Sampling method development and optimization in view of human hand odor analysis by thermal desorption coupled with gas chromatography and mass spectrometry

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Abstract:

Forensic profiling of human odor is challenging and would be useful to support information provided by dogs in courts of justice. Analyses of volatile compounds constitutive of human odor are commonly performed with gas chromatography coupled with mass spectrometry. All developed methods and sampling prototypes have to be easy-to-use in the field by crime scene investigators. This paper will focus on techniques for human hand odor sampling prior to analysis by a thermodesorption device coupled with gas chromatography and mass spectrometry. Thermodesorption and gas chromatography methods were developed using a sorbent phase spiked with a mixture of 80 compounds representative of human hand odor. Then the crucial sampling step was performed indirectly with a home-made device based on air suction and trapping on a sorbent. This indirect sampling device was evaluated with the same synthetic mixture for optimization. An innovative polymer sorbent called Sorb-Star® was compared to classic Tenax TA® packed tubes. Sorb-Star® provided similar recovery to Tenax TA® packed tubes and a smaller pooled coefficient of variation (6% vs 13%). Thus, it appeared to be fully suited to the indirect sampling of human hand odor. The developed methods were successfully applied to real samples, the ultimate aim being the comparison of a suspect's sample to a sample collected from a crime scene.

1. Introduction

Forensic profiling of human hands odor is challenging and requires the development of an entire strategy, from sampling to statistical data processing. Several studies have been carried out focusing on the analysis of hand odor (1–4) and the state of the art in this field is described in details in a review (5). Hands are the body part the most susceptible to be in contact with an element at the crime scene. It has been shown they generate volatile profiles which provide sufficient stability through time, and variations from one subject to another for differentiation (2,6). However, enough information has to be collected to allow a proper discrimination between individuals: thus, sampling step is critical for efficient and reliable collection of human hands odor for forensic purpose. There is no standardized protocol for the collection of odor for analytical purpose yet and two kinds of samplings, with or without contact, can be implemented. As for chromatographic separation and detection, gas chromatography coupled with mass spectrometry (GC-MS) appears to be one the most appropriate techniques and have a direct incidence on the sample preparation and injection.

Direct sampling procedure relies on the contact between a sorbent and the subject or at least an object left with its odor. Sorbent choice remains critical and has an influence on the trapping and release of compounds (7). Contact sampling with cotton material has been shown to be the collection method that yields the greatest number of volatile compounds and the highest scent mass amounts (7–10). Cotton swabs were also successfully used for the molecular cartography of the human skin surface (11). But a wide diversity of material can be used:glass beads were said to allow the preferential concentration of “oily” residues while minimizing collection of aqueous perspiration (3,12), Sorptive tape extraction (13,14)

57 which uses polymer “patches” made of a thin layer of flexible polymer put on a solid matrix,
58 hydrogels (15) (16). Many parameters (water content, temperature, composition, purity)
59 (17) are influential and make their use complicated. On the contrary, the use of commercial
60 sorbent, already conditioned, and ready-to-use could consequently facilitate the sampling
61 step, especially in the field. For instance, polydimethylsiloxane (PDMS) coated stir bars used
62 for stir bar sorptive extraction (SBSE) (18–20) eliminate the need for pads or cotton gauzes
63 which are not analytically clean and could lead to overloaded blanks. After conditioning, the
64 stir bar can be used directly on skin, or possibly stored and eventually analyzed.

65 However, contact sampling techniques are useless if the suspect was cautious, did not touch
66 anything at the crime scene and just left an odoriferous trail. In this case, the
67 implementation of non-contact/indirect sampling would be useful too, especially to sample
68 someone’s odor in a room or ambient air at a crime scene. “Scent Transfer Unit” is the most
69 commonly used in the USA for dog identification (21–23). This device is made of a vacuum
70 pump connected to a gauze, allowing the collection of volatile organic compounds. For such
71 an approach, many parameters need optimization and a validated protocol should be set up.
72 For instance, flow rate and time of collection have to be carefully chosen not to lose
73 compounds which can break through the collection material (24). Molecular backbone of the
74 sorbent, its chemical composition as well as the weave of the material used, play a part in
75 the trapping and release of compounds (9,25) and were studied in depth (26). Dormont et al.
76 evaluated several analytical preconcentration techniques such as dynamic head-space
77 sorbent tube sampling (DHS), head-space SPME, contact SPME –opposed to head-space
78 SPME (1,27). According to the authors, contact SPME sampling appeared to be faster and
79 more convenient for field experiments. However, only DHS allowed trapping more volatile
80 compounds and isolating trace-levels compounds that were not detected using SPME. The

81 “flow sampling chamber” is also a promising indirect sampling technique. Subject’s hand is
82 inserted into a hermetic device coupled with a pump (N_2 flow). Compounds carried by the
83 flow are trapped with a Solid Phase Micro Extraction (SPME) fiber, which is then
84 thermodesorbed in GC-MS (28).

85 Whatever the sampling method, with or without contact, desorbing directly samples into the
86 GC-MS reduces steps of sample preparation, compared with solvent based elution
87 techniques, and avoids losing compounds. Different materials and sorbent phases can be
88 thermally desorbed (20) and are suitable for human hands odor analysis. Thermodesorption
89 is then a relevant approach (29) and is a promising alternative to SPME. The latter was used
90 by Colon-Crespo et al. in a recent paper but the extraction time was set to 15 hours (30)
91 which would barely be suitable for routine analysis.

92 The present paper aims at developing and optimizing two methods for both direct and indirect
93 sampling involving a thermodesorption device.. It is essential to keep in mind that profiling
94 human odors use for forensic applications will not be possible as long as no standardized
95 sampling procedures are clearly defined and validated. In real cases, sampling steps won’t be
96 performed at the bench but directly at a crime scene. This is one of the reason why all
97 developed methods and prototypes have to be easily transferrable in the field. For now,
98 sampling methods are validated in the lab while testing in real conditions is carried out in
99 partnership with dog handlers.

100 In this paper, thermodesorption parameters for both direct and indirect sampling were
101 optimized. An original and easy-to-use polymeric sorbent phase called Sorb-Star® was
102 evaluated and compared to Tenax TA® packed tubes. Specifically for indirect sampling, a
103 prototype was developed with an industrial partner. This papers gives the results of the

preliminary study of the prototype device at our disposal. At this stage of the project, both thermodesorption device and indirect sampling methods were developed using experimental design and a synthetic mixture of 80 standards representative of the compounds likely to be present in human hand odor (5,31–33). Both GC-MS and comprehensive 2D gas chromatography coupled with mass spectrometry (GC×GC-MS) were used in this study. While GC-MS was sufficient enough to perform preliminary experiments, real samples are likely to be more complex and the use of GC×GC-MS will enhance the separation.

2. Material and methods

1) Chemicals and reagents

Standards were purchased from Sigma-Aldrich (Saint Louis, USA). The detailed composition of this mixture is reported in supplementary information (SI). Methanol was purchased from Carlo Erba Reagents (Cornadero, Italy). Standards were not purchased in solution. Ultra-pure water was produced using a Direct-Q UV 3 system (18.2 MΩ/cm) from Millipore (Darmstadt, Germany).

2) Thermodesorption and separation devices

The purge and trap system Versatile Sample Preparator (VSP4000) was purchased from Innovative Messtechnik GmbH (Vohenstrauß, Germany). Volatile substances are purged from the sample matrix by the carrier gas of the GC. This concentration step is done with adsorption on a suitable adsorbent in the system trap by freezing out at low temperatures. After completion of the purging process the concentrated substances are transferred by fast

126 thermal desorption from the trap onto a transfer line and then separated by gas
127 chromatography.

128 The thermodesorption device was coupled with a GC-MS Q2010Plus purchased from
129 Shimadzu (Kyoto, Japan). A ZB-1MS column (30 m x 0.25 mm, 0.25 µm) (Phenomenex,
130 Torrance, USA) was used to conduct the chromatographic separation. Initial temperature
131 was 40 °C, held 1 min, then raised to 250 °C at 2.5 °C/min, held 1 min. Mass spectrometer
132 was used with the electronic ionization source (70 eV) heated at 200°C. The acquisition was
133 made with scan mode. The scan range was 29-250 m/z. Data were acquired with GC Real
134 Time Analysis and processed with GC PostRun Analysis 4.20 (Shimadzu softwares).

135 For GC×GC-MS analyses, a ZB-1MS column (30 m x 0.25 mm, 0.25 µm) (Phenomenex,
136 Torrance, USA) coupled with a ZB-1701 column (1.5 m x 0.1 mm, 0.1 µm) (Phenomenex,
137 Torrance, USA) were used. Modulation was performed with a N_2 cooled Zoex ZX1 thermal
138 modulator (Zoex, Houston, USA). Modulation time was 8 seconds. The same gradient and
139 mass spectrometry conditions as the GC-MS method were used. Data were acquired with GC
140 Real Time Analysis 4.20 and processed with GC Image 2.5 (Shimadzu and Zoex softwares)
141 and ChromSquare 2.2 (Shimadzu and Chromaleont softwares).

142 All statistical comparisons and significance tests were carried out by ANOVA with a type I
143 error risk α set at 5% (JMP® software, SAS Institute).

144

145 ### 3) Analytical standards for optimization

146 To optimize analytical methods, a mixture of 80 standards was prepared in heptane at a
147 concentration of 10 mg/kg (6.84 mg/L) each. The detailed composition of this mixture is
148 reported in supplementary information (SI). The selected standards are compounds likely to

149 be found in human hand odor (5). A particular attention was given to covering a wide range
150 of compounds with different chemical properties.
151

152 ## 4) Sorbent phase for compounds trapping

153 Sorb-Star® were purchased from Action Europe (Sausheim, France). This sorbent is a
154 patented silicon-based polymeric phase and is subject to specific conditioning processes to
155 avoid contaminations as far as possible. The Sorb-Star® has a density of 1.12 g / cm^3 and is
156 compliant with FDA 177.2600. It is physiologically safe and even suitable for applications in
157 the food industry. It is meant to be used while direct sampling. People will be asked to rub it
158 in their hands during a defined period of time.

159 Sorb-Star® packaging, before and after sampling, could impact the final results by
160 introducing interfering compounds. The Sorb-Star® was conditioned in a 2mL vial in
161 controlled atmosphere. 6 different packaging were evaluated: 1 - amber glass vial with blue
162 polypropylene (PP) cap, aluminium foil seal; 2 - amber glass vial with blue PP cap, silicone
163 and PTFE seal; 3 - amber glass vial with blue PP cap, butyl and red PTFE seal; 4 - amber glass
164 vial, silicone and PTFE seal; 5 - PP vial with red polyamide (PA) cap, transparent silicone and
165 PTFE seal. 6 – amber cartridge – silver silicone aluminium cap

166 To optimize analytical methods, Sorb-Star® were spiked with 5 µL of the mixture of 80
167 standards prepared in heptane (10 mg/kg) to mimic a direct sampling. Spiked Sorb-Star® was
168 then placed into a stainless steel vial for analysis with VSP4000-GC-MS.

169 Optimization of desorption parameters was performed by monitoring 9 compounds – out of
170 80 – using only GC-MS separation (cf. table 1). The compounds were selected to cover the
171 whole chromatogram with a wide range of boiling point and different chemical families.

172

173 ## 5) Indirect sampling device

174 A lab sampling prototype (cf. fig 1) was provided by Action Europe (Sausheim, France).

175 This is the first step to the development of a field user-friendly sampling device. Despite the
176 fact that this device is not fully adapted to the use in the field yet, it provided crucial
177 preliminary results to methods development. It consisted of a glass chamber with one inlet
178 and one outlet: a gas inlet valve connected to a flowmeter and an outlet to connect solid
179 sorbent tubes and collect compounds. The whole device is connected to a portable pump
180 which can work at flowrates from 10 to 80 mL/min. Two different sorbents were used: Tenax
181 TA® and Sorb-Star®. Both Tenax TA® packed tubes and Sorb-Star® cartridges were purchased
182 from Action Europe (Sausheim, France). A plastic hand covered with a nitrile glove and 5 µL
183 of the mixture of 80 standards prepared in heptane (10 mg/kg) were put into the chamber.
184 The use of a plastic hand allowed to mimic real sampling conditions.

185 Glass chamber was washed carefully with perfume-free soap, rinsed with milliQ water and
186 dried at 150°C during one hour between analyses. The seal of the glass chamber were
187 disposable and changed before any experiment (cf. fig 1). Analyses were performed at
188 ambient temperature between 22 and 23 °C.

189 Optimization of the indirect sampling device was performed by monitoring 16 compounds –
190 out of 80 – using GC×GC-MS separation (cf. table 2). These compounds were selected
191 according to their retention time so that they are scattered all over the 2D chromatogram
192 (cf. fig 2).

193 With Tenax TA packed tubes, a vacuum sampling pump (Sigma Aldrich, St Louis, USA) was
194 used at a flowrate of 70 mL/min during 15 minutes. Higher values can increase breakthrough
195 phenomena and are hence not well suited (34) .

196 With Sorb-Star® cartridges, a specific prototype was developed by Action Europe (Sausheim,
197 France) to drill the cartridges and control the flowrate. A flowrate of 70 mL/min was used
198 during 15 minutes. For now, flowrates cannot be higher than 70mL/min because of the
199 prototype limitations.

200

201 3. Results and discussion

202 Prior to method development, the impact of the use of different vials seals for the
203 conservation of samples –sorbent- was evaluated using GC×GC-MS. Then, the desorption
204 conditions were optimized by TD-GC-MS using spiked Sorb-Star® in order to mimic a direct
205 sampling. Once the desorption method was developed, the indirect sampling procedure was
206 optimized by TD-GC×GC-MS.

207 The whole mixture was run and then a subset of peaks was selected to reduce data
208 processing, for both GC-MS and GC×GC-MS (cf. tables 1 and 2). They were selected so they
209 are scattered all over the chromatogram. Both GC-MS and GC×GC-MS were used in this
210 study. Because of technical issues, GC×GC-MS was not available to carry out every
211 experiment but it did not have an impact on the results as only data obtained with the same
212 technique were compared. The desorption conditions were optimized with 1D separation
213 while the indirect sampling procedure was developed using comprehensive 2D gas
214 chromatography. As sampling and desorption steps are totally independent of the

215 chromatographic separation, using 1D or 2D GC cannot modify the results and conclusions.
216 All comparisons and significance tests were carried out by ANOVA with a type I error risk α
217 set at 5%.

218

219 ### 1) Packaging and reduction of external contamination of samples

220 Six different vial seals (cf. fig 3-2 to 3-7) were tested to study and minimize their possible
221 effect on the contamination of Sorb-Star®. The analyses were performed on blank Sorb Star
222 with GC×GC-MS to compare the different packagings qualitatively, using the color plots
223 which were all drawn with the same intensity scale. The differences between the
224 chromatograms are significant and this figure highlights the importance of packaging
225 selection. It appeared essential to reduce matrix effects and seal number 2 (cf. fig 3-2) was
226 selected because it minimized the contamination (peaks of silica derivatives). Its
227 chromatogram was the closest to a "fresh" Sorb-Star® (cf. fig 3-1) – analysed directly after
228 conditioning process. Thus, the same seal was selected for Sorb-Star® stored in vials for
229 direct sampling and in cartridges for indirect sampling. Moreover, the impact of the use of
230 nitrile gloves during sample preparation was evaluated. A blank Sorb-Star® was put during
231 15 minutes inside a nitrile glove. No additional contaminants were noticed (cf. fig 3-8).

232

233 ### 2) Optimisation of desorption

234 The study was carried out by GC-MS coupled to the thermodesorption device. To optimize
235 analytical methods, Sorb-Star® were spiked with 5 µL of the mixture of 80 standards
236 prepared in heptane (10 mg/kg). Spiked Sorb-Star® was then placed into a stainless steel vial
237 for analysis with TD-GC-MS.

238 Eight parameters had to be optimized: sample temperature, valve temperature, purge time,
239 purge flow, trap temperature, trap desorption temperature, desorption time and split flow.

240 According to constructor recommendation, desorption time was set to 10 min. A shorter
241 time would not be enough to desorb the heaviest compounds whereas a longer one could
242 reduce the lifetime of the trap. Many parameters had to be taken into account so that, prior
243 to the experimental design, repeatability analysis and some preliminary tests were carried
244 out to determine the temperature conditions to be applied to the valve, the trap and for
245 desorption. Then, a design of experiment was conducted to optimize the sample
246 temperature, the purge time, the purge flow and the split flow with a reduced number of
247 experiment.

248 ## 2.1 Repeatability evaluation

249 Analyses were performed with GC-MS nine times to assess the repeatability of the peak
250 areas. The coefficients of variation (CV) of the compounds (10 mg/kg in heptane) were
251 calculated (cf. table 3). Thermodesorption induces many sources of variability which are not
252 easy to control, and CV under 10% are quite acceptable. The pooled CV is 7.6%.

253

254 ## 2.2 Preliminary tests

255 The valve, trap and desorption temperatures were determined with preliminary tests. Their
256 interactions with other parameters to be studied using the DOE were considered
257 unimportant. To evaluate the influence of these parameters, each sample was analyzed in
258 triplicate and ANOVA (35) was performed.

2.2.1 Valve temperature

The valve temperature can theoretically be set from 50°C to 280°C. In practice, temperatures higher than 210°C prevent the trap from being cooled at – 35°C. Thus, 150°C, 180°C, 210°C were tested to determine if this parameter had an influence, but no significant difference was observed (cf. table 3). Thus, the valve temperature was set to 210°C to avoid any cold point in the system.

2.2.2 Trap temperature

The trap temperature can theoretically be set from -40°C to -10°C. In practice, when the valve temperature was set to 210°C, it prevented the trap from being cooled at – 40°C. Thus, -30°C and -35°C were tested to determine if this parameter needed further optimization. There was no significant difference between these two temperatures except for octanoic acid, methyl ester (cf. table 3). The trap temperature was set to -30°C.

2.2.3 Desorption temperature

The trap desorption temperature can be theoretically set from 100°C to 400°C. According to the constructor recommendations, temperatures higher than 240°C could damage the trap and reduce its lifetime. Yet, Tenax TA was successfully used in other studies at temperature higher than 240°C (36). Two temperatures were tested: 240°C and 260°C. There was no significant difference between these two temperatures except for acetophenone (cf. table 3). Moreover, for both temperatures, a second analysis of the tubes showed that all

280 compounds were desorbed during the first analysis. Thus, the desorption temperature was
281 set to the recommended value of 240°C.
282

283 ### 2.3 Design of experiment

284 A 2^4 full factorial design was conducted in order to check whether sample temperature,
285 purge flow, purge time and split flow had a significant effect on the analytical process. The
286 peak areas were used as response in the experiments. The "+1" level was set as follows:
287 sample temperature = 220°C, purge flow = 30mL/min, purge time = 30 min, split =
288 50mL/min. The "-1" level was set as follows: sample temperature = 160°C, purge flow =
289 10mL/min, purge time = 10 min, split = 10mL/min. Four repeated measurements were
290 performed at the center of the domain (sample temperature = 190°C, purge flow =
291 20mL/min, purge time = 20 min, split = 30mL/min). This whole design was repeated twice to
292 increase the number of degrees of freedom. The lack of fit test was also performed to check
293 whether the proposed model was correct.

294 The split had a major effect on all compounds intensity. This effect is expected as only a
295 fraction of the injected sample goes into the column when a split is used. Unexpectedly, in
296 the tested conditions, the effects sample temperature, purge time and purge flow were not
297 significant. This thermodesorption method appears to be robust with respect to the other
298 experimental parameters (cf. fig 4). For the further analyses, the sample temperature, purge
299 time and purge flow were set to the "0" level – respectively 190°C, 20 minutes and 20
300 mL/min - while the split was decreased to the "-1" level - 10 mL/min.

301 In conclusion of this section, the direct sampling can be performed using Sorb-Star®: the
302 desorption parameters of this sorbent phase are now optimized and will be used for the
303 analysis of real samples collected with direct sampling procedures.

304 3) Indirect sampling

305 Two different solid sorbent phases were tested in this study: Tenax TA packed tubes and
306 Sorb-Star® cartridges. Considering that Tenax is one of the most classic sorbent phase used,
307 the comparison between Tenax and Sorb-Star was interesting. The same mixture of 80
308 standards was used, but separation was performed using comprehensive 2D gas
309 chromatography coupled with mass spectrometry (cf. fig 2). The 16 monitored compounds
310 are indicated with red circles. Since this method must be suitable to human hands as well as
311 to objects found at a crime scene, analyses were performed at room temperature (between
312 22°C and 23°C) and not at a human body's temperature.

313

314 3.1 Sorb-Star® cartridges

315 *3.1.1 Repeatability tests*

316 First of all, the repeatability of every part of the sampling device was checked. To evaluate
317 separately the different parts of the device, the glass chamber was replaced by a 20 mL glass
318 vial. The pump – without the glass chamber – provided quite good results at 70 mL/min
319 during 15 minutes since the coefficient of variation of target compounds did not exceed 6%
320 except for nonane (which is close to the solvent tail) and eicosane (which is close to its limit
321 of detection). However using the complete device - with the glass chamber - was the pooled
322 coefficient of variation rose up to 30%. Different hypotheses can be made to explain this
323 increase. First, the flow rate of 70 mL/min was not suited (too low) and needed a proper

324 optimization. To solve this problem, a new pump prototype is being designed and should
325 reach flow rates up to 200 mL/min. Also, the design of the glass chamber may not be
326 appropriate and could be improved. To this end, a new glass chamber is being designed to
327 create turbulences inside the chamber and to enhance repeatability.

328 ### *3.1.2 Preliminary tests for indirect sampling optimization*

329 The first tests showed that the complete device - glass chamber and pump – was not
330 optimized. For all preliminary tests – except for the fan - the glass chamber (cf. fig 1) was
331 replaced by a 20 mL glass vial to reduce sources of variability and get a better evaluation of
332 the tested effect. Effectively, as the geometry of the glass chamber is independent of the
333 cartridge optimization, it is possible to decouple the problems.

334

335 #### Use of a fan

336 Considering the bad repeatability of the first results, the use of a fan was evaluated during
337 sampling. Whereas agitation was expected to create turbulences which would enhance the
338 repeatability of the process, it was shown not to be decisive. Pooled coefficient of variations
339 reached 31% without the fan (CV ranged from 2 to 56%) and decreased to 28% with it (CV
340 ranged from 12 to 44%). These CV (cf. table 4) are high but are not dramatic and are close to
341 classic headspace CV (37,38). In the meantime, further testing was done to design the
342 cartridges containing the Sorb-Star®.

343

344 ## Use of a centering piece in the cartridge

345 To maximize the contact between the Sorb-Star® and the flow, the use of a centering piece
346 inside the cartridge was evaluated. It consisted in an aluminum cross which maintained the
347 Sorb-Star® at the center of the cartridges and avoided contact with the cartridge wall, thus
348 maximizing the available surface. Surprisingly, whereas it was expected to collect more
349 compounds as the available surface increased, centering or not the sorbent made no
350 difference. Moreover, the variability increased despite the fact that the position of the
351 sorbent in the cartridge was better controlled with the centering piece (cf. table 4): the
352 pooled coefficient of variations was 10% without the centering piece (CV ranged from 4 to
353 20%) and it rose up to 17% with the centering piece (CV ranged from 6 to 32%).

354 Thus, this centering piece will not be used for further experiments.

355

356 ## Pulsed mode

357 Unlike Tenax, with Sorb-Star® cartridges, the polymer did not fill the whole cartridge. It is
358 important that compounds adsorb on the sorbent phases and do not go through the
359 cartridge. The use of a pulsed mode of the pump was evaluated to create turbulences and
360 enhance the trapping of compounds. The pump is on during a predefined time, then turned
361 off during a predefined time, this cycle being repeated during all the sampling procedure.
362 The pulses aimed at creating some turbulences to stir in the cartridge and maximize the
363 contact between the Sorb-Star® and the compounds. 2mL cartridges were used with a
364 maximal flow rate of 80 mL/min. Pulses cannot be lower than 500 ms (device limitation) and
365 should not exceed 1500 ms (homogenous agitation).

366 Pulses of 500 ms and 1000 ms were evaluated. There were no significant differences with or
367 without pulse. The use of a pulsed mode increased the variability (cf. table 4), preventing us
368 from considering this mode of sampling. The pooled coefficient of variation was 10% without
369 the centering piece (CV ranged from 4 to 20%) and it rose up to 12% and 13% with the short-
370 pulsed mode (CV ranged from 0.1 to 34%) and the with the long-pulsed mode respectively
371 (CV ranged from 1 to 38%).

372

373 ### Use of restricted cartridges

374

375 Another attempt was to reduce the volume of the cartridges to trap more compounds on
376 the sorbent phase. Since the volume of the cartridge was reduced, the loss of compounds
377 going through the cartridge was expected to be limited. There were significant differences
378 between experiments with or without restriction. Surprisingly, more compounds were
379 collected without using the restricted cartridge.

380 As for the variability, pooled coefficient of variation (cf. table 4) was 10% without the
381 restricted cartridge (CV ranged from 4 to 20%) and it rose up to 17% with the restricted
382 cartridge (CV ranged from 2 to 42%).

383

384 ## 3.2 Tenax TA phase

385 First of all, the Tenax TA packed tubes repeatability was assessed (n=3) by measuring the
386 intensities of the 16 targeted compounds. The results were quite promising as there was no
387 significant difference between the three tubes.

388 Tenax TA packed tubes and Sorb-Star® cartridges performance used in indirect sampling
389 mode were compared to direct thermodesorption of Sorb-Star® spiked with the synthetic
390 mixture of 80 compounds. The pooled coefficient of variations was 19% for direct sampling
391 (CV ranged from 0.1 to 39%).

392 This experiment gave interesting results (cf. fig 5). First, the variability of recovered
393 compounds is more important with Tenax phase - pooled coefficient of variation was 13%
394 (CV ranged from 1 to 30%) – than with Sorb-Star® cartridges where the pooled coefficient of
395 variation was 6% (CV ranged from 1 to 16%).

396 As expected, for most compounds, there was a lower recovery between direct spiked Sorb-
397 Star® and indirect sampling process on the two phases (Tenax and Sorb-Star® cartridges).

398 Tenax and Sorb-Star® provided quite similar results for recovering our compounds of
399 interest. However, the developed methods have to be easily transferable in the field. The
400 use of a disposable, cheap and easy-to-use sampling phase such as Sorb-Star® will be
401 preferred by police forces. A Sorb-Star® costs around 3 euros while a Tenax packed tube is
402 much more expensive and costs around 100 euros. And even if it can be reused after a
403 proper conditioning procedure, Tenax can only be used for indirect sampling. Nevertheless,
404 the use of reconditioned sampling phases is not viable as it would weaken the probative
405 value of evidence brought in courts of justice. These are the reasons why we selected Sorb-
406 Star® for both direct and indirect sampling and gave up tests with Tenax phase.

407 ### 3.3 Application to real samples

408 The developed methods were applied to real samples. Several hundreds of peaks are detected
409 (cf. figure 6) showing that the developed protocol is relevant and efficient to collect volatiles
410 from human hands. The use of a bidimensional separation technique is also relevant

411 considering the number of compounds involved in human odor. GC×GC-MS provided an
412 increased peak capacity, thereby allowing the separation of compounds which would not have
413 been possible using classical GC-MS. A selection of 40 compounds identified in this
414 chromatogram using NIST library is given in the table 5. All of them were described at least
415 once as constitutive compounds of human hands odor (5). It should be noted that a large
416 diversity of chemical families is present, validating the choice of the 80 standards for previous
417 optimization.

418 For real samples, putting a hand in the confined space of the sampling chamber raises
419 substantially the temperature during sampling. It also increases the humidity as compared to
420 tests carried out with the synthetic mixture. Further experiments will be carried out to see to
421 what extent this parameter has an influence on the sampling procedure.

422

423 4. Conclusion

424 Forensic profiling of human hand odor is complex and needs the development of a validated
425 sampling procedure. Desorption optimisation and indirect sampling method using a thermal
426 desorption device coupled with GC×GC-MS were developed for human hand odor, from the
427 selection of the packaging seal to the optimization of indirect sampling and
428 thermodesorption conditions. To mimic human hand odor and perform proper comparison
429 between techniques, a synthetic mixture of 80 compounds representative of human hand
430 odor was used. The methods developed constitute a solid background to perform further
431 testing, to carry out analyses of real samples and to assess the transfer of the different
432 methods to crime scene investigators. Sorb-Star® was selected for both direct and indirect
433 sampling while tests with Tenax phase given up. Unlike Tenax, Sorb-Star® can be used for

434 both direct and indirect sampling. The use of a disposable, cheap, easy-to-use and unique
435 sampling phase will be preferred by police forces. While the pumping mode and the
436 cartridge were successfully optimized, a new prototype of glass chamber is being developed
437 and will be evaluated using the preliminary results obtained in this study. Moreover,
438 different tests would be performed with dogs to check if enough information was collected
439 with Sorb-Star® to individualize people. Whatever the sampling method – direct or indirect –
440 dogs should be able to trace back a person just by sniffing out a Sorb-Star®.

441 All the developed methods will be easily transferable in the field. This first study was
442 restricted to 80 compounds and GC-MS could be used in this preliminary approach. The
443 developed protocols were successfully applied to real samples. Several hundreds of
444 compounds were detected, making the use of GC×GC-MS a true asset at this stage. The
445 combination of increased selectivity, peak capacity, as well as sensitivity due to the
446 cryomodulation will enhance the separation and enable to collect more information. The
447 application to real samples will need a proper statistical processing to validate the relevance
448 of the collected information, to assess its repeatability given the variations of human odor,
449 and finally to perform proper individualization.

450

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455

Conflicts of interest

The authors declare having no conflict of interest of any kind.

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