amount of analyte was recovered for contact times of up to 3-5 min, after which the recovery slowly decreased. For example, for linalool the mean recoveries were between 18 to 25% for contact times of 10 s to 5 min, and around 10% for a contact time of 10 min. The lowest recoveries were at 10 min contact time for all six analytes.

As observed with the perfume ageing time experiment, there were big differences in the amounts of limonene and pinene recovered from the donor materials compared to lower volatility compounds. Generally, around 0.5% of pinene and 1% of limonene were recovered from the secondary piece of fabric, whilst for the other four VOCs the recoveries were generally between 15 and 35%.

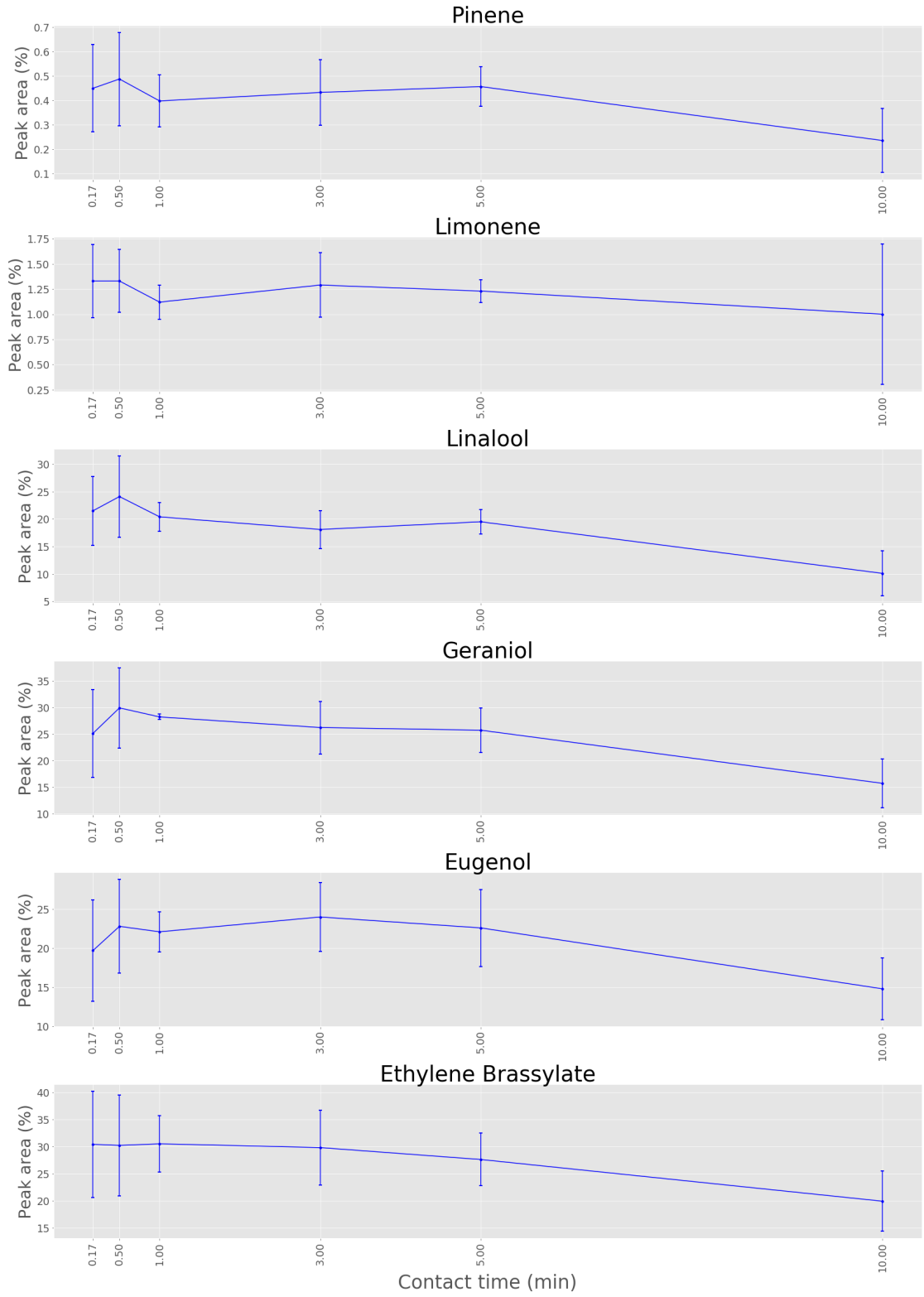

Figure 5-4 Recovery of the six analytes from the secondary piece of fabric when the contact between the fabrics varied from 10 s (0.17 min) to 10 min. The error bars are standard deviations (n=3). The difference in scaling between analytes should also be noted

5.2.3. Fabric type

5.2.3.a SEM analysis

For the last transfer experiment, seven different fabrics, shown in Figure 5-5, were employed as donor materials and two of these (polyester and cotton) as recipient materials.

The fabrics were examined under SEM to establish the fabric weave characteristics and to provide insight into how these characteristics might play a role in the transfer of fragrances. The SEM results (Figure 5-6) show the seven different fabrics using a 250x resolution. In terms of width, the wool fibres had the largest fibre width and nylon the smallest. Acrylic, nylon and polyester show smooth and regular surfaces, wool a scale-like structure, and cotton a ribbon like structure. Although denim is made of 100% cotton, there is a clear difference in the weave of these two materials, with denim showing tightly woven fibres. Also noticeable is the dissimilarity between the 100% polyester material and the 50/50 blend of polyester and viscose. Whilst the former presents more order and it has depth, the latter presents many loose fibres. Nylon, a waterproof material, shows a very ordered structure, with no gaps between the fibres.

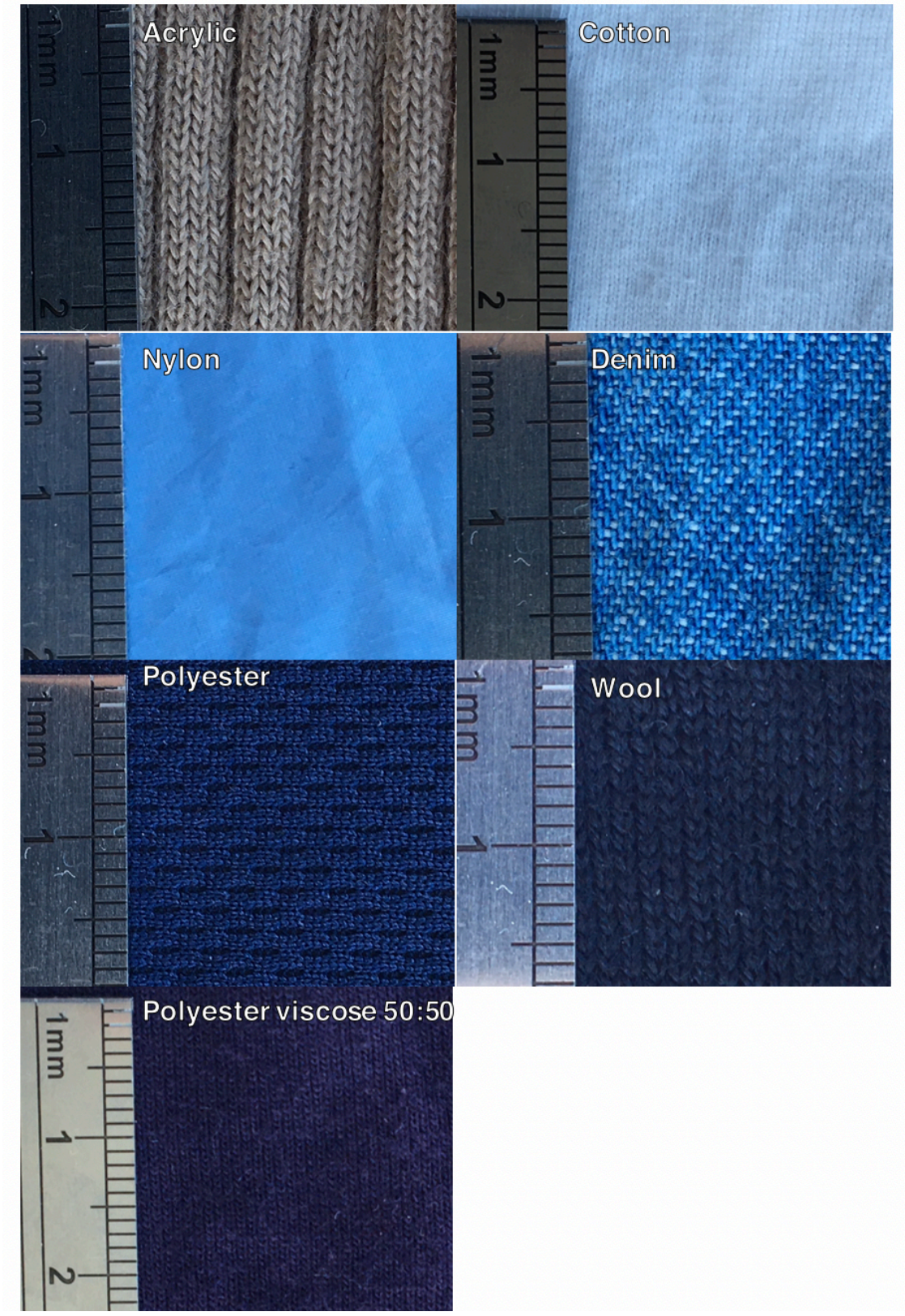

Figure 5-5 Close-up photographs of the seven fabrics employed. Each individual mark on the ruler represents 1 mm

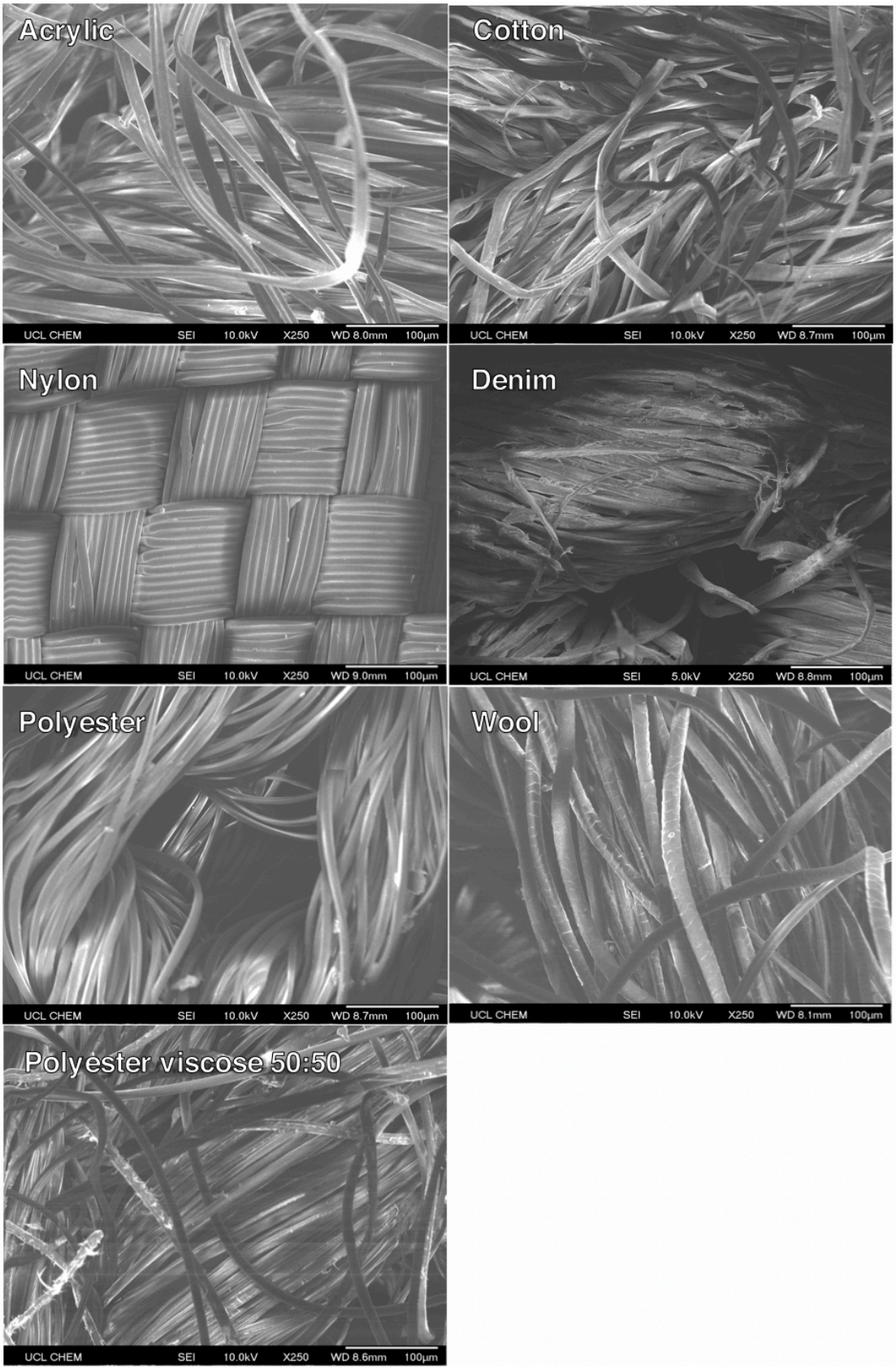

Figure 5-6 SEM images showing the seven fabrics using a 250x resolution with the man-made blends on the left side and the natural blends on the right side

5.2.3.b VOCs analysis

For the quantification of the six VOCs from the fabric type samples, calibration curves were carried out in both cotton and polyester. As the calibrants span a large range of concentrations from 0.2 to 200 μM, quadratic weighted curves were fitted which apply more emphasis to some concentration points than others, specifically to the lower concentration points. The weighting factors are generally between 0 and 1, and most commonly are $1/x$ or $1/x^2$, where x is the concentration (Gu et al., 2014). The results of fitting weighted curves are shown in Table 5-1. With the exception of α-pinene in polyester which was fitted to a linear curve, all other calibrations were fitted to quadratic curves. Good R^2 were obtained for all curves with values above 0.996, with the exception of ethylene brassylate in cotton for which the R^2 was 0.988.

Table 5-1 Calibration results for the transfer experiment on fabric type

Compound	Matrix	R^2	Calibration curve	Working range (μM)
α-Pinene	Cotton	0.998	Y=-0.00042*X2+0.62552*X+0.15118	0.5-200
	Polyester	0.996	Y=0.42279*X+0.00019	0.2-200
Limonene	Cotton	0.999	Y=-0.00225*X2+2.04015*X+0.48393	0.2-200
	Polyester	0.999	Y=-0.00107*X2+1.41214*X+7.15824	0.5-200
Linalool	Cotton	0.999	Y=-0.00041*X2+0.25149*X-0.00799	0.5-200
	Polyester	0.999	Y=-0.00019*X2+0.14212*X-0.03347	0.5-200
Geraniol	Cotton	0.999	Y=0.00019*X2+0.12994*X-0.03017	0.5-200
	Polyester	0.998	Y=0.00003*X2+0.11365*X-0.00327	0.5-200
Eugenol	Cotton	0.998	Y=0.00012*X2+0.03240*X+0.00682	0.5-200
	Polyester	0.999	Y=0.00005*X2+0.06418*X-0.00637	2.5-200
Ethylene brassylate	Cotton	0.988	Y=0.00002*X2+0.00317*X+0.03172	0.5-200
	Polyester	0.999	Y=0.00008*X2+0.03283*X+0.00476	0.2-200

For the fabric type experiment, a natural (cotton) and a synthetic (polyester) fabric were used as recipient fabrics, whilst six of the seven fabrics available were used as donor materials. The name of the samples starts with the name of the donor material, followed by the name of the recipient material, so that for the sample “acrylic_polyester” the fragrance mix was pipetted onto acrylic, and then transferred onto a polyester material using the crockmeter for a contact time of 1 min.

As noted for the two time-related experiments, very low amounts of α-pinene are transferred and recovered from recipient fabrics. This was also observed in the fabric type experiment, even though FM2 contained a higher concentration of VOCs compared to the FM1 used for the other two experiments. Many samples led to the recovery of this compound below the lower limit of the working range (0.5 μM in cotton and 0.2 μM in polyester), and so only the other five VOCs (limonene, linalool, geraniol, eugenol, and ethylene brassylate) were employed for data analysis.

To obtain an overview and a visual representation of the samples (6 blanks + 6 donor fabrics * 2 recipient fabrics * 3 replicates) principal component analysis (PCA) was employed. PCA is an unsupervised method (no labels are given) that reduces the dimension of the data with the aim of retaining as much information as possible. It creates an artificial set of variables, called principal components, that account for the most variance in the data set. PCA showed a clear separation of the blank samples in the set of 42 samples (Figure 5-7). The sum of three principal components accounted for 89.1% of the total variability.

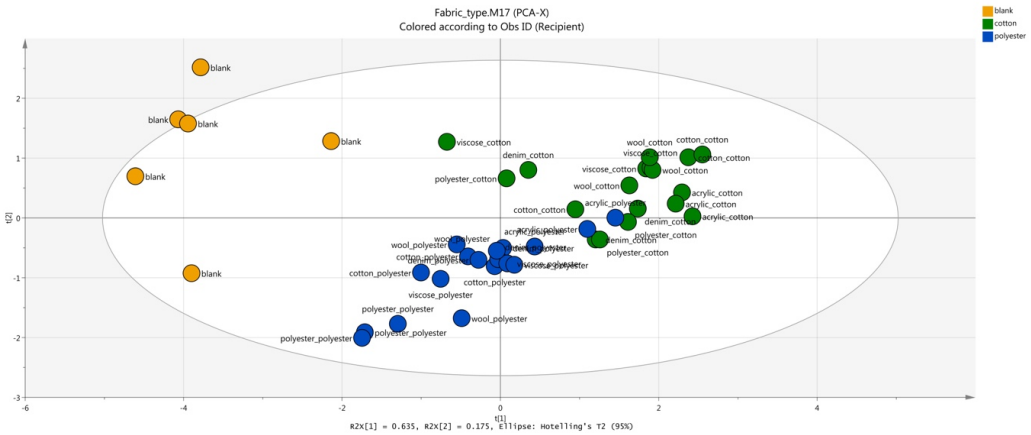

Figure 5-7 PCA representation of fabric type samples and blanks based on the peak area of five VOCs (limonene, linalool, geraniol, eugenol, ethylene brassylate)

Further statistical analysis was carried out without the blanks. It was noticed that when the 36 fabric type samples were used to create a new PCA model, the Distance to model (DModX) analysis identified the viscose_cotton_R3 sample as being an outlier (Figure 5-8). This sample displays the largest portion of unexplained variation as it is the farthest away from the model plane and above the critical distance.

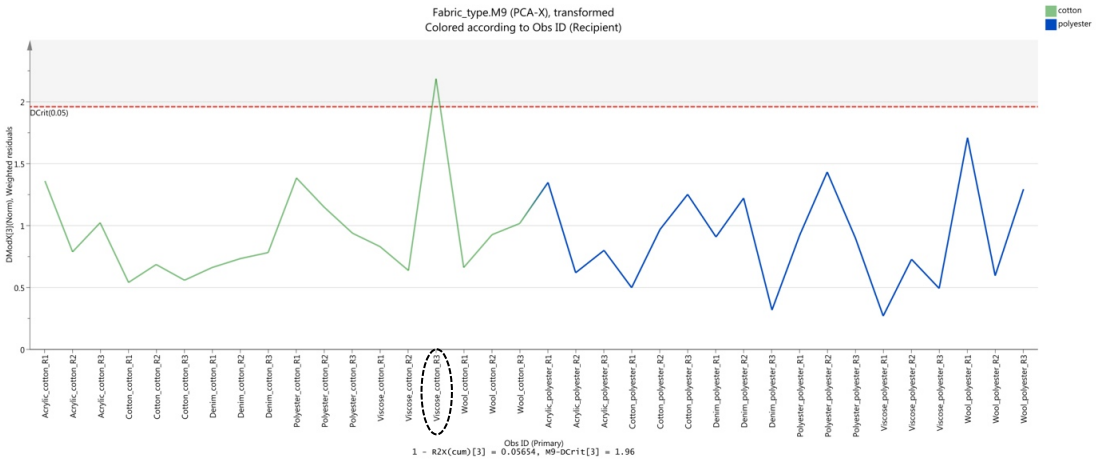

Figure 5-8 Standardised representation of distance to model results showing that sample viscose_cotton_3 is an outlier

The new PCA permitted a reduction of the 35 samples to three principal components. These three PCs were extracted explaining 98.1% of the total variance of fabric type samples. The obtained three-dimensional PCA representation is colour coded in Figure 5-9 according to the recipient material (polyester or cotton), the donor material (the six fabrics), or as a combination of recipient and donor material (twelve different groups). In Figure 5-9a it can be observed that the transfer of perfume from all different materials onto cotton is very different from the one onto polyester. The two groups are clearly distinguishable between each other, as emphasised by the dashed circles. In Figure 5-9b, it can be observed that the acrylic fabric as a donor it is clearly different from the rest of the fabrics studied, when transferred to polyester. Lastly, Figure 5-9c shows how the three natural fibres tested, cotton, denim, and wool exhibited a similar transfer behaviour when transferred onto polyester (highlighted by the ellipse), with the exception of one wool replicate. However, when the VOCs were transferred to cotton, the natural fibres were also close or overlapping on the PCA with some man-made fibres.

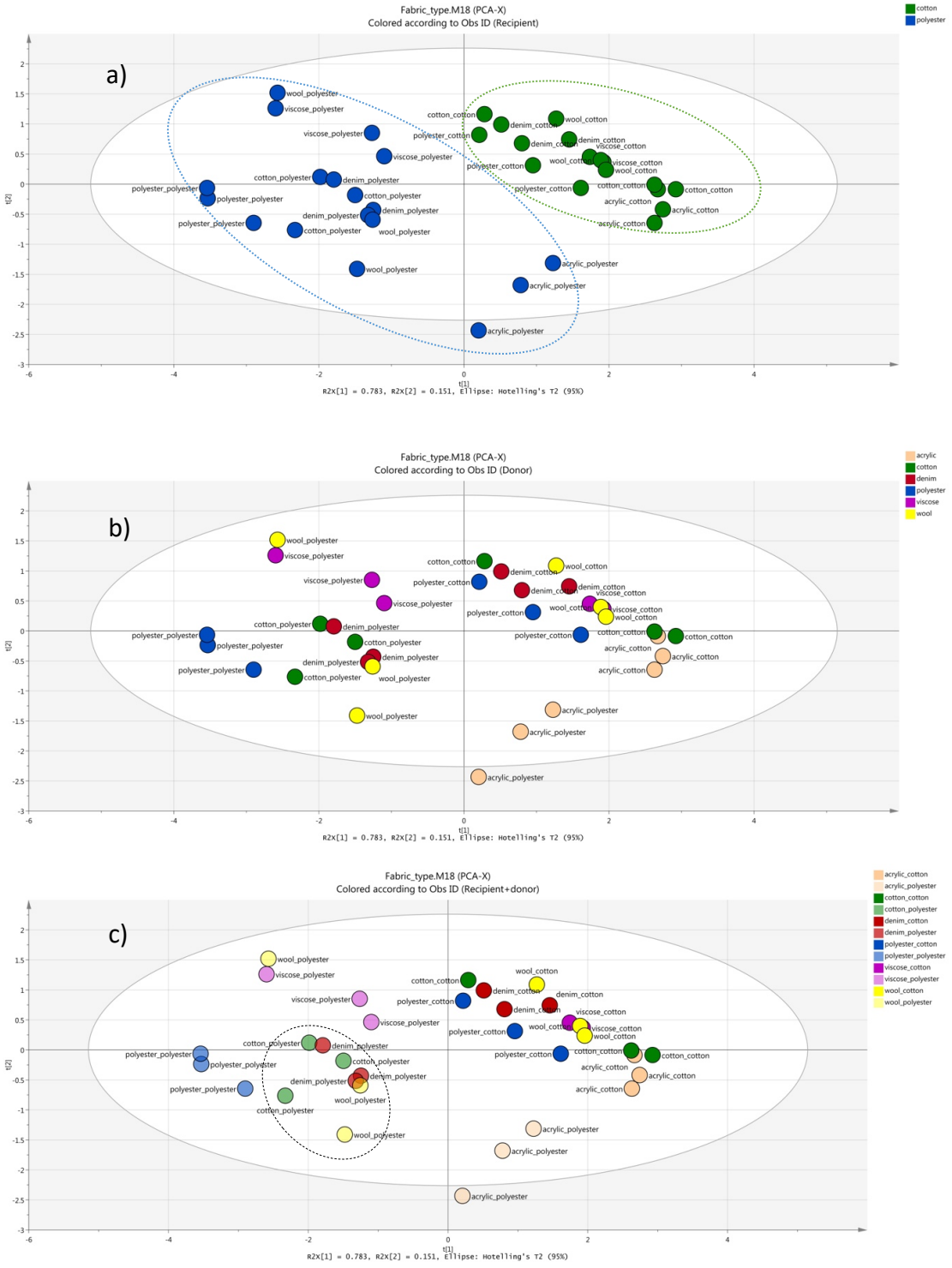

Figure 5-9 Three PCA representations using peak areas of five VOCs (limonene, linalool, geraniol, eugenol, ethylene brassylate) from the fabric type transfer data, and coloured by: a) the recipient fabric, b) the donor fabric, and c) the recipient and donor fabric. The ellipses were drawn on to facilitate identification

To explore and identify structures within the data gathered, hierarchical cluster analysis (HCA) was employed. HCA is an exploratory data analysis algorithm for the organisation of samples in groups and among groups depicting a hierarchy. The result of HCA is generally presented in a dendrogram (Figure 5-10). With one exception, the dendrogram shows that the samples separate clearly into two major groups according to the recipient fabric, cotton and polyester. The three acrylic to polyester samples were misassigned by the HCA as belonging to the cotton recipient samples. These samples were close to the cotton recipient samples in the PCA, as it can be observed in Figure 5-9b. Within these two major HCA groups, various subgroups are formed. It can be observed how all three polyester to polyester replicates are grouped together, showing little dissimilarity. However, it can be observed that not all triplicates are grouped together, and there is an overlap for some samples, for example the replicate 3 of wool to polyester samples is grouped with viscose to polyester samples. Such overlap in the triplicates can also be observed in the PCA in Figure 5-9c.

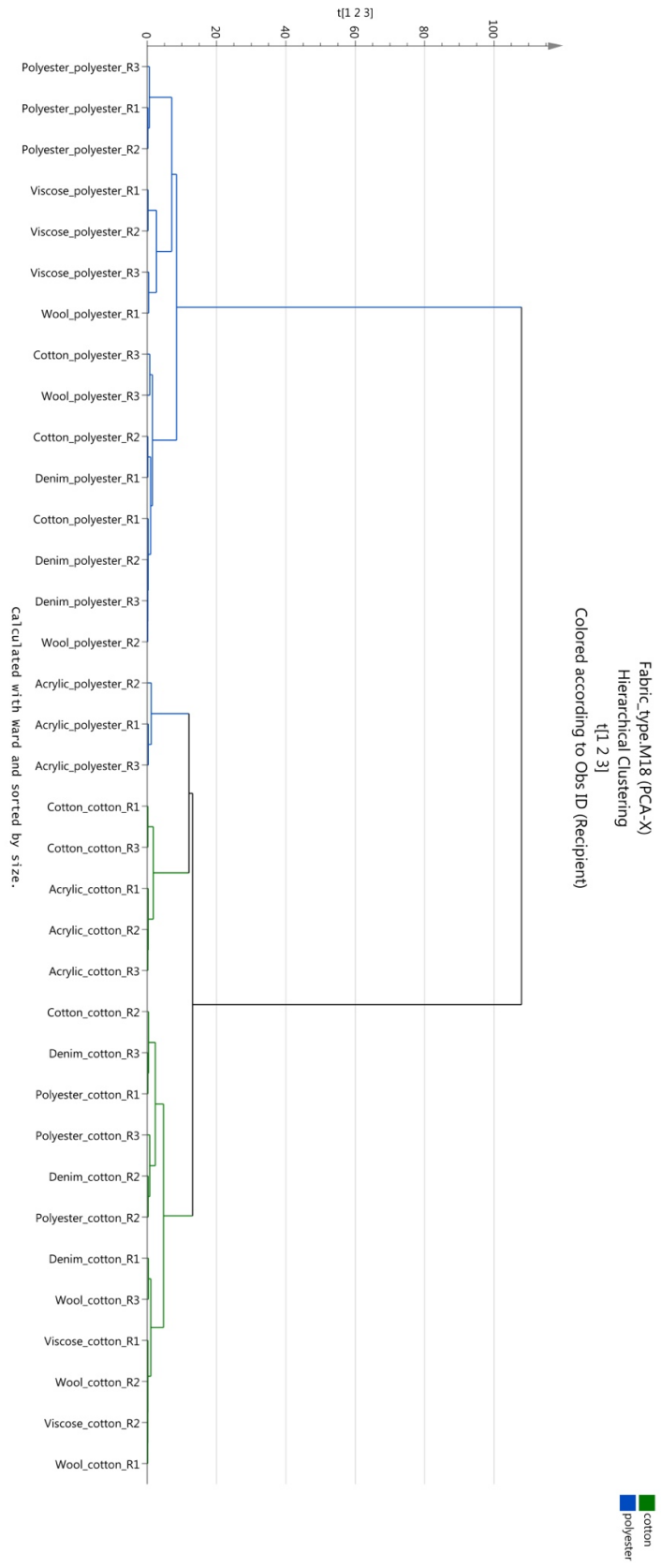

Figure 5-10 Dendrogram obtained by hierarchical cluster analysis (HCA) using the samples from the fabric type experiment showing a good sepration between cotton and polyester recipient fabrics

Finally, a PLS (partial least square) discriminant analysis was used to determine a model able to predict the nature of the recipient fabric depending on the transfer of the VOCs studied. The graphical result is shown in Figure 5-11. The model was able to successfully distinguish the two groups (cotton and polyester), accounting for 96% (R^2 value of 0.96) of the variance in the observed samples for the training set. The Q^2 value obtained was 88.8%. The Q^2 value is an estimate of the predictive ability of the model and is a result of the cross-validation, where a $1/7^{th}$ of the data is removed at a time, and the model's prediction ability is tested.

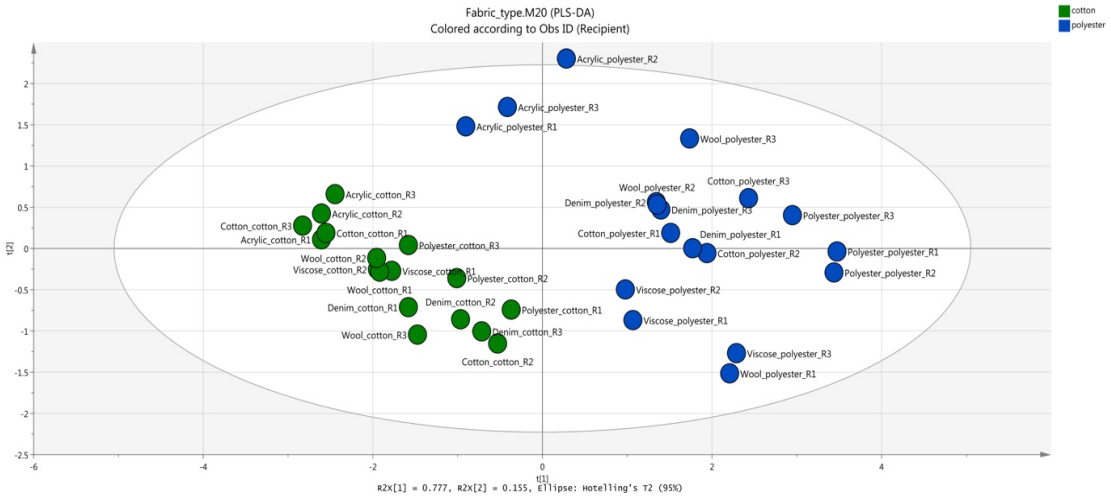

Figure 5-11 Partial least square (PLS) discriminant analysis representation showing the classification of the transfer type samples based on the donor material

5.3. Discussion

To our knowledge, these are the first experimental studies carried out on the transfer process of fragrances between fabrics for forensic applications using an optimised and validated SPME-GC-MS method. Three variables (fabric type, perfume ageing time, and fabric contact time) considered to have an important effect on the dynamics of the transfers were investigated, following the studies of Bull et al. (2006) and Hudson et al. (2009), which demonstrated the importance of material type and the dependence of VOC volatility with time. The experiments for this study were also designed to closely recreate the type of contact between fabrics relevant to a sexual assault case.

5.3.1. Perfume ageing time

As the samples were spiked with the VOCs and the internal standards at the same time, it was observed that the behaviour of the internal standards during the transfer process at various PAT was not constant. Therefore, the internal standards were deemed not suitable for the quantification of the VOCs. Instead, the amount of VOCs recovered from the recipient cotton swatches was calculated using the reference amounts of VOCs from a spiked cotton swatch, i.e. the amount of VOCs on a primary swatch.

Overall, longer perfume ageing times led to lower amounts of VOCs being recovered from the secondary piece of fabric. Lower volatility compounds, such as linalool, geraniol, and eugenol followed a typical decay curve, identified in many other forensic studies concerning the persistence of traces on clothing, such as by Dachs et al. (2003) for hair scalp and Robertson et al. (1982) for textile fibres. Additionally, it was observed that for all perfume ageing times very low amounts of high volatility compounds, such as pinene and limonene were recovered from the donor fabric, with values lower than 0.04% for pinene and 0.3% for limonene, when compared to a reference spiked fabric. For the other VOCs, the recovery was generally between 0.5 and 5%. This suggests that highly volatile compounds might not be suitable candidates for forensic VOCs analyses.

Moreover, when looking at the RSD values for the triplicate analytes at all ageing time, it can be observed that large RSD values were obtained for pinene, with values between 9.8 and 76%. This further consolidates the point of view that such high volatility

compounds may have limited value in forensic studies, firstly because they transfer in very small amounts for all ageing time studies, and secondly because of the high variability. On the other hand, lower volatility compounds presented lower RSD values, higher recovery rates, and steady decay curves. Ethylene brassylate, the least volatile compound, showed an initial spike in recovery for an ageing time of 1 h prior to the decay curve.

5.3.2. Contact time

Once again, the quantification was carried out using reference samples that had been directly treated with the VOCs. Generally, for contact times between fabrics from 10 s up to 3-5 min, no discernible differences in the amount of VOCs recovered from the secondary piece of fabric were observed. The recovery did decrease for longer contact times, such as 10 min. This is in contrast with work carried out by Gherghel et al. (2016), where an increase in the amount of VOC recovered from the donor fabric was observed for longer contact times between fabrics. This can be potentially explained by the difference in the transfer methods between the two studies. In Gherghel et al. (2016), the two fabrics were kept in contact under the pressure of a weight. The weight could act as a hermetic seal, trapping the VOCs between the two fabrics and not allowing their evaporation into the surrounding air. In the current study, a crockmeter was employed to better simulate the reality of a sexual assault case. The fabrics were rubbed against each other under high pressure, and thus generating heat by the friction. The increase in temperature could lead to the evaporation of some compounds with a longer contact time, and thus explain the lower recovery rates observed for the longest studied contact time of 10 min.

Similar to 5.3.1, small amounts of pinene and linalool were recovered from the recipient fabric, compared to the lower volatility compounds. So that, for shorter contact times, the recoveries for pinene and limonene were around 0.5 and 1.2%, respectively, whilst for the other VOCs the recoveries were around 20-35%.

5.3.3. Fabric type

Scanning electron microscopy was employed to obtain information about the structure of the fabric and the weaving patterns of the seven different fabrics, both natural and

man-made. The nylon fabric, originating from a waterproof jacket, was a tightly woven fabric, which can explain its natural low permeability. All other fabrics presented gaps between threads or bundles of threads, allowing for air or liquid flow. The fibre structure varied between the fabrics studied, with synthetic fibres generally showing smoother surfaces (Figure 5-6). However, studies such as DeGreeff et al. (2011) and Prada et al. (2011) have shown that both the fabric weave and the molecular structure, and in turn the interaction forces between analytes and fabrics play a role in the transfer of human scent to fabrics.

When looking at the transfer of VOCs onto cotton and polyester, clear differences were readily noticeable in the PCA plot (Figure 5-9c) and in the HCA dendrogram (Figure 5-10) with two distinctive groups formed based on the recipient material. Moreover, a PLS discriminant analysis model was able to discriminate between the two recipient fabric samples with a R^2 value of 96% (Figure 5-11).

Within these two major groups, various donor samples were distinctive. For example, when acrylic was used as the donor material this appeared on the 3-D PCA well discriminated from the rest of the samples, especially when polyester was used as the recipient fabric (Figure 5-9c). In spite of the weave differences observed by SEM analysis, the natural fabrics (cotton, denim, wool) showed overlap on the PCA, especially when the VOCs were transferred from these fabrics onto polyester.

5.4. Conclusions

The transfer behaviour of six VOCs between fabrics has been investigated in this study. All factors studied, the perfume ageing time, the contact time, and the fabric type, affected how volatile compounds are transferred onto a secondary piece of fabric.

The results from the perfume ageing time study showed that lower volatility compounds were recovered from recipient fabrics in higher amounts compared to higher volatility compounds. It was also observed that the longer the time period since fragrance VOCs have been applied to a piece of fabric, the lower the recovery of compounds from the secondary piece of fabric (Figure 5-2). This decrease follows a typical decay curve, where the recovery drops considerably for the samples when the fragrance mixture was aged

for 1 to 3 h, after which the recovery drops steadily. Perfume traces were recovered from the recipient fabric even when the perfume was aged on the donor fabric for as long as 48 h.

The data from the contact time experiment demonstrated that even for contacts as short as 10 s, VOCs were successfully transferred and recovered from recipient cotton swatches. Moreover, the amounts recovered for all six VOCs maintained rather constant for the first 5 min of contact, after which a decrease was noticed (Figure 5-4). This can be explained by the evaporation of VOCs with increased rubbing time, and thus increase in the heat generated by rubbing the fabrics together.

When looking at the fabric type, clear differences were observed in the VOCs behaviour when they were transferred onto cotton and onto polyester. Two distinctive groups were determined by the PCA based on the recipient fabric (Figure 5-9a). On the other hand, when looking at the influence of the primary fabric for each of these two groups, some samples were distinctive, whilst other samples displayed some overlap on the PCA plot (Figure 5-9c).

Overall, this study has confirmed that VOCs have the potential to be a helpful form of trace evidence as detectable amounts were recovered from a recipient fabric for fabric contacts as short as 10 s and when the perfume on the donor fabric was applied as long as 48 h prior to transfer. However, factors that affect the transfer, such as the ones studied here, should be taken into consideration when seeking to interpret the presence of VOCs traces recovered from fabrics. For forensic reconstructions where clothing from two individuals may have been in contact, such as a sexual assault, this study indicates that there may be value in testing the clothing items for VOCs. Depending on the type of fragrance originally applied, these findings indicate that it may be possible to identify specific fragrance VOCs on clothing exhibits which may provide trace specimens that can contribute to a forensic reconstruction seeking to establish whether a contact may have taken place.

6. Persistence of fragrances on fabrics after transfer

6.1. Outline

This chapter presents empirical data from experiments designed to assess the persistence of transferred fragrances. The experiments in this chapter were informed by sexual assault casework and designed taking into account the results obtained from chapter 5 on the transfer of fragrance VOCs between fabrics, as well as forensic literature on the persistence of VOCs and of trace evidence on clothing (Obendorf et al., 2006; Pounds and Smalldon, 1975c). The results from chapter 5 demonstrated that both fabric type and time, be it ageing time or contact time, affect the transfer behaviour of VOCs between fabrics. As such, the persistence experiments from this chapter incorporate the effect of persistence time (from 1 h to as long as 28 d), as well as that of fabric type. In addition, the effect of temperature was examined as VOCs, by definition, are dependent on the environmental temperature. The environmental conditions investigated were room temperature, fridge temperature, and freezer temperature. These temperatures were selected to incorporate likely temperatures at which assaults occur, as well as storage temperature of forensic evidence. Cotton was used for the secondary piece of fabric, that is the fabrics the VOCs were transferred to and left to persist on (as outlined in Table 3-3). For the room temperature experiment in addition to cotton, polyester was examined as another secondary piece of fabric (see Table 3-3) as transfer experiments carried out at room temperature showed that VOCs transfer differently to cotton than to polyester. The experiments for this study were also designed to closely recreate the scenario where a piece of garment subjected to secondary transfer of fragrance VOCs during a sexual assault is left exposed to air, for example hanging on the back of a chair. As such, the fabric samples with transferred VOCs were left exposed to air on Petri dishes.

6.2. Results

In a similar manner to the transfer experiments presented in Chapter 5, the chromatographic peak area data from this chapter (Table 6-1) were quantified using the calibration curves presented in section 5.3, Table 5-1. The resulting concentration (μM)

(Table 6-2) was further divided by the fabric weight (Table 6-3), which varied between 0.19 and 0.28 g for all samples (average weight was 0.24 g). The resulting data with the concentration of the VOCs determined in the fabrics is provided in Table 6-4. As such, for all trendline graphs presented in this paper, the x axis represents the persistence time (d) and the y axis is represented by the detected concentration of the analyte in the fabric divided by the fabric weight (μM/g). Nine, five and three different persistence times were sampled in triplicate for each of the three temperature experiments (as outlined in Table 3-3).

For the room temperature and fridge experiment, the recoveries of pinene and limonene were below the linear range of the calibration, which did not allow for reliable quantification of these two compounds. However, for the other four VOCs (linalool, geraniol, eugenol, and ethylene brassylate), all recoveries were within the calibration ranges reported Table 5-1, unless otherwise specified in Table 6-4.

Table 6-1 The peak area of the VOCs of interest and of the internal standards from the fabric samples for the persistence study

Environmental temperature		Peak area count (E+07)																							
		Room temperature												Fridge						Freezer					
Recipient fabric		Cotton						Polyester						Cotton						Cotton					
Compound		Linalool	Geraniol	Eugenol	Ethylene brassylate	Methyl nonanoate	Dibromobenzene	Linalool	Geraniol	Eugenol	Ethylene brassylate	Methyl nonanoate	Dibromobenzene	Linalool	Geraniol	Eugenol	Ethylene brassylate	Methyl nonanoate	Dibromobenzene	Limonene	Linalool	Geraniol	Eugenol	Ethylene brassylate	Methyl nonanoate	Dibromobenzene
Persistence time (d)	0.04	3.26	30.82	10.32	3.68	5.29	4.49	1.07	5.43	5.75	5.21	4.89	6.37	45.63	182.80	16.08	3.02	13.06	9.09	616.20	40.34	173.50	24.74	3.66	6.27	8.31
		2.30	21.20	11.49	3.44	4.22	4.59	1.58	6.30	5.32	5.72	4.58	6.37	42.62	182.30	17.90	3.27	11.66	8.28	500.30	34.47	174.20	24.34	3.84	5.58	7.66
		3.34	43.26	15.64	4.11	5.15	4.71	1.22	5.45	4.76	6.21	4.04	5.83	47.31	175.00	17.57	2.88	11.83	8.93	795.20	45.37	156.30	17.45	2.55	5.76	6.89
	0.33	2.05	8.53	2.57	2.77	6.17	5.40	1.07	4.21	3.17	5.92	4.99	7.95													
		3.67	9.53	3.14	2.30	5.39	5.64	0.93	3.51	3.27	3.77	4.68	7.40	-	-	-	-	-	-	-	-	-	-	-	-	-
		3.12	6.21	1.55	1.64	4.91	4.72	1.22	3.93	3.05	4.05	4.12	6.83													
	1	-	-	-	-	-	-	-	-	-	-	-	-	4.90	104.30	20.46	3.30	13.77	7.07	830.80	10.35	121.20	18.89	2.37	4.20	3.80
														4.21	119.40	24.73	4.04	14.54	6.94	927.90	7.87	104.60	19.05	2.15	4.42	4.49
														5.85	146.90	30.00	3.93	14.65	7.10	1239.00	1.31	168.00	25.73	2.04	3.57	3.50
	3	2.41	6.09	2.20	2.27	5.50	6.95	1.59	2.60	1.30	1.80	4.96	9.59	-	-	-	-	-	-	-	-	-	-	-	-	-

	3.07	5.68	1.26	1.56	5.45	6.09	1.21	2.31	1.12	2.06	4.71	10.00														
	2.77	4.05	0.90	1.00	4.47	5.48	1.12	1.90	1.10	1.94	4.70	9.22														
													3.01	33.67	7.03	1.83	14.80	3.98								
4	-	-	-	-	-	-	-	-	-	-	-	-	2.77	24.90	6.17	1.64	16.28	4.22	-	-	-	-	-	-	-	
													2.43	29.54	10.13	2.30	12.30	3.81								
	2.21	3.7	0.67	3.64	6.20	3.83	1.88	3.51	1.88	2.33	5.31	3.87														
5	2.57	4.17	0.67	2.80	6.55	4.05	0.69	1.34	1.24	1.87	4.94	3.74	-	-	-	-	-	-	-	-	-	-	-	-	-	
	2.04	4.54	1.06	4.02	4.83	3.49	1.35	2.40	1.51	2.94	4.99	3.24														
	5.30	5.45	0.57	1.55	7.07	3.43	2.46	3.69	1.67	2.28	5.57	4.48														
7	6.03	8.11	1.25	3.32	6.68	3.91	2.30	4.32	1.86	3.01	4.82	4.16	-	-	-	-	-	-	-	-	-	-	-	-	-	
	3.86	4.71	0.78	2.99	6.43	3.99	1.29	2.57	1.40	2.73	5.31	4.21														
	2.27	3.84	0.65	2.72	7.69	3.32	1.04	2.54	1.60	1.68	6.33	4.52	1.17	8.44	6.28	4.64	16.87	5.74	1170.00	15.84	98.25	15.29	3.20	6.44	3.65	
10	2.49	3.09	0.58	2.41	7.32	3.72	0.81	2.05	1.57	2.51	7.32	4.98	2.04	18.83	9.15	6.63	18.28	6.18	1092.00	16.29	141.50	20.15	3.10	5.88	3.11	
	2.10	2.99	0.47	1.9	6.73	3.45	1.54	2.97	1.52	2.04	6.92	4.46	2.27	18.31	6.94	2.71	17.68	6.06	958.10	19.86	204.60	32.04	3.65	5.96	327	
	1.42	2.45	0.33	1.09	5.53	2.83	0.52	0.76	0.57	0.86	5.13	3.27														
14	1.91	2.49	0.37	1.30	5.07	3.01	0.71	1.24	0.84	1.07	4.48	3.09	-	-	-	-	-	-	-	-	-	-	-	-	-	
	2.00	2.46	0.32	1.10	5.50	3.05	0.60	0.96	0.65	0.93	4.99	3.01														
													1.61	12.96	5.43	1.36	16.32	5.90								
15	-	-	-	-	-	-	-	-	-	-	-	-	1.28		5.46	1.84		5.66	-	-	-	-	-	-	-	

													1.21	8.31 8.76	5.92	1.96	13.91 14.44	5.97							
	1.93	1.75	0.40	0.60	5.02	2.51	1.63	1.58	0.55	0.84	4.88	2.88													
17	2.06	1.76	0.21	0.67	4.81	2.60	0.50	0.81	0.43	0.64	4.14	2.82	-	-	-	-	-	-	-	-	-	-	-	-	-
	2.68	2.10	0.23	0.61	5.39	3.07	0.87	1.20	0.48	0.75	4.59	2.53													
	2.86	2.62	0.22	0.95	3.85	2.19	0.53	0.68	0.38	0.81	3.79	2.57													
21	1.88	1.73	0.19	0.93	4.14	2.39	0.94	1.05	0.37	0.69	3.80	2.49	-	-	-	-	-	-	-	-	-	-	-	-	-
	2.09	1.59	0.12	0.68	4.25	2.61	0.69	0.72	0.35	0.63	3.89	2.27													
	2.57	1.69	0.14	0.45	3.51	2.12	1.00	0.93	0.28	0.81	3.56	2.29													
24	2.21	1.33	0.13	0.39	3.80	2.46	1.00	0.81	0.28	0.95	3.62	2.26	-	-	-	-	-	-	-	-	-	-	-	-	-
	2.79	2.25	0.22	0.51	3.66	2.37	0.52	0.51	0.25	0.64	3.21	1.90													
	2.09	2.61	0.35	1.20	3.63	2.33	0.49	0.54	0.26	0.52	3.27	1.99													
28	1.33	1.44	0.19	1.12	3.87	2.43	0.30	0.58	0.27	0.54	3.16	2.05	-	-	-	-	-	-	-	-	-	-	-	-	-
	1.94	2.01	0.39	0.81	3.74	2.51	0.31	0.51	0.19	0.56	3.21	1.98													

Table 6-2 Concentration of the analytes in the fabric samples for the persistence study

		Concentration (μM)																
Environmental temperature		Room temperature								Fridge				Freezer				
Recipient fabric		Cotton				Polyester				Cotton				Cotton				
Compound		Linalool	Geraniol	Eugenol	Ethylene brassylate	Linalool	Geraniol	Eugenol	Ethylene brassylate	Linalool	Geraniol	Eugenol	Ethylene brassylate	Limonene	Linalool	Geraniol	Eugenol	Ethylene brassylate
		2.5	42.4	58.4	137.8	1.8	9.8	14.0	23.4	14.3	94.7	46.6	67.8	37.7	26.8	170.2	72.6	85.9
	0.04	2.2	36.9	62.8	129.0	2.7	12.1	13.0	25.6	14.9	104.4	55.4	78.5	33.0	25.7	188.1	76.6	95.0
		2.6	59.7	79.4	143.9	2.4	11.9	12.7	30.0	16.4	99.5	51.1	66.0	60.4	33.1	167.6	63.4	74.4
		1.4	10.7	13.8	96.8	1.7	7.4	6.3	21.4									
	0.33	2.8	13.6	16.0	80.6	1.6	6.6	6.9	14.8	-	-	-	-	-	-	-	-	-
		2.6	9.8	9.6	70.3	2.3	8.4	7.0	17.2									
										1.4	54.2	70.9	89.8	123.8	10.0	176.4	109.7	112.5
Persistence time (d)	**1**	-	-	-	-	-	-	-	-	1.2	58.4	84.2	106.9	116.0	7.2	149.4	96.9	91.7
										1.6	70.1	96.5	102.7	233.6	15.1	261.5	147.9	107.0
		1.8	8.6	9.2	66.9	2.3	4.3	4.8	12.3									
	3	2.3	8.2	6.1	53.7	2.0	4.1	4.5	15.2	-	-	-	-	-	-	-	-	-
		2.5	7.1	4.8	38.7	1.8	3.4	4.8	15.5									
										0.8	17.3	46.5	88.6					
	4	-	-	-	-	-	-	-	-					-	-	-	-	-
										0.7	11.8	39.4	77.6					

									0.8	18.2	66.2	109.9							
	1.5	4.8	5.1	153.1	2.7	5.8	7.6	17.4											
5	1.6	5.1	4.8	121.6	1.2	2.4	5.2	14.5	-	-	-	-	-	-	-	-	-		
	1.7	7.4	8.9	175.0	2.1	4.3	7.3	25.8											
	3.0	6.1	4.9	87.6	3.4	5.8	5.9	14.8											
7	3.6	9.4	9.3	141.1	3.6	7.9	7.0	20.8	-	-	-	-	-	-	-	-	-		
	2.4	5.8	5.7	128.8	1.9	4.3	5.3	18.8											
	1.2	4.1	5.7	137.4	1.4	3.5	5.6	10.9	0.3	4.1	30.3	136.4	202.2	10.0	102.2	96.0	144.6		
10	1.4	3.5	4.5	115.6	1.0	2.5	5.0	14.6	0.5	8.1	39.8	87.0	230.1	11.3	151.6	134.4	158.0		
	1.3	3.6	3.9	102.5	1.8	3.8	5.4	13.3	0.5	8.1	31.6	104.5	178.7	13.6	203.4	182.3	171.1		
	1.1	3.6	3.4	76.6	1.0	1.3	2.8	7.7											
14	1.5	4.0	3.5	84.8	1.4	2.5	4.3	10.1	-	-	-	-	-	-	-	-	-		
	1.5	3.7	3.0	72.6	1.1	1.7	3.5	9.1											
									0.4	6.3	25.8	48.8							
15	-	-	-	-	-	-	-	-	0.4	4.8	27.0	66.5	-	-	-	-	-		
									0.4	4.9	27.6	67.2							
	1.6	2.9	4.6	50.1	2.6	2.9	3.1	8.6											
17	1.7	3.0	2.3	53.8	1.1	1.8	2.5	6.6	-	-	-	-	-	-	-	-	-		
	2.0	3.2	2.1	42.3	1.6	2.3	3.1	8.8											
	3.0	5.4	2.8	85.1	1.2	1.6	2.4	9.3											
21	1.8	3.4	2.2	77.7	2.0	2.5	2.5	8.2	-	-	-	-	-	-	-	-	-		

	2.0	3.1	1.2	54.8	1.5	1.7	2.5	8.2											
	3.0	3.9	1.8	44.9	2.2	2.3	2.0	10.3											
24	2.4	2.9	1.4	33.5	2.2	2.0	2.1	12.3	-	-	-	-	-	-	-	-	-		
	3.1	4.9	2.6	45.7	1.4	1.4	2.2	9.9											
	2.3	5.7	4.3	97.5	1.3	1.5	2.1	7.7											
28	1.4	3.1	2.2	88.9	0.9	1.6	2.1	7.8	-	-	-	-	-	-	-	-	-		
	2.1	4.4	4.6	66.6	0.9	1.4	1.6	8.4											

Table 6-3 Weight of the recipient fabric samples (g) for the persistence study

		Fabric weight (g)			
Environmental temperature		Room temperature		Fridge	Freezer
Recipient fabric		Cotton	Polyester	Cotton	Cotton
		0.25	0.21	0.24	0.24
	0.04	0.27	0.24	0.25	0.27
		0.25	0.23	0.21	0.27
		0.21	0.21		
	0.33	0.27	0.24	-	-
		0.27	0.23		
				0.23	0.28
	1	-	-	0.24	0.25
				0.25	0.27
Persistence time (d)		0.27	0.25		
	3	0.24	0.20	-	-
		0.22	0.23		
				0.24	
	4	-	-	0.25	-
				0.21	
		0.27	0.23		
	5	0.24	0.21	-	-
		0.27	0.20		
	7	0.25	0.23	-	-

	0.25	0.27		
	0.26	0.28		
	0.26	0.23	0.25	0.26
10	0.27	0.22	0.26	0.24
	0.28	0.22	0.27	0.24
	0.28	0.20		
14	0.22	0.23	-	-
	0.21	0.19		
			0.26	
15	-	-	0.24	-
			0.27	
	0.24	0.22		
17	0.21	0.22	-	-
	0.22	0.22		
	0.23	0.20		
21	0.25	0.21	-	-
	0.25	0.22		
	0.26	0.19		
24	0.24	0.21	-	-
	0.25	0.22		
	0.22	0.19		
28	0.24	0.23	-	-
	0.28	0.24		

Table 6-4 The recoveries (average ± standard deviation (n=3)) of the four VOCs of interest for the three environmental conditions studied from the persistence study

		Concentration (µM/g)															
Environmental temperature		Room temperature								Fridge				Freezer			
Recipient fabric		Cotton				Polyester				Cotton				Cotton			
Compound		Linalool	Geraniol	Eugenol	Ethylene brassylate	Linalool	Geraniol	Eugenol	Ethylene brassylate	Linalool	Geraniol	Eugenol	Ethylene brassylate	Linalool	Geraniol	Eugenol	Ethylene brassylate
Persistence time (d)	**0.04**	9.6 ± 1.2	181.7 ± 52.1	261.3 ± 48.9	534.8 ± 50.9	10.0 ± 1.4	49.5 ± 2.6	58.6 ± 7.0	116.1 ± 12.7	65.7 ± 10.6	428.6 ± 40.6	219.6 ± 24.7	303.7 ± 18.3	109.7 ± 13.8	675.5 ± 47.8	273.8 ± 34.9	328.4 ± 46.1
	0.33	8.7 ± 2.0	45.8 ± 8.2	53.5 ± 16.0	340.0 ± 106.6	8.4 ± 1.6	33.1 ± 4.9	29.8 ± 0.8	79.4 ± 20.4	-	-	-	-	-	-	-	-
	1	-	-	-	-	-	-	-	-	5.9 ± 0.8	253.1 ± 24.0	348.4 ± 38.9	415.6 ± 27.8	40.1 ± 14.0	732.0 ± 205.4	442.4 ± 91.2	388.4 ± 18.8
	3	9.1 ± 2.4	32.8 ± 1.0	27.1 ± 6.4	215.8 ± 36.7	9.0 ± 1.0	17.6 ± 3.0	20.9 ± 1.6	64.2 ± 13.6	-	-	-	-	-	-	-	-
	4	-	-	-	-	-	-	-	-	3.4 ± 0.5	68.7 ± 20.0	222.0 ± 82.5	401.1 ± 110.0	-	-	-	-
	5	6.1 ± 0.7	22.1 ± 4.9	24.0 ± 7.8	573.9 ± 71.0	9.5 ± 3.2	19.4 ± 7.1	31.5 ± 5.9	72.5 ± 4.5	-	-	-	-	-	-	-	-
	7	12.0 ± 2.6	28.2 ± 8.4	26.2 ± 9.7	470.0 ± 109.2	11.7 ± 4.1	23.3 ± 7.2	23.5 ± 4.1	69.5 ± 6.6	-	-	-	-	-	-	-	-
	10	4.8 ± 0.3	13.8 ± 1.5	17.6 ± 4.1	440.8 ± 81.9	6.3 ± 1.8	14.7 ± 3.0	23.8 ± 1.0	58.2 ± 9.8	1.7 ± 0.4	25.8 ± 8.3	130.3 ± 19.8	433.9 ± 158.1	47.3 ± 9.1	624.1 ± 227.4	562.9 ± 195.3	642.5 ± 79.6

14	5.9 ± 1.9	16.2 ± 2.8	14.1 ± 2.0	334.8 ± 56.7	5.5 ± 0.6	8.8 ± 2.1	17.0 ± 2.6	43.4 ± 4.5	-	-	-	-	-	-		-	-		
15	-	-	-	-	-	-	-	-	1.5 ± 0.2	20.7 ± 3.1	104.7 ± 6.9	238.0 ± 45.7	-		-		-		-
17	8.0 ± 1.3	13.7 ± 1.4	13.4 ± 5.2	219.1 ± 33.2	7.9 ± 3.5	10.5 ± 2.6	13.0 ± 1.6	36.3 ± 5.4	-	-	-	-	-		-		-		-
21	9.5 ± 3.1	16.6 ± 6.1	8.6 ± 3.8	300.1 ± 75.9	7.5 ± 1.8	9.1 ± 2.3	11.7 ± 0.4	40.8 ± 4.9	-	-	-	-	-		-		-		-
24	11.2 ± 1.3	15.6 ± 3.8	7.8 ± 2.2	165.1 ± 22.6	9.4 ± 2.8	9.4 ± 2.9	10.1 ± 0.5	52.6 ± 6.8	-	-	-	-	-		-		-		-
28	8.0 ± 2.4	18.1 ± 7.0	15.1 ± 5.3	350.5 ± 104.1	4.8 ± 1.7	7.0 ± 1.0	9.0 ± 2.4	36.4 ± 3.7	-	-	-	-	-		-		-		-

6.2.1. Room temperature

For the room temperature experiments two fabrics were tested to assess persistence on both natural (cotton) and synthetic (polyester) fibres.

6.2.1.a Room temperature - cotton

When the VOCs were transferred from cotton to cotton and the secondary fabric was left to dry at room temperature, it was observed that geraniol and eugenol followed a typical decay curve (Figure 6-1), with the highest recoveries obtained for the shortest time studied of 1 h (0.04 d) persistence time; at 8 h (0.33 d) their recoveries diminished by a factor of 4 and 5 respectively, followed by a steady, but slower decay. For linalool and ethylene brassylate, the trends were not as clear. Ethylene brassylate exhibited an initial decrease in concentration from 1 h to 3 d persistence time, followed by a spike at 5 d, and then a more regular decay curve of slower loss.

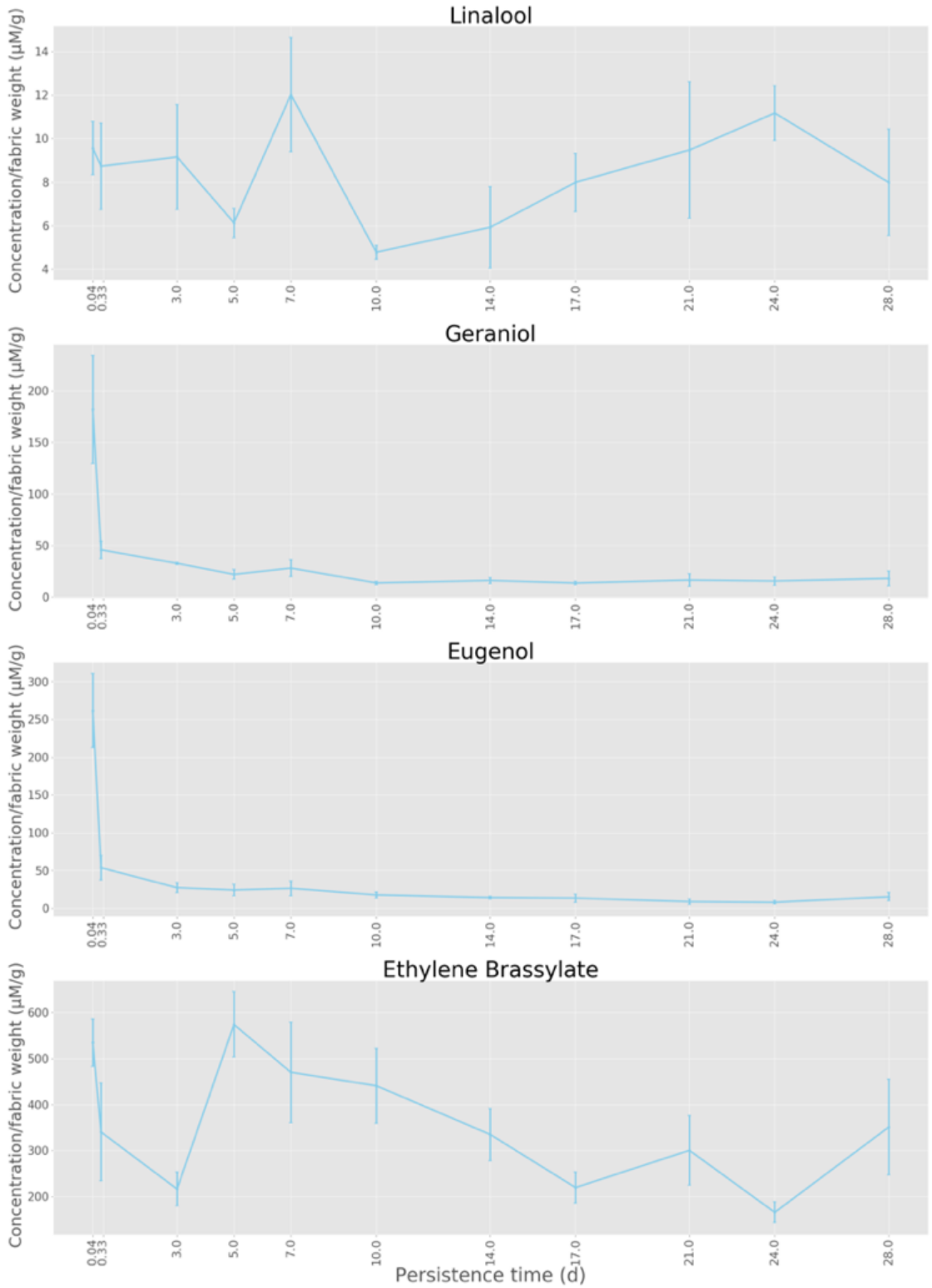

Figure 6-1 Persistence of four transferred VOCs on cotton at room temperature for periods between 1 h and 28 d. The error bars are standard deviations (n = 3). The persistence for geraniol and eugenol follows an exponential decay curve, and the persistence trend for linalool and ethylene brassylate is not as defined

6.2.1.b Room temperature - polyester

For the polyester samples left to dry at room temperature, the results were very similar to the cotton samples, with linalool displaying a rather undefined pattern and geraniol and eugenol following a trend similar to a decay curve (Figure 6-2).

However, geraniol and eugenol were released at a faster rate from cotton than from polyester. For polyester, the initial concentration at 1 h was only about two times higher than at 8 h (49.5 μM/g in comparison to 33.1 μM/g for geraniol, and 58.6 μM/g in comparison to 29.8 μM/g for eugenol) (Table 6-4). On the other hand, for cotton, the initial concentration at 1 h for these two compounds was about 4-5 times higher than at 8 h (181.7 μM/g in comparison to 45.8 μM/g for geraniol, and 261.3 μM/g in comparison to 53.5 μM/g for eugenol). Lastly, ethylene brassylate at 1 h persistence time was recovered in the highest amounts, unlike in in the cotton samples, where the recovery was high at 1 h, but also after 5 d (Figure 6-2).

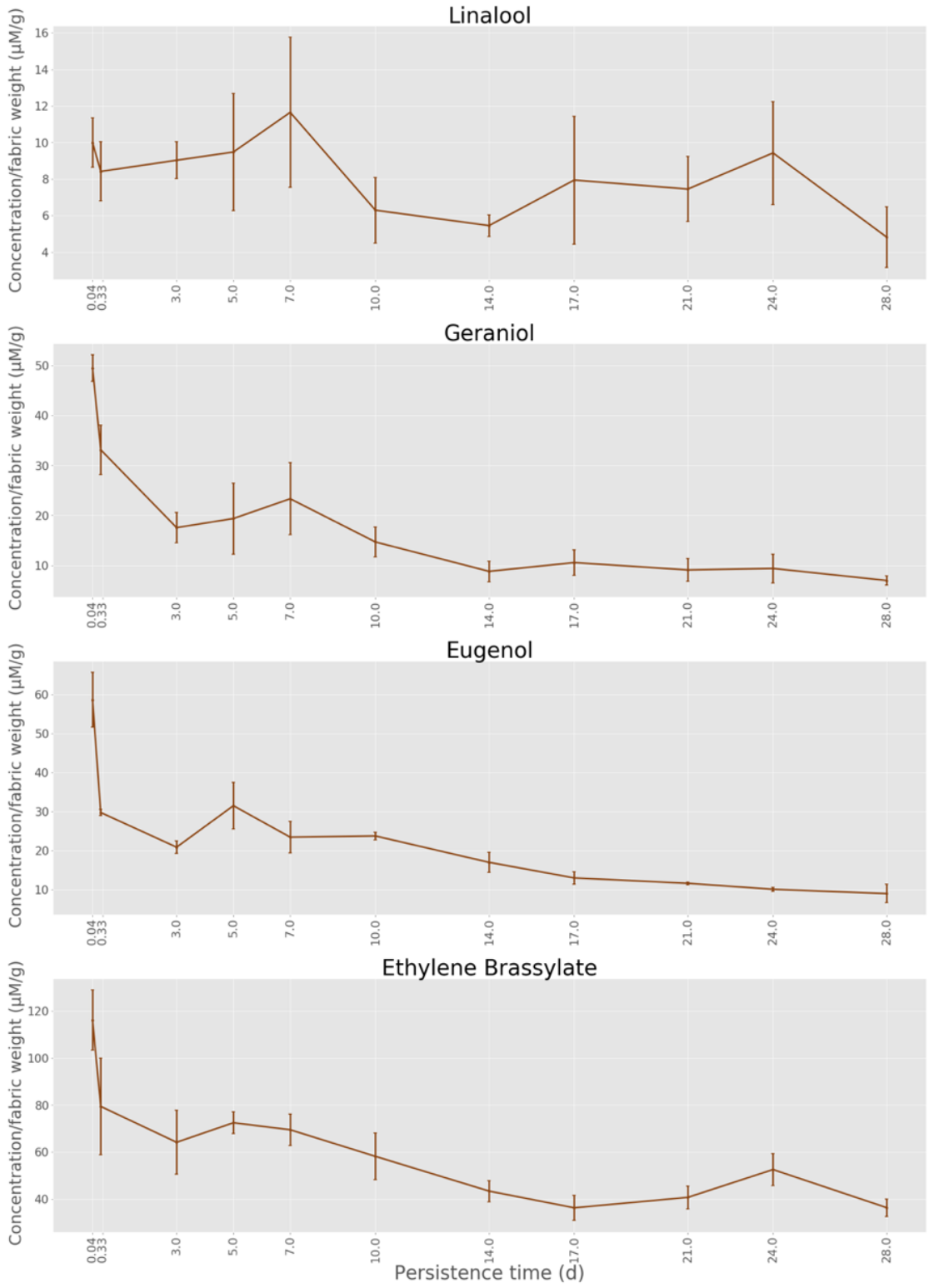

Figure 6-2 Persistence of four transferred VOCs of interest on polyester at room temperature for periods between 1 h and 28 d. The error bars are standard deviations (n = 3). The persistence for geraniol, eugenol and ethylene brassylate follow a decay curve-like trend, and the persistence trend for linalool is not as defined

6.2.1.c Room temperature - cotton and polyester

When compared alongside the results for cotton and polyester at room temperature (Figure 6-3), it can be observed that with the exception of linalool, higher amounts of VOCs were obtained generally from cotton (Figure 6-3,light blue), especially for geraniol (181.7 μM/g in comparison with 49.5 μM/g) and eugenol (261.3 μM/g in comparison with 58.6 μM/g) at persistence of 1 h and for ethylene brassylate at all persistence times (Table 6-4). Linalool, despite not displaying a clear trend for each material, when compared together the trends were similar, with a peak at 1 h, at 7 d, and at 24 d (Figure 6-3).

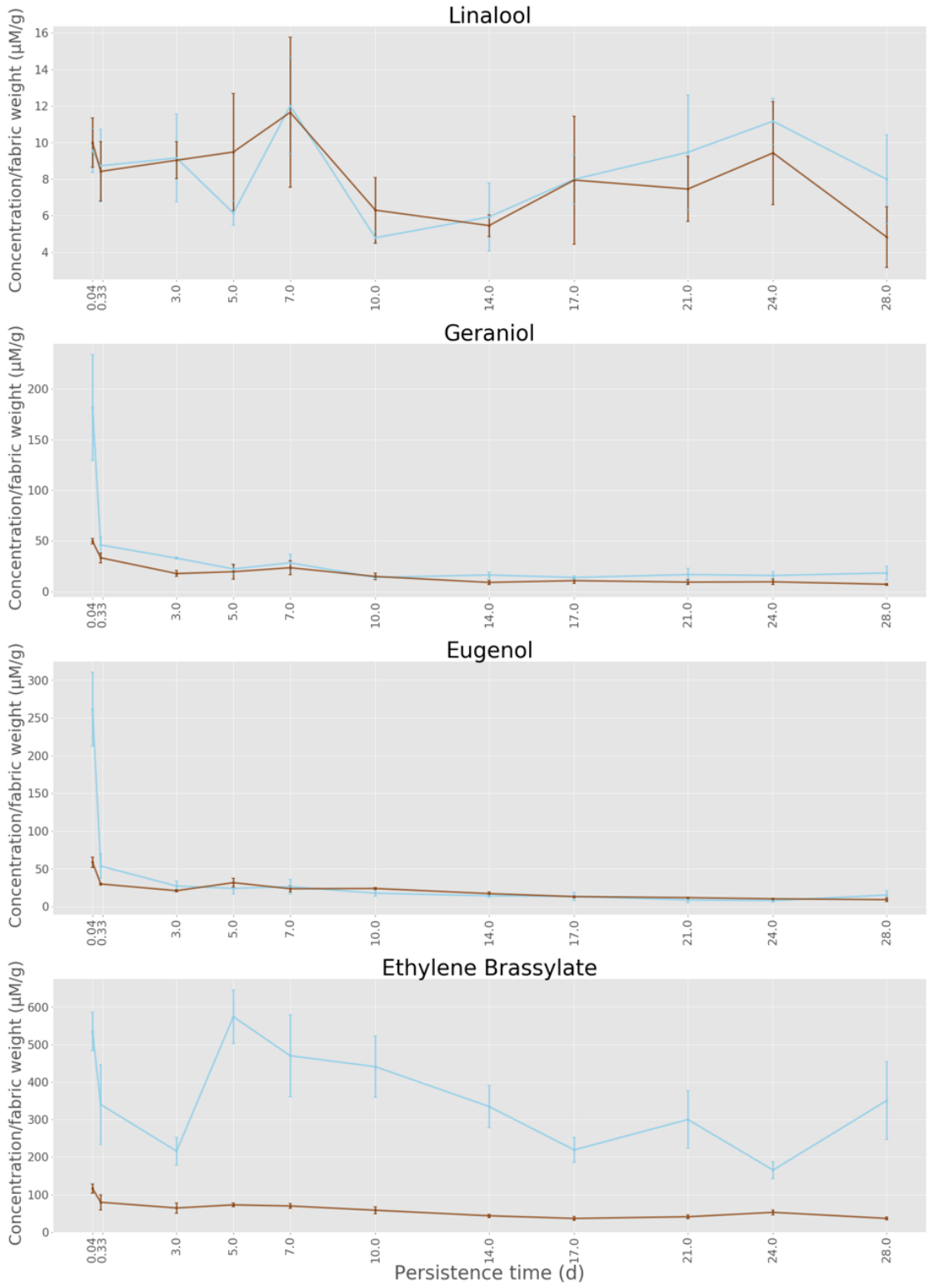

Figure 6-3 Persistence of four transferred VOCs of interest on cotton (light blue) and polyester (brown) at room temperature for periods between 1 h and 28 d. The error bars are standard deviations (n = 3). The persistence trend for linalool is similar between the two materials, and the persistence of geraniol, eugenol, and ethylene brassylate is generally higher for shorter persistence times

6.2.2. Fridge temperature

For the cotton samples with transferred VOCs left in a fridge, due to time constrictions, the experiment was carried out for a shorter time frame, from 1 h to 15 d. The results showed that for linalool, the most volatile compound out of the 4 VOCs, there was a considerably higher amount determined at 1 h (65.7 μM/g) compared to the other persistence times (>5.9 μM/g) (Table 6-4). However, for 10 and 15 d persistence time, the limonene concentration reported is estimated as it was below the lower limit of quantification (Table 6-4). Geraniol exhibited a decay curve too, albeit with a more gradual decrease (Figure 6-4). Eugenol displayed an initial increase in concentration from 1 h to 1 d persistence time (from 219.6 μM/g to 384 μM/g), after which the concentration steadily decreases up to 15 d (104.7 μM/g) (Table 6-4). For ethylene brassylate, the trend was not very clear (Figure 6-4).

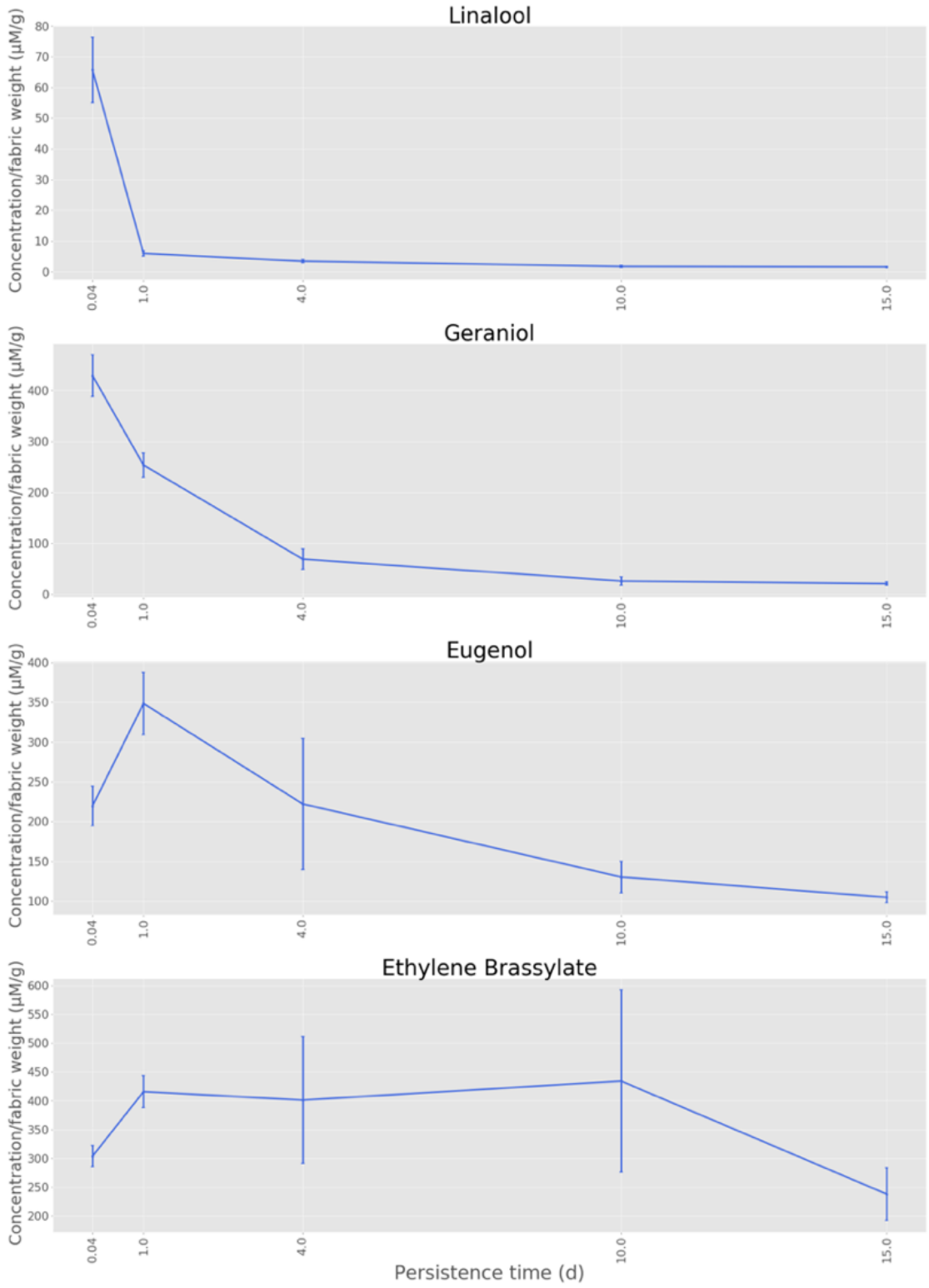

Figure 6-4 Persistence of four transferred VOCs of interest on cotton at fridge temperature for periods between 1 h and 15 d. The error bars are standard deviations (n = 3). The persistence for linalool and geraniol follow a decay curve trend, and the persistence trend for ethylene brassylate is not as defined

6.2.2.a Fridge temperature and room temperature

When comparing the room and fridge conditions, higher amounts of linalool, geraniol, and eugenol were generally recovered for the samples kept in the fridge (dark blue) compared to the samples left at room temperature (light blue) (Figure 6-5). This was especially true for shorter persistence times for linalool (at 1 h, 65.7 μM/g in comparison with 9.6 μM/g) and geraniol (at 1 h, 428.6 μM/g in comparison with 181.7 μM/g), and for all persistence times studied for eugenol with the exception of the shortest time of 1 h (Table 6-4). The concentrations determined for ethylene brassylate were consistent between the two temperatures (Figure 6-5).

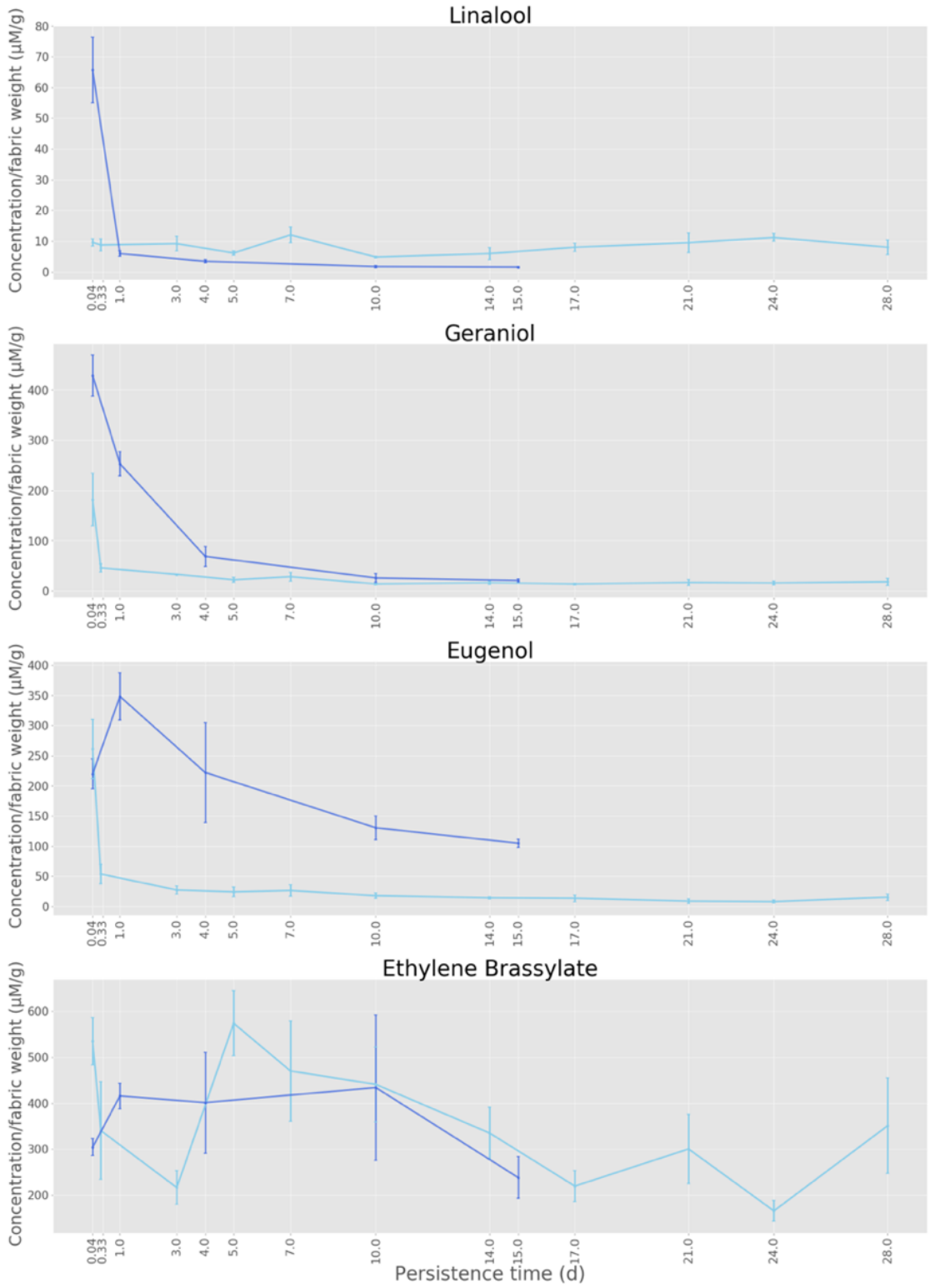

Figure 6-5 Persistence of four transferred VOCs of interest on cotton at room temperature (light blue) and fridge temperature (dark blue) for periods between 1 h and 28 d. The error bars are standard deviations (n = 3). Generally higher amounts of VOCs were obtained from the samples kept in the fridge compared to the samples kept at room temeprature. The recoveries for ethylene brassylate are similar between the two temperatures

6.2.3. Freezer temperature

For the freezer temperature, three persistence times were studied: 1 h, 1 d and 10 d. Whilst for the room temperature and fridge experiments, the two most volatile compounds, pinene and limonene were recovered in quantities below the lower limit of the calibration range, for the freezer experiment limonene was successfully recovered, and pinene was above the limit of detection. Figure 6-6 displays the gas chromatograms for pinene and for limonene at 1 h, 1 d and 10 d persistence time at the three different experimental temperatures. It can be observed how the chromatographic peaks for the freezer sample (green) are considerably higher than for the other two environmental conditions. Moreover, when looking at the y axis, representing the peak height, for pinene this value is expressed in kCps (kilo Counts per second) or MCps (Mega Counts per second) and for limonene in GCps (Giga Counts per second). What is even more interesting, is that for both analytes, the amount recovered increased with persistence time, for example the peak height for limonene at 1 h was c. 1.25 GCps and at 10 d c. 2.5 GCps.

This observed effect, where considerably higher amounts of VOCs are recovered for the samples kept at freezer temperature in comparison to the sample kept at fridge or room temperature, was not encountered for any of the other VOCs. For ethylene brassylate, the recoveries did not considerably differ between the three temperatures sampled at 1 h, 1 d or 10 d persistence time (Table 6-4). For linalool, geraniol and eugenol, the lowest amounts of VOCs were generally recovered for the samples with transferred perfume kept at room temperature (Table 6-4).

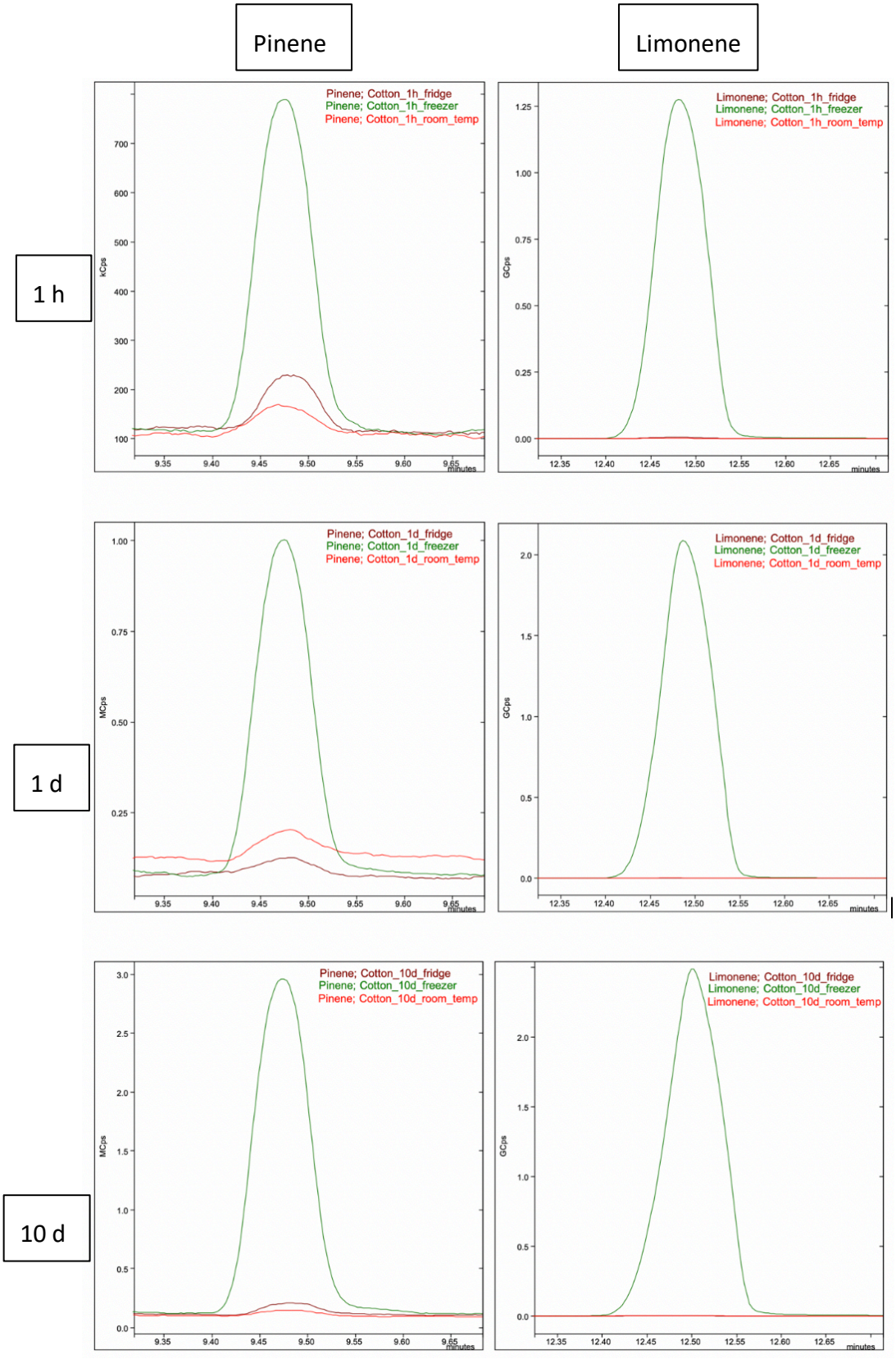

Figure 6-6 Gas chromatograms of pinene (left side) and limonene (right) at 1 h (top), 1 d (middle) and 10 d (bottom) persistence time for three environmental conditions: room temperature (red), fridge (brown) and freezer (green). Pinene and linalool were recovered in considerably higher amounts from the samples left in the freezer compared to the samples kept in the fridge and at room temperature

6.3. Discussion

This is the first empirical study addressing the persistence of transferred fragrance VOCs on clothing for forensic applications. With casework application in mind, and based on previous research addressing the persistence of VOCs and other forms of trace material transferred to clothing (Obendorf et al., 2006; Pounds and Smalldon, 1975c; Bull et al., 2006), as well as work from chapter 5 looking at the transfer of VOCs between clothing, three variables were investigated to assess the persistence of transferred fragrance VOCs: environmental temperature (room, fridge, and freezer temperature), fabric type (cotton and polyester) and persistence time (1 h to as long as 28 d).

6.3.1. Room temperature

Generally, with longer persistence times, lower amounts of VOCs were recovered from the fabric samples left at room temperature, as the compounds volatilise and evaporate from the fabrics. Geraniol and eugenol displayed typical exponential decay curves, with a high initial recovery followed by a rapid loss in recovery and then a steadier loss. This behaviour is consistent with the published literature for the persistence of forensic traces, such as fibres (Pounds and Smalldon 1975b), pollen and flint (Bull et al., 2006) on fabrics .

In terms of persistence of different VOCs on fabrics, Obendorf et al. (2006) showed that compounds with lower vapour pressure resulted in increased retention on the two fabrics studied, cotton and polyester. This observation is consistent with the results in this current study, as it can be noted for example in the y scale from Figure 6-3, as well as in the inability to quantify the two compounds with the highest vapour pressure.

Obendorf et al. (2006) also found that the recoveries of the VOCs at 70 min did not differ as much between cotton and polyester as they did at 480 min. At the extended time, the VOCs were released at a faster rate from polyester than from cotton (Obendorf et al., 2006). However, in this present study, for the shortest time of 1 h, higher amounts of VOCs were consistently recovered from cotton compared to polyester, with the exception of linalool, where the recoveries were similar between the two fabrics (Figure 6-3). This behaviour has been previously observed for the persistence on fabrics of other

traces, such as hair, pollen, and fibres (Dachs, McNaught and Robertson, 2003; Bull et al., 2006; Lepot et al., 2015). For example, in a study focused on the persistence of human scalp hair on fabrics for times as long as 8 h, it was demonstrated that with the exception of wool, the greatest persistence of hair was observed on a cotton t-shirt (maximum of around 5 h) and the shortest persistence on a polyester top (maximum 1 h) (Dachs, McNaught and Robertson, 2003).

6.3.2. Fridge temperature

There are currently no published studies comparing the persistence of VOCs on fabrics at various temperatures for forensic science applications. The findings from this study showed that generally, the VOCs from the fabric samples were preserved better at fridge temperature than at room temperature, so that higher recoveries were obtained for the extracted VOCs (Figure 6-5). The approximately 20 °C cooler temperatures between the fridge and room conditions helped reduce the evaporation of VOCs, especially the VOCs with higher volatility such as linalool and geraniol, at shorter persistence times. These lower boiling point compounds are more sensitive to environmental conditions. For ethylene brassylate, the least volatile compound, there were no major differences in the obtained recovery for the two temperatures. These findings demonstrate the value of storing garments that have been seized in suspected assault cases in fridge conditions. The lower temperature can help preserve the samples until instrumental analysis.

6.3.3. Freezer temperature

Pinene and limonene are the compounds with the lowest boiling point from this study, and in turn with the highest volatility. The freezer temperature was the only temperature that allowed the recovery of limonene for all three persistence times tested at amounts above the lower range of the calibration curve and of pinene above the limit of detection (Figure 6-6). Moreover, longer persistence times led to higher recoveries for both analytes.

Whilst the fridge temperature experiment demonstrated that cooler temperatures of 2 - 4 °C help increase the recovery of compounds such as linalool and geraniol, the freezer experiment showed that temperatures as low -28 °C led to the recovery of the

compounds with even higher volatility. It might be assumed that compounds with vapour pressure in the range of the compounds from this study ($4.4E^{-7}$ - 2.1 mmHg at 23.5 °C) will have similar behaviour under the studied storage conditions. These findings demonstrate the utility of storing garments that have been seized in suspected assault cases in freezer conditions over fridge conditions where possible. The lower temperature can help preserve the samples until instrumental analysis.

6.4. Conclusions

The persistence behaviour of six VOCs on a secondary fabric was examined. All the factors studied, persistence time, fabric type, and environmental temperature affected how the volatile compounds persisted on a secondary piece of fabric after transfer.

The persistence time of transferred VOCs on fabrics is a crucial factor in the potential to recover these compounds. In a sexual assault case, there is often a period of time from the crime taking place until an exhibit is recovered and the laboratory analysis is undertaken. The findings from this study showed that the highest recoveries are generally obtained for shorter persistence times of up 1 d. It was found that it was possible to successfully recover four of the six transferred VOCs from fabrics even after as long as 28 d since the initial fragrance transfer. Therefore, these results show the applicability of this technique for forensic cases such as sexual assault, even when the crime is reported weeks after it has been committed. This is especially important as a Home Office report from 2007 (Feist et al., 2007) found that 46% of rapes were reported on the same day on which they occurred, 23% between 1 d to 1 week, 17% between 1 week to 6 months, and only 14% after more than 6 months. However, in line with other forms of trace materials, the length of persistence also raises questions on how to discern materials transferred pre-, syn- and post- forensic event and provide evaluative interpretations regarding activity level propositions.

The fabric medium onto which the perfumes were transferred and then persisted, led to differences in VOCs recovery especially for shorter timeframes. At 1 h persistence time, considerably higher amounts of VOCs were generally recovered from cotton compared to polyester. This trend where cotton enables higher recoveries compared to polyester has been previously reported in the published forensic science literature for

the persistence of multiple traces such as hair, pollen, and fibres (Dachs, McNaught and Robertson, 2003; Bull et al., 2006; Lepot et al., 2015). After 1 h, similar quantities of VOCs were recovered from both fabrics.

Temperature had a greater effect in recoverability especially for the VOCs with lower boiling points. For temperatures between 4 and 22 °C, the recovered quantity of the two most volatile compounds was below the lower limit of the calibration range, whilst for the other four VOCs, the shorter the time frame the higher the recovery of VOCs from fabrics. For temperatures of approximately -28 °C, an additional fifth VOC, limonene, was also successfully recovered.

In conclusion, the findings from this study have confirmed that transferred VOCs can persist on different fabrics at different environmental temperatures for times as long as 28 d. For fragranced fabrics kept at temperatures between 2 and 22 °C, medium and low volatility compounds are most likely to be recovered and to be of value in forensic investigations. For samples kept at freezing temperatures of approximately -28 °C, high volatility compounds may also be recoverable. These data provide additional support for the potential of being able to use fragrance analysis in the investigation and detection of sexual assault cases, especially for the cases where the clothing is available for analysis even up to a month after the assault took place.

7. Summary and conclusions

7.1. Outline

This chapter provides an evaluation of the main research findings from the three experimental chapters from this thesis that address: the development of an analytical method able to recover fragrance traces from fabrics, the transfer of fragrances between clothing, and the persistence of transferred fragrance on clothing. Key contributions to the field are then discussed, with a consideration of limitations and opportunities for further development.

7.2. A summary of findings

As highlighted in Chapter 1, the aim of this thesis was to develop the empirical foundation on the ability of fragrance volatile organic compounds (VOCs) to be a form of trace. More specifically, this research sought to understand the process through which fragrance VOCs transfer and persist between and on fabrics. Thereby, experimental studies were carried out where specific variables (fabric type, contact time, environmental conditions) were investigated. To enable these experimental studies addressing transfer and persistence, first an investigation into the optimal instrumental conditions for the recovery and analysis of VOCs from fabrics was carried out.

7.2.1. Research question 1: Method development for the analysis of fragrance traces from clothing (Chapter 4)

A combination of headspace solid phase micro-extraction (HS-SPME) and gas chromatography-mass spectrometry (GC-MS) was used for the analysis of VOCs. It was determined that out of the five SPME fibres examined for the extraction of five VOCs (limonene, linalool, geraniol, eugenol, and coumarin) and of a commercially available perfume, the 50/30 µm DVB/CAR/PDMS was the optimum fibre overall, both in terms of the amounts of VOCs extracted and the reproducibility between samples (Table 4-1). The CAR/PDMS and PDMS/DVB fibres were the next best performers, showcasing how fibres made of multiple phases are better suited for the analysis of fragrance VOCs.

Using the DVB/CAR/PMDS fibre for the analysis of fragrance mixture spiked onto a cotton fabric, three different SPME extraction conditions were investigated simultaneously, namely incubation time, extraction time, and extraction temperature, using a central composite design. It was determined that the optimal extraction values were an incubation time of 5 min, an extraction time of 60 min, and an extraction temperature of 58 ˚C, where the extraction time was the only significant factor (p=0.001).

Using these conditions, the method was validated and the main figures of merit of the proposed method (calibration range, limits of detection and quantification, trueness, and precision) showed that the method was fit for the proposed application. The validated method was further successfully used for the quantification of commercial perfumes diluted as much as 1500 times and spiked onto cotton and polyester, showing considerably higher sensitivity compared to a previously documented method in the published literature.

The HS-SPME GC-MS method provides a robust, simple and sensitive method for the analysis of traces of fragrance VOCs from clothing that can be used to obtain an insight into the transfer and persistence of these compounds between/on fabrics. This has important implications for forensic science laboratories, as it uses an instrument commonly found in these labs, it has a short preparation and analysis time, which allows more samples to be analysed and to provide quick turnaround times.

7.2.2. Research question 2: Transfer of fragrances between clothing (Chapter 5)

Using this validated SPME GC-MS method, experimental data was obtained on key factors thought to affect the transfer of VOCs between fabrics. The factors were chosen based on previous studies for other forms of trace evidence, especially based on human scent research. As such these factors included perfume ageing time, contact time, and fabric type.

For the perfume ageing time, that is the period of time that a fragrance has been on a donor fabric prior to transfer, it was identified that the shorter this time period, the higher the amounts of VOCs recovered from the secondary fabric. VOCs were recovered

from the secondary fabric even after they have been aged on the primary fabric for as long as two days.

For the contact time, that is the period of time that two fabrics were rubbed against each other using a crockmeter, it was determined that the amount of analyte recovered from the secondary fabric was consistent for times of up to less than 5 min, after which the recovery decreased. This decrease in recovery at 10 min was attributed to the heat generated by the continuous friction between fabrics resulting in increased volatility and therefore loss of compounds.

For the fabric type experiment, clear differences were observed in the behaviour of the VOCs tested when they were transferred onto cotton and onto polyester. Two distinctive groups were determined by the PCA based on the two recipient fabrics. Additionally, a PLS discriminant analysis model was able to discriminate between the two recipient fabric samples with a R^2 value of 96% (Figure 5-11). The differences between the fabrics employed as donor fabrics were not as clear as for the recipient fabrics.

An observation drawn from all three transfer studies was that VOCs with higher volatility were recovered in lower amounts. For example, in the contact time experiment, where the recovery for each analyte was consistent for contact times of up 5 min, there were differences in the recovery % between analytes. So that, pinene and limonene, the first and second most volatile compounds in this study, were recovered at c. 0.4-0.5% and 1.2-1.3% compared to a spiked sample, whilst the other 4 VOCs with lower volatility were recovered at c. 18-32% (Figure 5-4).

7.2.3. Research question 3: Persistence of fragrances on fabrics after transfer (Chapter 6)

Following the studies addressing the transfer of VOCs between fabrics, the next step was to investigate how that transferred fragrance persists on the secondary fabrics. The experiments showed that the highest overall analyte recoveries were obtained for persistence times of up to 1 d. However, analytes were successfully recovered even for the longest persistence time studied of 4 weeks.

Further results showed that the recovery of most analytes was higher from cotton than from polyester for a persistence time of 1 h, after which the recoveries were similar. An exception was linalool, where there were no differences in recoveries, and ethylene brassylate, where considerably higher amounts were recovered from cotton for all persistence times studied (up to 4 weeks).

As seen in the transfer studies, high volatility compounds transfer into low amounts, and in turn for the persistence studies, the quantification of pinene and limonene was not possible for most persistence experiments. However, at freezer temperature limonene was successfully recovered and quantified, whilst pinene was recovered in considerably higher amounts than at room temperature and fridge temperature.

7.3. Key conclusions and contributions to the field

No other published research has investigated the optimal extraction of fragrance traces from fabrics for forensic reconstruction applications. In this thesis, a SPME GC-MS extraction method to recover fragrances from fabrics that were diluted as much as 1500 times prior to spiking of the fabrics was developed, optimised, and validated. This simple, highly sensitive, and robust method has the potential to represent a powerful approach for forensic reconstructions where perfumes have transferred between individuals, such as during assaults and sexual assaults.

The findings from the experimental studies on the transfer of fragrances between clothing has demonstrated that there is value in using this method even for assault cases where contact between clothing may have been brief, as all six VOCs were recovered in similar amounts for fabric contact times from 10 s up to 5 min. Additionally, it was highlighted that although the sooner the transfer occurs after the spiking of the donor fabric, the higher the recoveries from the recipient fabric, VOCs were recovered even when that time was as long as 2 d. This time translates into the period of time from the application of perfume on the offender's clothing to the commission of the assault, and it illustrates that recovery of analytes from a victim's clothing can be carried out even when the perfume application on the offender's clothing is not recent.

The findings from the experimental studies on the persistence of transferred fragrance from clothing showed that quantification of VOCs was successfully carried out for four out of six compounds when the persistence times were as long as 4 weeks for room temperature conditions. These results corroborate the applicability of this technique for sexual assault cases, even when the crime is reported several weeks after it has been committed. Limonene, one of the high volatility compounds for which the recovery at room temperature was not possible, was successfully quantified when the persistence happened at freezer temperature. These findings demonstrate that more information on fragrance traces can be obtained when clothing from casework is preserved in a freezer.

7.4. Limitations

In line with other forms of trace materials, the transfer and persistence of fragrance traces are clearly complex phenomena. The present studies represent the first steps into understanding the trace evidence dynamics and they have demonstrated that all variables studied, such as length of time since application, fabric type, and environmental conditions play a role in the evidence dynamics of fragrance traces. These studies managed to assess what is happening under specific conditions, and so similar VOC recovery values can only be expected for scenarios with analogous conditions. This is consistent with experimental studies for other forms of trace evidence, such as fibres and pollen.

For applications to real world interpretation, it is important to test the transfer and persistence of fragrance traces under a variety of other scenarios to better understand the variables involved. For reliable inferences on how fragrance traces arrived and persisted on clothing, and for accurate interpretation when considering activity level propositions, a case-by-case approach should be followed. The circumstances in which a crime occurs will be different from any other crime, and consequently the properties of the fragrance traces available will likely differ. Consideration of experimental variables pertinent to a specific case can contribute to the previous body of literature, in addition to obtaining a more accurate collection, analysis and interpretation of the fragrance traces pertinent to that case.

In this work, a crockmeter was employed to replicate the friction between clothing during a sexual assault. The crockmeter applies a constant force of 9 N. In reality, the pressure applied will differ from one person to another, but the use of a crockmeter provided a systematic way to generate and compare data on the transfer and persistence of fragrance traces.

7.5. Avenues for future research

There are three main areas in which further research could be examined to build upon the findings of this research. Based on the experimental studies from this work, population studies on the prevalence of fragrance traces on clothing should be carried out, followed by casework studies on the prevalence of fragrance traces on clothing from sexual assault cases. These additional data can then inform further experimental studies that will improve our understanding of the dynamics of fragrance trace transfer and persistence and will aid forensic reconstructions that involve close contact between individuals. This approach should be an iterative process as highlighted in Figure 7-1.

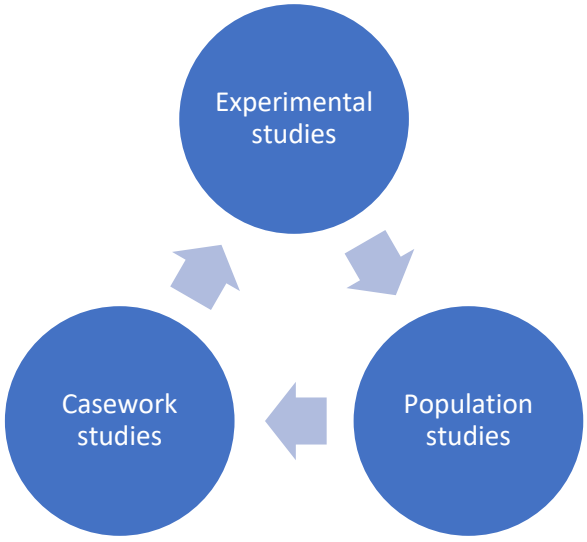

Figure 7-1 The process for a more holistic research on fragrance traces for forensic applications

The empirical findings from this thesis addressing the transfer and persistence of fragrances on clothing lay a valuable foundation in establishing the degree to which fragrances transfer and persist on clothing. One of the next steps needed will be a survey of the background quantities of fragrance VOCs on clothing of the general population in

order to gain greater insights into the prevalence of this kind of VOCs on clothing so as to assist in a more accurate interpretation of fragrance traces on clothing under source and activity level propositions. Additionally, sampling various parts of the clothing can provide an understanding of the types of amounts of VOC traces expected.

Informed by experimental and population studies, casework studies should then be carried out by examining clothing exhibits from sexual assault cases. This will allow a comparison with the studies from the general population, and thus enable better assessments of the likelihood of having fragrance traces on clothing as well as in specific clothing areas.

Analysis of exhibits from sexual assault casework can help refine experimental studies and pinpoint the next variables. For example, potential emerging research topics could be the study of more diverse VOCs, or the study of longer persistence times (beyond the four weeks study from this work).

7.6. Final conclusion

This current thesis has made the case for the potential use of fragrance traces from clothing recovered from sexual assault cases. Sexual assault has the lowest conviction rate amongst all offence types and part of the reason is a lack of the requisite evidence that the crime has occurred. Trace materials can provide valuable information for establishing contact and potential information about the timing of contact. Fragrances contain a large number of different VOCs, they are commonly used by both men and women, and they were suspected to transfer between individuals during contact. Moreover, VOCs from various matrices have been extensively analysed in published forensic literature. Nevertheless, prior to this work, there was no published research on the analysis of fragrances from clothing for forensic application.

The present experimental studies have opened up a new area of trace evidence area by developing an analytical method able to quantify fragrances that have been diluted up to 1500 times prior to spiking on clothing. This method enabled insights into how fragrances transfer to a secondary piece of clothing and how they persist. Experimental studies demonstrated that fragrances transfer between clothing for contact as short as

10 s, and even when the perfume was aged on the primary fabric for as long as 48 h before transfer took place. In terms of persistence, the findings indicated that the highest VOC amounts are generally obtained from fabric after shorter persistence times of up to 1 d, however VOCs were successfully quantified for persistence times of up to 4 weeks. This research represents a big step forward to the point of fragrance being a viable form of trace for forensic reconstruction approaches, where it can provide an additional line of enquiry that is independent of traditional traces such as fibres, DNA, etc.

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