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Headspace Solid-Phase Microextraction Gas Chromatography-Mass Spectrometry Analysis of Scent Profiles from Human Skin

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Abstract: Volatile organic compounds (VOCs) emanating from human skin contribute to an individual's body odour. Understanding the modulation of human odour by a fragrance is of significant importance to the cosmetic sector in the design, development and evaluation of new products. The present research describes an in vivo approach for passive headspace sampling of skin volatile emissions in human participants. A wearable headspace solid-phase microextraction (HS-SPME) method has been employed to investigate baseline endogenous skin volatiles and the subsequent modulation of skin volatile profiles after application of a fragrance to skin. Coupled with gas chromatography-mass spectrometry (GC-MS) this method enables characterisation of scent profiles and fragrance longevity in vivo. A total of 51 compounds were identified in participants' skin, including 19 endogenous and 32 fragrance-derived compounds. The temporal variation in volatile profiles at different times after fragrance application was investigated. Fragrance diffusion from skin varied between participants resulting in diversified scent profiles over time. This non-invasive approach could be employed during cosmetic product development for in vivo evaluation of fragrance profiles and for assessment of the retention of fragrance components in skin to reduce reliance on expert panels during product development.

Keywords: volatile organic compounds; human odour; fragrance; non-invasive

1. Introduction

Human skin is a constant source of volatile organic compounds (VOCs) which generates a complex mixture of compounds often referred to as human odour [1]. VOCs emitted by human skin are gaining interest in the cosmetics sector, in areas such as the design and development of fragranced products to modulate body odour [2] and the investigation of skin barrier properties in vitro and in vivo [3]. Applications are also emerging in disease diagnosis [4], ecology of blood-sucking insect vectors of human disease [5], forensics and criminal investigations [6], and safety and security [7]. Studying volatile skin emissions requires a high degree of analytical sensitivity, and headspace solid-phase microextraction (HS-SPME) with gas chromatography-mass spectrometry (GC-MS) has emerged as the method of choice in this regard, owing to the sensitivity and identification capabilities of MS and the ease of performing HS pre-concentration with SPME [5,8,9].

Over 500 volatiles have been reported isolated from skin secretions [10] and studies employing HS sampling typically recover between 20 and 90 volatiles that are airborne at normal body temperature [11]. Recovered compounds span a variety of chemical classes including aldehydes, hydrocarbons, carboxylic acids, alcohols, esters, ketones, and amines. Gland distribution and the

diversity of microbial flora at the skin surface varies across the body, and this is partially reflected in the regional diversity of skin volatiles. For instance, volatile profiles from feet are often dominated by short chain fatty acids, while axillary volatiles primarily contain $C_{6\text{-}11}$ carboxylic acids and alkanes, and hand and forearm volatiles tend to exhibit a greater distribution of aldehydes and ketones [8,11–14]. Furthermore, the chemical profile of an individual's skin VOCs can vary with pathophysiological status [15], and it is thought that some VOCs may derive from the potential passage of compounds from blood vessels [16]. Diet can affect gland secretions and consequently VOC profiles [17,18], and age-related metabolic activity has also been linked to changes in odour profiles [8]. Environmental factors and personal habits also have a significant influence on an individual's VOC profile, including level of physical activity, personal hygiene, and cosmetic or fragrance usage [6]. The use of fragrances to modulate body odour dates back to ancient times and has experienced remarkable growth throughout the centuries. In 2016, the global fragrance market was estimated to be worth US$40 billion [19].

Understanding the modulation of skin volatile profiles by a fragrance is of significance to the cosmetic sector in the design, development, and evaluation of new products [2,20]. Efficacy testing is most often reliant on subjective sensory evaluation employing panel testers or on the self-evaluation of volunteer users. The use of panels is time-consuming and costly. Analytical characterisation techniques such as gas chromatography (GC) and gas chromatography-olfactometry (GC-O) can provide a more objective measurement of odour which is important during product development and efficacy testing, but these techniques are typically applied to in-vial analyses. Cosmetic testing is most informative when carried out in human participants, and there is a growing interest in the development of non-invasive analytical tools in this area [3,21,22]. Our group have recently reported a non-invasive approach for in vivo profiling of skin VOCs that employs HS-SPME in a wearable format with subsequent gas chromatography-mass spectrometry (GC-MS) analysis [23]. The present research employs this approach for identification of the major skin VOCs present in healthy human participants and investigation of the modulating effect fragrance application has on skin volatile profiles. HS-SPME enables trapping of VOCs on adsorbent coated fibres followed by direct thermal desorption into a GC injector, and provides signal magnitudes that are proportional to the free concentration of target analyte [24]. The present method involves direct extraction from human participants, which minimises the number of preparation steps prior to analysis. It is simple, sensitive, and cost-effective and could permit testing and evaluation of fragrance profiles in vivo reducing reliance on expert panels during product development. Moreover, this approach could be applied to assessing the retention of fragrance components on skin to ensure integrity of retention in a diverse range of cosmetic products.

2. Materials and Methods

2.1. Participant Profile

Healthy volunteers were recruited (4 males, 4 females, age range 20–30 years) having given their informed consent. Ethical approval for skin volatile sampling was obtained from Dublin City University Research Ethics Committee (DCUREC/2016/053), and the study was carried out according to the Declaration of Helsinki. In order to minimise contributions from exogenous sources, participants were instructed not to apply perfumes or cosmetics to their arms on the day of skin sampling, and a skin pre-treatment step was included in the sampling protocol. Participants washed their hands and arms with tap water and pure olive oil soap (Oliva, A.B.E.A Anatoli S.S., Crete, Greece) and dried the skin with paper towels prior to commencement of sampling. No special dietary regimes were applied.

2.2. Skin Sampling Method and Investigation of Endogenous Skin Volatile Emisisons

Skin volatile sampling was performed using SPME fibres comprising 50/30 µm divinylbenzene/carboxen/polydimethylsiloxane (DVB/Car/PDMS) Stableflex (2 cm) assemblies (Supelco Corp., Bellefonte, PA, USA). The adsorptive DVB/Car/PDMS coating is recommended for detection of a wide range of compounds comprising a broad range of volatility and polarity. Larger

analytes will be retained in the DVB layer, while smaller analytes will migrate through this to be retained by the Carboxen extending the analyte range compared to other available fibre coatings, while still enabling extraction of analytes at trace levels [25]. SPME fibres were conditioned at 250 °C for 25 min before use each day. A wearable pre-concentration approach [3,23] was used for collection of human skin volatile samples. The SPME fibre was held within a glass housing (3 mL internal volume, Pyrex®) using 2 septa (Supelco Thermogreen LB-2 Septa plug, Sigma Aldrich, Ireland). The glass housing was affixed to the skin on the volar forearm using surgical tape (Leukosilk®, BSN Medical GmbH, Hamburg, Germany) serving to create an enclosed area of skin headspace. SPME extraction time profiles were investigated during method development by collecting skin headspace samples for varying lengths of time (5–30 min) and the resulting volatile profiles were evaluated. Fifteen minutes were required to reach equilibrium, so this time was selected as the time for sampling herein. HS-SPME samples were collected from participants' volar forearms for 15 min and analysed immediately afterward by GC-MS to identify the major skin VOCs present. The volar forearm is an easily accessible area both to clean and dry and to sample from, and the potential for skin-mediated chemical transformation of perfume is low [26]. SPME control samples were also collected including fibre blanks, background air, and tape to exclude exogenous compounds from the analysis. All sampling in this study was performed in the same location, where the average humidity was 62% and room temperature was 21 °C. Tissue dielectric constants were measured on the volar forearm using a Delfin MoistureMeter D (Delfin Technologies, Kuopio, Finland) where each measurement was repeated 3 times at an effective measurement depth of 0.5 mm.

2.3. Gas Chromatography-Mass Spectrometry

HS-SPME samples were desorbed within an SPME inlet liner (Supelco, Bellefonte, PA, USA) in the injector of an Agilent 6890 gas chromatograph equipped with a Merlin Microseal (Merlin Instrument Co., Newark, DE, USA). The system was connected to an Agilent 5973 mass selective detector. Sample desorption was performed at 250 °C for 2 min in splitless mode. Separations were performed on an SLB-5ms column (30 m × 0.25 mm × 0.25 μm d_f, Supelco, Bellefonte, PA, USA) with helium carrier gas used at a constant flow rate of 1 mL min^{-1}. The initial GC oven temperature was set to 30 °C for 1 min, after which the oven was programmed at a rate of 5 °C min^{-1} to 150 °C, followed by a rate of 2.5 °C min^{-1} to 200 °C with a hold for 2 min, and a final temperature ramp at a rate of 15 °C min^{-1} to 270 °C. The MS was operated at a scan rate of 3.94 s^{-1} and a range of 35–400 m/z. An ionising energy of 70 eV was utilised, and the ion source temperature was maintained at 230 °C. Data processing and analysis was carried out using Agilent GC/MSD ChemStation and OpenChrom® [27]. The identification of compounds was performed using the National Institute of Standards and Technology library (2005) to >80% match factor and was supported by retention index (RI) matching (tolerance of ≤10 RI units), for which a standard mixture of saturated alkanes (C_7–C_{30}, Sigma Aldrich, Arklow, Ireland) was used. The Good Scents Company Information System Database was used to classify recovered fragrance volatiles into different odour types. Tabulated chromatographic peak areas of reliably identified compounds were imported into Microsoft Excel and Origin (2018b, OriginLab Corp., Northampton, MA, U.S.A) for statistical analysis and generation of figures.

2.4. Investigating Modulation of Skin Volatile Profiles after Fragrance Application

The impact of a women's eau fraîche on skin volatile profiles was investigated. The ingredients indicated in the composition were alcohol, aqua, perfume, limonene, linalool, butyl methoxydibenzoylmethane, ethylhexyl methoxycinnamate, citral, coumarin, citric acid, hydroxycitronellal, geraniol, citronellol, isoeugenol, CI 19,140 yellow, and CI 42090/Blue 1. For investigating fragrance evolution on participants' skin, 1 μL of the fragrance was applied to the volar forearm. HS-SPME samples were collected at three intervals following fragrance application. Participants kept this application site uncovered throughout the study. They were permitted to perform

regular office work, but they ensured not to wipe off the fragrance. For collection of the first HS-SPME sample, the wearable housing was affixed to the sampling site 30 s after fragrance application. Sample collection lasted 15 min and samples were analysed by GC-MS immediately afterward. Samples were collected from the same site again 1 and 2 h after fragrance application. Reliably identified compounds were ranked according to chromatographic peak areas, and their Spearman rank correlation coefficients (r_s) were calculated by Equation (1):

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

where d is the difference between ranked components, and n is the total number of components. The data from the 8 participants was considered, resulting in 28 pairings for each of the 3 time points under investigation.

3. Results and Discussion

3.1. Endogenous Skin Volatile Profiles

Analysis of VOCs following thermal desorption from SPME fibres revealed a variety of classes of compounds emanating from the skin. There were 24 compounds identified in endogenous skin volatile profiles across all participants as shown in Table 1, where confirmation of compound identities was performed using retention index matching with a tolerance of ±10 RI units. Compounds deemed to be contaminants (e.g., siloxanes from SPME fibres, column bleed) were excluded from the table. The predominant species were acids and aldehydes (Figure S1) including nonanal, decanal, hexadecanoic acid, and tetradecanoic acid. Many other major skin VOCs [11] were also present including octanal, undecanal, 6-methyl-5-hepten-2-one, and geranyl acetone. Squalane 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 VOCs common to the volatile profile of all participants comprising 6 aldehydes, 5 acids, 2 ketones, and 1 ester.

The primary constituents (acids and aldehydes) are derived from the oxidative degradation of fatty acids on the skin. Sebaceous secretions can also impact many VOCs emanating from skin, including hydrocarbons (e.g., squalane) and ketones (e.g., 6-methyl-5-hepten-2-one and geranyl acetone that derive from the oxidative degradation of squalene) [28–30]. Esters such as isopropyl palmitate in skin VOCs are linked to cosmetic usage, and their persistence in the skin can vary [9]. Variations in individual volatile profiles were expected due to the influence of underlying biochemical processes, skin gland secretions, and skin microbiota on VOC emissions, so variation across intra- and inter-participant volatile samples was investigated to understand potential of the method to detect differences within the population. The repeatability of the majority of compounds was very good with intra-participant samples, suggesting that these VOCs could be useful for differentiating participants from one another. However, a small number of compounds showed marked variance in intra-participant samples making them less valuable for such categorisation of participants. Of the compounds that demonstrated good intra-participant repeatability, the inter-participant variance was substantial as shown in Figures S2–S9 for hexanal (Figure S2), octanal (Figure S3), nonanal (Figure S4), *n*-decanoic acid (Figure S5), tetradecanoic acid (Figure S6), *n*-hexadecanoic acid (Figure S7), isopropyl palmitate (Figure S8), and squalane (Figure S9). Several compounds showing marked variance in intra-participant samples (6-methyl-5-hepten-2-one; Figure S10 and geranyl acetone; Figure S11) derive from the oxidative degradation of squalene. Squalene is the most abundant oxidisable component of skin surface lipids, with concentrations in adult skin reaching up to 20% [31]. The observed variation for both ketones may be due to variations in the fast oxidative degradation of this abundant skin surface lipid, which can occur upon the action of both environmental oxidants and skin microbial residents to give rise to a wide spectrum of by-products [31]. In contrast, squalane is not subject to the same auto-oxidation that squalene is due to its complete saturation, which may account for the better intra-participant repeatability as seen in Figure S9.

Table 1. Compounds identified in the skin headspace of human participants after 15 min sample collection using a wearable concentration method employing headspace solid-phase microextraction (HS-SPME) followed by thermal desorption to gas chromatography-mass spectrometry (GC-MS). Compounds are shown in order of increasing retention time, where × indicates the presence of a compound in a sample. (F—female, M—male).

Compound	CAS	F1	F2	F3	F4	M1	M2	M3	M4
Hexanal	66-25-1	×	×	×	×	×	×	×	×
Benzaldehyde	100-52-7	×	×		×	×		×	
6-Methyl-5-hepten-2-one	110-93-0	×	×	×	×	×	×	×	×
2,6-Dimethyl-2,6-octadiene	2792-39-4	×			×		×	×	
Octanal	124-13-0	×	×	×	×	×	×	×	×
Nonanal	124-19-6	×	×	×	×	×	×	×	×
Octanoic acid	124-07-2							×	
Decanal	112-31-2	×	×	×	×	×	×	×	×
2-Decenal	2497-25-8	×			×	×	×	×	×
Nonanoic acid	112-05-0	×	×	×	×	×	×	×	×
Undecanal	112-44-7	×	×	×	×	×	×	×	×
2-Undecenal	2463-77-6	×			×			×	
n-Decanoic acid	334-48-5	×	×	×	×	×	×	×	×
Geranyl acetone	689-67-8	×	×	×	×	×	×	×	×
1-Dodecanol	112-53-8	×					×	×	
Tetradecanal	124-25-4	×	×	×	×	×	×	×	×
Pentadecanal	2765-11-9				×			×	
Tetradecanoic acid	544-63-8	×	×	×	×	×	×	×	×
Pentadecanoic acid	1002-84-2		×	×		×	×	×	×
9-Hexadecenoic acid	2091-29-4	×	×	×	×	×	×	×	×
n-Hexadecanoic acid	57-10-3	×	×	×	×	×	×	×	×
Isopropyl palmitate	142-91-6	×	×	×	×	×	×	×	×
Octadecanoic acid	57-11-4				×			×	
Squalane *	111-01-3	×		×	×	×		×	×

* Tentative identification, retention index (RI) match >10 RI units.

3.2. Investigating the Modulation of Skin Volatile Profiles after Fragrance Application

Comparison of total ion chromatograms obtained after fragrance application (Figure 1) revealed distinct temporal differences as fragrance evaporation took place on participants' skin. There were 51 compounds identified in skin volatile profiles comprising 19 endogenous and 32 fragrance-derived compounds, as outlined in Table 2. The suppression of several endogenous compounds was apparent, where 5 endogenous VOCs (2-decenal, 2-undecenal, 1-dodecanol, pentadecanal, and octadecanoic acid) present in baseline skin volatile profiles were not detected in the presence of the eau fraîche. Fragrances can modulate skin volatile profiles by reducing the formation of compounds arising from air oxidation and microbial transformation of skin gland secretions [2]. The observed suppression of endogenous VOCs was most prominent immediately after fragrance application where a number of oxidation products of skin gland secretions were not detected including aldehydes, acids, ketones, and hydrocarbons. There were seven compounds common to the volatile profile of all participants across all times investigated including two endogenous compounds (2,6-dimethyl-2,6-octadiene and *n*-hexadecanoic acid) and five fragrance-derived compounds (linalool acetate, γ-decalactone, methyl dihydrojasmonate, isopropyl tetradecanoate, and galaxolide). Other volatiles displaying a high frequency of occurrence within the group of participants included hexanal, β-myrcene, *cis*-linalool oxide, β-linalool, undecanal, geranyl acetone, 5-cyclohexadecen-1-one, and 2-ethylhexyl-4-methoxycinnamate. The data shown in Table 2 reflects the ongoing evaporation of fragrance components on skin, where several species were no longer present in skin HS after 1 h including β-pinene, α-pinene, β-terpineol, *trans*-2-pinanol, longifolene, and cedrene. Several other volatiles were no longer present in the majority of participants after 2 h, including α-citral, β-citral, isosafrole, nerol acetate, and dihydro-β-ionone.

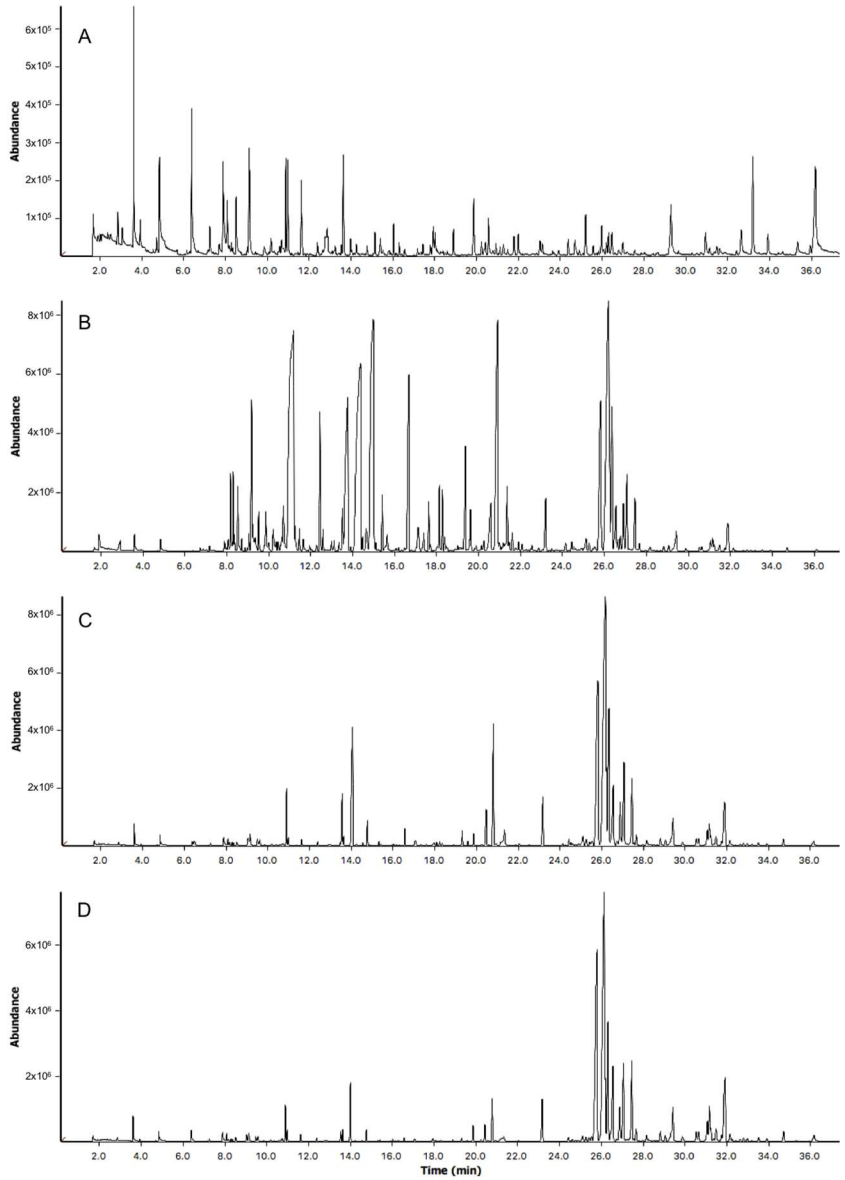

Figure 1. Overlaid total ion chromatograms showing recovered skin volatiles. (**A**) The baseline skin volatile profile; (**B**) the volatile profile immediately after fragrance application; (**C**) skin volatiles 1 h after fragrance application and (**D**) 2 h after fragrance application.

Table 2. Compounds identified in the skin headspace of human participants 0, 1, and 2 h after fragrance application. Samples were collected using headspace solid-phase microextraction (HS-SPME) in a wearable format followed by thermal desorption to gas chromatography-mass spectrometry (GC-MS). Compounds are shown in order of increasing retention time, where × indicates the presence of a compound in a sample. (F—female, M—male).

Compound	CAS	0								1										2							
		F1	F2	F3	F4	M1	M2	M3	M4	F1	F2	F3	F4	M1	M2	M3	M4	F1	F2	F3	F4	M1	M2	M3	M4
Hexanal [+]	66-25-1	×	×	×	×	×	×	×		×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
Citronellene [^]	10281-56-8	×	×	×	×	×		×		×	×														
Benzaldehyde [+]	100-52-7				×	×		×	×		×	×	×	×		×	×		×	×	×	×	×	×	×
β-Pinene [^]	127-91-3	×	×	×	×	×	×	×																	
6-Methyl-5-hepten-2-one [+]	110-93-0									×		×	×	×	×	×	×	×	×	×	×	×	×	×	×
β-Myrcene [^]	123-35-3	×	×	×	×	×	×	×	×	×	×		×	×	×	×		×	×		×	×	×	×	×
2,6-Dimethyl-2,6-octadiene [+]	2792-39-4	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
Octanal [+]	124-13-0									×					×	×		×			×		×	×	
3-Hexen-1-ol, acetate [^]	3681-71-8	×	×	×	×	×	×	×	×		×	×	×	×			×		×	×		×			×
o-Cymene [^]	527-84-4	×	×	×	×	×	×	×	×		×	×		×					×	×		×			
D-Limonene [^]	5989-27-5	×	×	×	×	×	×	×	×	×	×	×		×	×		×	×		×		×			×
α-Pinene [^]	80-56-8	×		×	×	×	×	×	×	×															
γ-Terpinene [^]	99-85-4	×	×	×	×	×	×	×	×	×			×	×			×	×							×
Cis-Linalool oxide [^]	5989-33-3	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	
Nonanal [+]	124-19-6					×				×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
β-Linalool [^]	78-70-6	×	×	×	×	×	×	×	×		×	×	×	×		×	×		×	×	×	×		×	×
Phenylethyl alcohol [^]	60-12-8	×	×	×	×	×	×	×	×	×	×	×	×	×		×	×		×		×	×			×
β-Terpineol [^]	138-87-4	×			×	×	×	×																	
Trans-2-pinanol [^]	4948-29-2	×	×	×		×	×	×	×																
Octanoic acid [+]	124-07-2										×	×	×		×	×	×	×	×	×	×	×	×	×	×
Decanal [+]	112-31-2									×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
β-Citral [^]	106-26-3	×	×	×	×	×	×	×	×	×	×	×	×	×		×		×				x			
Linalool acetate [^]	115-95-7	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
α-Citral [^]	141-27-5	×	×	×	×	×	×	×	×		×	×	×	×	×	×	×	×			×	×			
Nonanoic acid [+]	112-05-0						×			×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
Hydroxycitronellal [^]	107-75-5	×			×			×		×															
Isosafrole [^]	120-58-1	×	×	×	×	×	×	×	×	×			×	×		×	×	×							
Undecanal [+]	112-44-7		×	×		×	×		×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
Methyl anthranilate [^]	134-20-3					×			×	×	×	×	×	×	×	×	×	×	×		×	×		×	×
Nerol acetate [^]	141-12-8	×	×	×	×	×	×	×	×	×			×			×	×	×							
n-Decanoic acid [+]	334-48-5			×			×			×	×	×	×	×	×	×		×	×	×	×	×	×	×	×
Cedrene [^]	11028-42-5	×		×	×	×		×	×																
β-Damascenone [^]	23726-93-4	×	×	×	×	×	×	×	×				×			×		×							
Longifolene [^]	475-20-7	×	×	×		×	×	×																	
α-Ionone [^]	127-41-3	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×				×		×	

Table 2. *Cont.*

Compound	CAS	0								1								2							
		F1	F2	F3	F4	M1	M2	M3	M4	F1	F2	F3	F4	M1	M2	M3	M4	F1	F2	F3	F4	M1	M2	M3	M4
Dihydro-β-ionone ^	17283-81-7	×	×	×	×	×	×	×	×	×			×	×	×	×	×	×							
Geranyl acetone +	689-67-8	×	×	×		×	×			×		×	×	×	×	×	×	×		×	×	×	×	×	×
γ-Decalactone ^	706-14-9	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
α-Calacorene ^	21391-99-1	×	×	×	×	×	×	×										×	×			×			
Tetradecanal +	124-25-4									×									×	×			×		
Methyl dihydrojasmonate ^	24851-98-7	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
Tetradecanoic acid +	544-63-8	×					×		×		×	×			×				×	×		×	×	×	
Isopropyl tetradecanoate ^	110-27-0	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
Pentadecanoic acid +	1002-84-2									×	×	×							×	×	×				
5-Cyclohexadecen-1-one ^	37069-25-9	×	×	×	×	×	×	×	×	×	×		×	×	×	×	×	×			×	×	×	×	×
Galaxolide ^	1222-05-5	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
9-Hexadecenoic acid +	2091-29-4				×					×			×					×	×			×			
n-Hexadecanoic acid +	57-10-3	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
Isopropyl palmitate +	142-91-6				×					×			×		×	×					×		×	×	
2-Ethylhexyl 4-methoxycinnamate ^	5466-77-3	×	×	×		×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
Squalane *,+	111-01-3	×	×			×					×		×	×	×	×	×	×	×	×	×	×	×	×	×

* Tentative identification, retention index (RI) match >10 RI units; + indicates a compound present in baseline skin volatile samples; ^ indicates a fragrance-derived compound.

This approach can provide an insight into the longevity of individual fragrance components on skin. For instance, a number of fragrance-derived compounds with lower vapour pressures remained present in all participants' skin volatile profiles 2 h after application (e.g., methyl dihydrojasmonate, galaxolide, linalool acetate, and γ-decalactone shown in Table 2). Isopropyl tetradecanoate (solvent) and 2-ethylhexyl 4-methoxycinnamate (UVB absorber) were also observed to remain after 2 h, while several other compounds exhibited longevity on the majority of participants including 5-cyclohexadecen-1-one, β-myrcene, and *cis*-linalool oxide. Many other fragrance components displayed varied substantivity between participants, such as phenylethyl alcohol, isosafrole, and nerol acetate after 1 h (Table 2). Inter-participant differences in the persistence of fragrance components were apparent. For instance, isosafrole and β-damascenone exhibited greater tenacity on F1 compared to other participants. Similarly, cymene persisted on F2, F3, and M1, nerol acetate on F1, α-ionone on F1, M1, and M3, and dihydro-β-ionone on F1 after 2 h. Differences in compound tenacity can be attributed to variations in the diffusion of the fragrance from skin which is known to vary from individual to individual depending on skin properties, air movement, and the concentration of fragrance on the skin [32]. Local tissue hydration levels were investigated in participants as a function of tissue dielectric constants (TDC) and all values were within the normal range for healthy skin [33] (Table S1). Water present on the surface of the skin can influence the retention and rate of evaporation of hydrophilic components such as *cis*-linalool oxide and β-linalool, which remained after 2 h in the majority of participants. In the presence of moisture, the more hydrophobic moieties may be driven off the skin as seen for several terpene hydrocarbons including citronellene, α-pinene, β-pinene, and cymene, while moderately hydrophobic compounds may have some affinity for the lipid phase in the epidermis and would be expected to exhibit greater longevity as observed for linalool acetate.

Inter-participant variance was investigated for all endogenous and fragrance-derived volatiles and is shown in Figure 2. The highest concentrations of VOCs detected in participants' skin HS were present immediately after fragrance application at 0 h, where β-linalool, linalool acetate, limonene, and methyl dihydrojasmonate were among the most abundant across the participant group (Figure 2). These and several other prevalent volatiles, namely α-citral, nerol acetate, and α-ionone, exhibited the greatest variance between participants at 0 h. When all participants were clustered together, there was a significant reduction ($p \leq 0.01$) in the majority of endogenous VOCs after fragrance application (0 h) including octanal, nonanal, decanal, tetradecanal, 2,6-dimethyl-2,6-octadiene, 6-methyl-5-hepten-2-one, nonanoic acid, 9-hexadecenoic acid, and isopropyl palmitate. Tetradecanoic acid, pentadecanoic acid, squalane, and *n*-decanoic acid also underwent a significant reduction ($p \leq 0.05$) in participants' skin HS immediately after fragrance addition to skin.

As fragrance evaporation proceeded the modulation of endogenous volatiles became less apparent, with a significant increase ($p \leq 0.01$) observed for 6-methyl-5-hepten-2-one, nonanal, decanal, nonanoic acid, and *n*-decanoic acid after 1 h. Conversely, a significant reduction ($p \leq 0.01$) occurred in numerous fragrance-derived compounds after 1 h, including citronellene, β-myrcene, 3-hexen-1-ol acetate, cymene, limonene, α-pinene, γ-terpinene, *cis*-linalool oxide, β-linalool, phenylethyl alcohol, *trans*-2-pinanol, linalool acetate, α-citral, nerol acetate, β-damascenone, longifolene, α-ionone, dihydro-β-ionone, and α-calacorene. Further reduction ($p \leq 0.01$) in fragrance components occurred after 2 h, as observed for phenylethyl alcohol, α-citral, γ-decalactone, methyl dihydrojasmonate, and 2-ethylhexyl-4-methoxy cinnamate, accompanied by a reduction ($p \leq 0.05$) in linalool acetate, isosafrole, α-ionone, and dihydro-β-ionone. A number of compounds exhibited a certain degree of temporal stability in skin volatile profiles, including hexanal, undecanal, geranyl acetone, galaxolide, and squalane, where no statistically significant differences were observed within the group as time after fragrance application elapsed.

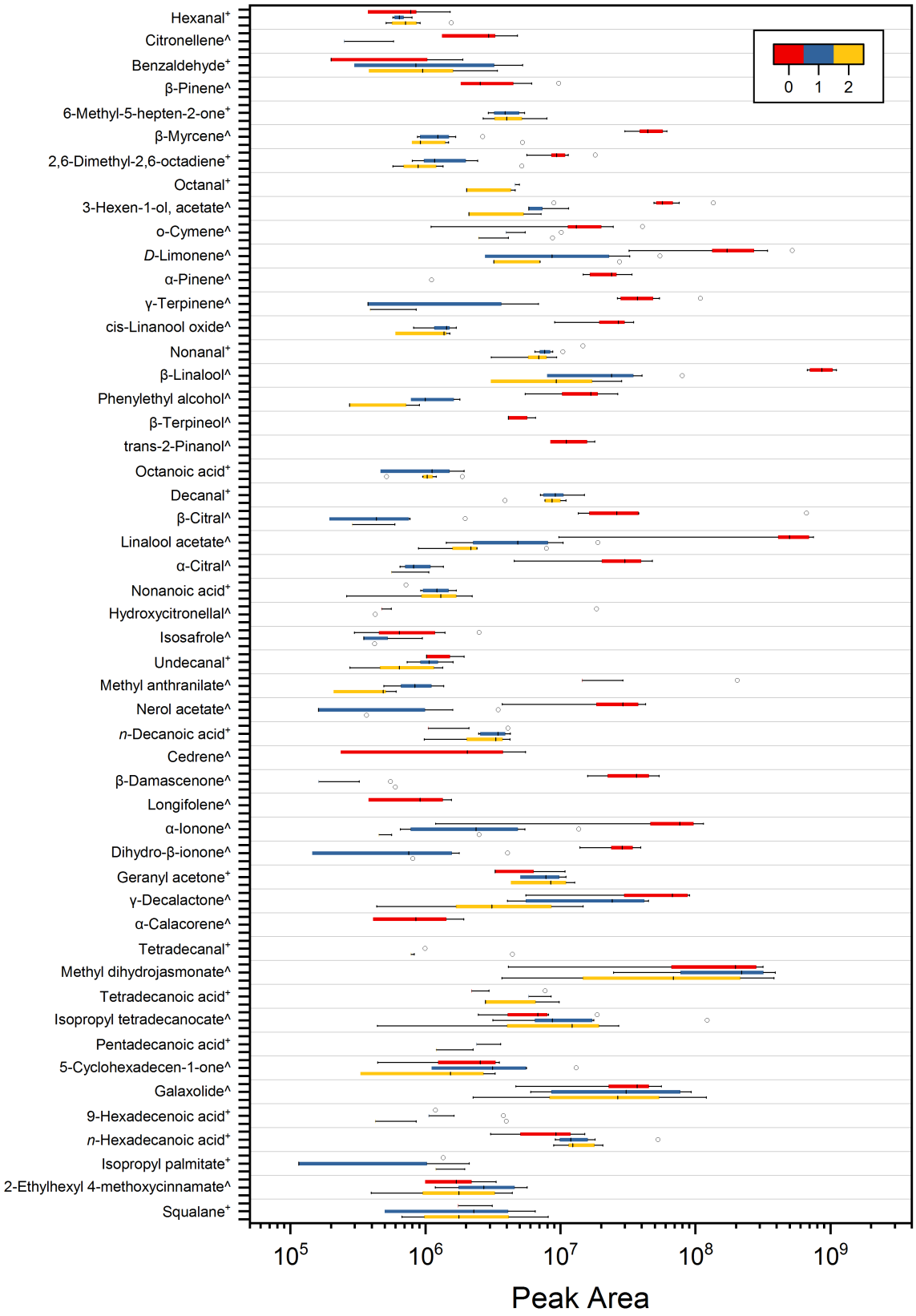

Figure 2. Box plot showing the inter-participant variance for fragrance-derived and endogenous volatiles recovered from participants' skin headspace at 0 h (red), 1 h (blue), and 2 h (yellow). Box shows middle 50% of data with lines indicating the median. Whiskers represent range within 1.5 IQR. (○ represents outliers; $^{+}$ indicates compound present in baseline skin volatile samples; $^{\wedge}$ indicates that the compound is fragrance-derived).

Inter-participant diversity was considered in terms of olfactory qualities. Fragrance-derived compounds were classified according to their olfactory family. The 10 olfactory groups identified consisted of floral, woody, herbal, citrus, musk, fruity, spicy, green, earthy, and terpenic groups. Figure S12 shows the percentage contribution of each olfactory family to individual scent profiles at the times investigated based on peak areas obtained by HS-SPME GC-MS. Floral odours were dominant within scent profiles, where compounds such as methyl dihydrojasmonate and β-linalool were the main contributors. At 0 h, the predominant floral compounds were accompanied by a secondary herbal note attributed to the presence of α-pinene, β-pinene, and linalool acetate, in addition to a discernible citrus component (limonene, α-citral, and β-citral). There were minor contributions from all other constituents. After 1 h had elapsed, the predominant floral compounds were accompanied by a diversified composition, with the emergence of musk (galaxolide, 5-cyclohexadecen-1-one), fruity and citrus scents, while herbal and terpenic notes were diminished. After 2 h, the floral note was primarily accompanied by musk and citrus, and there were distinct differences evident between participants' scent profiles (Figure S12). Inter-participant diversity was investigated through comparison of Spearman correlation coefficients for data shown in Figure 2. Correlation analysis can be used to investigate the relationship between two or more variables, and Spearman rank correlation is a common nonparametric approach to measure correlation. It has previously shown potential for distinguishing between individuals based on their skin VOC profiles [34]. The analysis was performed on all 8 participants and generated 28 pairs for each time investigated (0, 1, and 2 h). An overall analysis of all samples (84 pairings) resulted in 88% discrimination at the 0.9 correlation threshold. At a correlation threshold of 0.9, participants were distinguished in 64% of cases immediately after the addition of fragrance at 0 h. This increased to 100% discrimination between participants after 1 and 2 h had elapsed from time of perfume application, thus supporting the presence of diverse scent profiles among participants.

4. Conclusions

The present research has shown that in vivo HS-SPME GC-MS analysis is a valuable method to investigate the volatile composition of human odour. Profiling VOCs offers a non-contact method of chemical interrogation that is repeatable and can easily be performed on human participants and is also suitable for use in vivo on skin models. The method permits repeated evaluation of scent profiles during fragrance evaporation on skin. Fifty-one VOCs derived from fragrance and endogenous sources were identified in participants' skin in this study. The information generated was used to assess the diversity across the participant population, where the potential to discriminate between individuals based on Spearman rank correlation was demonstrated, suggesting diverse scent profiles existed amongst participants over time. This study has a number of limitations including the low number of participants and the fact that only reliably identified VOCs were reported, thus a number of potentially notable compounds were not yet considered in the context of this investigation. In future research, it will be important to address regional diversity in skin volatiles (owing to the diverse distribution of glands on human skin), and the contribution of skin flora to volatile emanations and their influence on fragrance evolution. This approach could permit testing and evaluation of fragrance profiles in vivo reducing reliance on expert panels during product development and it could be applied to assessing the retention of fragrance components on skin to ensure integrity of retention in a diverse range of cosmetic products. Moreover, recent evidence has demonstrated the role that deodorant residues may affect the attractiveness of volatile skin emanations to the malaria mosquito *Anopheles coluzzii* [35]. This highlights the need for further investigation into the impact of cosmetics on the human scent profiles towards development of novel vector control tools.

Supplementary Materials: The following are available online at http://www.mdpi.com/2079-9284/5/4/62/s1. Figure S1: Distribution of compound classes in human skin volatile samples. Figure S2: Box plot comparing intra- and inter-participant variance for hexanal; Figure S3: Box plot comparing intra- and inter-participant variance for octanal; Figure S4: Box plot comparing intra- and inter-participant variance for nonanal; Figure S5: Box

plot comparing intra- and inter-participant variance for *n*-decanoic acid; Figure S6: Box plot comparing intra- and inter-participant variance for tetradecanoic acid; Figure S7: Box plot comparing intra- and inter-participant variance for *n*-hexadecanoic acid; Figure S8: Box plot comparing intra- and inter-participant variance for isopropyl palmitate; Figure S9: Box plot comparing intra- and inter-participant variance for squalane; Figure S10: Box plot comparing intra- and inter-participant variance for 6-methyl-5-hepten-2-one; Figure S11: Box plot comparing intra- and inter-participant variance for geranyl acetone; Table S1: Measured tissue dielectric constants for participants' skin; Figure S12: Participant scent profiles after addition of fragrance to skin.

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