Fragrance transfer between fabrics for forensic reconstruction applications

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Abstract

Sexual assault is a serious crime that often has low conviction rates. Recent literature has demonstrated that there is potential for fragrances to be valuable in forensic reconstructions where there has been contact between individuals. However, developing appropriate evidence bases for understanding the nature of fragrance transfer in these contexts is needed. This article presents three experiments that address the transfer process of fragrances that have been transferred from a primary piece of fabric onto a secondary piece of fabric, in a manner that could occur during an assault. 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 primary material and the recipient material). The transfer was evaluated using a validated solid phase micro-extraction gas chromatography-mass spectrometry (SPME GC-MS) method. The findings demonstrated that all three variables had an impact on the transfer of fragrances between clothing fabrics. Generally, lower volatility compounds were transferred and recovered in larger amounts than higher volatility compounds. All fragrance compounds were successfully recovered from a secondary piece of fabric even when the contact time was as short as 10 s, and even when the perfume was aged on the primary fabric for as long as 48 h. The nature of the fragrance transfer also depended on the fabric type, so that a clear discrimination was observed between the fragrance transfer that occurred onto a natural fabric (cotton) and onto a synthetic fabric (polyester).

Abbreviations:

FM (Fragrance mix)
HCA (Hierarchical cluster analysis)
PAT (Perfume ageing time)
PCA (Principal component analysis)
PLS (Partial least square)
RSD (Relative standard deviation)
SEM (Scanning electron microscope)
SPME GC-MS (Solid phase micro-extraction gas chromatography-mass spectrometry)
VOCs (Volatile organic compounds)

1. Introduction

In the last decade, the number of sexual offences reported to police in England and Wales has

increased [1]. For the year ending March 2017, the Crime Survey for England and Wales [2]

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) and 41,186 rapes (approximately 1 in 4 of the total) were

2

62 recorded by the police [2]. These incidents are at the highest level recorded since record

63 tracking started in 2002. However, many of these cases do not go to court. For example, in

64 2016, 12,572 defendants were prosecuted for sexual offences, and 7,511 were sentenced [1].

65 The c. 60% conviction rate was the lowest amongst all offence types. The disparity between

66 cases recorded by the police and those leading to a prosecution has been explained by

67 reference to various factors, including the vulnerability of victims, the relationship between

68 victim and suspect, and the lack of the requisite evidence for a crime to have occurred [3].

69 The purpose of this current study is to address this latter issue of a lack of physical evidence.

70   Clothing is often collected in the course of an investigation into sexual assault and it is

71 generally tested for various trace materials [4]. Transferred trace materials can be used to

72 reconstruct activities and can help to identify contacts between objects, people, or locations.

73 Perfumes are traces that are invisible to the eye, and are widely used by both men and

74 women. It is therefore possible that during a contact between individuals, components of

75 those fragrances can transfer. The recovery of relevant fragrance components from clothing

76 has the potential to be a form of trace evidence that can be used to assess the likelihood of a

77 contact between individuals.

78   Gherghel et al. [5] developed, optimised, and validated a method able to identify volatile

79 organic compounds (VOCs) from fabrics that had been spiked with perfumes diluted as much

80 as 1500 times in the first study to demonstrate the potential of fragrances for forensic

81 reconstructions. However, a correct identification of VOCs from fabrics is only the first step

82 towards the ability to offer a reliable interpretation of fragrance evidence from clothing [6]. A

83 second important step is undertaking studies to establish how the transfer of fragrance

84 between clothing occurs, and what the limitations to that transfer occurring can be [7].

85   The first experimental studies undertaken to understand the evidence dynamics of

86 forensic traces evidence were carried out by Pounds and Smalldon [8–10], who investigated

87 the transfer, persistence, and the mechanisms involved during the transfer of fibres between

3

88   items of clothing. In their initial study involving the transfer of textile fibres to wool and

89   acrylic clothing [8], they determined that both the fabric type of the recipient garment and the

90   force of contact affect the amount of fibres transferred, so that high pressures and coarse

91   recipient garments lead to the transfer of shorter fibres. In a further study exploring the

92   persistence of transferred fibres [9], the authors determined that the loss of transferred fibres

93   from the surface follows an exponential decay, with a rapid loss initially followed by a

94   subsequent slower loss. An initial loss of approximately 80% was observed after 4 h, which

95   increased to around 95% after 24 h.

96   Pounds and Smalldon were the first to show empirically that the transfer of trace

97   evidence is a dynamic process. They highlighted the importance of considering any potential

98   changes to the trace evidence since its formation and transfer when interpreting the presence

99   of a specific form of trace. Following their work, numerous studies have been carried out to

100 investigate the evidence dynamics of a number of different forms of trace materials, such as

101 glass [11–13], paint [14–16], hair [17–19] and pollen [4,20,21]. Similar patterns of loss to the

102 two-stage model outlined by Pounds and Smalldon [9] have been shown in these studies; for

103 example Bull et al. [4] determined a significant loss followed by a slower decay for pollen

104 grains. Their study also showed that the decay was more influenced by the recipient material

105 than the particle type when conducting experiments using pollen grains, powder and metal

106 particulates. Looking at airborne particulates, rather than solid phase particles, Prada et al.

107 [22] have shown that the highest number of volatile organic molecules and the highest scent

108 mass amounts were transferred from the hands of individuals to fabrics when cotton was used

109 as a collection material. However, there are currently no studies evaluating how the primary

110 (donor) and the recipient material type impact the transfer of volatile organic compounds

111 between clothing.

112   VOCs differentiate in their physical state from most forms of trace material, such as

113 fibres, pollen, hair, because of their high vapour pressure at ordinary room temperature. For

114   example, Hudson et al. [23] investigated the persistence of human scent VOCs collected on

115   cotton under various environmental conditions over a 7-week period and identified a change

116   in the human scent profile over time. Thus, it is paramount when designing experimental

117   studies to test the ability of VOCs to be a form of trace evidence, to investigate the effect of

118   time on the amount (number and abundance) of VOCs present at different stages in the

119   transfer and persistence process. Therefore, in addition to fabric type, this study addressed the

120   period of time that passed from the application of perfume onto the primary fabric until

121   transfer, referred to as the "perfume ageing time" [24], and the period of the time two fabrics

122   are in contact for, the "contact time" [24].

123   A full understanding of how trace materials transfer and persist is important for forensic

124   reconstruction purposes. Establishing an empirical evidence base upon which to draw

125   conclusions about the activities of a particular form of trace material is critical for a

126   transparent and robust interpretation of what the evidence means in a specific case [6].

127   Availability of empirical data on the dynamics of trace materials can aid the forensic scientist

128   at a crime scene, in the laboratory, and during the interpretation process.

129   Given the importance of understanding the changes that happen to trace materials

130   throughout the forensic process, and how that information can offer insights to inform the

131   effective collection, analysis, and interpretation of that evidence, this current study presents

132   the findings from a series of experimental studies investigating the transfer of fragrances

133   between clothing materials. The three variables studied were perfume ageing time, contact

134   time, and fabric type. Empirical studies of this nature can enable a robust interpretation of

135   what a particular form of trace evidence means in a specific case scenario and can offer

136   valuable intelligence and evidence [4].

137

2. Material and methods

2.1 Chemical and materials

This study followed the method outlined by Gherghel et al. [5] and utilised reference standards ((+)-α-pinene (98.5% purity), (R)-(+) limonene (97%), linalool (97%), geraniol (98%), eugenol (99%), and ethylene brassylate (97%)) and internal standards (1,4-dibromobenzene and methyl nonanoate), as well as methanol (HPLC grade, 99.9% purity). The stock solutions were prepared in methanol on a monthly basis at concentrations between 10 – 25 mM.

The headspace solid phase micro-extraction (HS-SPME) method used divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibres that were purchased from Supelco (Bellefonte, PA, USA). Prior to first use and daily when used, the fibre was conditioned according to the manufacturer's recommendations. For all SPME analyses, 20 mL screw cap vials sealed with 18 mm pre-fitted PTFE-silicon septum were used.

The garments used in this study were purchased from a popular UK male clothing retailer, and were a beige jumper made of 100% acrylic, a white long sleeve T-shirt made of 100% cotton, a blue jacket made of 100% nylon, a long sleeve denim jacket made of 100% cotton, a blue tank top made of 100% polyester, a black jumper made of 100% wool, and a purple T-shirt made of polyester:viscose 50:50 (Figure 1). Prior to the analyses, the garments were washed together in a conventional washing machine without adding any detergent. The dried garments were stored together in a closed plastic bag at ambient temperature.
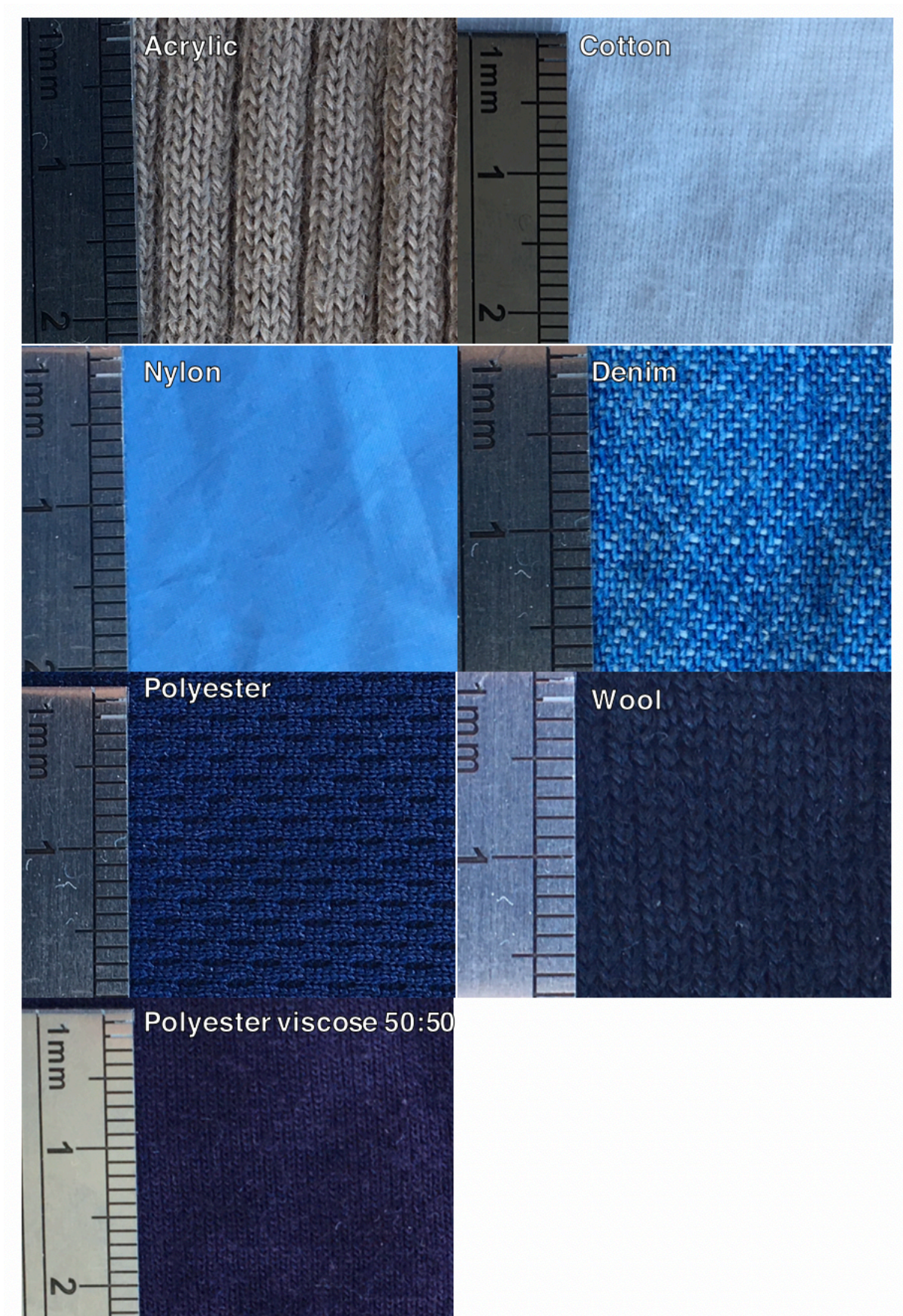
160   Figure 1 Close-up photographs of the seven fabrics employed. Each individual mark on the ruler represents 1
161   mm.

162

2.2   Instrumentation

163

164   A Jeol JSM-6700F field emission scanning electron microscope (SEM) was used for the

165   characterisation of the fabrics. Samples were coated with gold and placed on carbon tape to

166   minimise electrical charging.

167   A previously validated chromatography-mass spectrometry (GC-MS) method was used

168   for the VOCs extraction and instrumental analysis of the samples [5]. A Scion GC system

169   coupled to a Scion QqQ-MS/MS instrument (Bruker Corporation, Freemont, CA, USA) and

170   equipped with a Combi Pal autosampler (CTC Analytics, Switzerland) was used for all

171   samples. The separation of the analytes was achieved on a VF-5ms capillary column (30 m ×

172   0.25 mm, 0.25 μm film thickness) from Varian (Palo Alto, CA, USA). Helium carrier gas

173   (99.9999% purity) was maintained at a flow rate of 1 mL·min-1 using an electronic flow

174   controller. The GC oven temperature was programmed from 35 °C (held for 1 min) to 180 °C

175   (held for 1 min) at 5 °C/min rate, and then to 300 °C (held for 2 min) at 25 °C/min rate. The

176   transfer line and the ion source temperatures of the mass spectrometer were set to 280 °C. As

177   outlined in Gherghel et al. [5], the values for the SPME extraction of the VOCs from fabrics

178   was an incubation time of 5 min, an extraction time of 60 min, and an extraction temperature

179   of 58 ˚C. The thermal desorption of the SPME fibre was carried out using an injector

180   temperature of 250 °C in splitless mode for 3 min. The mass spectrometer was operated in

181   Electron Ionisation (EI) mode at -70 eV. Data acquisition rate was of 5 spectra/s and the mass

182   range was scanned from m/z 40 to 250.

183   A portable thermometer-hygrometer (model 82021, VWR Scientific) was employed for

184   measuring the laboratory temperature and humidity. Throughout this study, the laboratory

185   ambient temperature ranged between 19 and 23 °C, and the humidity ranged between 25 and

186   48%.

8

187   Work carried out by Gherghel et al. [24] looked at the transfer of fragrances between

188   fabrics using two different transfer methods, one that involved hand rubbing, and one that

189   involved the two fabrics being left in contact under the pressure of a heavy weight. The

190   authors identified that the two procedures led to similar number of perfume ingredients being

191   transferred, however lower reproducibility was observed for the hand rubbing method. To

192   overcome this issue, and to simulate closer to reality a sexual assault case where friction

193   between fabrics occurs, a motorised crockmeter (Figure 2) was used to simulate the transfer

194   between fabrics. The crockmeter, also called a fabric abrasion tester, is generally used in the

195   textile industry to determine the amount of colour transferred from fabrics to other surfaces

196   by rubbing, however it has also been used in the published forensic science literature to

197   simulate fabric contact in a reproducible way [25,26].The crockmeter finger covered with a

198   piece of the primary fabric spiked with the VOCs was rubbed against a blank recipient

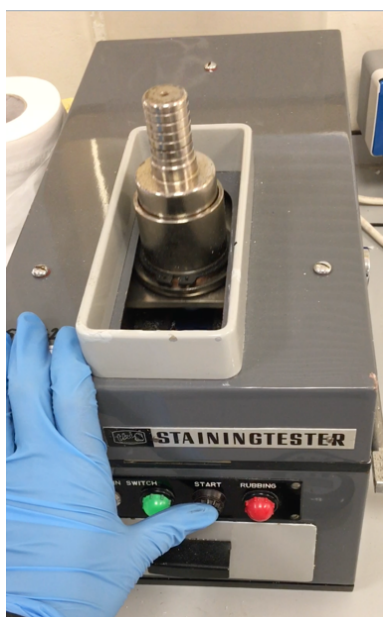199   sample fabric under a fixed load of 9 Newtons.

200

201   Figure 2 Crockmeter employed to carry out the fragrance transfer between fabrics

202   **2.3   Preparation of solutions and experiments**

203   A quality control process was applied by the analysis of blank samples after every three

204   replicate samples in order to verify that no contamination of the fabrics, lab materials or

205 instrument was observed. The series of three experiments were carried out as presented in

206 Figure 3. A swatch of primary material was spiked with VOCs, left to dry for a period of time

207 called perfume ageing time (PAT), followed by transfer of fragrances onto a secondary piece

208 of fabric for a period of time (referred to as contact time) using a crockmeter. The time from

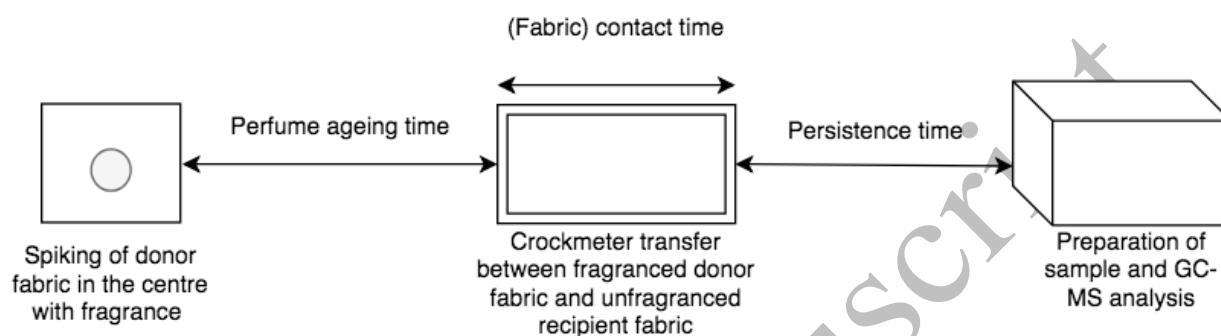209 the transfer until analysis (the persistence time), was kept at 0 s for the purpose of this study.

211 Figure 3 Schematic of the fragrance transfer experimental process

212
213   2.3.1 Perfume ageing time and contact time experiments

214   For the perfume ageing time and fabric contact time experiments, a fragrance mix (FM1)

215 containing 500 μM of each of the six VOCs, as well as 200 μM of the two internal standards

216 was prepared in methanol. The fragrance levels in a typical personal-care perfume are in the

217 range 0.5-2 wt% [27]. The fragrance levels for FM1 were between 0.008 and 0.009 wt%, 50

218 times lower than the regular lower limit of 0.5 wt%.

219   A cotton swatch of approximately 3 x 3 cm (primary material) placed in a clean Petri

220 dish was spiked in the centre with 10 μL of the FM1 using a Hamilton syringe, following the

221 method of Gherghel et al. [5]. For the ageing time experiments, the fabrics, in triplicates,

222 were left on the Petri dish from 10 min up to 24 h, with a leftover sample being used for 48 h.

223 For the contact time experiments, the fabrics, in triplicates, were left on the dish for a

224 constant ageing time of 10 min. After the appropriate ageing time, the spiked primary fabric

225 was placed onto the finger of the crockmeter for the fragrance transfer onto a secondary piece

226 of cotton swatch (the recipient material). For the perfume ageing time experiment, the contact

227 time was kept constant at 1 min, while for the contact time experiment the time varied from

228 10 s to 10 min. After the appropriate contact time, the recipient fabric was removed from the

229 crockmeter, weighed, then added to a 20 mL vial, to which 10 mL of water were added, prior

230 to agitating the vial using a vortex for 30 s.

231

232   2.3.2 Fabric type experiment

233   The fabric type experiment was carried out in a similar manner to the first two

234 experiments with a few modifications. The spiking fragrance mixture (FM2) contained 2mM

235 of each of the six VOCs in methanol. The internal standard mixture was prepared separately

236 at a concentration of 20μM of the two IS in methanol. The FM2 was spiked onto the primary

237 piece of fabric as above, however the internal standard mix was added to the secondary piece

238 of fabric (the recipient) prior to adding it to the vial and adding the 10 mL water. In this way,

239 the internal standards were not submitted to the transfer process and they were only used for

240 the GC calibration step. Quantitative curves were verified when standards were submitted to

241 the SPME GC-MS analysis checking the consistency of the extraction efficiency and

242 instrumental response along the calibration range.

243   The ageing time was kept at 0 min, and the contact time at 1 min. Whilst the two time-

244 related experiments above, used cotton for both the donor and the recipient material, for the

245 fabric type experiment, all seven fabrics available were used as primary materials, and cotton

246 and polyester were used as recipient materials. However, when the nylon fabric was used as a

247 donor material it kept slipping off the crockmeter finger, even for contact times as short as 1

248 min, and therefore it could not be used.

249   The inclusion of an internal standard just prior to extraction allowed for quantification

250 using calibration curves. Six different calibrant concentrations of the six studied VOCs were

251 prepared in methanol, ranging from 0.2 to 200 μM. The same internal standard mixture of

252 20μM in methanol used for the samples was used for the calibration. The calibrants were

11

253 spiked directly onto cotton or polyester swatches, followed by spiking with the internal

254 standards mixture. The fabrics were then added to a 20 mL vial, to which 10 mL of water

255 were added, prior to agitating the vial using a vortex for 30 s.

256 **2.4   Data analysis**

257   The quantitative composition of the VOCs recovered from the primary fabrics was

258 evaluated by hierarchical cluster analysis (HCA), principal component analysis (PCA), and

259 partial least square (PLS) analysis using the software programme SIMCA-P 12.0.1. (Umetrics

260 AB, Umeå, Sweden).

261

262 **3. Results**

263 **3.1   Perfume ageing time**

264   For this experiment, the internal standards were added to the FM1 used to spike the

265 primary piece of fabric. It was observed that by varying the perfume ageing time (PAT),

266 besides the expected change in the amounts of VOCs recovered, there was also a

267 considerable change in the amounts of internal standards recovered. Figure SI-1 presents

268 scatter plots with error bars for the internal standards, with each point representing the mean

269 peak area, whilst the error bars represent the relative standard deviation (RSD) of the three

270 replicates. This figure shows how for both dibromobenzene and methyl nonanoate, very large

271 chromatographic peak areas were obtained using an ageing time of 1 h, compared to all

272 perfume ageing times investigated. This behaviour might be due to time needed to reach

273 equilibrium and therefore, it was deemed that the internal standards as used here are not

274 appropriate for quantitation.

275   Instead, the recoveries of the VOCs were calculated using as control two reference

276 samples. These reference samples were fabric swatches spiked with the FM1 and analysed at

277 the beginning and at the end of the batch of the sampling sequence. Average peak area values

278   were considered for comparison with those obtained experimentally. Additionally, the fabric

279   weight was taken into consideration for a more accurate calculation. And so, the amounts of

280   analytes recovered from the recipient fabrics are reported in % as sample analyte peak area

281   per fabric weight divided by the reference analyte peak area per fabric weight.

282   By varying the period of time that a fragrance mix has been on a primary piece of fabric,

283   prior to transfer, considerable differences in the amount of VOCs recovered from the

284   secondary piece of fabric were observed (Figure 4). Generally, and especially for lower

285   volatility molecules, it was noted that a short PAT lead to higher amounts recovered from the

286   secondary fabric. Linalool, geraniol, and eugenol follow a typical decay curve, where high

287   amounts were recovered for short times followed by a steady decrease until a plateau was

288   reached. For example, for linalool approximately 3% of the total 100% reference peak area

289   was recovered from the secondary fabric using a 10 min PAT, and then the recovery slowly

290   decreased with the time increasing, so that at 1 h PAT around 1% was recovered, at 3 h

291   around 0.4%, after which it levelled off until 48 h PAT. Limonene and ethylene brassylate

292   display an initial spike prior to the decay curve, at 3 and 1 h PAT, respectively. For pinene

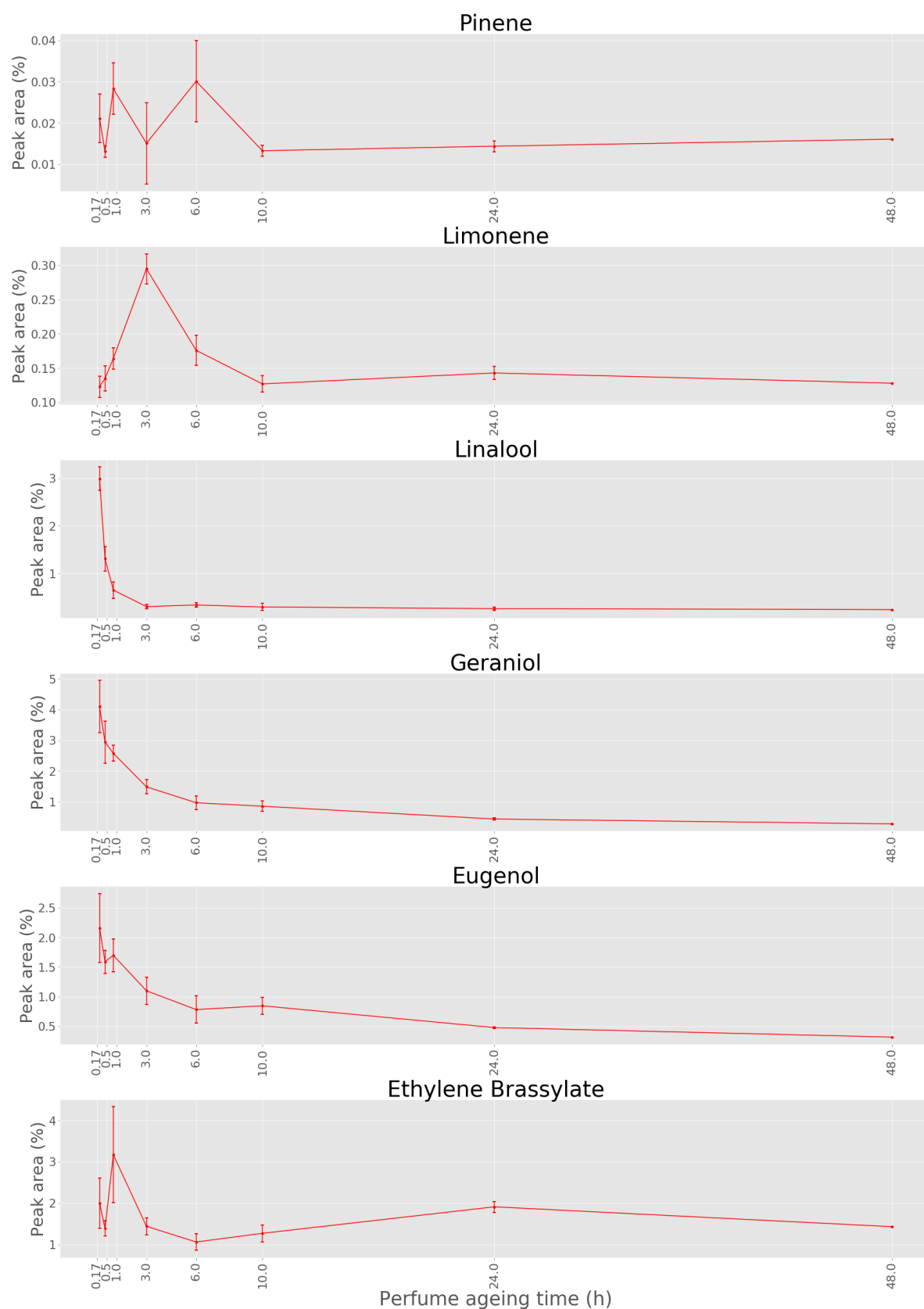293   the trendline is not very clear.

294

Figure 4 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 to 48 h) prior to contact. The error bars are standard deviations
(n=3). The difference in scaling between analytes should also be noted.

298

299   Moreover, it was noted that different VOCs were recovered at different rates as it can be

300   noted on the y scale in Figure 4. For example, for pinene, very small amounts were recovered

301   from the recipient fabric for all ageing times, between 0.005 and 0.05% of the original spiked

302   amount. In comparison, the lower volatility compounds were recovered in higher levels (2-

303   5% of the amount spiked on the original fabric), especially for shorter PAT.

304   Additionally, when looking at the error values for the triplicate samples, it can be

305   observed that good RSD values (n=3) were obtained for most samples. Pinene displays

306   higher RSD, with values between 9.8 and 76% for PAT lower than 6 h. The pinene recoveries

307   are generally around 100 times lower compared to low volatility compounds, which can

308   explain the higher RSD. It should be noted that only one sample was available for the 48 h

309   time, and so no RSD values are available for this time.

310
311   **3.2   Contact time**

312   For the contact time study, the donor and recipient fabrics were rubbed against each

313   other using the crockmeter for a series of different lengths of time ranging from 10 s (0.17

314   min) to 10 min. It was noticed that with the longest contact time (10 min), there was a higher

315   risk of the primary piece of fabric becoming detached from the crockmeter finger, in which

316   case a new sample was prepared.

317   Similar to the PAT experiment, the internal standards added to the fragrance mix

318   behaved differently depending on the contact time (Figure SI-2), thus the quantification of

319   VOCs from the samples was again carried out without the use of internal standards, but rather

320   using reference samples spiked with FM1, and thus the amounts of VOCs recovered are

321   expressed as percentages.

322   As can be seen in Figure 5, for all six VOCs, with an increase in the contact time

323   between the fragranced fabric and the fragrance-free fabric, a consistent amount of analyte

324   was recovered for contact times of up to 3-5 min, after which the recovery slowly decreased.

15

325   For example, for linalool the mean recoveries were between 18 to 25% for contact times of

326   10 s to 5 min, and around 10% for a contact time of 10 min. The lowest recoveries were at 10

327   min contact time for all six analytes.

328   As observed with the perfume ageing time experiment, there were big differences in the

329   amounts of limonene and pinene recovered from the donor materials compared to lower

330   volatility compounds. Generally, around 0.5% of pinene and 1% of limonene were recovered

331   from the secondary piece of fabric, whilst for the other four VOCs the recoveries were

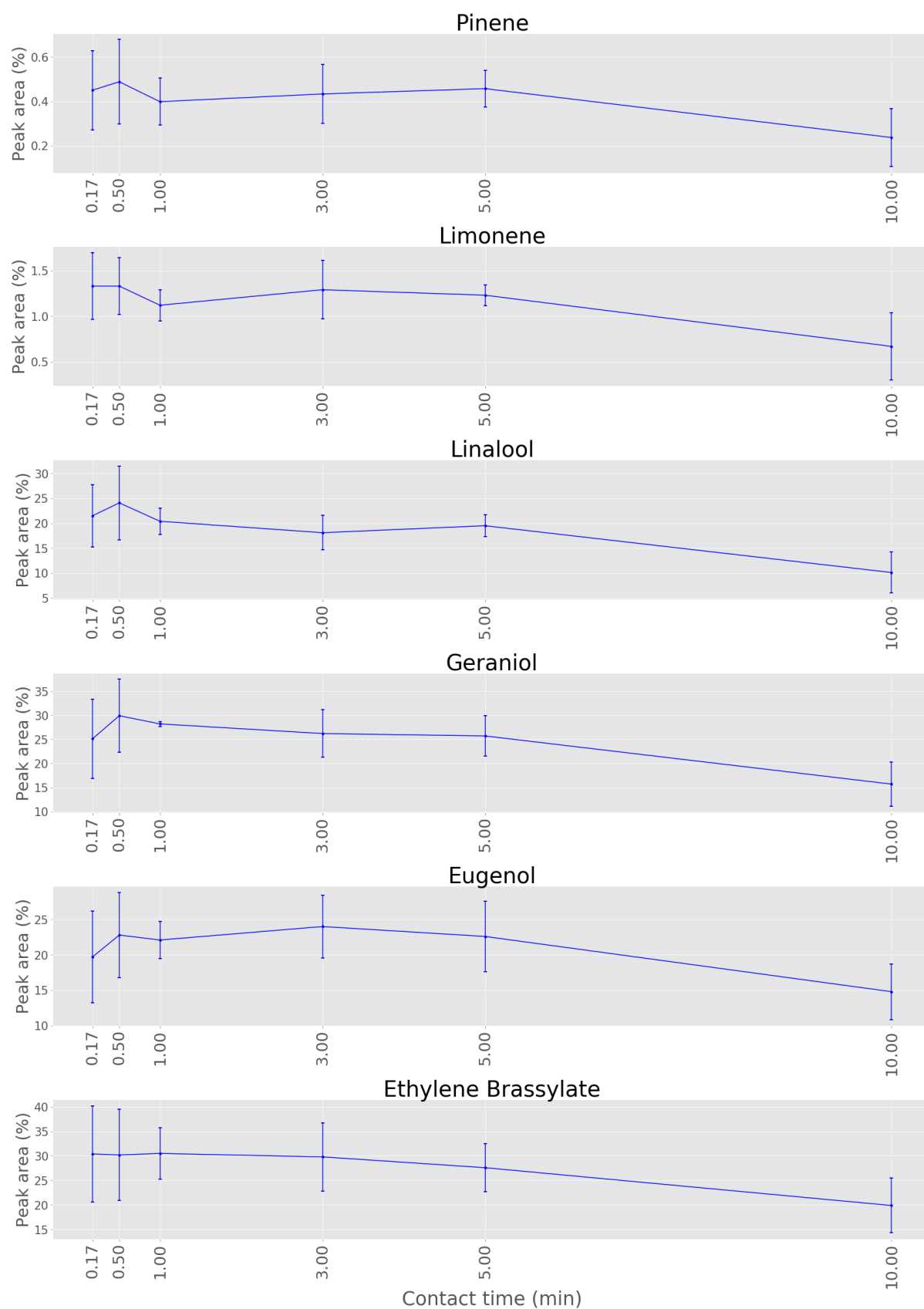332   generally between 15 and 35%.

333

Figure 5 Recovery of the six analytes from the secondary piece of fabric when the contact between the fabrics

varied from 10 s to 10 min. The error bars are standard deviations (n=3). The difference in scaling between

analytes should also be noted.

3.3   Fabric type

337

338   For the last transfer experiment, seven different fabrics, shown in Figure 1, were

339   employed as donor materials and two of these (polyester and cotton) as recipient materials.

340   • SEM analysis

341   The fabrics were examined under SEM to establish the fabric weave characteristics and

342   to provide insight into how these characteristics might play a role in the transfer of

343   fragrances. The SEM results (Figure 6) show the seven different fabrics using a 250x

344   resolution. In terms of width, the wool fibres had the largest fibre width and nylon the

345   smallest. Acrylic, nylon and polyester show smooth and regular surfaces, wool a scale-like

346   structure, and cotton a ribbon like structure. Although denim is made of 100% cotton, there is

347   a clear difference in the weave of these two materials, with denim showing tightly woven

348   fibres. Also noticeable is the dissimilarity between the 100% polyester material and the 50/50

349   blend of polyester and viscose. Whilst the former presents more order and it has depth, the

350   latter presents many loose fibres. Nylon, a waterproof material, shows a very ordered

351   structure, with no gaps between the fibres.
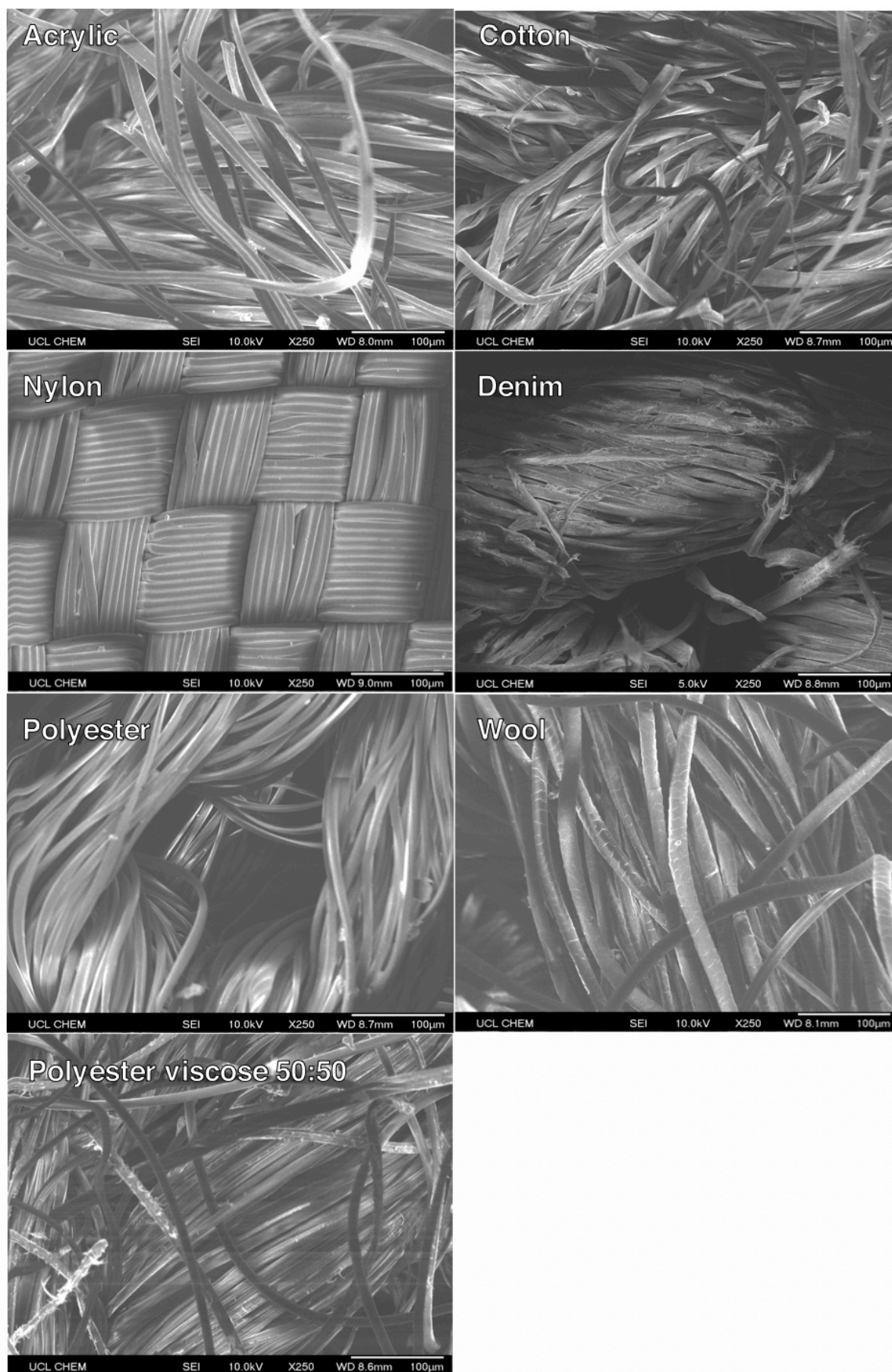
353   Figure 6 SEM images showing the seven fabrics using a 250x resolution with the man-made blends on the left

354   side and the natural blends on the right side.

355 • VOCs analysis

356 For the quantification of the six VOCs from the fabric type samples, calibration curves

357 were carried out in both cotton and polyester. As the calibrants span a large range of

358 concentrations from 0.2 to 200 μM, weighted curves were fitted which apply more emphasis

359 to some concentration points than others, specifically to the lower concentration points. The

360 weighting factors are generally between 0 and 1, and most commonly are $1/x$ or $1/x^2$, where x

361 is the concentration [28]. The results of fitting weighted curves are shown in Table 1. With

362 the exception of α-pinene in polyester which was fitted to a linear curve, all other calibrations

363 were fitted to quadratic curves. Excellent R^2 were obtained for all curves with values above

364 0.996, with the exception of ethylene brassylate in cotton for which the R^2 was 0.988.

365

366 Table 1 Calibration results for the fabric type experiment

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

367

20

368   For the fabric type experiment, a natural (cotton) and a synthetic (polyester) fabric were

369   used as recipient fabrics, whilst six of the seven fabrics available were used as donor

370   materials. The name of the samples starts with the name of the donor material, followed by

371   the name of the recipient material, so that for the sample "acrylic_polyester" the fragrance

372   mix was pipetted onto acrylic, and then transferred onto a polyester material using the

373   crockmeter for a contact time of 1 min.

374   As noted for the two time-related experiments, very low amounts of α-pinene are

375   transferred and recovered from recipient fabrics. This was also observed in the fabric type

376   experiment, even though FM2 contained a higher concentration of VOCs compared to the

377   FM1 used for the other two experiments. Many samples led to the recovery of this compound

378   below the lower limit of the working range (0.5 μM in cotton and 0.2 μM in polyester), and

379   so only the other five VOCs (limonene, linalool, geraniol, eugenol, and ethylene brassylate)

380   were employed for data analysis.

381   To obtain an overview and a visual representation of the samples (6 blanks + 6 donor

382   fabrics * 2 recipient fabrics * 3 replicates) principal component analysis (PCA) was

383   employed. PCA is an unsupervised method (no labels are given) that reduces the dimension

384   of the data with the aim of retaining as much information as possible. It creates an artificial

385   set of variables, called principal components, that account for the most variance in the data

386   set. PCA showed a clear separation of the blank samples in the set of 42 samples (Figure SI-

387   3). The sum of three principal components accounted for 89.1% of the total variability.

388   Further statistical analysis was carried out without the blanks. It was noticed that

389   when the 36 fabric type samples were used to create a new PCA model, the Distance to

390   model (DModX) analysis identified the viscose_cotton_R3 sample as being an outlier (Figure

391   SI-4). This sample displays the largest portion of unexplained variation as it is the farthest

392   away from the model plane and above the critical distance.

393   The new PCA permitted a reduction of the 35 samples to three principal

394   components. These three PCs were extracted explaining 98.1% of the total variance of fabric

395   type samples. The obtained three-dimensional PCA representation is colour coded in Figure

396   7a-b according to the recipient material (polyester or cotton), the donor material (the six

397   fabrics), or as a combination of recipient and donor material (twelve different groups). In

398   Figure 7a it can be observed that the transfer of perfume from all different materials onto

399   cotton is very different from the one onto polyester. The two groups are clearly

400   distinguishable between each other, as emphasised by the dashed circles. In Figure 7b, it can

401   be observed that the acrylic fabric as a donor it is clearly different from the rest of the fabrics

402   studied, when transferred to polyester. Moreover, Figure 7b shows how the three natural

403   fibres tested, cotton, denim, and wool exhibited a similar transfer behaviour when transferred

404   onto polyester (highlighted by the ellipse), with the exception of one wool replicate.

405   However, when the VOCs were transferred to cotton, the natural fibres were also close or

406   overlapping on the PCA with some man-made fibres.

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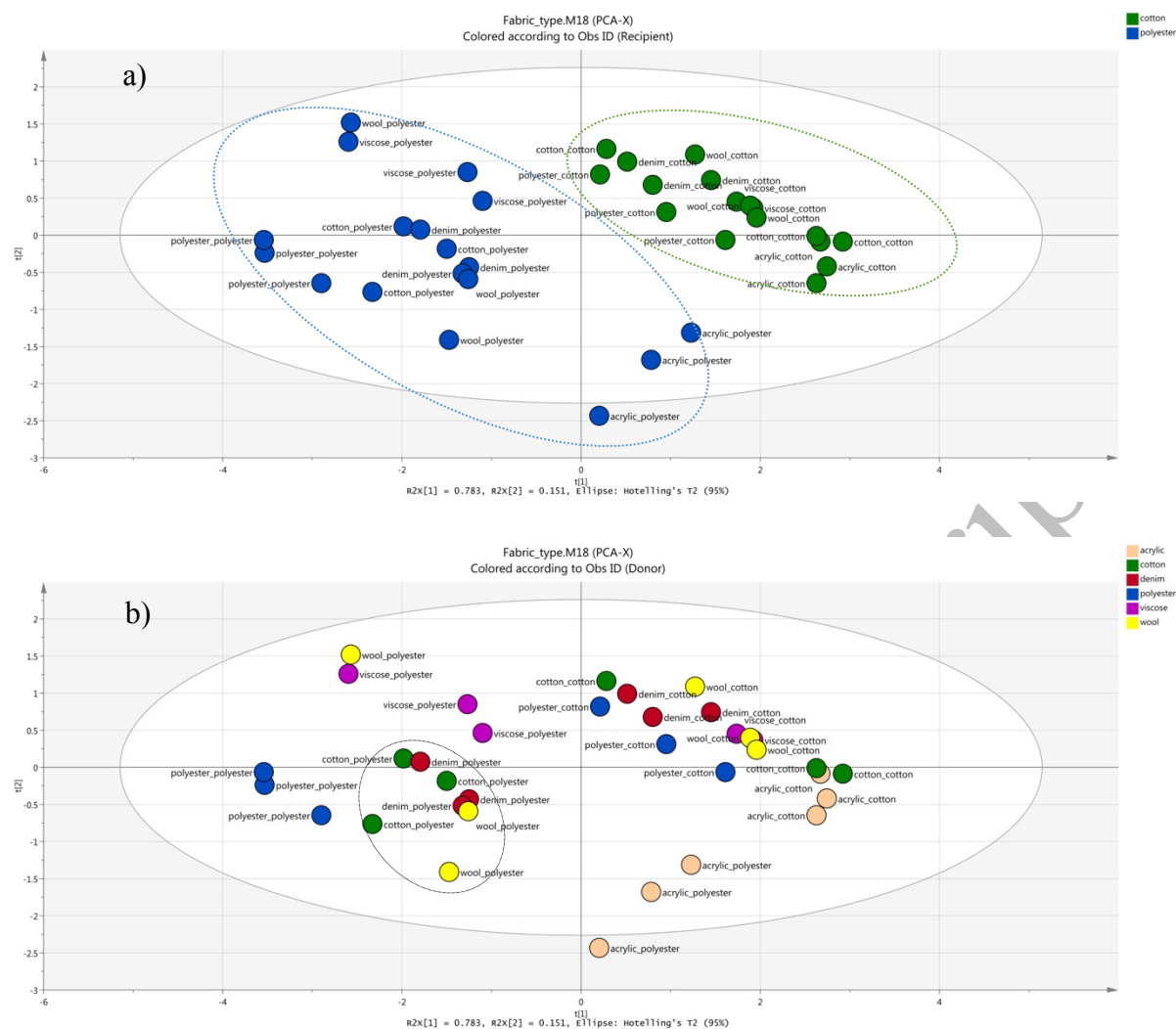409

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Figure 7 PCA representations using peak areas of 5 VOCs (limonene, linalool, geraniol, eugenol, ethylene brassylate) from the fabric type transfer data, and coloured by: a) the recipient fabric, b) the 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 8). 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 7b. Within these two major HCA groups, various

431   subgroups are formed. It can be observed how all three polyester to polyester replicates are

432   grouped together, showing minimal dissimilarity. However, it can be observed that not all

433   triplicates are grouped together, and there is an overlap for some samples, for example the

434   replicate 3 of wool to polyester samples is grouped with viscose to polyester samples. Such

435   overlap in the triplicates can also be observed in the PCA in Figure 7b.
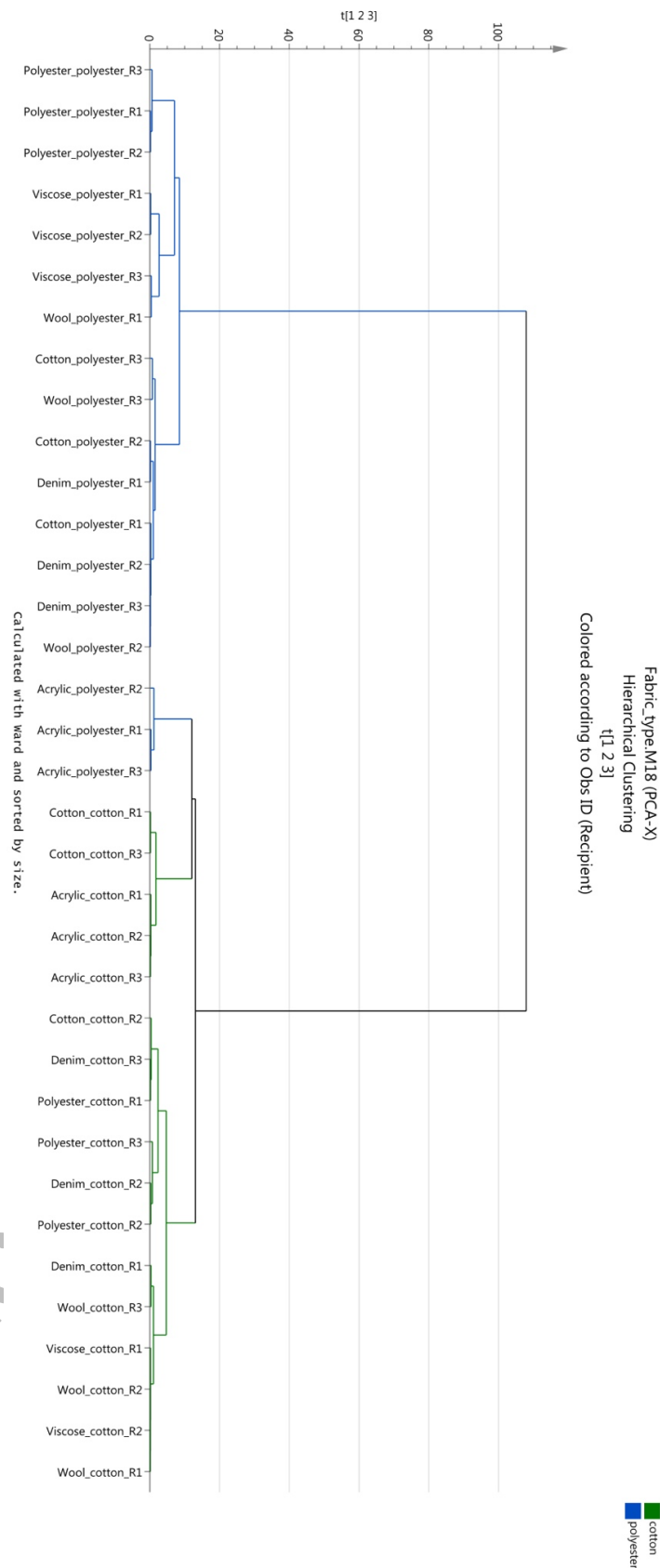
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Figure 8 Dendrogram obtained by hierarchical cluster analysis using the samples from the fabric type

440 experiment

441   Finally, a PLS (partial least square) discriminant analysis was used to determine a model

442   able to predict the nature of the recipient fabric depending on the transfer of the VOCs

443   studied. The graphical result is shown in Figure 9. The model was able to successfully

444   distinguish the two groups, accounting for 96 % (R^2 value of 0.96) of the variance in the

445   observed samples for the training set. The Q^2 value obtained was 88.8%. The Q^2 value is an

446   estimate of the predictive ability of the model and is a result of the cross-validation, where a

447    $1/7^{th}$ of the data is removed at a time, and the model's prediction ability is tested.
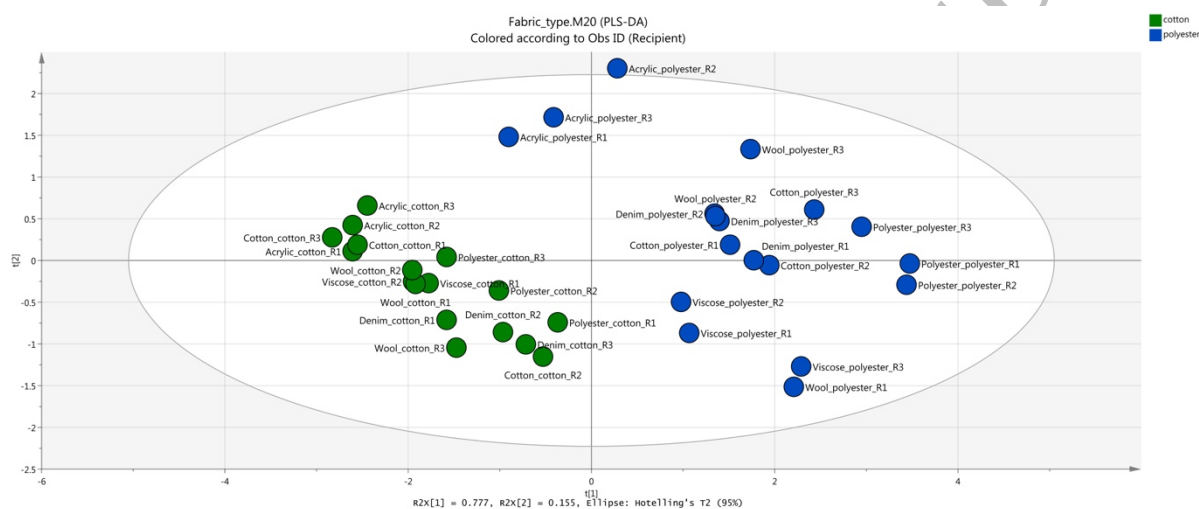

448

449   Figure 9 Partial least square (PLS) discriminant analysis representation showing the classification of the transfer
450   type samples based on the donor material

451

4. Discussion

453   To our knowledge, these are the first experimental studies carried out on the transfer

454   process of fragrances between fabrics for forensic applications using an optimised and

455   validated SPME-GC-MS method. Three variables (fabric type, perfume ageing time and

456   fabric contact time) considered to have an important effect on the dynamics of the transfers

457   were investigated, following the studies of Bull et al. [4] and Hudson et al. [23], which

458   demonstrated the importance of material type and the dependence of perfume volatility with

26

459 time. The experiments for this study were also designed to closely recreate the type of

460 contact between fabrics relevant to a sexual assault case.

461
462 **4.1 Perfume ageing time**

463 As the samples were spiked with the VOCs and the internal standards at the same time, it

464 was observed that the behaviour of the internal standards during the transfer process at

465 various PAT was not constant. Therefore, the internal standards were deemed not suitable for

466 the quantification of the VOCs. Instead, the amount of VOCs recovered from the recipient

467 cotton swatches was calculated using the reference amounts of VOCs from a spiked cotton

468 swatch, i.e. the amount of VOCs on a primary swatch.

469 Overall, longer perfume ageing times led to lower amounts of VOCs being recovered

470 from the secondary piece of fabric. Lower volatility compounds, such as linalool, geraniol,

471 and eugenol followed a typical decay curve, identified in many other forensic studies

472 concerning the persistence of traces on clothing, such as by Dachs et al. [29] for hair scalp

473 and Robertson et al. [25] for textile fibres. Additionally, it was observed that for all perfume

474 ageing times very low amounts of high volatility compounds, such as pinene and limonene

475 were recovered from the donor fabric, with values lower than 0.04% for pinene and 0.3% for

476 limonene, when compared to a reference spiked fabric. For the other VOCs, the recovery was

477 generally between 0.5 and 5%. This suggests that highly volatile compounds might not be

478 suitable candidates for forensic VOCs analyses.

479 Moreover, when looking at the RSD values for the triplicate analytes at all ageing time, it

480 can be observed that large RSD values were obtained for pinene, with values between 9.8 and

481 76%. This further consolidates the point of view that such high volatility compounds may

482 have limited value in forensic studies, firstly because they transfer in very small amounts for

483 all ageing time studies, and secondly because of the high variability. On the other hand, lower

484 volatility compounds presented lower RSD values, higher recovery rates, and steady decay

485 curves. Ethylene brassylate, the least volatile compound, showed an initial spike in recovery

486 for an ageing time of 1 h prior to the decay curve.

487 **4.2   Contact time**

488   Once again, the quantification was carried out using reference samples that had been

489 directly treated with the VOCs. Generally, for contact times between fabrics from 10 s up to

490 3-5 min, no discernible differences in the amount of VOCs recovered from the secondary

491 piece of fabric were observed. The recovery did decrease for longer contact times, such as 10

492 min. This is in contrast with work carried out by Gherghel et al. [24], where an increase in the

493 amount of VOC recovered from the donor fabric was observed for longer contact times

494 between fabrics. This can be potentially explained by the difference in the transfer methods

495 between the two studies. In Gherghel et al. [24], the two fabrics were kept in contact under

496 the pressure of a weight. The weight could act as a hermetic seal, not allowing the

497 evaporation of VOCs. In the current study, a crockmeter was employed to better simulate the

498 reality of a sexual assault case. The fabrics were rubbed against each other under high

499 pressure, and thus generating heat by the friction. The increase in temperature could lead to

500 the evaporation of some compounds with a longer contact time, and thus explain the lower

501 recovery rates observed for the longest studied contact time of 10 min.

502   Similar to section 4.1, small amounts of pinene and linalool were recovered from the

503 recipient fabric, compared to the lower volatility compounds. So that, for shorter contact

504 times, the recoveries for pinene and limonene were around 0.5 and 1.2%, respectively, whilst

505 for the other VOCs the recoveries were around 20-35%.

506 **4.3   Fabric type**

507   Scanning electron microscopy was employed to obtain information about the structure of

508 the fabric and the weaving patterns of the seven different fabrics, both natural and man-made.

509 The nylon fabric, originating from a waterproof jacket, was a tightly woven fabric, which can

510 explain its natural low permeability. All other fabrics presented gaps between threads or

511   bundles of threads, allowing for air or liquid flow. The fibre structure varied between the

512   fabrics studied, with synthetic fibres generally showing smoother surfaces (Figure 6).

513   However, studies such as DeGreeff et al. [30] and Prada et al. [22] have shown that both the

514   fabric weave and the molecular structure play a role in the transfer of human scent to fabrics.

515   When looking at the transfer of VOCs onto cotton and polyester, clear differences were

516   readily noticeable in the PCA plot (Figure 7a) and in the HCA dendrogram (Figure 8) with

517   two distinctive groups formed based on the recipient material. Moreover, a PLS-DA model

518   was able to discriminate between the two recipient fabric samples with a R^2 value of 96%

519   (Figure 9).

520   Within these two major groups, various donor samples were distinctive. For example,

521   when acrylic was used as the donor material this appeared on the 3-D PCA well

522   discriminated from the rest of the samples, especially when polyester was used as the

523   recipient fabric (Figure 7b). In spite of the weave differences observed by SEM analysis, the

524   natural fabrics (cotton, denim, wool) showed overlap on the PCA, especially when the VOCs

525   were transferred from these fabrics onto polyester.

526

527   5. Conclusions

528   The transfer behaviour of six VOCs between fabrics has been investigated in this study.

529   All factors studied, the perfume ageing time, the contact time, and the fabric type, affected

530   how volatile compounds are transferred onto a secondary piece of fabric.

531   The results from the perfume ageing time study showed that lower volatility compounds

532   were recovered from recipient fabrics in higher amounts compared to higher volatility

533   compounds. It was also observed that the longer the time period since fragrance VOCs have

534   been applied to a piece of fabric, the lower the recovery of compounds from the secondary

535   piece of fabric (Figure 4). This decrease follows a typical decay curve, where the recovery

536 drops considerably for the samples when the fragrance mixture was aged for 1 to 3 h, after

537 which the recovery drops steadily. Perfume traces were recovered from the recipient fabric

538 even when the perfume was aged on the donor fabric for as long as 48 h.

539   The data from the contact time experiment demonstrated that even for contacts as short

540 as 10 s, VOCs were successfully transferred and recovered from recipient cotton swatches.

541 Moreover, the amounts recovered for all six VOCs maintained rather constant for the first 5

542 min of contact, after which a decrease was noticed (Figure 5). This can be explained by the

543 evaporation of VOCs with increased rubbing time, and thus increase in the heat generated by

544 rubbing the fabrics together.

545   When looking at the fabric type, clear differences were observed in the VOCs behaviour

546 when they were transferred onto cotton and onto polyester. Two distinctive groups were

547 determined by the PCA based on the recipient fabric (Figure 7a). On the other hand, when

548 looking at the influence of the primary fabric for each of these two groups, some samples

549 were distinctive, whilst other samples displayed some overlap on the PCA plot (Figure 7b).

550   Overall, this study has confirmed that VOCs have the potential to be a helpful form of

551 trace evidence as detectable amounts were recovered from a recipient fabric for fabric

552 contacts as short as 10 s and when the perfume on the donor fabric was applied as long as 48

553 h prior to transfer. However, factors that affect the transfer, such as the ones studied here,

554 should be taken into consideration when seeking to interpret the presence of VOCs traces

555 recovered from fabrics. Further studies will be beneficial to test the variability in the number

556 and abundance of VOCs that transfer and persist under different environmental conditions.

557 For forensic reconstructions where clothing from two individuals may have been in contact,

558 such as a sexual assault, this study indicates that there may be value in testing the clothing

559 items for VOCs. Depending on the type of fragrance originally applied, these findings

560 indicate that it may be possible to identify specific fragrance compounds on clothing exhibits

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561   which may provide trace specimens that can contribute to a forensic reconstruction seeking to

562   establish whether a contact may have taken place.

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569   References:

570   [1]   Office for National Statistics, Criminal Justice Statistics quarterly, England and Wales,
571   2016 (final), 2017. https://www.gov.uk/government/statistics/criminal-justice-system-
572   statistics-quarterly-december-2016.
573   [2]   Office for National Statistics, Sexual offences in England and Wales: year ending
574   March 2017, 2018.
575   https://www.ons.gov.uk/peoplepopulationandcommunity/crimeandjustice/articles/sexu
576   aloffencesinenglandandwales/yearendingmarch2017.
577   [3]   M. Hester, S.J. Lilley, Rape investigation and attrition in acquaintance, domestic
578   violence and historical rape cases, in: J. Investig. Psychol. Offender Profiling, 2017:
579   pp. 175–188. doi:10.1002/jip.1469.
580   [4]   P.A. Bull, R.M. Morgan, A. Sagovsky, G.J.A. Hughes, The transfer and persistence of
581   trace particulates: experimental studies using clothing fabrics, Sci. {&} Justice. 46
582   (2006) 185–195.
583   [5]   S. Gherghel, R.M. Morgan, J. Arrebola-Liébanas, R. Romero-González, C.S.
584   Blackman, A. Garrido-Frenich, I.P. Parkin, Development of a HS-SPME/GC–MS
585   method for the analysis of volatile organic compounds from fabrics for forensic
586   reconstruction applications, Forensic Sci. Int. 290 (2018) 207–218.
587   doi:10.1016/j.forsciint.2018.07.015.
588   [6]   R.M. Morgan, Conceptualising forensic science and forensic reconstruction. Part I: A
589   conceptual model, Sci. Justice. (2017) 0–4. doi:10.1016/j.scijus.2017.06.002.
590   [7]   R.M. Morgan, J. Cohen, I. McGookin, J. Murly-Gotto, R. O'Connor, S. Muress, J.
591   Freudiger-Bonzon, P.A. Bull, The relevance of the evolution of experimental studies
592   for the interpretation and evaluation of some trace physical evidence, Sci. Justice. 49
593   (2009) 277–285. doi:10.1016/j.scijus.2009.02.004.
594   [8]   C.A. Pounds, K.W. Smalldon, The transfer of fibres between clothing materials during
595   simulated contacts and their persistence during wear. Part I—fibre transference, J.
596   Forensic Sci. Soc. 15 (1975) 17–27. doi:http://dx.doi.org/10.1016/S0015-
597   7368(75)70932-5.
598   [9]   C.A. Pounds, K.W. Smalldon, The transfer of fibres between clothing materials during
599   simulated contacts and their persistence during wear. Part II—fibre persistance, J.
600   Forensic Sci. Soc. 15 (1975) 29–37.
601   [10]   C.A. Pounds, K.W. Smalldon, The transfer of fibres between clothing materials during
602   simulated contacts and their persistence during wear. Part III--a preliminary

603 investigation of the mechanisms involved., J. Forensic Sci. Soc. 15 (1975) 197–207.
604 doi:10.1016/S0015-7368(75)70985-4.
605 [11] F. Brewster, J. Thorpe, G. Gettinby, B. Caddy, The retention of glass particles on
606 woven fabrics, J. Forensic Sci. Soc. 30 (1984) 798–805. doi:10.1520/JFS11012J.
607 [12] T.J. Allen, J.K. Scranage, The transfer of glass - part 1. Transfer of glass to individuals
608 at different distances, Forensic Sci. Int. 93 (1998) 167–174. doi:10.1016/S0379-
609 0738(98)00041-3.
610 [13] J.M. Curran, C.M. Triggs, J.S. Buckleton, K. Walsh, T. Hicks, Assessing transfer
611 probabilities in a Bayesian interpretation of forensic glass evidence., Sci. Justice. 38
612 (1998) 15–21. doi:10.1016/S1355-0306(98)72068-4.
613 [14] E.F. Pearson, R.W. May, M.D. Dabbs, Glass and paint fragments found in men's outer
614 clothing--report of a survey., J. Forensic Sci. 16 (1971) 283.
615 [15] P. Buzzini, G. Massonnet, S. Birrer, N.M. Egli, W. Mazzella, A. Fortini, Survey of
616 crowbar and household paints in burglary cases—population studies, transfer and
617 interpretation, Forensic Sci. Int. 152 (2005) 221–234.
618 doi:http://dx.doi.org/10.1016/j.forsciint.2004.09.116.
619 [16] R. Moore, D. Kingsbury, J. Bunford, V. Tucker, A survey of paint flakes on the
620 clothing of persons suspected of involvement in crime, Sci. Justice. 52 (2012) 96–101.
621 doi:http://dx.doi.org/10.1016/j.scijus.2011.08.002.
622 [17] B.D. Gaudette, A.A. Tessarolo, Secondary transfer of human scalp hair, J. Forensic
623 Sci. 32 (1987) 1241–1253.
624 [18] M.J. Mann, Hair transfers in sexual assault: a six-year case study, J Forensic Sci. 35
625 (1990) 951–955.
626 http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Cit
627 ation&list_uids=2391483.
628 [19] D.L. Exline, F.P. Smith, S.G. Drexler, Frequency of pubic hair transfer during sexual
629 intercourse., J. Forensic Sci. 43 (1998) 505–8.
630 http://www.ncbi.nlm.nih.gov/pubmed/9608688.
631 [20] D.C. Mildenhall, Hypericum pollen determines the presence of burglars at the scene of
632 a crime: An example of forensic palynology, Forensic Sci. Int. 163 (2006) 231–235.
633 doi:http://dx.doi.org/10.1016/j.forsciint.2005.11.028.
634 [21] R.M. Morgan, G. Davies, F. Balestri, P.A. Bull, The recovery of pollen evidence from
635 documents and its forensic implications, Sci. Justice. 53 (2013) 375–384.
636 doi:http://dx.doi.org/10.1016/j.scijus.2013.03.004.
637 [22] P.A. Prada, A.M. Curran, K.G. Furton, The evaluation of human hand odor volatiles
638 on various textiles: A comparison between contact and noncontact sampling methods,
639 J. Forensic Sci. 56 (2011) 866–881. doi:10.1111/j.1556-4029.2011.01762.x.
640 [23] D.T. Hudson, A.M. Curran, K.G. Furton, The stability of collected human scent under
641 various environmental conditions, J. Forensic Sci. 54 (2009) 1270–1277.
642 doi:10.1111/j.1556-4029.2009.01153.x.
643 [24] S. Gherghel, R.M. Morgan, C.S. Blackman, K. Karu, I.P. Parkin, Analysis of
644 transferred fragrance and its forensic implications, Sci. Justice. 56 (2016) 413–420.
645 doi:10.1016/j.scijus.2016.08.004.
646 [25] J. Robertson, C.B.M. Kidd, H.M.P. Parkinson, The Persistence of Textile Fibres
647 Transferred During Simulated Contacts, J. Forensic Sci. Soc. 22 (1982) 353–360.
648 doi:10.1016/S0015-7368(82)71511-7.
649 [26] S. Bennett, C.P. Roux, J. Robertson, The significance of fibre transfer and persistence
650 – A case study, Aust. J. Forensic Sci. 42 (2010) 221–228.
651 doi:10.1080/00450611003758256.
652 [27] A. Van Asten, The importance of GC and GC-MS in perfume analysis, TrAC - Trends
653 Anal. Chem. 21 (2002) 698–708. doi:10.1016/S0165-9936(02)00807-5.

654   [28]   H. Gu, G. Liu, J. Wang, A.F. Aubry, M.E. Arnold, Selecting the correct weighting
655   factors for linear and quadratic calibration curves with least-squares regression
656   algorithm in bioanalytical LC-MS/MS assays and impacts of using incorrect weighting
657   factors on curve stability, data quality, and assay perfo, Anal. Chem. 86 (2014) 8959–
658   8966. doi:10.1021/ac5018265.
659   [29]   J. Dachs, I.J. McNaught, J. Robertson, The persistence of human scalp hair on clothing
660   fabrics, Forensic Sci. Int. 138 (2003) 27–36. doi:10.1016/j.forsciint.2003.07.014.
661   [30]   L.E. DeGreeff, A.M. Curran, K.G. Furton, Evaluation of selected sorbent materials for
662   the collection of volatile organic compounds related to human scent using non-contact
663   sampling mode, Forensic Sci. Int. 209 (2011) 133–142.
664   doi:10.1016/j.forsciint.2011.01.010.
665