Filetti et al. Egyptian Journal of Forensic Sciences (2019) 9:35
https://doi.org/10.1186/s41935-019-0139-1

Egyptian Journal of Forensic Sciences

ORIGINAL ARTICLE Open Access

Volatile organic compounds: instrumental and canine detections link an individual to the crime scene

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Abstract

Background: Whenever a crime is committed, forensic personnel are requested to collect every kind of evidence to establish the relationship between the suspects and the crime. When any evidence is accidentally destroyed or not found, there is one type of latent evidence that is always deposited at the crime scene: unique human scent. Recently, the use of trained canines to detect selective human scent at a crime scene has increased. To consolidate this kind of evidence, it is essential to have an exact knowledge and an awareness of the chemical signature of the volatile compounds that could indicate the presence of the alleged offender at the crime scene.
This experimental study aims to detect the volatile organic compounds (VOCs) released from subjects who handled scent-articles to imprint their odor on. After handling, each scent-article was wrapped in sterile and VOC-free cotton gauzes for 48 h for secondary transfer. VOCs were detected by headspace/solid-phase microextraction-gas chromatography/mass spectrometry (HS/SPME-GC/MS) and well-trained dogs, at different time points (up to 15 days). Furthermore, the possibility of further DNA detection after contact was also investigated to propose a novel approach able to identify a subject from this latent forensic trace.

Results: Data show that inter-individual human scent composition includes different VOCs, but dogs were able to discriminate the individual who touched the object at the crime scene. The dog training procedure showed excellent sensitivity (between 99.48 and 100%) and specificity (between 60 and 100%), having a positive predictive value (PPV) ranging between 97.94 and 100% and a negative predictive value (NPV) ranging between 85.71 and 100%. Preliminary work on DNA analysis released after contact yielded positive results, even if further studies are necessary, expanding the same experimentation to a larger sample with the aim of obtaining a statistically significant result.

Conclusion: Data show that human scent is a good source of VOCs and a good target for canine training. The well-trained dog represents a specialized biological device able to discriminate personal human odor from any contaminants in the mixture detected by instrumental analysis. Furthermore, this study proposes the use of human scent as a forensic latent trace for DNA profiling.

Keywords: Forensic science, Volatile organic compounds, Human scent, Gas chromatography/mass spectrometry, Dog training, DNA analysis

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Background

Human scent consists of a mixture volatile organic compounds (VOCs) released from the body. VOCs are chemical compounds such as aliphatic, aromatic and chlorinated hydrocarbons, aldehydes, terpenes, alcohols, esters, and ketones. These are defined as organic compounds, which at 293.15 K (20 °C) have a vapor pressure of 0.01 kPa or higher (Waring and Wells 2015). This characteristic of passing easily to the aeriform state at room temperature and ambient pressure makes them perceptible to smell.

"Odorology" is the set of investigative methods to extract and analyze human scent to establish its association with an object/subject or if the subject had handled an object (Pomara et al. 2015; Maglietta et al. 2017; Sessa et al. 2018; Ferrara et al. 2019; De Simone et al. 2019). The importance of this application in the forensic field is the possibility to link a suspect to a crime scene. Moreover, it is very useful in cases of missing persons when "odorology" could be very important for the investigation. Both cases involve the use of dogs to target a specific scent. In the first case, detailed sampling is carried out, comparing the human scent found at the crime scene, with the suspect's scent. In the second case, the dog follows the trace of human scent by comparing the smell of a target with the missing person's scent (Marchal et al. 2016). In one case, the trace is attributed to the author of the crime, while in the other the victim's trace is followed. Two operational systems correlated to protocols for human scent identification in forensic field are generally used in many countries (Schoon and Haak 2002).

In various studies, the VOCs of human scent were detected after direct contact between the palms of the subject's hands and absorbent gauzes (Curran et al. 2007; Colón-Crespo et al. 2017), or glass beads (Doležal et al. 2017). Other studies reported the use of a non-contact device, known as the Scent Transfer Unit 100 (STU-100), to detect the VOCs from an object or subject (Caraballo et al. 2016; DeGreeff et al. 2011).

The first experiments on human scent stored in containers were carried out in the Soviet Union in the 1960s. Subsequently, this specialty was developed in the German Democratic Republic. Based on the Russian experience, in 1989, an "Odorology Laboratory" was established in Cuba. To date, "odorology" is used in Cuba, Denmark, Holland, Belgium, Sweden, Germany, Hungary, Argentina, and Poland (Gajjar and Kasting 2014; Intarakumhaeng et al. 2018).

Human skin VOCs are aliphatic hydrocarbons, aldehydes, ketones, alcohols, fatty acids, esters (Curran et al. 2005; Haze et al. 2001; Spagnolo et al. 2018; Bernier et al. 2000; Munk et al. 2000; Zeng et al. 1991, 1996), steroids, unsaturated acids (Kanda et al. 1990; Messina et al. 2018), aromatic hydrocarbons, amides, and amines (Curran et al. 2007; Prada and Furton 2008). Their composition is influenced by genetics, the environment, daily activities, diet, and physiological secretions. Furthermore, various factors, such as humidity, temperature, bacterial flora, pH of the skin surface (Pandey and Kim 2011; Sperandeo et al. 2018), illnesses, the onset of puberty, and menstrual cycle in females (Ensminger et al. 2010) contribute to the unique human scent.

Several studies have been conducted studying the VOCs released from human skin. However, to the best of our knowledge, no studies have been performed on secondary transfer linked to human scent. In other words, even if the opportunity to transfer biological traces indirectly has been well described, no references have been found related to human scent. Indeed, secondary transfer is well described as the event that occurs when any biological trace is deposited on an item or subject, and then transferred to another item or person (Goray et al. 2010). A biological sample that has been transferred multiple times, if detectable, will often appear as components of complex DNA profiles (Wickenheiser 2002; Ladd et al. 1999).

Aim of the work

This experimental study aimed to detect the VOCs released from subjects that stimulate canine olfactory discrimination alerts, using gas chromatography-mass spectrometry (GC-MS) after secondary transfer. Thus, the main goals were to analyze the chemical composition of human scent, discriminating it from contaminants and determining the scientific basis for dog training. Finally, DNA analysis was performed to verify if it is possible to obtain a profile that could be useful for the identification after a secondary transfer.

Methods

Preparation of human scent samples

Human scent samples were collected from four Caucasian subjects (two males and two females) between 18 and 50 years old. The exclusion criteria for subject recruitment were the presence of skin diseases or disorders. All subjects gave written informed consent in accordance with the latest version of the declaration of Helsinki. The protocol was approved by the Research Committee (University of Foggia). The subjects washed their hands with water, using the same antiseptic soap. Subsequently, the hands were dried off naturally, without contact with any object or other subject. Then each subject handled two scent-articles for 5 min to imprint their odor on, for a total of eight items. The scent-articles were made up of different substrates in groups of two: ceramic, plastic, treated wood, and wax. Different scent-articles were used in the experimental design to verify if human scent could be sampled by the different substrates at the experimental

conditions. After handling, each scent-article was wrapped in ten sterile and VOC-free cotton gauzes (10 × 10 cm) for 48 h, for secondary transfer. The gauzes did not affect the results because they are inert and VOC-free. At each time (0, 5, 10, 15 days), a new gauze was analyzed using the headspace/solid-phase microextraction-gas chromatography/mass spectrometry (HS/SPME-GC/MS) method to verify the background.

After 48 h, the gauzes were removed. Ten gauzes were prepared and only nine were used for the successive phases. Each gauze for GC/MS analysis was divided into two parts and inserted into clear glass vials closed with a sterile and VOC-free film for subsequent analysis in duplicate. This sampling method was used to preserve the headspace of each sample. The gauzes for the following DNA profiling were inserted into vials previously pretreated with UV light for 20 min. They were subdivided into three groups. The samples were all prepared at the same time (time 0 of the experiment) for a total of 98 samples (32 gauzes in duplicate for GC-MS detection, 32 for canine training, 2 gauzes for DNA profiling). The first group (four gauzes for each scent-article) was used to detect the VOCs using HS/SPME-GC/MS; the second group (four gauzes for each scent-article) was presented to a dog (Labrador Retriever) to sniff and stimulate discriminating alerts during the training procedure described below. These gauzes were placed in 40 ml clear glass vials closed with a sterile and VOC-free film. The vials were stored at a room temperature of 22 °C and relative humidity of 45%.

The third group (one gauze from two scent-articles) was used for DNA extraction. A negative control was taken for each gauze before experimentation and each sample was analyzed in duplicate. Two buccal swabs (called "MAN A" and "MAN B") were taken from the subjects who participated in this study. All the samples for the genetic analysis were stored at − 20 °C.

SPME-GC/MS analysis

The gauzes were analyzed at different extraction times (0, 5, 10, and 15 days) in duplicate. At each time point, a "white fiber" (PDMS/DVB) with empty vial and a "white sample" with new sterile and VOC-free cotton gauze were performed to verify the siloxane peaks and background. The siloxane peaks that resulted from the SPME fiber coating and from the column were removed. The first extraction of the gauzes was assessed at time 0 of the experiment. The SPME extractions were repeated every 5 days for 15 days. For all samples, four analysis times were investigated.

After several optimization tests, SPME extraction time and temperature and GC method parameters were chosen in order to obtain a chromatogram with an optimal signal to noise (S/N) ratio. Polydimethylsiloxane/divinylbenzene (PDMS/DVB) 65 μm fiber was exposed to the headspace of the vials containing the gauzes to extract the VOCs at the different times of analysis with an incubation time of 10 min at 40 °C. The samples were analyzed in a Thermo Scientific Trace GC Ultra with a DSQ II mass selective detector (Thermo Fisher Scientific, Waltham, MA, USA). The column used was a 30 m × 0.25 mm ID, 0.25 μm film thickness DB-5-MS. Helium was the carrier gas, flow controlled at 1.0 ml/min. The VOCs were desorbed in the injection port of the GC using an inlet temperature set at 200 °C. The 28-min GC method consists of three steps: at the first step, the temperature of 50 °C was maintained for 3 min, followed by an increment of 10 °C/min up to 200 °C, and ending with a 10-min hold. The quadrupole mass analyzer was operated in the electron ionization (EI) mode and scanned over a mass range of *m*/*z* 50–650 in full-scan mode (Table 1).

After extraction and analysis of the collected human scent samples, the National Institute of Standards and Technology (NIST) mass spectral library and the extracted ion chromatograms were used to identify the compounds in the headspace of the samples. The qualitative method was based on a previously reported GC-MS method that analyzed human VOCs (Urbanová et al. 2012; Stránský et al. 2006; Doležal et al. 2017; Vass 2012; Hoffman et al. 2009; Rendine et al. 2018).

Dog training for human scent discrimination

In this experimental model, two trained Labrador Retrievers were used. All experimental procedures were

Table 1 Analytical parameters used for VOCs extraction, separation, and detection by HS/SPME-GC/MS

Gas chromatography/head space	
Fiber	Polydimethylsiloxane/ divinylbenzene (65 μm)
Incubation time	10 min
Constant incubation temperature	40 °C
Column	DB-5-MS (30 m × 0.25 mm ID, 0.25 μm)
Carrier gas	Helium
Constant flow	1.0 ml/min
Inlet temperature	200 °C
Ramp	50 °C for 3 min
	10 °C/min until 200 °C
Hold time	10 min
Oven run-time	28 min
Mass detector	
Ionization mode	Electron ionization
Full-scan mass range	50–650 *m/z*

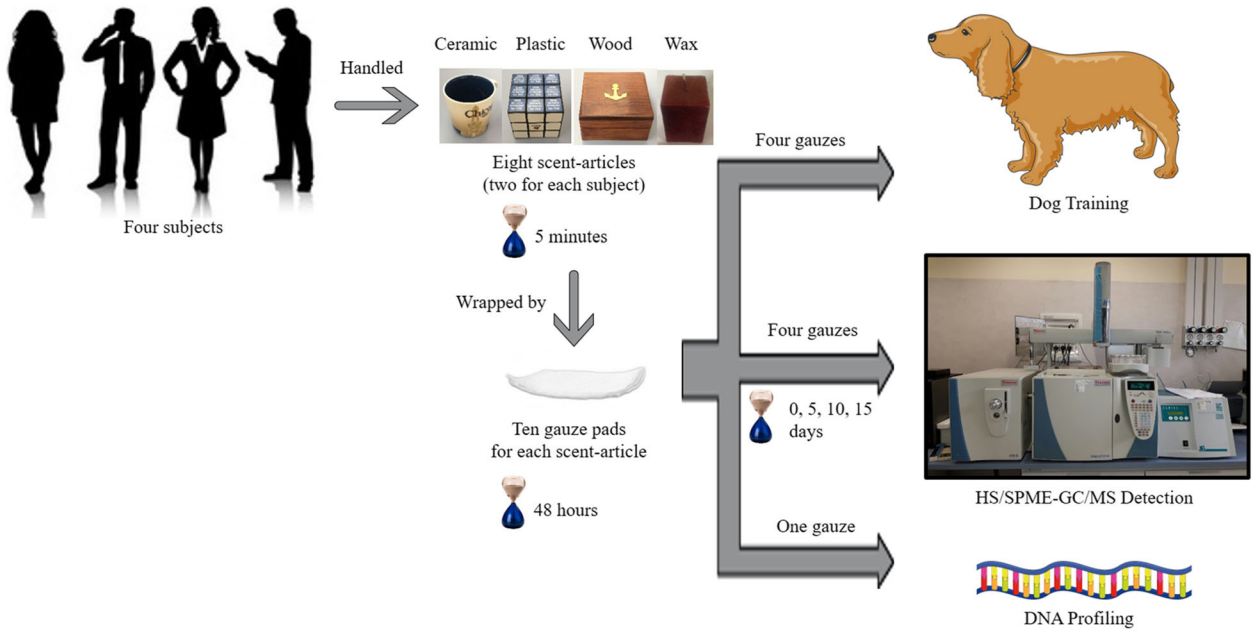

Fig. 1 The experimental model

performed in strict accordance with the Italian and EU regulation on animal welfare and were previously approved by the Animal Research Committee of the University of Foggia. The gauzes were presented to the first dog, after having being in contact with the scent-articles for 48 h. Two hundred trials were carried out for each scent-article (50 trials with each gauze for four sampled gauzes). The subjects were asked to position themselves in a straight line in front of the dog with its handler (Riezzo et al. 2014). After smelling the gauzes, the dog approached the subjects with its handler. The dog sat in front of the corresponding subject whenever it matched the scent. Following each correct choice, the handler rewarded the dog with a treat. After an incorrect choice, or if the dog did not make a choice at all, the dog was not rewarded. The standard protocol for trained dogs changes based on the type of training. In all cases, you have to reward the dog with a cookie or with its favorite toy because the dog associates the prize with a successful action. A second dog (Labrador Retriever) was used to repeat the protocol and confirm the results of the first (Fig. 1).

DNA analysis

DNA extraction of the reference samples (buccal swabs called "MAN A" and "MAN B") was obtained with QIAmp DNA mini kit (Qiagen, Venlo, the Netherlands). The gauzes were sampled with the cut-out technique;

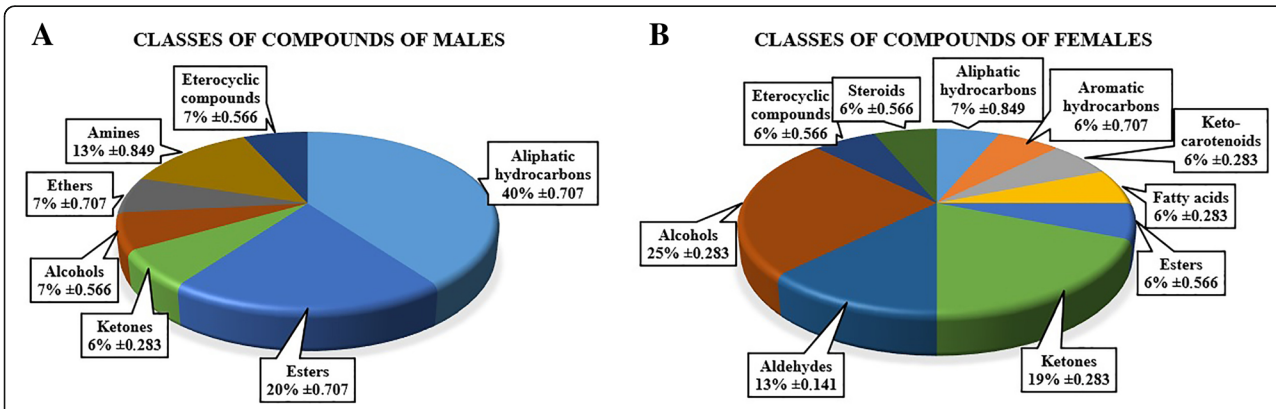

Fig. 2 Classes of compounds detected from male (**a**) and female (**b**) subjects are presented as percentage average (± S.D.)

Table 2 The chemical composition of the human scent samples of the four subjects

Compounds	Man A	Man B	Woman A	Woman B
Canthaxanthin (keto-carotenoid)				✓
2,2-Dimethoxy-ethanol (alcohol)	✓		✓	
Protoporphyrin IX dimethylester (ester)				✓
Tridecane (aliphatic hydrocarbon)		✓		
Ethanone, 2-(4-hydroxy-5,7-dimethylpyrido[2,3-d]pyrimidin-2-ylsulfanyl)-1-(4-(eterocyclic compound)				✓
Benzo [b] thiophene, 6-methyl (eterocyclic compound)	✓			
Propane (aliphatic hydrocarbon)	✓			
Ethylhexanol (alcohol)				✓
Decane (aliphatic hydrocarbon)		✓		
14-Octadecenal (aldehyde)				✓
Linalyl alcohol (alcohol)				✓
Camphor (ketone)				✓
Octadecane, 1,1-dimethoxy (ether)	✓			
Silanamine, N-phenyl (amine)		✓		
Sulfurous acid, cyclohexylmethyl hexyl ester (ester)	✓			
Cyclohexane, 1,4-dimethyl-2-octadecyl (aliphatic hydrocarbon)	✓			
Naphthalene, decahydro-2,6-dimethyl-3-octyl (aromatic hydrocarbon)			✓	
1,4-Benzenediol, 2,5-dimethyl (alcohol)				✓
2-Thiopheneacetic acid, undec-10-enyl ester (ester)	✓			
3-Dodecylcyclohexanone (ketone)	✓			
Dodecane (aliphatic hydrocarbon)	✓			
Cedr-8-ene (terpene)				✓
Thujopsene (terpene)				✓
Undecane (aliphatic hydrocarbon)	✓			
Octadecadienoic acid (fatty acid)			✓	
Tetrahydrocortisone (steroid)			✓	
1,4-Naphthoquinone (ketone)			✓	
9-n-Hexylheptadecane (aliphatic hydrocarbon)				✓
Propanoic acid, dimethylester (ester)		✓		
Benzaldehyde (aldehyde)			✓	
Beta- Methylionone (ketone)				✓
Methyl-3-phenylpyridine (amine)		✓		

therefore, a small portion of each gauze previously in contact with the scent-article was cropped to proceed with DNA extraction. Each sample was analyzed using the double-blind method. The DNA was obtained using the QIAmp DNA Investigator kit (Qiagen, Venlo, Netherlands) following the protocol used for paper or similar material. The AmpFlSTRIdentifiler PCR Amplification kit (Applied Biosystems, Foster City, CA, USA), which simultaneously amplifies 15 markers, was utilized. A negative control of the PCR reaction and a positive control (control DNA 9947A) were added as a quality control. The fragments were determined by capillary electrophoresis through the ABI Prism 3130 Genetic Analyzer (AppliedBiosystems, Foster City, CA, USA) and the GeneMapper ID v.4.0 software, which allows assignment of alleles, comparing them with an allelic ladder.

Results and discussion

The human scent samples were extracted at four different times: 0, 5, 10, and 15 days. Over 30 VOCs were identified with different functional groups. Only VOCs that had been previously cited in the literature as

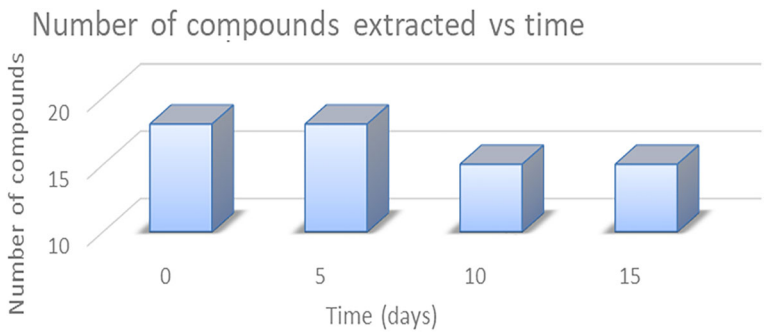

Fig. 3 Number of compounds extracted from fresh and aged samples

originating from human specimens were used in the analysis of our samples.

The chemical compounds detected in males' scent included seven different functional groups: aliphatic hydrocarbons, esters, amines, alcohols, ethers, heterocyclic compounds, and ketones (Fig. 2a). The chemical compounds detected in the females' samples included ten different classes: alcohols, ketones, aliphatic hydrocarbons, aldehydes, esters, steroids, heterocyclic compounds, fatty acids, keto-carotenoids, and aromatic hydrocarbons (Fig. 2b). Both graphs contain the average percentage of the compounds (± S.D.) of the subjects detected within each gender. Aliphatic hydrocarbons and alcohols were found to be the most prevalent, contributing 40% and 22% of scent-articles from males and females, respectively. 2,2-Dimethoxy-ethanol was detected both in males and females.

Table 2 shows the complete experimental data on the chemical composition of the human scent samples of the four subjects involved in the study (two males and two females) and the differences in composition depending on the individual. Inter-individual human scent composition includes different VOCs, independently of sex.

Furthermore, 2,2-dimethoxy-ethanol was detected both in fresh (time = 0–5 days) and in old samples (time = 10–15 days). As previously described, the probability that compounds with greater volatility are detected is higher for the fresher scent-articles with respect to older samples. In older samples, these compounds could evaporate or be transformed by microbial action (Curran et al. 2007), becoming undetectable. Loss by evaporation has been supported anecdotally from the behavior of trained dogs when following a scent trail. A fresh trail is followed with the head in an upright position suggesting that more volatile compounds are being utilized, whereas an old trail is followed with the nose to the ground suggesting that less volatile compounds are being utilized (Curran et al. 2007). In agreement with this theory, in this study, 18 human

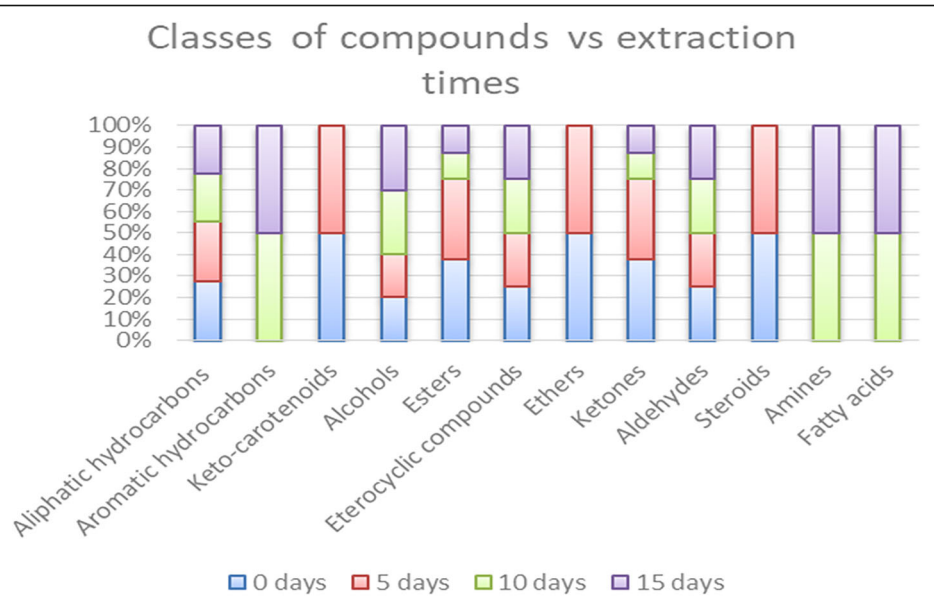

Fig. 4 Distribution of the classes of compounds at the different analysis times

scent VOCs were extracted from fresh samples, and 15 from aged samples suggesting that in older samples some compounds became undetectable (Fig. 3).

Figure 4 shows the various classes of compounds detected at the different analysis times. Some compounds were detected at each sampling time. However, keto-carotenoids, ethers, and steroids were only detected in fresh samples (time = 0–5 days), while aromatic hydrocarbons, amines, and fatty acids were only detected in aged samples (time = 10–15 days). Some compounds are no longer detectable in aged samples because they evaporate or turn into other compounds. In fact, new molecules appear in aged samples, probably transformed by microbial action.

Only 26 VOCs were considered constituents of human secretions. Compounds were selected on the basis of abundance, and whether they were previously investigated in recent literature. These were used in the analysis of our samples as key markers of the presence of the suspect at the crime scene. Infact, recent research has provided information that supports the feasibility of using hand odor as a means for subject identification and differentiation in forensic investigations. The use of the VOC marker combinations may facilitate the association of individuals to criminal activity, by reducing the number of suspects in a crime (Colón-Crespo et al. 2017). The VOCs that were detected, in accordance with the literature, and could be related to the composition of human secretions, were divided into 11 groups: aliphatic and aromatic hydrocarbons, aldehydes, ketones, alcohols, fatty acids, esters, ethers, amines, steroids, and heterocyclic compounds. The compounds, ordered according to their affiliation in chemical groups, are listed in Table 3.

Some of the VOCs detected, namely keto-carotenoids, terpenes, and terpenoids (Table 4), are not constituents of human secretions because they are likely to be "primary odor" and "tertiary odor." Therefore, after several bibliographic searches, these compounds were considered as contaminants. In particular, Canthaxanthin is a natural pigment belonging to the class of carotenoids. Its presence in tissues is related to the individual's dietary intake, as animals are unable to synthesize them. The coloring of most food, be it vegetarian or otherwise, is the cause for the presence of these classes of compounds in human VOCs (Ytrestøyl et al. 2004; Beaulieu et al. 2013). Terpenes are made up of hydrocarbon chains organized in isoprenic units, while terpenoids are characterized by the introduction of heteroatoms along the hydrocarbon chains. Therefore, this study showed that they possess different functional groups, such as ketones and alcohols. These compounds belong to the classes of dyes, essential oils, and fragrances. In particular, Cedr-8-ene and Camphor are essential oils extracted from Juniperus procera Endl wood, belonging to the family of cypresses that grow in Kenya (Akeng'a and Chhabra 1997).

Table 3 VOCs related to the composition of human secretions

Chemical functional groups	Compounds
Aliphatic hydrocarbons	Tridecane
	Propane
	Decane
	Cyclohexane, 1,4-dimethyl-2-octadecyl
	Dodecane
	Undecane
	9-n-Hexylheptadecane
Aromatic hydrocarbons	Naphthalene, decahydro-2,6-dimethyl-3-octyl
Aldehydes	14-Octadecenal
	Benzaldehyde
Ketones	3-Dodecylcyclohexanone
	1,4-Naphthoquinone
Alcohols	2,2-Dimethoxy-ethanol
	Ethylhexanol
	1,4-Benzenediol, 2,5-dimethyl
Fatty acids	Octadecadienoic acid
Esters	Protoporphyrin IX dimethylester
	Sulfurous acid, cyclohexylmethyl hexyl ester
	2-Thiopheneacetic acid, undec-10-enyl ester
	Propanoic acid, dimethylester
Ethers	Octadecane, 1,1-dimethoxy
Amines	Silanamine, N-phenyl
	Methyl-3-phenylpyridine
Steroids	Tetrahydrocortisone
Eterocyclic compounds	Ethanone, 2-(4-hydroxy-5,7-dimethylpyrido [2,3-d]pyrimidin-2-ylsulfanyl)-1-(4-
	Benzo [b] thiophene, 6-methyl

The persistence of a particular substance on the substrate after contact is strictly related to the donor's characteristics, distinguishing them as good or bad donors, depending on the amount of secretion product they release on the substrate they touch (Vanderkolk 2011). It is also related to storage conditions of the samples, the time passed from the contact, and the nature of the substrate (Curran et al. 2007; DeGreeff et al. 2011). In this

Table 4 VOCs related to "primary odor" and "tertiary odor"

Primary odor	Tertiary odor	
Keto-carotenoids	Terpenes	Terpenoids
Canthaxanthin	Cedr-8-ene	Beta-methylionone
	Thujopsene	Camphor
		Linalylalcohol

study, the storage conditions and the time passed from the contact were monitored, eliminating such variables.

This experimental model also analyzed secondary transfer. Donor subjects touched an object, which was placed in contact with gauzes; finally, these were used as samples and analyzed at different time points. Therefore, several compounds could be lost in the transfer from the palms of the hands to the gauze. During secondary transfer, some biological traces as well as some compounds may not be transferred. In this case, we would have only primary transfer (from the subject to the object) and not secondary (from the object to the gauze). The detected compounds were present in traces, in the range of ppb-ppt.

Recent studies suggest that human scent is unique to each person. As previously described, well-trained dogs can positively match the scents of subjects, distinguishing both identical and non-identical twins (Pinc et al. 2011). Dogs have the ability to detect odor concentrations of both organic and inorganic compounds in the range of ppb-ppt. Corroborating our work, previous studies reported that even though each subject does not have the same mixture of VOCs at all times of analysis, dogs were able to match the scent-article with the corresponding subject at all time-points. In the present study, the dog always discriminated the subjects regardless of the contact substrate (two for each subject). Therefore, scent-discriminating dogs demonstrate an advantage over instrumental methods by being able to perform recognition regardless of whether or not the same subject expresses different scent mixtures (Angle et al. 2016). It is important to note that human scents are subjective, and that the dog needs to identify the individual who touched the object at the crime scene. Thus, it is very important to have proper dog training procedures. In Europe, the general method can be divided into two protocols: the tube-retrieving system and the cloth responding system (Prada and Furton 2012).

The professionally trained scent-identification dog is an outstanding biological device for collecting invisible traces at a crime scene. This dog training procedure showed excellent sensitivity (between 99.48 and 100%) and specificity (between 60 and 100%), having a positive predictive value (PPV) ranging between 97.94 and 100% and a negative predictive value (NPV) ranging between 85.71 and 100% (Table 5).

DNA analysis of the two samples tested in duplicate allowed us to obtain an interpretable profile (12/16 loci) corresponding to the reference (Fig. 5), and three uninterpretable profiles. The DNA concentration on the gauzes was very low, between 0.035 and 0.065 μg/ml.

The relationship between the different analyses conducted in this work is that human scent is a good source of VOCs and a good target for canine training. Furthermore, human scent is a latent trace that if sampled in an appropriate manner could allow DNA profiling. The main goal is to identify the perpetrator of the crime, starting with the VOC findings and obtaining a human DNA profile.

Conclusions

An HS/SPME method was developed, using PDMS/DVB fiber to extract human scent samples after secondary transfer, for the subsequent identification of the VOCs at different time points within a period of 15 days. The GC/MS analysis provided qualitative information about human scent VOC mixtures, which are a good target for canine training. The compounds identified after contact of the gauzes with the scent-articles touched by four different subjects can be divided into 11 groups: aliphatic and aromatic hydrocarbons, aldehydes, ketones, alcohols, fatty acids, esters, ethers, amines, steroids, and heterocyclic compounds. These compounds are constituents of human secretions. Some of these compounds were considered as contaminants, in particular from diet and certain daily activities.

Table 5 Full experimental data on the human scent discrimination by the dogs

	Trials	Scent-article	Positive	True positive (TP)	False positive (FP)	PPV	Negative	True negative (TN)	False negative (FN)	NPV	Sensitivity	Specificity
Man A	200	Ceramic	194	190	4	97.94%	6	6	0	100.00%	100.00%	60.00%
	200	Plastic	194	193	1	99.48%	6	6	0	100.00%	100.00%	85.71%
Man B	200	Ceramic	193	191	2	98.96%	7	6	1	85.71%	99.48%	75.00%
	200	Plastic	198	198	0	100.00%	2	2	0	100.00%	100.00%	100.00%
Woman A	200	Treated wood	192	191	1	99.48%	8	7	1	87.50%	99.48%	87.50%
	200	Wax	200	200	0	100.00%	0	0	0		100.00%	
Woman B	200	Treated wood	196	195	1	99.49%	4	4	0	100.00%	100.00%	80.00%
	200	Wax	199	199	0	100.00%	1	1	0	100.00%	100.00%	100.00%

PPV = TP/(TP + FP); NPV = TN/(TN + FN); Sensitivity = TP/(TP + FN)*100; Specificity = TN/(TN + FP)*100

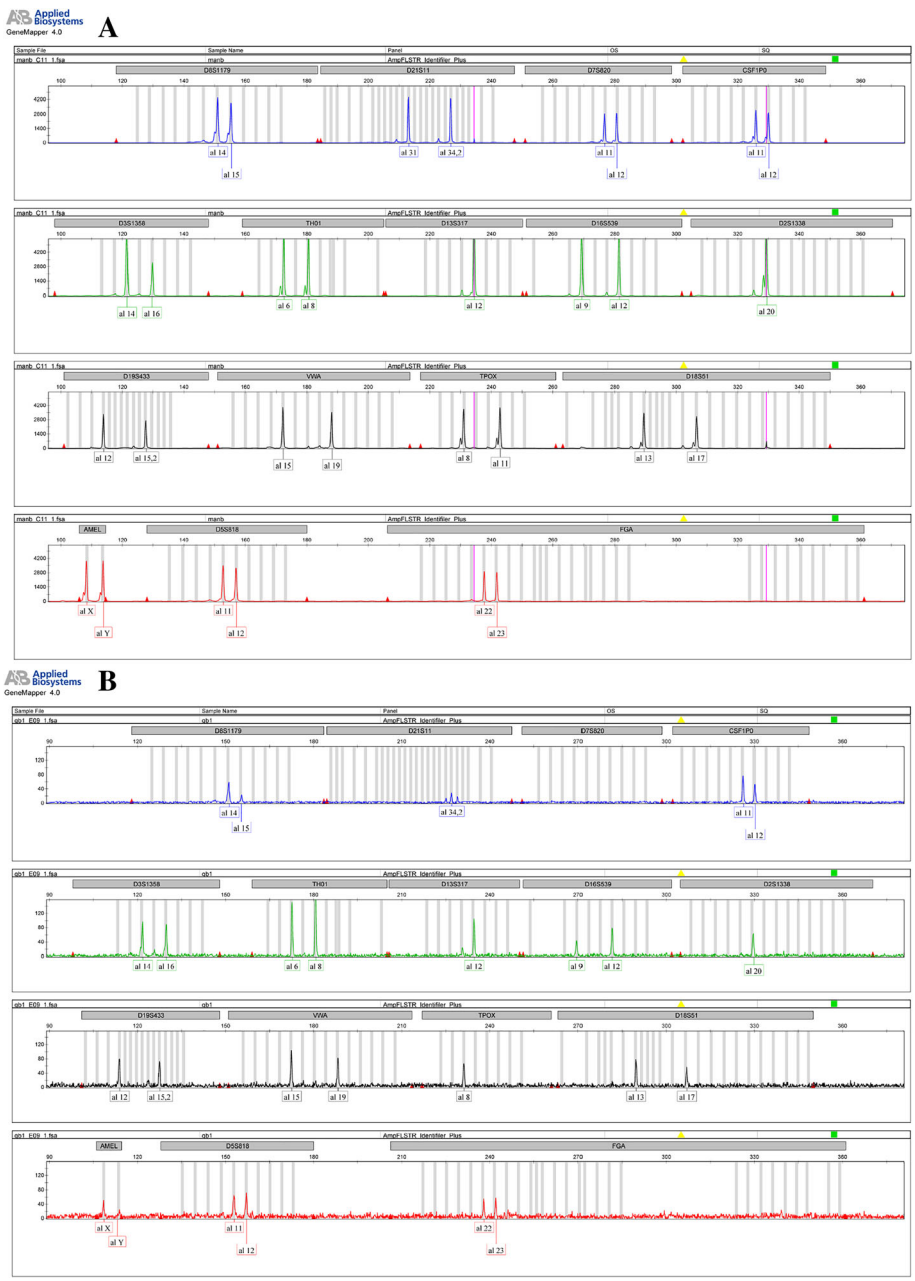

Fig. 5 Electropherograms of the interpretable profile (12/16 loci): reference profile (**a**) and profile obtained from gauzes (**b**)

Storage time of human scent samples led to the detection of different VOCs, which were found to have different properties. Some compounds were transferred to the gauze, others were absorbed by the contact substrate, and others evaporated.

The experimental model proposed is sampling the human scent and storing the sample in an appropriate manner, and subsequently explore the analytical technique (GC-MS) and the olfactory capacity of dogs to identify which VOCs are detected by GC-MS from human scent. These trials demonstrate the ability of well-trained dogs to detect human scent and to associate the odor emanation source with the touched objects. Furthermore, this study proposes the use of human scent as a forensic latent trace for DNA profiling. The positive result obtained in the present experimental model could be proposed in a real forensic case, linking the suspect to the crime scene, even if it does not provide any evidence that the suspect was the perpetrator of the crime. Finally, as highlighted in this paper, associating "touch DNA" analysis with human scent evidence could be considered a useful tool to solve a criminal case, increasing the weight of evidence.

The relationship between the different analyses is that human scent is a good source of VOCs and a good target for canine training. Furthermore, human scent is a latent trace that if sampled in an appropriate manner could allow DNA profiling.

The main limitation of the present study is related to the sample size (two dogs, four humans). The small sample size in this study depends on the type of investigation: a well-trained dog is not easy to find. For this reason, it was not possible to perform statistical tests that normally require a larger sample size, to ensure a representative distribution of the population and to be considered representative of groups of people to whom results will be generalized or transferred. Nevertheless, it is well known that sample size is generally less relevant in qualitative research if explained in the context of the research problem. Moreover, another limitation of the present study is related to the age criteria used to enroll the subjects: indeed, Mitro et al. (2012) described differences in VOCs obtained from human scent according to age criteria. Furthermore, this experimental work aimed to explore the inter-individual differences in VOCs, without analyzing the difference in relation to the different substrates: in fact, the sample was too small to obtain significant information about this.

Finally, this research represents a pilot study to perform further investigation in the future.

Abbreviations

EI: Electron ionization; GC: Gas chromatographic; GC-MS: Gas chromatography-mass spectrometry; HS/SPME-GC/MS: Headspace/solid-phase microextraction-gas chromatography/mass spectrometry; NIST: National Institute of Standards and Technology; PDMS/DVB: Polydimethylsiloxane/divinylbenzene; STU-100: Scent Transfer Unit 100; VOCs: Volatile organic compounds

Acknowledgements

We wish to thank the Scientific Bureau of the University of Catania for language support.

Funding

The authors received no specific funding for this work.

Availability of data and materials

All relevant data are inserted in the manuscript. Please contact the author for further data requests.

Authors' contributions

VF, GDM, MR, PR, CP, IR, MS, and FS: conceived the study and participated in its design. VF, GDM, MR, PF, PR, CP, IR, MS, and FS: contributed to the conception and design. VF, GM, AM, CZ, MS, and FS wrote the manuscript. CZ, PR, CP, IR, MS, and FS drafted the article and revised it critically for intellectual content; VF, GDM, PR, CP, FS: final approval of the version to be published. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All procedures performed in the study were in accordance with the ethical standards of the University of Foggia and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all participants. The protocol was approved by the Research Committee (University of Foggia).

Moreover, all experimental procedures were performed in strict accordance with the Italian and EU regulation on animal welfare and were previously approved by the Animal Research Committee of the University of Foggia.

Consent for publication

The authors declare to have received written consent from all persons involved in this experimental study, to publish the article mentioned above in the Egyptian Journal of Forensic Sciences.

Competing interests

The authors declare that they have no competing interests.

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Received: 13 March 2019 Accepted: 17 June 2019
Published online: 02 July 2019

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