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Review article

Human scent characterization: A review

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ABSTRACT

Human scent has long been cited as a probable parameter that can be exploited as a biometric measure. Identifying the scent of individual persons using specially trained canines is a well-known forensic method which is frequently used in criminal investigations. To date there has been limited research on the chemical components present in human scent and their usefulness in distinguishing between people. This review delivers insight into studies which have dealt with human scent in forensics. Sample collection methods, sample preparation, instrumental analysis, compounds identified in human scent and data analysis techniques are discussed. Methods for sample collection and preparation are presented, but to date, there is no available validated method. Instrumental methods are presented and from the overview it is clear that gas chromatography combined with mass spectrometry is the method of choice. New developments such as two-dimensional gas chromatography offer exiting possibilities to collect more information. Given the amount and complexity of data, data processing is used to extract the relevant information to discriminate people. Finally, sensors offer new opportunities for the characterization of human scent.

Contents

1. Introduction

Human scent profile analysis and comparison is an area of analytical research that has attracted a great deal of interest in the last twenty years. The medical community's interest lies in the possible use of the volatile organic compounds (VOCs) released by the human body as a diagnostic tool for disease while the forensic community's interest is primarily in the use of human scent evidence as an investigative tool. The latter is sometimes referred to as forensic volatolomics and it aims to detect, analyze and characterize VOCs released by an object, a substance or a human being. These mixtures of VOCs form an odor or a scent and can be detected or monitored using analytical instrumentation or an electrochemical sensor. Human scent has long been cited as a probable parameter that can

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be exploited as a biometric measure. Identifying the scent of individual persons using specially trained canines is a well-known forensic method which is frequently used in criminal investigations [1–3]. However, the scientific basis for their use is not well developed. In the Netherlands identification using canines in the so called "geurproef" has been discarded in 2011 due to the fact that it would be unscientific [4]. In the United States the use of canines for scent detection has not gained widespread acceptance due to the lack of studies demonstrating the reliability of this approach [3,5,6]. Hence, the development of an objective, instrumental method that will enable comparative scent identifications is highly desirable.

The human scent signature is a complex mixture of probably several thousand volatile, semi-volatile and non-volatile compounds. Several hundreds of these chemical compounds have been successfully identified in the past [7,8] and include alkanes, aldehydes, ketones, amines, alcohols, amides, fatty acids as well as their esters [9–14]. However, the chemical analysis of such a complex mixture, where some compounds have distinctively varied concentrations while others have concentrations below the detection limits of any analytical technique, is difficult and some understanding of the human scent signature is necessary. Curran et al. classified the human scent into primary, secondary, and tertiary categories [3]. The primary scent of an individual consists of endogenous compounds that are stable over time regardless of diet or environmental factors. This primary scent is believed to be as specific as a fingerprint and therefore is assumed to be genetically conditioned. The secondary scent contains skin scent compounds which are also endogenous but whose relative composition depends on internal factors such as diet, illness, medication, emotional state, and environmental factors such as weather, temperature and humidity. Finally, the tertiary scent originates from exogenous sources, such as cosmetics, lotions, soaps, smoking and scents of the workplace. Since especially cosmetics contain a large amount of different scent chemicals these exogenous compounds can be a challenge in any analysis as they may hide the primary scent. From the above definitions of the scent categories the primary scent is of most interest for identification of humans and it is therefore of importance to know which compounds comprise the primary scent. However, these primary scent compounds have not yet been unambiguously recognized. From earlier research one could conclude that the human scent profile is formed primarily by VOCs [10,15–17]. However, more recent research shows that semi-volatile compounds may also play a role [13,18]. In this context the sampling procedure is crucial in the collection of human scent compounds. Different sampling techniques, with or without skin contact, have been used and seem to lead to different characterizations of the scent profile (see Sampling section). Gas chromatography coupled with mass spectrometry (GC/MS) seems to be the most employed technique to analyze samples [19]. More recently comprehensive two-dimensional gas chromatography with mass detection (GCxGC/MS) has been used to elucidate the human scent profile [20]. Finally, chemical sensors have been introduced to measure and characterize the human scent [21]. This review aims at collecting studies on human scent profiling, focusing on hand sampling and covering its analytical characterization, data processing and future developments.

2. Sampling of the human scent

Every living person has his own scent. It is composed of a static part that is said to be constant over time [22,23] and characteristic of an individual [24], and a more variable part that depends on the environment and life conditions. Current practice in forensic science is to first preserve the crime scene to prevent contamination and then to collect scent evidence within the first 72 h by placing cotton gauze on objects susceptible to contact with the subject [23]. Forensically, the scent collected from the hands of a subject is of primary interest as this is the region of the body where samples of human scent are most often collected by law enforcement. Hand odor is a combination of eccrine and sebaceous gland secretions without the involvement of the apocrine gland, which is responsible for the odors generated by the armpit region. Different scent profiles are obtained depending on the body location considered and the technique of odor compound extraction [25]. While mostly the hands are considered for sampling, profiling of skin odors using hair [8] and armpit [26] samples have also been performed. In practice, two types of sampling methods are used to collect human scent, contact (or direct) sampling and non-contact (or indirect) sampling. In most studies (see below), sampling is performed by direct contact between the sampler and a person's skin. Non-contact sampling is possible by passive or active sampling of the air close to the skin. Whatever method is used one should always be aware of factors affecting the deposition of odor. In general it is noted that there is a lack of standardized collection procedures. In addition to the validation of a standardized protocol, sampling materials and pretreatment methods of the collection materials should be tested also. The latter has been done in recent years. In the past, methods, contact and non-contact, were focused on the sampling of VOCs while in the more recent years there is also an interest in the sampling of semi-, or even non-volatile organic compounds (contact sampling only).

Contact sampling consists of bringing an adsorbent directly into contact with a person's skin to collect scent compounds. Most studies mention protocols to prevent contamination by exogenous compounds and these mostly consist of washing the hands with tap water and perfume-free soap for a pre-defined time followed by air drying [10,15,24,27]. Contact sampling with cotton material has been shown to be the collection method that yields the highest number of VOCs [28–31]. Prada et al. evaluated a range of textile substrates on their retention properties and subsequent release of VOCs associated with human scent [31]. The recovery of a set of target compounds was investigated via a direct spiking method as well as through an indirect method using an airflow collection device. The direct spike experiment showed that natural, cellulosic fibers such as viscose rayon have the best ability to produce a reproducible volatile odor profile. Rayon demonstrated the highest recoveries of the scent compounds which were around 50%, followed by wool and polyester. Somewhat surprisingly, cotton (often used in the past for scent sampling) with 10% showed the lowest recovery. This may be a consequence of the complex fiber morphology of the cotton material, which enhances the possibility of scent compounds to be retained at a higher rate within the structure of the cotton fiber. It was also found that samples collected on the same fabric material showed a reproducible odor profile which is important in forensic biometric measurements. In the indirect air sampling study of Prada et al., a "Scent Transfer Unit", STU-100 was used and a gauze pad of the different fabric materials is used as the adsorbent or trapping medium [31]. For all fabric materials the recovery of the scent compounds was < 20%, i.e. lower than most in the direct spiking study. In this study the cotton material showed the highest recovery which is in agreement with the higher retaining properties of cotton found in the direct spiking experiment. These findings indicate that chemical retention is strongly affected by fiber type and environmental variables such as airflow. Hydrogels may be an alternative for cotton gauze as they are hydrophilic three-dimensional networks and are able to absorb large amounts of water and biological fluids [32]. Penn et al. used polydimethylsiloxane (PDMS) coated stir bars for collecting human scent [24]. These stir bars are rolled over the skin to adsorb scent compounds and can be directly thermally desorbed which is a great advantage compared to the gauze pads which need an additional extraction step, usually with solid phase microextraction (SPME, see the section of Sample Preparation). Cuzuel et al. also used stir bars for the collection of scent compounds from hands [20]. They used so-called Sorb-Stars which is a patented

silicon-based polymeric sorbent that is subjected to specific conditioning processes to avoid contamination. The Sorb-Stars are thermally desorbed to release the absorbed compounds and thus the focus is on the detection of volatile scent compounds. Glass beads are also used as a sampling medium and are said to allow preferential concentration of "oily" residues while minimizing the collection of aqueous perspiration [33,34]. More recently glass beads have been used to sample the semi- or non-volatile fraction of the scent profile as they may have a special significance in scent identification [18,35]. In many previous studies these semi-volatile compounds were marginalized by the choice of the sampling method and consequent analytical sample preparation. Another advantage of glass beads for sampling is that these materials can be cleaned easily, unlike cotton gauzes that are not analytically clean and can be contaminated by exogenous contaminants. Analysis of blank cotton gauzes showed the presence of nonanal and decanal, compounds that were found to be characteristic of human odor [19]. Pojmanova et al. tested different materials as sorbents for the sample collection, namely glass beads, different textile fabrics including nano-textiles, cotton gauze, and compresses from non-woven fabric [35]. One of the tested materials, Aratex, is a textile fabric consisting of cotton, viscose and polyester. According to the Czech Police regulations, Aratex is used as a sorbent for scent sample collection for individual identifications of persons by specially trained dogs. However, the study of Pojmanova et al. shows that this fabric is not sufficiently cleanable and is therefore unsuitable for instrumental analysis. It should be mentioned however that all impurities were found in the semi- and non-volatile fraction and probably don't hinder the identification of VOCs. Cotton gauze and the non-woven material also produced similar interferences in the blank analysis. Interestingly they also studied a number of nano-textiles made of acetate cellulose (ACC), polyurethane (PUR) and polyvinylidene fluoride (PVDF) which all produced interferences in the blank analysis including in the more volatile fraction of the analysis. Glass beads were selected as the best sorbent for the human scent collection, since the possibility of its faultless purification allowed a minimalization of the undesirable contaminations of the scent samples. Another interesting option is the use of polymer patches composed of a thin layer of flexible PDMS polymer bound to a solid matrix forming an adsorptive tape extractor [36]. While this sampling technique was developed for a different purpose, it may be transferred to the analysis of human scent allowing the extraction of not only the volatile fraction but also the non-volatile fraction. For contact sampling, the patch is directly in contact with the skin for a predefined time. An advantage is that either solvent or thermal desorption can be used both for analysis and conditioning of the sample material. To focus on volatiles, a more complex system based on this concept was developed by sandwiching the PDMS membrane between two stainless steel meshes resulting in non-contact sampling [37]. Brown et al. studied the storage of scent samples trapped in cotton gauze and stored in glass vials over time up to 12 months [25]. The results of their study suggest that the VOC fraction in the scent samples changed only minimal during the storage period. Glass containers were found to be the optimal type of storage for human scent samples because they are not permeable for gases, show low absorption of vapors and can be cleaned easily.

The scent transfer unit STU-100 was already mentioned as an example of non-contact sampling [38–40]. It consists of a vacuum pump connected to a piece of gauze for the collection of VOCs. Studies were conducted to determine the recovery of compounds sampled at different flow rates. Since the adsorbent consists of no more than a piece of gauze it showed that higher flow rates resulted in a substantial breakthrough and lower yields of captured VOCs [41]. Another indirect technique is the use of a "flow sampling chamber" where a body part of the subject is isolated and purified nitrogen gas is flowing through the chamber to a pump connected in-line after an adsorption tube. The tube is then analyzed with thermal desorption GC/MS [42]. In a similar set-up the body part is isolated in a plastic bag and dynamic headspace sampling combined with thermal desorption GC/MS of the adsorbent tube or SPME-GC/MS is used to isolate and analyze VOCs [43]. As expected dynamic headspace sampling followed by thermal desorption GC/MS allowed for more and lower concentrations of volatiles to be trapped compared to SPME [44]. More recently passive sampling has been used in combinations with an electronic nose sensor [21]. In that situation the sensor was built in a kind of bracelet and is held at a certain distance to the skin of the test person. VOCs diffuse from the skin into the carbon nanotube based sensor and an odor analysis is carried out.

Recently, Kim and Bae developed a new technique, called in-needle microextraction (INME), using a graphene oxide:polyaniline/zinc nanorods/zeolitic imidazolate framework-8 (GO:PANI/ZNRs/ZIF-8) adsorbent for the indirect sampling of scent compounds [45]. Comparable to SPME the adsorbent is bound to a wire in a needle and is analyzed using thermal desorption in the injection port of the gas chromatograph. The method was tested using five target compounds, trans-2-nonenal, benzothiazole, hexyl salicylate, a-hexyl cinnamaldehyde and isopropyl palmitate, all of which are odor compounds emitted from human skin and associated with aging. Recoveries of these compounds varied from 91% to 103% with good reproducibility.

In general, the method of sampling depends on which group of compounds are analyzed. For volatile compounds, contact sampling with gauzes seems to be the method of choice although care has to be taken to avoid contamination. It should be added that such contaminations are generally semi-volatile and non-volatile compounds which will not interfere with the analysis of the volatiles. For non-volatile compounds, contact sampling with glass beads is the method of choice.

3. Sample preparation

In general, gas chromatography combined with mass spectrometry (GC/MS) is used for the analysis of samples. The first step is the transfer of the sampled volatiles or non-volatiles to the chromatographic column and this can be achieved with solvent extraction or SPME. The disadvantage of solvent extraction is that the sample is diluted making the identification of trace compounds difficult. In addition, the use of a solvent may interfere with the analyses of VOCs. Since there has been a strong focus on the analyses of volatiles, SPME extraction has been used in most cases to eliminate the disadvantages of solvent extraction [8,10,15,25,28,31,38–40,43,44,46–52]. Solid phase microextraction consists of a modified syringe containing a coated fused silica fiber inside the needle. This fiber can be immersed in a liquid or gaseous phase to collect VOCs. In the practice of the scent analysis, the sampling materials (patch, gauze or glass beads) are collected in a headspace vial and heated with or without agitation. The SPME fiber is then exposed to the headspace in the vial for a defined equilibration time and at a certain extraction temperature. After the equilibration time the SPME fiber is thermally desorbed in the injection port of the GC, again for a certain time and at a certain desorption temperature. Typical equilibration times range from 30 min to 15 h while extraction temperatures range from room temperature to 50 °C. Higher extraction temperatures may result in the loss of volatile compounds during the headspace extraction. The composition of the coating on the silica fiber is of influence on which compounds are adsorbed best. In the reviewed studies, the SPME fibers were mostly divinylbenzene on polydimethylsiloxane (DVB/PDMS) or divinylbenzene/carboxen on polydimethylsiloxane (CAR/DVB/PDMS). Especially the latter has been proven to be the most

relevant for the range of studied VOCs. Saito et al. studied the determination of the body odor component 2-nonenal using SPME-GC/MS. They experimented with the coatings DVB/PDMS, CAR/DVB/PDMS, CAR/PDMS and polyacrylate and found that DVB/PDMS gave the best recovery [52]. SPME provides good sensitivity of VOCs. The technique is able to determine the molecules which are released into the headspace above the sampled sorbent. However, the sensitivity is significantly worse for semi- and non-volatile compounds which may also contribute to human body odor [18].

An alternative is direct thermal desorption of gauzes or sorbents in desorption tubes. In that case the tube holding the adsorbent is heated and the volatile compounds are usually trapped on a micro-trap packed with a small amount of adsorbent. Next, the micro-trap is heated and the compounds are transferred to the chromatographic column. As with SPME no solvent is used and it would eliminate one potentially discriminating step (HS-SPME) in comparison to the SPME method. While with SPME quantification remains a challenge, this is relatively simple with thermal desorption. In several studies the use of direct thermal desorption was mentioned [24,26,33,37,41,44,53] although it was not found in combination with human skin volatiles in the literature of the last 5 years.

While solvent extraction of human skin odor compounds was one of the first techniques used it has not been used for many years. Only recently the interest has shifted again to solvent desorption to include the semi-volatile and non-volatile compounds that may be a part in body odor composition. Solvent extraction in the past was carried out using acetone and hexane as solvents [36,54]. In more recent publications the recovery of odor compounds from different type of adsorbents is tested using different solvents including hexane, ethanol, methanol and acetonitrile [35]. Ethanol and hexane were evaluated as the most suitable solvents, however, overall hexane showed the best results, especially for the more volatile compounds. Dolezal et al. used hexane extraction to recover odor compounds from glass beads and used preparative GC to split the odor components in three fractions, volatile, semi-volatile and non-volatile, for further testing [13,18]. Pojmanova et al. used a more elaborate solvent extraction using a double ethanol extraction supported by shaking and ultrasonication. For instrumental analysis, the extract was evaporated to dryness and redissolved in 1:1 mixture of hexane and ethanol. From the solvent evaporation step it is clear that the VOCs will be lost and that the results focus on the semi- and non-volatile compounds only [27].

The choice of sample preparation depends on the nature of the sample. In case of gauzes, sample preparation using SPME is most often used since this allows the identification of even traces of volatile compounds. For glass beads solvent extraction is used to recover the non-volatiles. Since these extracts can be concentrated, low levels of non-volatiles can be determined.

4. Instrumental analysis

GC/MS is the method of choice for identifying marker molecules in odor analysis, volatile as well as non-volatile [8,9,13,15,27,28,35,40,45–47,49–56]. In all cases 30 m or 60 m non-polar capillary columns are used in the analysis with more or less identical GC methods. Typically the chromatographic method is optimized to start compound separation at a temperature of 40 °C. This temperature is held for 5 min, after which the temperature is increased at a rate of 10 °C/min to a temperature of 220 °C. Subsequently, the temperature is increased again at a rate of 30 °C/min until reaching 300 °C. The temperature is then held at 300 °C for 5 min. The GC/MS transfer line is maintained at 300 °C, while the MS ion source is maintained at 250 °C. Mass spectra are scanned for a mass range of 45–400 amu and the NIST spectral reference library is used to tentatively identify all compounds during analyses. For standard-confirmed identifications each compound's retention time is compared to the retention time of chemical reference standards.

Even though GC/MS has proven to be a reliable technique, for the analysis of volatile and non-volatile compounds in complex samples, such as human scent, its separation power remains the limiting factor. In the last years, a number of publications in forensic research have emphasized the benefits of comprehensive two-dimensional gas chromatography (GCxGC), such as increased peak capacity, higher sensitivity, and the possibility of group-type analysis, in comparison to conventional 1D GC [55,57,58]. Nevertheless, the use of GCxGC in forensic applications remains limited and its moderate use is in strong contrast with the wide and successful use in other science areas [57]. The number of publications investigating the human scent for forensic purposes with GCxGC is limited [13,20,27,59]. Dolezal et al. were the first to use GCxGC in a non-targeted approach of female skin scent profiling [13]. They collected hand samples from nine female volunteers using glass beads and solvent extraction. They used GCxGC combined with time-of-flight mass spectrometry (GCxGC-TOFMS) for the instrumental analysis focusing on the semi-volatile compounds. Already in standard mode the GCxGC technique demonstrated its high peak capacity and good resolution, however, when the authors compared GCxGC-TOFMS with the single GC/MS technique where the deconvolution of peaks was carried out, the qualitative difference was not so obvious. The main results of the study was the identification of 137 semi-volatile organic compounds observed from the scent samples of the nine volunteers. Of these compounds 76 were observed for the first time in human scent samples and 33 were tentatively identified. As mentioned before, the importance of these semi- and non-volatile scent compounds is that they probably contribute to the so-called human signature which is crucial in the scent identification performed by specially trained dogs [16]. Cuzuel et al. optimized a GCxGC method for the profiling of hand odor by focusing on the assessment of orthogonality criteria of 27 column combinations. The study was limited to the analysis of a synthetic mixture and no real odor samples were analyzed. The results suggested that a conventional phase column set (non-polar x polar) is most suitable for the analysis of complex mixtures of volatile compounds [59]. Pojmanova et al. also used a synthetic mixture containing 98 compounds commonly found in human skin scent to optimize a GCxGC method using five different column configurations [27]. The best results were obtained for the reverse system, consisting of a 2 m pre-column RTX-200 MS, a 30 m primary column RTX-200 MS and a 1 m secondary column TG-5HT. On the reverse system, using the chromatographic method with a temperature gradient of 5 °C/min, the highest utilization of the separation space and the highest theoretical and conditional peak capacities of the system were achieved, and the smallest number of coelutions occurred. This optimized system was then used for subsequent analysis of real scent samples. Concluding, the results show that the GCxGC technique is a higher resolution technique for forensic applications.

More recently, several chromatography companies have begun developing miniaturized gas chromatography-mass spectrometry devices, which can potentially conduct full chemical analysis of volatile components in remote conditions. These devices were originally developed for screening specific volatiles in the fields of environmental sciences but are now starting to be used in the field of animal chemosignaling [60,61]. While these portable GC/MS devices are less sensitive than benchtop models, they represent a viable alternative to laboratory-based methods for the analysis of samples under field conditions.

From the literature review it is clear that GC/MS is the only method used. In fact, often the GC/MS conditions used by different researchers are more or less identical. During the last five years GCxGC/MS has become more popular since this technique is better

Table 1
Summary of studies for human scent composition with respective analytical conditions.

Year	Reference	Study description	Sample type	Sampling	Extraction	Analytical technique	Analytical column
2000	Bernier et al.[33]	Analysis of human skin emanations by identification of VOCs.	Hands	Glass beads	TDU	GC/MS	HP5 (25 m x 0.20 mm, 0.33 µm)
2001	Haze et al.[62]	2-Nonenal analysis in body odor related to aging.	Skin	Cotton shirt	Tenax TA/Solvent	GC/MS	HP Innowax (60 m x 0.25 mm, 0.25 µm
2004	Hasegawa et al.[26]	Identification of new odor compounds in human axillary sweat.	Axillea	Stirbar	TDU	GC/MS	DB-1 (60 m x 0.25 mm, 0.25 µm)
2005	Curran et al.[3]	Analysis of the uniqueness and persistence of human scent.	Armpit	Dukal gauze	SPME (DVB/ Car/PDMS)	GC/MS	HP5-MS (30 m x 0.25 mm, 0.25 µm)
2005	Zhang et al.[63]	Fingerprint characteristics of the emanations from human arm skin.	Hands	Emanation sampling device	SPME (PDMS/DVB)	GC/MS	HP-VOC (60 m x 0.32 mm, 1.8 µm)
2006	Eckenrode et al.[41]	Testing performance of the STU-100.	Spiked mixture	STU-100	TDU	GC/MS	DB-5 (30 m x 0.25 mm, 0.25 µm)
2006	Sisalli et al.[36]	Sorptive tape extraction of VOCs from biological matrices.	Forehead	PDMS tape	TDU	GC/MS	CP-WAX58 (25 m x 0.25 mm, 0.20 µm)
2007	Bicchi et al.[64]	Sorptive tape extraction of VOCs from biological matrices.	Hands	PDMS tape	TDU	GC/MS	FSOT Carbowax (30 m x 0.25 mm, 0.25 µm)
2007	Curran et al.[46]	Frequency of occurrence of VOCs in human scent.	Hands	Dukal gauze	SPME (DVB/ Car/PDMS)	GC/MS	HP5-MS (30 m x 0.25 mm, 0.25 µm)
2007	Penn et al.[24]	Individual and gender specific fingerprints in human body odor.	Axillea	Stirbar	TDU	GC/MS	DB-5 MS (20 m x 0.18 mm, 0.18 µm)
2008	Gallagher et al.[10]	Analysis of VOCs from human skin.	Hands	SPME (DVB/Car/PDMS)	-	GC/MS	Stabilwax (30 m x 0.32 mm, 1 µm)
2010	Curran et al.[38]	Canine human scent identifications.	Hands	Dukal gauze	SPME (DVB/ Car/PDMS)	GC/MS	HP5-MS (30 m x 0.25 mm, 0.25 µm)
2011	De Greeff et al.[43]	Selection of sorbent materials for VOC analyses with STU-100.	Hands	STU-100	SPME (DVB/ Car/PDMS)	GC/MS	HP5-MS (30 m x 0.25 mm, 0.25 µm)
2011	De Greeff et al.[39]	Testing the scent transfer unit STU-100.	Spiked gauzes	STU-100	SPME (DVB/ Car/PDMS)	GC/MS	DB-225 MS
2013	Brown et al.[25]	Applicability of VOC eminations and identification for profiling.	Hands	Cotton gauze	SPME (DVB/ Car/PDMS)	GC/MS	HP5-MS (30 m x 0.25 mm, 0.25 µm)
2013	Dormont et al.[44]	Methods for field collection of human skin volatiles.	Foot	SPME	-	GC/MS	CPSil-8CB (30 m x 0.25 mm, 0.25 µm)
Year	Reference	Study description	Sample type	Sampling	Extraction	Analytical technique	Analytical column
2013	Jiang et al.[37]	A non-invasive method for in vivo skin VOC sampling.	Skin	PDMS membrane	TDU	GC/MS	SLB-5 (30 m x 0.25 mm, 0.25 µm)
2013	Kusano et al.[47]	Comparison of VOCs for profiling potential.	Hands	Dukal gauze	SPME (DVB/ Car/PDMS)	GC/MS	HP5-MS (30 m x 0.25 mm, 0.25 µm)
2013	Mess et al.[65]	Identification of endogenous skin surface compounds.	Skin	DIP-it samplers	-	DART-TOFMS	-
2013	Omolo et al.[66]	Analysis of human foot odors.	Foot	Adsorbent pads	Solvent	GC/MS	CPSil-5 (50 m x 0.2 mm, 0.33 µm)
2014	Dutkiewicz et al.[32]	Sampling and profiling of skin metabolites.	Forearm	Hydrogel pads	-	Direct MS	-
2014	Giannoukos et al.[67]	Analysis of the human chemical signature using MIMS.	Diverse	Solvent extraction	-	PDMS membrane inlet MS	-
2016	Caraballo et al.[40]	Collection of human scent using a non-contact sampling device.	Hands	Dukal gauze	SPME (DVB/ Car/PDMS)	GC/MS	DB-225 (30 m x 0.25 mm, 0.25 µm)
2016	Colon-Crespo et al.[50]	VOC markers for classification by gender/race.	Hands	Cotton gauze	SPME (DVB/ Car/PDMS)	GC/MS	HP-5 MS (30 m x 0.25 mm, 0.25 µm)
2017	Dolezal et al.[13]	Semi-volatile molecules from female skin scents.	Hands	Glass beads	Solvent	GCxGC/ TOFMS	1:ZB-5 MS (28 m x 0.25 mm, 0.25 µm) 2:BPX-50 (1.29 m x 0.1 mm, 0.1 µm)
2017	Grabowska et al.[53]	Analysis of VOCs from human skin.	Forearm	Carbotrap X	TDU	GC/MS	DB-1 (60 m x 0.32 mm, 5 µm)
2017	Jha et al.[49]	Body odor classification with filtering approaches.	Diverse	Cotton gauze	SPME (PEG)	GC/MS	(60 m x 0.32 mm)
2018	Cuzuel et al.[59]	Human odor and forensics.	Hands	Stirbar	TDU	GCxGC/MS	1:ZB-1 MS (30 m x 0.25 mm, 0.25 µm) 2:ZB-1701 (1.5 m x 0.1 mm, 0.1 µm)
2018	Duffy et al.[51]	Analysis of scent profiles from human skin	Forearm	SPME (DVB/Car/PDMS)	-	GC/MS	SLB-5 ms (30 m x 0.25 mm, 0.25 µm)
Year	Reference	Study description	Sample type	Sampling	Extraction	Analytical technique	Analytical column

(*continued on next page*)

Table 1 (*continued*)

Year	Reference	Study description	Sample type	Sampling	Extraction	Analytical technique	Analytical column
2019	Dolezal et al.[18]	Fractionation of human scent using preparative GC	Hands	Glass beads	Solvent	GC/FID	Rxi-5 ms (30 m x 0.53 mm, 1.5 µm)
2019	Pojmanova et al.[35]	Optimization of sorbent/solvent parameters	Hands	Glass beads	Solvent	GC/MS	SLB-5 MS (30 m x 0.25 mm, 0.25 µm)
2019	Tavares et al.[8]	Profiling human skin odors using hair samples	Hair	SPME (PDMS/DVB)	-	GC/MS	DB-1 MS (30 m x 0.25 mm, 0.25 µm)
2021	Poirier et al.[48]	Field analysis of animal scent using portable GC/MS	Swabs	SPME (PDMS/DVB)	-	portable GC/MS	MXT-5
2021	Pojmanova et al.[27]	Analyses of human scent samples using 2D GC	Hands	Glass beads	Solvent	GCxGC/MS	1:Rtx-200 MS (30 m) 2:TG-5HT (1 m)
2021	Saito et al.[52]	Optimization of a method for 2-nonenal in body odor analysis	Hands	Cotton gauze	SPME (diverse)	GC/MS	DB-1 (60 m x 0.25 mm, 1 µm)
2022	Kim & Bae[45]	In vitro and in vivo human body odor analysis	Forearm	GO:PANI/ZNRs/ZIF-8 adsorbent	TDU	GC/MS	HP-5 MS (30 m x 0.25 mm, 0.25 µm)

Car: carboxen; DART: direct analysis in real time; DVB: divinylbenzene; FID: flame ionization detector; GC: gas chromatography; MS: mass spectrometry; PDMS: polydimethylsiloxane; SPME: solid phase micro-extraction; TDU: thermal desorption unit; VOC: volatile organic compound; TOFMS: time-of-flight mass spectrometry; 2D GC: two-dimensional gas chromatography.

capable of separating the often complex mixtures, especially in the case of non-volatiles.

5. Identification of characteristic human hand odor compounds

In general, the odor analysis is performed by a two-step process consisting of: 1) a marker molecules identification and 2) a discrimination or classification of odors based on the identified specific markers. This section focuses on the first step in the process, the identification of odor compounds that are characteristic of the human body, while the next section (data analysis) will focus on the second step in the process, the discrimination and classification of odors based on specific markers. The identification of compounds, volatile and non-volatile is an important step. Often it is also difficult because sometimes the compounds responsible for the odor are not the most intense peaks in the GC chromatogram. That makes peak picking and peak identification with tools such as the NIST database challenging. It should also be noted that peak identification using databases is only a tentative identification. To confirm the identification a comparison with a chemical standard, analyzed under the same analytical conditions, is required (standard-confirmed identity), however, such standards are not always commercially available.

In a review paper from 2017 Cuzuel et al. published a compilation of compounds that were identified in hand odor samples and that were reported in 11 publications in the period 2000–2011 [19]. In total the list contains 273 compounds consisting of the groups; alkanes, alkenes, acid/acid ester, alcohols, aldehydes, halogens, aromatic hydrocarbons, ketones, esters and others. Most of the reported compounds originate from the studies from Curran et al. (60 subjects) [46], Callagher et al. (25 subjects) [10] and Bernier et al. (4 subjects) [33]. These publications originate from 2007, 2008 and 2000, respectively. A more recent study that can be added to these was performed by Caraballo et al. (20 subjects) [40]. Curran, Callagher and Caraballo used contact sampling with gauze and HS-SPME followed by GC/MS for the analysis while Bernier used contact sampling with glass beads followed by thermal desorption of the glass beads and on-line GC/MS. From this we can conclude that these authors focused on the identification of volatile compounds. The data of the mentioned studies was combined and Table 2 gives an overview of those compounds that were reported in minimal 5 of the 12 studies and which therefore may be regarded as characteristic volatiles for hand odor.

Colon et al. collected hand odor samples from 105 individuals using a cotton gauze and SPME-GC/MS analyses [50]. They identified over 100 VOCs and selected compounds from this list to generate a hand odor profile. Compounds were selected on the basis of their occurrence in hand odor profiles and their potential for comparison across the different groups (race/ethnicity and gender). This resulted in a hand odor profile consisting of a list of 26 VOCs. While the analytical methods used are very similar to those of Curran and Galagher the final profile is very different from Table 2. Surprisingly, the list also contains a number of semi-volatile compounds such as the hydrocarbon pristane and the synthetic musk fragrances pentadecanolide (exaltolide) and galaxolide. The scent profile list of Colon et al. contains a number of semi-volatile fatty acid esters that were identified also in the study of Dolezal et al. [13].

Duffy et al. conducted a study into the identity of human skin volatiles using 8 individuals [51]. Sampling was done by non-contact SPME and analyses with GC/MS. The results revealed a variety of classes of compounds emanating from the skin. There were 24 compounds identified in endogenous skin volatile profiles across all participants. The predominant species were acids and aldehydes including nonanal, decanal, tetra- and hexadecanoic acid. Many other major skin volatiles were also present including octanal, undecanal, 6-methyl-5-hepten-2-one and geranyl acetone (6,10-

Table 2
Possible VOC profile for a human hand's odor.

Classification	Compounds	CAS nr.	MW (g/mol)	BP °C	Frequency (out of 12 studies)
Alkanes.	Undecane	1120–21–4	156.31	196	6
	Dodecane	112–40–3	170.34	216	7
	Tridecane	629–50–5	184.36	234	6
	Tetradecane	629–59–4	198.39	254	7
	Pentadecane	629–62–9	212.42	271	5
	Hexadecane	544–76–3	226.44	287	6
	Heptadecane	629–78–7	240.47	302	5
Acid/Acid ester.	Octanoic acid methyl ester	111–11–5	158.24	193	5
	Dodecanoic acid	143–07–7	200.32	299	6
Alcohols.	Phenol	108–95–2	94.11	182	5
	2-Furanmethanol	98–00–0	98.10	170	5
	Benzyl alcohol	100–51–6	108.14	205	7
	Nonanol	143–08–8	144.25	214	5
	3,7-Dimethyl-1,6-octadien-3-ol (linalool)	78–70–6	154.25	198	7
Aldehydes.	Benzaldehyde	100–52–7	106.12	178	7
	Heptanal	111–71–7	114.19	153	5
	Octanal	124–13–0	128.21	171	5
	2-Nonenal	18829–56–6	140.22	188	5
	Nonanal	124–19–6	142.24	195	11
	Decanal	112–31–2	156.27	207	10
	Undecanal	112–44–7	170.29	226	5
	Dodecanal	112–54–9	184.32	257	6
Ketone.	6-Methyl-5-hepten-2-one	110–93–0	126.20	173	9
	2-Decanone	693–54–9	156.27	211	6
	6,10-Dimethyl-5,9-undecadien-2-one (geranyl acetone)	689–67–8	194.32	247	9
Others.	Pyridine	110–86–1	79.10	115	5

dimethyl-5,9-undecadien-2-one). Squalene and 2,6-dimethyl-2,6-octadiene were the principal hydrocarbon species identified, and one alcohol, 1-dodecanol, and ester, isopropyl palmitate, were also present. There were 14 volatiles common to the volatile profile of all participants comprising 6 aldehydes, 5 acids, 2 ketones and 1 ester, and these show some resemblance to the profile in Table 2. Another observation in this study was that the repeatability of the majority of compounds was very good with intra-participant samples, suggesting that these volatiles could be useful for differentiating participants from one another.

An excellent review was published by Mochalski et al. [68]. While many studies dealing with the human scent have reported a large number of compounds comprising the human scent, the majority of them yield only qualitative data (i.e., names of identified compounds and in a few cases their relative occurrence based on peak areas in chromatograms). Moreover, the GC/MS-based studies provide mainly tentative identification of these compounds based on peak spectra that were checked against commercial mass spectral libraries (e.g., NIST). Based on sparse data Mochalski et al. collected not only compound identifications but also quantitative data (i.e. emission rates) of these compounds, mostly determined for peripheral skin such as hand, arm, or leg. Fig. 1 shows the emission rates of compounds emitted from skin. In general, the mentioned compounds show a strong resemblance with the compounds presented in Table 2.

Dolezal et al. used glass bead sampling and solvent extraction followed by instrumental analyses with GCxGC-TOFMS to analyze the semi-volatile compounds in the samples from 9 females [13]. Altogether 137 different compounds were observed in the scent samples and identified based on a comparison of the measured mass spectra with the NIST library mass spectra. The observed compounds were sorted into basic groups: hydrocarbons and aromatic compounds, alcohols, aldehydes and ketones, organic acids, fatty acid esters, steroid alcohols and miscellaneous. While a number of the observed compounds are similar to the volatile compounds presented in Table 2, a large group of fatty acid esters was identified. These fatty acid esters comprised 64 of the 137 identified compounds and are important since they mostly belong to the so-called

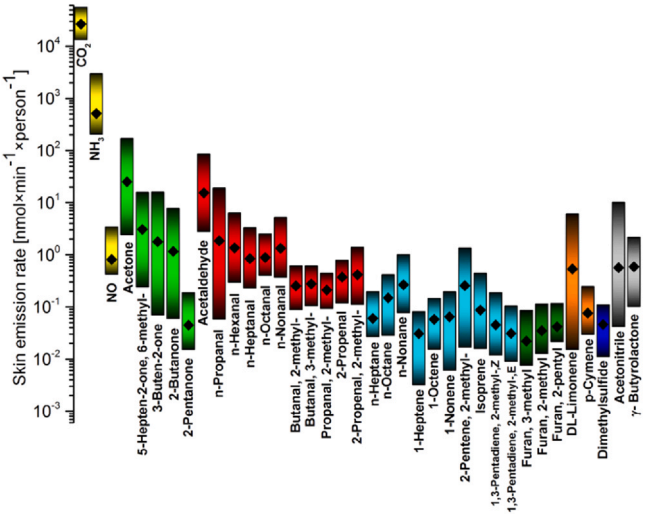

Fig. 1. Ranges and means of emission rates of potential skin-borne markers of the human body. The colors correspond to the different chemical classes of compounds [68].
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primary scent molecules [16] and some of them constitute the human scent signature. Other compounds found in high concentrations in all chromatograms were squalene and cholesterol.

Pojmanova et al. also identified a rather large group of semi-volatile fatty acids and fatty acid esters in hand samples of 10 individuals [35]. They used glass beads and extraction with hexane and ethanol to isolate the scent molecules, resulting in 20 sample extracts. Using GC/MS they observed over 500 compounds in total in the extracts of these 20 samples. In all, 218 different chemical compounds were unambiguously identified, specifically 175 in hexane and 172 in ethanol extracts. Of all the compounds observed in the scent samples, the compounds present in the scent samples of the majority of the individuals (9 out of 10 samples for each solvent) were searched with the aim to delimit the molecules of the primary scent. In all, 28 and 42 such compounds were found in the hexane

and the ethanol extracts, respectively. Interestingly the list of the hexane extracts also contains nonanal and 6,10-dimethyl-5,9-undecadien-2-one, two compounds that are also important representatives in the VOCs listed in Table 2. Other volatiles are not present in the results of Pojmanova which can be explained by the fact that the extracts were evaporated to dryness during the sample preparation which will result in the loss of the volatiles.

Based on the findings we conclude that it is reasonably clear which the major volatile compounds are that compose the primary odor. These compounds are summarized in Table 2. For the non-volatiles this is less clear and only a few large datasets were published. Further research is needed to determine the role of these compounds in the primary odor.

6. Data analysis

The second step in odor analysis is a discrimination or classification of odors based on the identified specific markers or even on the entire profile pattern. A statistical approach appears essential not only for determining which compounds of odor are significant to allow discrimination but also for matching the odor of a suspect with one collected at a crime scene. Before any use of data, preprocessing should be carried out to subtract a baseline, normalize the chromatograms, and align and identify the peaks. The use of internal standards is relevant to achieve a good set of data, especially to perform pattern recognition. Algorithms may be applied to highlight the differences or similarities between samples. Tools such as principal component analysis [24,63], partial least-squares regression [69], or correlation analysis [15,46,50] are used for this purpose. Other statistical techniques used are principal component analysis in combination with support vector machine analysis and kernel principal component analysis [70,71], and even bio-inspired techniques like artificial neural networks [72].

That some kind of automation is necessary is illustrated by the work of Penn et al. [24]. They collected samples from 197 individuals in five-fold (over a 10 week period) and analyzed those with GC/MS. After the removal of chromatograms with analytical problems this resulted in 965 chromatograms. It is- impractical to analyze each chromatogram manually since that would take a lot of time (19 years according to the authors). Instead they developed semi-automated methods for data processing, including alignment, peak picking and integrating, based on the work of Dixon et al. [73]. Peaks with similar mass spectra and retention times were aligned across the chromatograms, and all peaks identified in less than five chromatograms were removed resulting in a data table consisting of 965 samples and 4941 peaks. Of these peaks, 373 peaks were detected in at least one individual in four out of five samples and using a variety of pattern recognition techniques they found strong evidence for individual fingerprints for human scent. They calculated pairwise similarities between GC/MS chromatograms of all 965 samples, using a qualitative presence/absence criterion, and found that intra-individual samples were significantly more similar than inter-individual samples. In addition to individual fingerprints, they also tried to identify characteristic peaks that distinguish the sexes and the most significant gender-specific compounds. Interesting is that they concluded that qualitative indicators of similarity (presence/absence) were more effective than quantitative ones (variations in the relative ratios of compounds).

Curran et al. collected 3 samples over a 12 h period from 10 individuals resulting in 30 GC/MS analysis [15]. Across the chromatograms 37 previously reported human volatile compounds were extracted and ranked according to their peak areas in ascending fashion for each subject. These ranked data arrays were then compared using the Spearman correlation, as seen below, where d is the difference between the ranked compounds and n is equal to the number of compounds being compared.

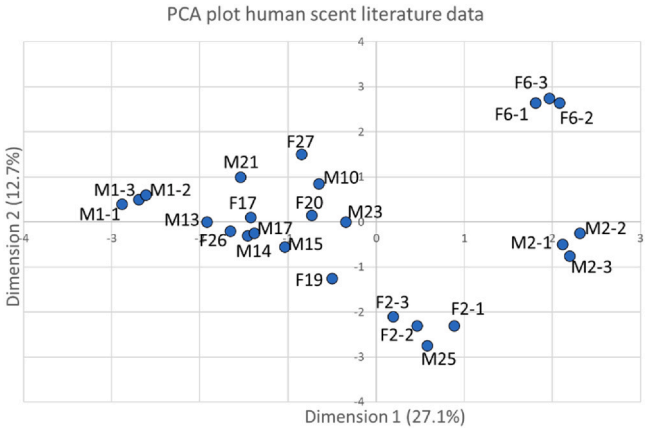

Fig. 2. PCA plot of human scent data published by Curran et al. [15] and which shows that intra-individual samples (M1–1, M1–2, M1–3 etc.) are significantly more similar than inter-individual samples. On the other hand there is no clear distinction between male (Mx) and female (Fx) scent samples.

$$r_s = 1 - \frac{6\sum d^2}{n(n^2-1)}$$

The Spearman rank correlation coefficient r_s ranges from −1 to +1 and supplies information about the strength and direction of relationships. Three samples of 10 individuals were considered producing 435 possible pairings. When considering a correlation threshold of 0.8 the individuals were correctly distinguished and identified in 89.66% of the cases. Narrowing the compounds for each individual to only those determined to be present in all three intra-day samples resulted in 24 compounds across the 10 individuals, a profile that was termed the individuals “primary odor”. When running the Spearman correlation using only these 24 primary odor compounds, and considering a correlation threshold of 0.8, the individuals were correctly discriminated and identified in 99.54% of the cases. This indicates that it is important to identify the compounds comprising the primary odor. Spearman ranking was also used by Duffy et al. to differentiate between 8 individuals after applying fragrances [51]. An overall analysis of all samples resulted in 88% discrimination at the 0.9 correlation threshold. Fig. 2 shows the result of a principal component analysis (PCA) of data published by Curran et al. [15] which indicates that intra-individual samples (M1–1, M1–2, M1–3, etc.) are significantly more similar than inter-individual samples. This confirms the earlier results of Penn et al. [24].

Colon-Crespo et al. selected 26 VOCs of over 100 identified compounds in the chromatograms of 105 individuals [50]. Compound amounts were determined by comparison with external calibration curves for each sample. The potential classification of each individual on the basis of their traits and their expressed VOCs, was evaluated using linear discriminant analysis (LDA). This allowed the creation of canonical plots to display the potential impact of hand odor VOCs in individual classification by gender or race/ethnicity, independently. The LDA canonical plot revealed a clear differentiation of the groups by displaying individuals of equal races/ethnicities clustering together, and with a tendency to appear separated from those of other groups. The results showed that Caucasian individuals were classified with an accuracy of 72%, while East Asians and Hispanics were classified with accuracies of 82% and 67%, respectively. For gender, an overall accuracy of 80% was obtained for the classification of individuals. However when a validation was carried out the accuracies for race/ethnicity and gender were 57% and 71%, respectively. Differences were explained by the choice of VOC markers. A limitation of many statistical approaches is the large number of dimensions, e.g. the presence of numerous chemical compounds in

body odor, compared to the number of samples which is often limited. Selecting the most significant peaks in GC/MS chromatograms of body odor samples as was done manually by Curran [15] and Colon-Crespo [50] is a solution to that. Jha et al. have suggested filter-based approaches for the selection of the most significant peaks of chemical compounds which may result in a better classification accuracy of body odor samples [74].

Other odor studies used a non-metric multidimensional scaling (NMDS) ordination and analysis of similarity (ANOSIM) approach, based on presence/absence of compounds of interest [48]. However, due to shortcomings of the analytical technique the identification of compounds of interest in the samples was limited and as a consequence it was difficult to find clear differences in the chemical compositions of the different odors in the samples. While the focus of this study was on animal odor, the same statistical approach could also be used for human odor.

With the advent of GCxGC/MS analysis of human body odor samples, a new approach was proposed for the interpretation of the analysis results that does not aim to classify subjects by gender or ethnicity but aims to identify subjects [20]. The approach is based on the definition of a distance between odor chromatograms and the application of Bayesian hypothesis testing. Using a calibration panel of subjects for whom several odor chromatograms are available, the densities of the distance between chromatograms of the same person, and between chromatograms of different persons are estimated. Given the distance between a reference and a query chromatogram, the Bayesian framework provides an estimate of the probability that the corresponding two odor samples come from the same person. Cuzuel et al. used 600 compounds in their study to characterize hand odor samples not knowing whether all were relevant for identification [20]. The first results are promising considering that the compound selection is purely statistical, however, more work needs to be done on the compound selection technique. Bayesian classifiers based on a distance between chromatograms proved to be very efficient and the method is hence likely suitable for forensic applications.

Conventional odor discrimination is generally performed on the basis of GC/MS analysis that identifies specific marker compounds as described above. Unless some statistical tools are used the marker identification process can be labor intensive and the limited number of markers identified may be insufficient to discriminate complex odors. Jirayupat et al. described a new method for discriminating complex odors with GC/MS data by combining texture image analysis and machine learning [75]. Texture features like contrast, energy, homogeneity, correlation and dissimilarity, were extracted from two-dimensional (2D) MS maps by texture image analysis and were used as datasets for machine learning. Using this technique they successfully performed the discrimination of breath samples collected from persons with different blood glucose levels with higher performances and reliability than in the conventional approach. This technique may also be used for the discrimination of human body odor samples to classify or identify subjects.

Typically, statistical techniques as Spearman correlation and PCA analysis are used to compare the results of instrumental analysis and classify odors. Published results seem to confirm that doing so it is possible to identify individuals based on the analysis of volatile compounds. Since the amount of data can be large, automated or semi-automated methods are needed. More recently, Bayesian hypothesis testing and texture image analysis combined with machine learning have been developed.

7. Sensors to detect body odor

Based on the methods presented in Table 1, GC/MS is the most efficient method for human odor identification. However, several factors restrict its widespread use, such as large equipment size, long analysis time and high cost [48]. An electronic nose is suggested as an appropriate tool for on-body analysis of VOCs of human odor [76,77]. Jha published a review paper about aldehydes in human body odor and the detection of these using chemical sensor-array based systems. The most common types of chemical sensors used in VOC sensing applications in past studies include metal-oxide semiconductors and conducting composite polymer chemiresistors, quartz crystal microbalance and surface acoustic wave sensors [14,77–81]. Shang et al. used the phenomenon of localized surface plasmon resonance (LSPR), which results from the plasmonic response of nanoparticles by incident electromagnetic waves, in the sensing of volatile organic acids [82]. Compared to other chemical sensors mentioned above, the superiorities of LSPR are high-speed response and rapid recovery. However, the single LSPR sensors are not specific and therefore a molecular imprinted polymer (MIP) was employed as the sensitive layer on the LSPR sensor to make it specific for volatile organic acids. By changing the template molecules in the MIP the sensor could be made specific for four different volatile organic acids, pentanoic, hexanoic, heptanoic and octanoic acid.

Zheng et al. developed a wearable electronic nose for human skin odor identification [21]. The gas sensor array in this device consists of two functionalized carbon nanotubes (CNTs) encapsulated by three different polymers effectively forming six sensing materials. An important obstacle for identifying human skin odor is that the VOCs are mainly present at a trace level in the gaseous phase above the skin and therefore detection limits need to be low. The detection limits of the electronic nose for the test compounds hexanoic acid, dodecane and decanal are in the low ppm range (1–2 ppm) which may be low enough if the sensor array can be placed in close contact (headspace) with the skin. However, many compounds in the primary odor are present at ppb concentrations, too low to detect for present sensors. Other potential problems are humidity and temperature because chemisensors are generally sensitive to changes in these. This sensor showed to be stable up to 65% relative humidity after which the sensor had a very sluggish change in sensitivity between 65%– 92% relative humidity. For temperature the sensor was stable up to 70 °C which is far above the skin temperature of 35 °C and should therefore not be a problem.

It is interesting to see that the sensor array, because of the six different sensing materials, can differentiate between different compounds. To illustrate this, Fig. 3 shows the radar plots of the response pattern of the sensor array for the three test compounds hexanoic acid, dodecane and decanal as derived from the work of Zheng et al. [21]. Different patterns result and such images may be used in machine learning to differentiate between different odors.

Zheng et al. tested the wearable electronic nose on eight subjects and the experiment was repeated on four consecutive days. To perspire enough sweat (and collect sensing signals from different physical states) the subjects walked briskly on a treadmill. The sensor was mounted in a bracelet on the upper arm, close to the skin. Because of changes in the amount of sweating by the participants the changes in resistance of the individual sensors were found to be unrepeatable and a different way of signal processing, based on contrast response, needed to be used. The results indicated that the sensor array could differentiate and identify individual subjects.

Recently, Fang et al. proposed a smart electronic nose enabled by an all-feature olfactory algorithm [83]. The work presents an e-nose which consists of a simple combination of six metal-oxide-semiconductor gas sensors and a deep-learning-based algorithm that mimics the mammalian olfactory system in identifying odors. The tailored all-feature extraction method and the proposed data augmentation method seem to offer a superior advantage in complex odor discrimination over feature-based methods. The authors expect that the high integration of the gas sensor array and the intelligent algorithm will make the e-nose surpass the biological nose.

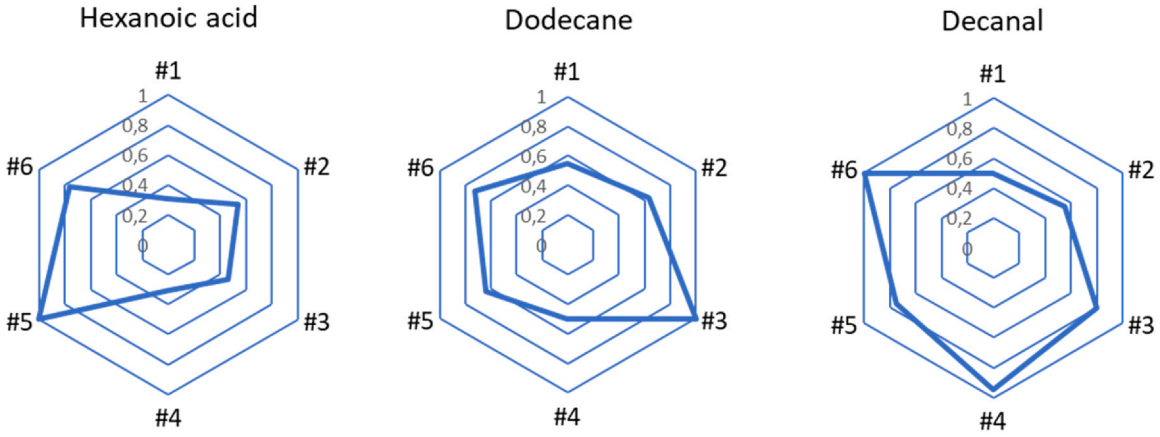

Fig. 3. Radar plots of the response pattern of the sensor (#1-#6) array to hexanoic acid, dodecane and decanal.

8. Conclusions and outlook

Looking back we can distinguish two routes, one which determines VOCs as the major compounds of the primary human scent, and another, more recent one, which focuses more on the semi-volatile compounds as the compounds comprising the primary human scent. For the VOCs it is more or less clear which compounds comprise the primary human scent and those may well be the compounds presented in Table 2. The accuracy with what these sets of compounds can identify a subject is 80% or better. For the semi-volatile compounds this is not so clear. Publications so far generally contain a long list of mostly tentatively identified compounds without a clear indication which are believed to belong to the primary human scent and why. On the other hand, Dolezal et al. showed that canines were better capable of identifying a subject based on the semi-volatile fraction of an odor sample [18]. The conclusion may be the observation that the human scent contains more than one group of compounds that allow for the individual scent identification of persons. This would mean that several human scent signatures co-exist in one scent trace, what Dolezal et al. termed as the multiplicity of the human scent signature.

In general, the instrumental methods used in the study of human odor are much the same, in almost all cases GC/MS. However, the composition of the odor samples is very complex making a clear identification with GC/MS difficult, certainly for the semi-volatile fraction. For that reason in recent years a development towards the use of two-dimensional GC has become evident since many more compounds can be resolved in that way. If the acceptation of GCxGC/MS is still to be considered at an early stage in this field, based on the long procedure to obtain a sufficient scientific standard to be used as expert testimony, no doubt that GCxGC/MS will rapidly become the method of choice for odor characterization. The very exhaustive description of sample compound profiles offers unique opportunities in sample characterization and therefore permits to enhance our level of confidence in terms of evidence-based sample differentiation as more subtle changes can be highlighted.

The development of sensors, and especially the “artificial intelligence”-added software (AI) interpreting the sensor signals is going fast. Several examples of this are presented above. While the limited detection capability of the sensors might still be a problem this will likely improve in the near future. As a result sensors may replace the classical GC/MS analysis.

The intention of this review was to compile the information in the literature about the determination of human scent and to present the state of the art. Analytical methods to determine scent were described as well as statistical methods to process the data and classify scents. The results show that the volatile compounds comprising the primary odor are likely identified while this is not yet the case for the non-volatiles. For the latter more research is needed. While data processing seems to allow the identification of individuals, further development using AI-added software is still ongoing. Finally, sensors may be on their way to take over the role of GC/MS in current analysis. Whether this will become true should be the subject of further work in this area.

CRediT authorship contribution statement

Ruud Peters: Conceptualization, Investigation, Writing – original draft. **Rick Veenstra**: Desk research, reviewing. **Karin Heutinck**: Desk research, reviewing. **Albert Baas**: Desk research, reviewing. **Sandra Munniks**: Desk research, reviewing. **Jaap Knotter**: Conceptualization, Writing – review & editing. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors have no conflicts of interest; including involvement, financial or otherwise, that might potentially bias the subject of this article.

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