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ORIGINAL PAPER

Criminalistics

Can latent fingerprint disclose the sex of the donor? A preliminary test study using GC–MS analysis of latent fingerprints

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Abstract

While fingerprints are a highly used means of identification, not every fingerprint left behind on a potential crime scene can be used for identification purposes. In some cases, the fingerprint may be smudged, partially preserved or overlapping with other prints hence distorting the ridge pattern and may therefore be not appropriate for identification. Further, fingermark residue yields a very low abundance of genetic material for DNA analysis. In such cases, the fingermark may be used to retrieve basic donor information such as sex. The focus of this paper was to assess the possibility of differentiating between the sexes of the donor of latent fingermarks. Analytical method was GC–MS analysis of the chemical compounds of latent fingermarks using 22 male and 22 female donors. Results showed 44 identified compounds. Two alcohols, octadecanol C_{18} and eicosanol C_{20}, were found to show a difference that was statistically significant between male and female donors. There is also some evidence for the possibility of distinguishing sex of the fingermark donor based on the distribution of branched chain fatty acids, as free compounds or esterified in wax esters.

KEYWORDS
chemical analysis, classification, fingermarks, mass spectrometry, profiling

Highlights

- Fingermarks that cannot be used for ridge pattern analysis can be analyzed chemically.
- GC–MS analysis is a promising method to determine the sex of the donor of latent fingermarks.
- Fingermarks can be used for identification in criminal investigations to suggest sex of donor.

1 | INTRODUCTION

The human fingerprint derives from the corrugated ridge and furrow pattern on the skin of the fingertips and is currently used as one of biometric traits for identification of individuals, even identical twins. Fingerprints have been used for identification purposes for more than 150 years [1]. The latent fingerprint is composed of the natural secretions from the glands in the skin, mainly eccrine and sebaceous excretions [2], as well as environmental contaminants. Because the openings of the excretory glands are located at the ridges rather than the furrows, the excretion transferred from the fingertip to a surface upon touch leaves behind the distinctive fingerprint pattern.

This study has previously been presented at the British Association for Human Identification (BAHID) Conference, November 22-24, 2019, in Manchester, United Kingdom.

This is considered a 'latent' fingerprint that can be visualized for recording and analysis and then used for identification purposes. The visualization of latent fingerprints employs numerous techniques including chemical, physical, optical, and electrochemical techniques [3, 4].

Eccrine excretions consist of 98% water mixed with inorganic and water-soluble organic substrates such as urea, amino acids, and proteins while sebaceous excretions consist largely of fat-soluble organic compounds such as fatty acids, sterols, squalene, glycerides, and wax esters [2, 5–8]. Environmental contaminants may be in the form of food residue, dust, bacterial spores, cosmetics, or nicotine [2, 7, 9]. However, not every fingerprint left behind on a potential crime scene can be used for identification purposes from comparison of the ridge patterns. In some cases, the fingerprint may be smudged, partially preserved, or overlapping with other prints hence distorting the pattern and may therefore not be appropriate for identification from the ridge pattern, and instead be a "fingermark," rather than a fingerprint. Fingermark residue yields very low abundance of genetic material for DNA analysis and is extremely challenging even for just sex determination analysis [10]. In such cases, the fingermark may be used to retrieve additional information and basic donor information. Several studies have been conducted on the chemical composition of latent fingermarks using techniques such as gas chromatography–mass spectrometry (GC–MS), advanced mass spectrometry techniques such as DESI, MALDI, or SALDI or chemical imaging techniques such as FTIR and Raman [6, 11–16].

The chemical composition of a fingermark has been analyzed for time since deposition [2, 17–24], age of donor [25–30] lifestyle [19, 31–33], and sex [8, 14, 27, 34–38]. Studies on the sex determination from the chemical compounds of latent fingermarks have revealed conflicting results, with some of these studies finding it was not possible to differentiate between the sex of the donor [34, 35, 37, 38] while other studies found it was possible to distinguish between the sexes based on some compounds such as urea and fatty acids [6, 8, 11, 28, 36, 38].

The focus of this paper was to assess the possibility of differentiating between the sexes of the donor of latent fingermarks using GC–MS analysis of the chemical compounds of latent fingermarks. Using this data, the difference in chemical composition of latent fingermarks is examined to provide a method for analysis of fingermarks at crime scenes that are not conducive to be lifted for identification from the visualized ridge and furrow pattern and may instead be useful for obtaining details on the donor profile.

2 | MATERIALS AND METHODS

2.1 | Collection of fingermarks

Fingermarks were collected from 44 adult donors (22 males and 22 females) with a minimum age of 20 years. See Table 1 for donor demographics. The project received ethical approval from the University of Dundee. Donors provided informed consent in writing and their data, and all samples, were anonymized to be identified only by a number (101–122) with a pre-fix of "M" for male and "F" for female. Participants were excluded as donors if receiving steroid or hormone treatment.

TABLE 1 Donor demographics for collected fingermark samples.

Sex	N	Mean age (years)	Range (years)
Male	22	33.0	22–68
Female	22	32.8	20–62

Donors were asked to wash their hands using only water and rinse them in distilled water. The hands were patted dry using tissue of the standard institutional brand "Lotus Professional (100% UK and Ireland recovered fibers)". Donors were requested not to touch anything and let 5 min pass for the regeneration of fingerprint residue and then requested to rub all 10 fingertips on the skin behind the ears. The fingertips of all 10 digits were then rubbed on disinfected microscope slide and placed in a glass jar. This process was repeated three times such that each donor provided three replicates, all of which were placed in the same jar for transport.

2.2 | Extraction of samples

Both sides of the slides were then dripped with dichloromethane and the extract was collected in a culture tube. The three replicates provided by the participants were all treated separately so that for each participant three samples were produced. Fifty microliters of the internal standard, methyl nonadecanoate (10.3 mg in 50 mL of dichloromethane), was added to each sample. The dichloromethane containing the fingermark residue was evaporated down by a gentle stream of nitrogen and a weak heating source to a volume of about 120 µL. The sample was then transferred to a 200 µL autosampler vial and evaporated down to approximately 60 µL. Each vial was labeled with the anonymized donor number and number of sample replicate.

2.3 | Analysis of samples by gas chromatography–mass spectrometry

The samples were analyzed using a DSQ II quadrupole mass spectrometer coupled to a Trace Ultra gas chromatograph (Thermo Electron Corporation) fitted with a CTC Combi Pal Autosampler (CTC Analytics). The GC column used was a 15 m × 0.25 mm i.d. × 0.25 µm film DB-5MS fused silica capillary column (J & W; Crawford Scientific). Samples (1 µL) were injected into a programmable temperature vaporizing (PTV) injector operating in splitless mode. The PTV conditions were as follows: Injection temperature 132°C held for 1 min, transfer rate 14.5°C/s, transfer temperature 320°C for 1 min, clean rate 14.5°C/s, and clean temperature 400°C for 2 min. Helium (99.999% purity, British Oxygen Company) was

used as carrier gas in constant flow mode at 1.5 mL/min. The GC oven temperature program was 100°C, held for 2.1 min, then increasing at 25°C/min to 320°C followed by a hold at that temperature for 3.5 min. The interface temperature was 250°C and MS was used in EI mode at 70 eV over the mass range 50–900 amu with a source temperature of 200°C. MS data were acquired at 6 scans/s after an initial 4 min delay.

Between each sample, a "blank" sample was inserted (pure solvent) to minimize carryover. The samples were all analyzed by the GC–MS on the same day as collection. One sample with three replicates was produced to act as controls. The data were acquired using the Xcalibur software package (V.2.07 Thermo Electron Corporation). The mass spectra of each peak in the TIC of several representative raw data files were examined manually and specific ions characteristic of each compound in the samples were selected using Xcalibur (Table S1). Selection criteria were that the ions should be of high relative abundance and should be unique to the compound and/or be well resolved from other ions with the same *m/z* [39]. A defined time window centered on the chromatographic peak apex, along with the selected characteristic ions, was used for compound detection and quantification in a processing method created in Xcalibur. A summed selected ion chromatogram (SIC) for all of the ions within the appropriate time window was then generated and integrated which constituted the absolute abundance of each compound, including the internal standard. Processed data were checked for correct peak assignment and adjusted where necessary. Compounds were identified by comparison of their mass spectra and retention times with reference standards (straight chain *n*-C_{16}, *n*-C_{18}, *n*-C_{20} alcohols and *n*-C_{16}, *n*-C_{18}, *n*-$C_{18:1}$, *n*-$C_{18:2}$ fatty acids, squalene, and cholesterol, 95% purity, Sigma Aldrich), with published data and entries in commercially available Mass spectral databases (Palisade Complete Mass Spectral Library, 600 K Edition, Palisade Corporation; NIST05, National Institute of Standards) and by comparison with retention and MS data for known compounds (all other compounds).

Summed SIC peak areas for each compound were normalized to the internal standard, by dividing the SIC area with that of the internal standard. The values quoted are therefore peak area ratios.

2.4 | Statistical analysis

MANOVA analysis was conducted to investigate the statistical differences between the mean peak area ratios of two sexes included in the study (considered significant when $p \leq 0.05$), followed by a discriminant function analysis. Analysis was performed in SPSS version 25.

3 | RESULTS

Initially, 132 compounds were detected, of which most were identified. However, many were alkyl esters in such low abundance that they were not included in further analysis. Obvious contaminants were also excluded; this was the case for several identified compounds, Vitamin E (α–tocopheryl) acetate, Parsol MCX and an isomeric form of identical mass spectrum, Parsol 1789 and a number of alkyl benzoates and glycerol esters. Unidentified compounds were also excluded. The 44 compounds which therefore remained for analysis can be seen in Table 2 where the mean of the three replicates for the 44 donors was used for data analysis. A large proportion of compounds were esters and fatty acids with only three alcohols, a terpene and sterols. A full list of compounds found in the samples is given in Table S1.

Free fatty acids consisted primarily of straight chain (*n*-) saturated (C_{14} – C_{18}) and unsaturated ($C_{16:1}$, $C_{18:1}$ $C_{18:2}$) homologs. In addition, *iso*-branched (*i*-) components (*i*-C_{16}, *i*-C_{17}; *i*-$C_{16:1}$) and *anteiso* branched (*a*-) components (*a*-C_{15}, *a*-C_{17}) were also present. *Iso*-branched chains have a methyl group (CH_3) located one carbon in from the carbon (CH_3) terminus of the acid, whereas in *anteiso*-branched compounds the methyl group is two carbons in from the C-terminus. Branching positions are determined by calculating an Equivalent Chain Length (ECL) value for each compound, for example, C_{14}, C_{15}, and C_{16} *n*- compounds are given ECL values of 14, 15, and 16, respectively. If a plot is made of ECL against Log of retention time (R_t), the ECL of branched (*br*-) components can be determined by interpolation. Then the relative spacing of *br*- components between adjacent *n*- components specifies the branching point. The normal elution order is *i*- then *a*- then *n*- for a given C_n for the type of low polarity GC column used in this study [40, 41]. The free fatty alcohols present were the *n*- C_{16}, C_{18}, and C_{20} homologs, no branched chain alcohols were detected. The predominant monounsaturated fatty acids in human sebum, unique to this secretion, have the double bond in the Δ6 position and it is, therefore, likely that the *n*-$C_{16:1}$ and $C_{18:1}$ acids found in the samples are (*Z*)-6-Hexadecenoic acid (sapienic acid) and (*Z*)-6-octadecenoic (petroselinic acid) acid [42–45]. The $C_{18:2}$ acid is thus probably (*Z*)-5, (*Z*)-8-octadecadienoic acid (sabaleic acid), also unique to human sebum, which is formed biosynthetically by a C_2 elongation of the precursor sapienic acid followed by insertion of the second double bond between C5 and C6.

Saturated esters consisted of both *n*-saturated C_{28}-C_{34} homologs, *i*-branched even carbon C_{28}, C_{30}, and C_{34} homologs and *a*-branched odd carbon C_{29}, C_{31}, and C_{33} homologs. In addition, branched (*br*-) odd carbon C_{29}, C_{31}, and C_{33} components of undetermined branching point were also present. The presence of *i*- and *a*- branched free acids and apparent absence of branched alcohols suggests that in the esters the branching occurs in the acid moiety. From examination of the mass spectral fragmentation patterns of the esters, it was apparent that for an ester of a given carbon number (C_n), several homologs were usually present with different acid/alcohol combinations, but with the same overall chain length (CL). The isomeric distribution within each overall CL was not determined in detail, although the identities of the homologs within each CL are listed in Table S1. Acid (*n*-, *i*-) and alcohol (*n*-) moieties in the range of C_{12}-C_{18} and C_{20} were present within the esters with C_{14}, C_{16}, and C_{18} most abundant, and *a*- and unidentified *br*- acids were in the range C_{13}-C_{17} with C_{15} and C_{17} most abundant.

TABLE 2 The 44 identified compounds with details of male and female data.

Compound	Males[a]				Females[a]				
	Min.	Max.	Range	Mean	Min.	Max.	Range	Mean	M/F[b]
Alcohols									
(1) *n*-$C_{16:0}$	0.0000	0.0301	0.0301	0.0045	0.0019	0.7943	0.7924	0.0684	0.07 (*0.05*)
(2) *n*-$C_{18:0}$	0.0010	0.0328	0.0318	0.0057	0.0065	0.1840	0.1775	0.0379	0.15 (*0.11*)
(3) *n*-$C_{20:0}$	0.0002	0.0047	0.0046	0.0011	0.0004	0.0124	0.0119	0.0029	0.38 (*0.28*)
Fatty acids									
(4) *n*-$C_{14:0}$	0.0081	0.3521	0.3441	0.0828	0.0049	0.2321	0.2272	0.0564	1.47 (*1.07*)
(5) *n*-$C_{16:0}$	0.0284	1.1709	1.1425	0.2547	0.0169	0.7890	0.7721	0.1863	1.37 (*1.00*)
(6) *n*-$C_{16:1}$	0.0098	0.9535	0.9437	0.1973	0.0094	0.7032	0.6937	0.1526	1.29 (*0.95*)
(7) *n*-$C_{18:0}$	0.0017	0.0898	0.0881	0.0199	0.0011	0.0459	0.0449	0.0151	1.32 (*0.96*)
(8) *n*-$C_{18:1}$	0.0097	1.1377	1.1280	0.1831	0.0060	0.3856	0.3796	0.1082	1.69 (*1.24*)
(9) *n*-$C_{18:2}$	0.0002	0.0381	0.0379	0.0072	0.0002	0.0612	0.0610	0.0101	0.71 (*0.52*)
(10) *i*-$C_{16:0}$	0.0001	0.0089	0.0088	0.0016	0.0003	0.0160	0.0157	0.0027	0.59 (*0.43*)
(11) *i*-$C_{16:1}$	0.0002	0.0408	0.0406	0.0065	0.0000	0.0412	0.0412	0.0070	0.93 (*0.68*)
(12) *n*-$C_{17:0}$	0.0009	0.0675	0.0666	0.0131	0.0007	0.0224	0.0217	0.0067	1.96 (*1.43*)
(13) *a*-$C_{15:0}$	0.0010	0.0986	0.0976	0.0168	0.0006	0.0558	0.0552	0.0148	1.14 (*0.83*)
(14) *i*-$C_{17:0}$	0.0018	0.1315	0.1297	0.0266	0.0008	0.0770	0.0761	0.0191	1.39 (*1.02*)
(15) *n*-$C_{15:0}$	0.0044	0.2925	0.2882	0.0595	0.0019	0.1466	0.1446	0.0332	1.79 (*1.31*)
(16) *a*-$C_{17:0}$	0.0012	0.1455	0.1443	0.0304	0.0005	0.0595	0.0589	0.0154	1.97 (*1.44*)
Esters (saturated)									
(17) *n*-C_{28}	0.0001	0.0033	0.0032	0.0006	0.0001	0.0076	0.0075	0.0009	0.67 (*0.49*)
(18) *n*-C_{30}	0.0001	0.0078	0.0077	0.0013	0.0003	0.0053	0.0050	0.0016	0.81 (0.59)
(19) *n*-C_{32}	0.0001	0.0071	0.0070	0.0015	0.0004	0.0088	0.0084	0.0023	0.65 (*0.48*)
(20) *n*-C_{34}	0.0002	0.0084	0.0082	0.0015	0.0001	0.0062	0.0061	0.0018	0.83 (*0.61*)
(21) *i*-C_{28}	0.0000	0.0004	0.0004	0.0001	0.0000	0.0011	0.0010	0.0002	0.50 (*0.37*)
(22) *i*-C_{30}	0.0000	0.0008	0.0008	0.0002	0.0000	0.0010	0.0010	0.0003	0.67 (0.49)
(23) *i*-C_{34}	0.0001	0.0017	0.0016	0.0004	0.0001	0.0016	0.0016	0.0004	1.00 (*0.73*)
(24) *n*-C_{29}	0.0000	0.0012	0.0011	0.0002	0.0000	0.0007	0.0006	0.0002	1.00 (*0.73*)
(25) *n*-C_{31}	0.0001	0.0030	0.0030	0.0005	0.0001	0.0020	0.0020	0.0005	1.00 (*0.73*)
(26) *n*-C_{33}	0.0000	0.0015	0.0015	0.0003	0.0000	0.0011	0.0010	0.0003	1.00 (*0.73*)
(27) *br*-C_{29}	0.0000	0.0005	0.0005	0.0001	0.0000	0.0005	0.0005	0.0001	1.00 (*0.73*)
(28) *br*-C_{31}	0.0000	0.0011	0.0011	0.0002	0.0000	0.0005	0.0005	0.0002	1.00 (0.73)

TABLE 2 (Continued)

Compound	Males[a]				Females[a]				
	Min.	Max.	Range	Mean	Min.	Max.	Range	Mean	M/F[b]
(29) *br*-C_{33}	0.0000	0.0010	0.0010	0.0001	0.0000	0.0005	0.0005	0.0001	1.00 (*0.73*)
(30) *a*-C_{29}	0.0000	0.0010	0.0010	0.0002	0.0000	0.0007	0.0007	0.0002	1.00 (*0.73*)
(31) *a*-C_{31}	0.0000	0.0025	0.0025	0.0004	0.0000	0.0012	0.0012	0.0004	1.00 (*0.73*)
(32) *a*-C_{33}	0.0000	0.0040	0.0039	0.0006	0.0001	0.0015	0.0015	0.0003	2.00 (*1.46*)
Esters (unsaturated)									
(33) *n*-$C_{30:1}$ (*n* – 16:1/14:0)	0.0002	0.0082	0.0079	0.0018	0.0001	0.0066	0.0065	0.0017	1.06 (*0.77*)
(34) *n*-$C_{32:1}$ (*n*-16:1/16:0)	0.0002	0.0119	0.0118	0.0023	0.0001	0.0077	0.0076	0.0023	1.00 (*0.73*)
(35) *n*-$C_{34:1}$ (*n*-16:1/18:0)	0.0004	0.0184	0.0180	0.0033	0.0002	0.0115	0.0113	0.0031	1.06 (*0.78*)
(36) *n*-$C_{36:1}$ (*n*-16:1/20:0)	0.0006	0.0227	0.0221	0.0035	0.0004	0.0148	0.0144	0.0032	1.09 (*0.80*)
(37) *i*-$C_{30:1}$ (*i*-16:1/14:0)	0.0000	0.0004	0.0004	0.0001	0.0000	0.0008	0.0008	0.0001	1.00 (*0.73*)
(38) *i*-$C_{32:1}$ (*i*-16:1/16:0)	0.0000	0.0020	0.0020	0.0004	0.0001	0.0033	0.0032	0.0006	0.67 (*0.49*)
(39) *i*-$C_{34:1}$ (*i*-16:1/18:0)	0.0000	0.0021	0.0021	0.0005	0.0001	0.0027	0.0026	0.0007	0.71 (*0.52*)
(40) *i*-$C_{36:1}$ (*i*-16:1/20:0)	0.0001	0.0054	0.0052	0.0009	0.0001	0.0035	0.0034	0.0008	1.13 (*0.82*)
(41) *n*-$C_{31:1}$ (*n*-16:1/15:0)	0.0000	0.0025	0.0025	0.0005	0.0000	0.0014	0.0014	0.0003	1.67 (*1.22*)
(42) *n*-$C_{33:1}$ (*n*-16:1/17:0)	0.0001	0.0074	0.0074	0.0013	0.0000	0.0035	0.0034	0.0009	1.44 (*1.06*)
Sterols and Terpenes									
(43) Squalene	0.4236	3.8863	3.4628	1.2205	0.2294	4.3848	4.1554	1.6826	0.73 (*0.53*)
(44) Cholesterol	0.0095	0.1459	0.1364	0.0441	0.0076	0.0807	0.0731	0.0374	1.18 (*0.86*)

[a]Abundance is the ratio of Summed SIC peak areas for compound/internal standard.

[b]Ratio of mean abundance values for M/F. Value in italic is (M/F)/1.37, the equivalent M/F ratio for abundances of compound/abundance of hexadecanoic acid (C16:0) in each sample.

Unsaturated esters consisted of *n*- $C_{30:1}$, $C_{31:1}$, $C_{32:1}$, $C_{33:1}$, $C_{34:1}$, and $C_{36:1}$ and *i*- $C_{30:1}$, $C_{32:1}$, $C_{34:1}$, and $C_{36:1}$ homologs. From the MS it was clear that the $C_{16:1}$ acid (sapienic acid) or *i*-$C_{16:1}$ acid were virtually the only acid moieties in each ester with alcohols in the range C_{14}-C_{18} and C_{20}, and that the branching was in the acid as found for the saturated esters. The sterol, cholesterol, and its biosynthetic precursor the terpene, squalene, were both present.

The overall distribution of compounds found within the fingerprint samples, by class and structure, was in agreement with that reported for sebaceous gland lipids [42–45]. Examples of chromatographic traces for samples from a female and two males are shown in Figure S1. The upper trace shows a typical chromatogram of a sample from a female. The lower traces show chromatograms from males exhibiting lower and higher levels of primary alcohols. None of the samples shown lie at the upper or lower extremes of analyte abundance found across all samples.

Multivariate analysis of variance showed there were no significant differences between male and female participants on a linear combination of dependent variables ($F(42, 1) = 84.085$, $p = 0.086$). However, univariate analyses of variance indicated that only two compounds (both alcohols) showed a statistical significance between the sexes, namely; octadecanol C_{18} ($F(1, 42) = 12{,}672$, $p = 0.001$), compound number 2 in Table 2 and Figure 1 and eicosanol C_{20} ($F(1, 42) = 7.249$, $p = 0.010$), compound number 3 in Table 2 and Figure 2. All three alcohols from Table 2 had a higher mean value for females than males.

The canonical correlation for compound number 2, octadecanol (C_{18}), was 0.481 and Wilks' lambda of 0.768 was found to be statistically significant ($p = 0.001$) with an Eigenvalue of 0.302. Compound number 2, octadecanol, therefore, indicates a prediction of male sex with 90.9% accuracy, while for females this is only 50%, Figure 3.

The canonical correlation for compound number 3, eicosanol (C_{20}), was 0.384 and Wilks' lambda of 0.853 was found to be statistically significant ($p = 0.010$) with an Eigenvalue of 0.173. Compound number 3, eicosanol, therefore, indicates a prediction of male sex with 95.5% accuracy while for females this is only 59.1%, Figure 4.

4 | DISCUSSION

The analysis showed that of the 44 compounds identified in latent fingermarks using GC–MS analysis using the method described above, only two compounds, both alcohols, had quantities that allowed differentiation between sexes with a statistical significance of less than 0.05. This result provides a valid avenue for further research into setting up a method that may be of use for establishing certain aspects of a donor profile from latent fingermarks. Profiling a donor using latent fingermarks could be applied in cases when fingerprints available at potential crime scenes are not conducive for identification purposes from their pattern. Certain fingerprints may be smudged or consist of only a partial print or overlap with other prints hence distorting the pattern and may therefore not be appropriate for identification from the ridge pattern. In such cases, the fingermark may be used to retrieve information such as the sex of donor where fingerprint visualization techniques and destructive nature of GC–MS analysis allow for its application [46].

Latent fingermarks degrade after deposition, however, it has not yet been possible to establish a rate of degradation [19]. For this

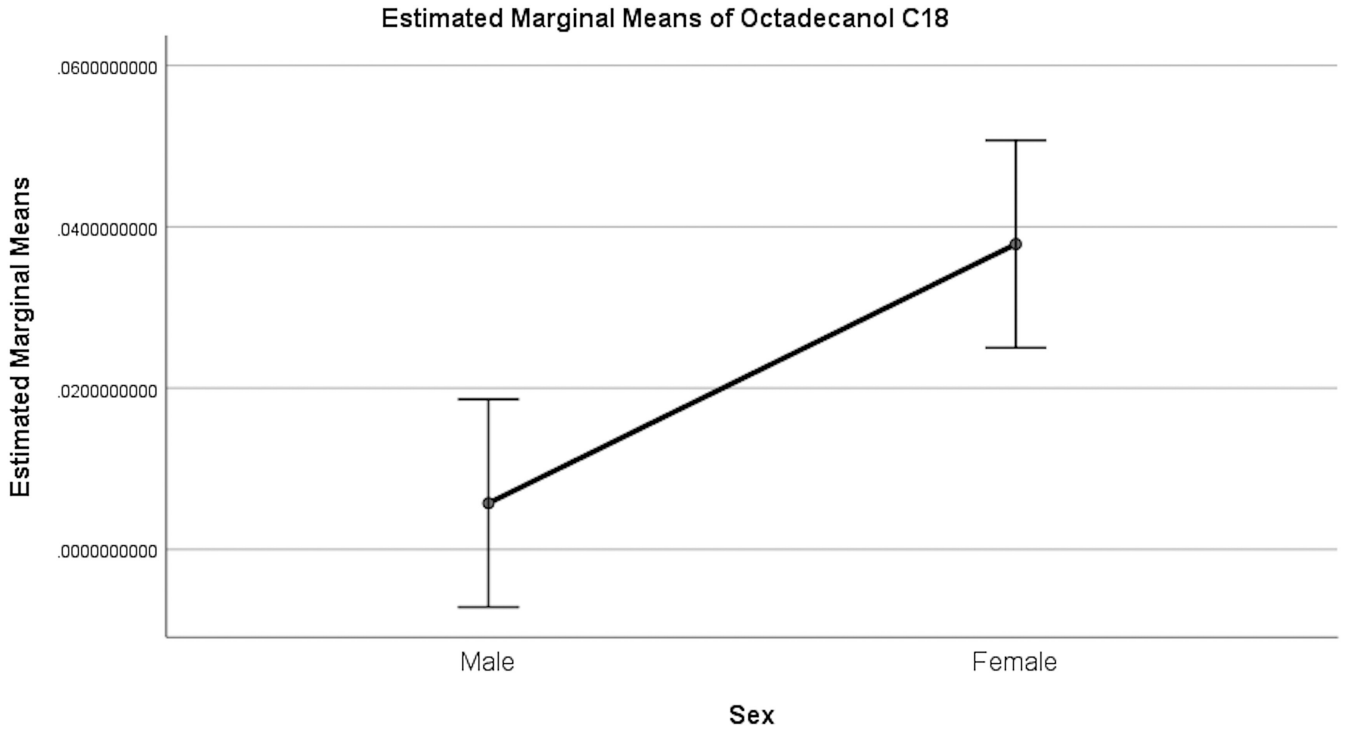

FIGURE 1 Estimated means with error bars (95% CI) for octadecanol C18 (compound 2 in Table 2).

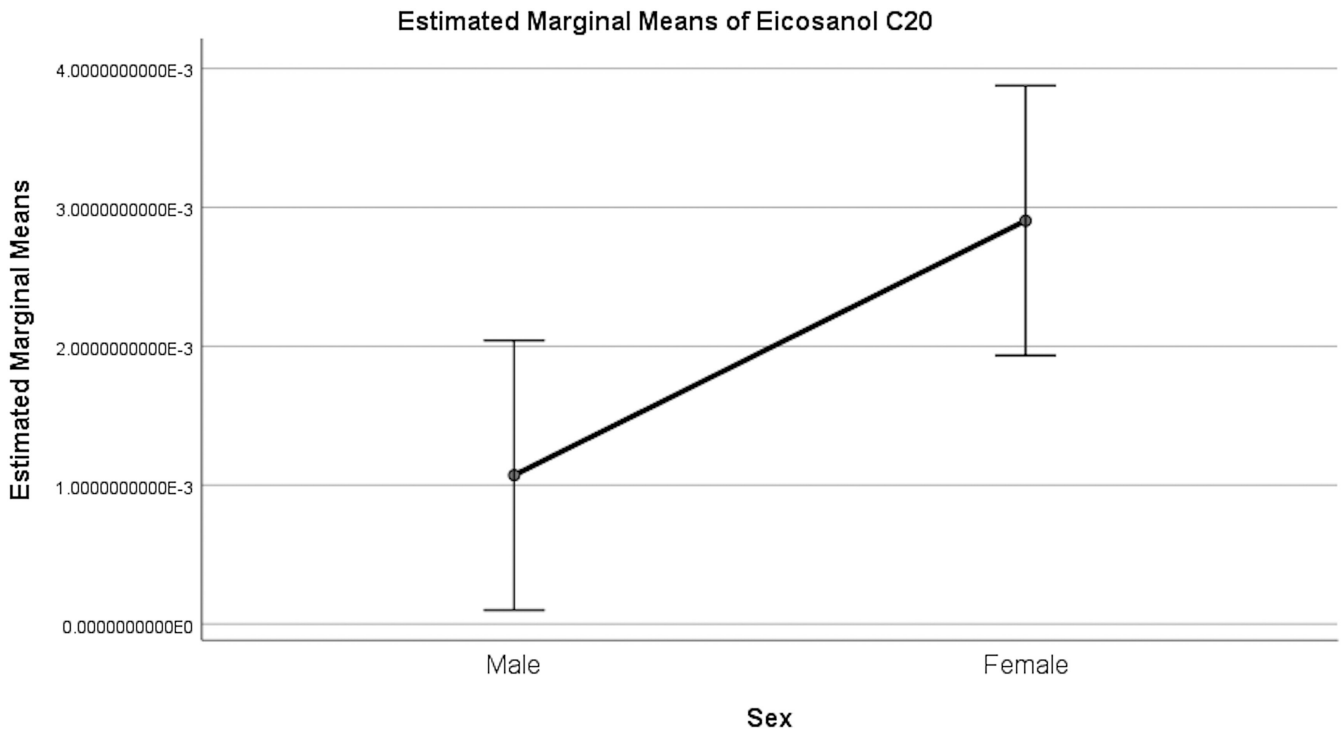

FIGURE 2 Estimated means with error bars (95% CI) for eicosanol C20 (compound 3 in Table 2).

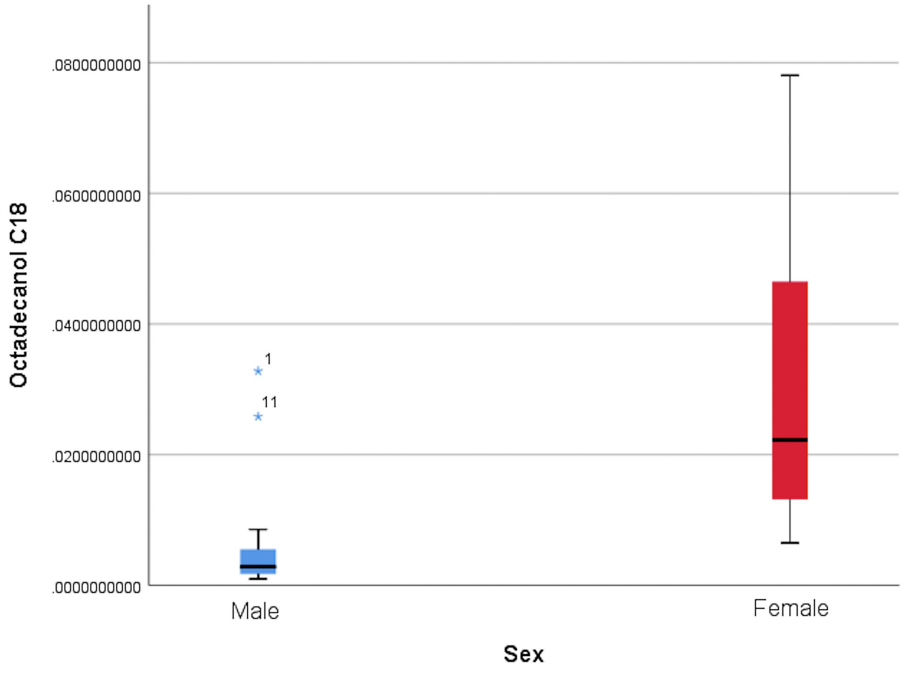

FIGURE 3 Box plot for male and female values of compound number 2, octadecanol (C18) (outliers and extreme outliers are included in analysis but not shown for values above 0.08).

research, it was not possible to collect all the samples at the same time and avoid a certain level of degradation. Samples from different donors were collected with some hours in between. However, to limit the effect of fingermark degradation the samples were analyzed the same day as collection. Further research is needed to establish a rate of fingermark degradation in order to make it more applicable to crime scene scenarios [19].

Fingerprints collected from a crime scene would most likely have higher levels of contaminants and hence could exhibit different chemical compositions compared to the results from this

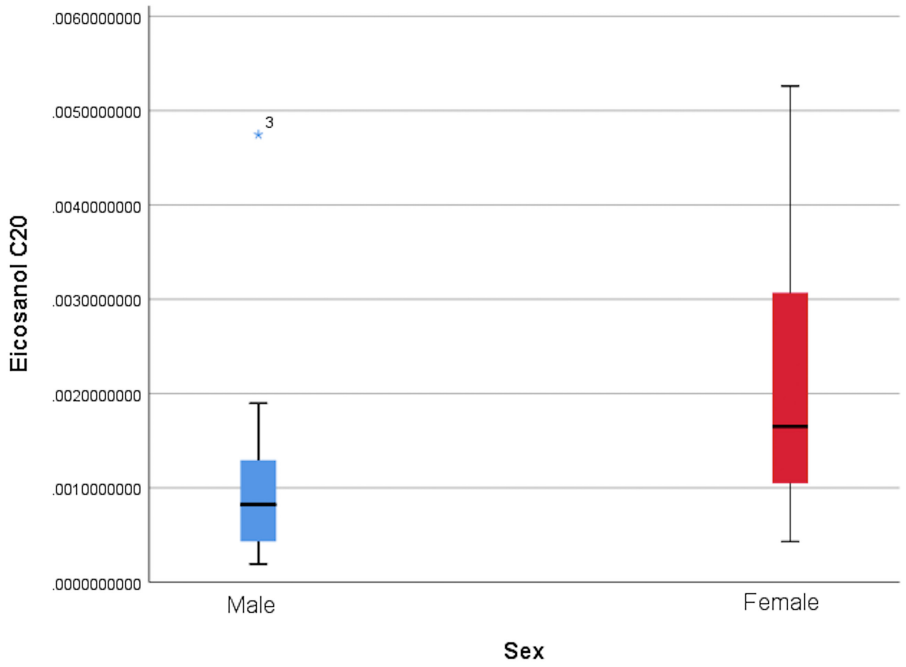

FIGURE 4 Box plot for male and female values of compound number 3, eicosanol (C20) (outliers and extreme outliers are included in analysis but not shown for values above 0.006).

research. Even newly washed hands will be contaminated by fatty acids from soap which are likely to contain some of the same fatty acids as found in this study [47]. However, it was anticipated that when the donors wiped the skin behind the ears to enhance analyte levels, this would to an extent mimic the residue somewhat to be found on 'dirty' fingerprints. It was initially hoped that wiping behind the ears, rather than the face as other studies have done, would eliminate contaminants such as make-up, moisturizer, and shaving foam. However, some obvious contaminants, such as Parsol MCX (2-Ethylhexyl-4-methoxycinnamate, two forms likely *cis* and *trans* positional or stereo isomers), Parsol 1789 (4-*t*-butyl-4′-methoxydibenzoylmethane), and alkyl (C_{12}–C_{15}) benzoates were found in this study. Parsol MCX is an ingredient in skin care products, predominantly as a UVB filter [48] and Parsol 1789 is a UVA absorber. Alkyl (C_{12}–C_{15}) benzoates are emollients and are found in many personal care products. The antioxidant Vitamin E (principally α-tocopherol) is a constituent of sebum secretions and it has been suggested that sebum may serve to deliver α-tocopherol to the skin surface where it functions as the main skin antioxidant [43]. However, Vitamin E acetate (α-tocopheryl acetate) is an ingredient in skin care products as an anti-aging product [49], consequently, we treated it as a potential contaminant. Some of the unknown compounds showed mass spectral characteristics similar to squalene but could not be characterized further on the basis of spectral matching with MS databases or other data. The controls showed non-detectable levels of analytes. There is also the possibility that skin care products may have contributed to the primary alcohols present in the samples since these compounds can be present in such products. However, there does not appear to be any correlation between the total abundances of the alcohols and the abundance of the other known contaminants, Parsol MCX and Alkyl benzoates in the samples. Plots of the ratios of alcohols to alkyl benzoates and to Parsol MCX for individual sample means are shown in Figure S2 and the variation in the ratios between samples appears random. Although the distribution of primary alcohols may not mirror those of other contaminants if present in skin care products, the randomness of the inter-sample variation in their ratios suggests that variation in alcohol abundance between samples from males and females is not driven primarily by exogenous sources There is also considerable variation in the ratio of Alkyl benzoates to Parsol MCX in the samples although there is evidence of clustering of some sample groups, particularly for males, see Figure S3. This may be indicative of the use of skin care products having different formulations.

This study only included adults with an age above 20 years, as this is when puberty is considered to have ceased [50, 51] and thus there should be less fluctuation in hormone levels which have proven to be evident from fingermark residue [28, 52]. Individuals receiving hormone or steroid treatment for illness, ailments, or for the purpose of gender affirmation [53–55] were also excluded as it was uncertain how this might influence fingermark residue. Androgens are known to increase secretion of sebum, whereas estrogens can have the opposite effect. Uptake of circulating lipids is a significant step in the production of sebum lipids. However, some constituents such as wax esters which are unique to sebum and the *a*- and *i*-branched chain fatty acids, uncommon in other organs, are produced locally in the sebaceous gland. Also, the Δ6 desaturase involved in production of sapienic and sebaleic acid is uniquely active in the sebaceous gland. Cholesterol and squalene are constituents of sebaceous secretions although squalene formed in

sebaceous cells is not converted to cholesterol as happens readily in other tissues [43]. The majority of the compounds formed in the sebaceous gland are the products of acyl chain synthesis and chain modification, fatty acids being direct products and alcohols being formed by subsequent reduction steps. It remains unclear how steroid or other treatments might affect these processes and the balance between different products. Chemical inhibitors and mutations can affect these processes and some of the steps may be influenced by transcription factors. This is usually seen as a change in chain length distribution within a compound class or a block in the formation of specific classes or a change in the distribution of different compound classes.

It was not possible to exclude females taking oral contraception, rather it was presumed that the majority were. It was also noted if the donors used asthma inhalers as some of these contain steroids. This was only the case for three individuals. However, none of the three individuals utilized "prevent inhalers" which are used every day to prevent an asthma attack. Prevent inhalers use steroids as the main drug. The three donors all used "reliever inhalers" to relieve symptoms such as breathlessness. The main drugs for reliever inhalers are bronchodilators [56]. Therefore, for these three individuals, no difference was expected to be apparent, and none was sought.

Certain outliers in the data were noticed with particularly one female for compound 1 having a much higher value (the maximum of 0.7943, Table 2) than the female with the lowest value (the minimum of 0.0019, Table 2). However, as all three replicates for this female showed equally high values (the same were true for other outliers) it was considered that outliers were a natural part of human variation for fingermark residue components. This was confirmed by the studies of [23, 57] who both found reproducible outliers which could be explained by variability in skin surface secretion due to differences in age, sex, race, disease, and activity levels. It was therefore decided not to manipulate the data by deleting certain outliers.

While some studies on the chemical analysis of latent fingermarks were not able to distinguish between males and females [34, 35, 37] others did find equally encouraging results [6, 8, 11, 36, 38]. The study by [6] analyzed fingermarks residue from 18 donors, nine males and nine females, using GC–MS. They found that mean levels of amino acids where higher in females but only statistically significant for asparagine, while the mean level of most fatty acids where higher in males although not at a level that was statistically significant. In our study, we found similar results for fatty acids, with mean male values higher than females (Table 2), and equally found this to be statistically non-significant.

The use of liquid chromatography–mass spectrometry (LC–MS) [8] allowed for analysis of the much less abundant amino acid components from fingermark residue which were not detected in our study. They found that the amino acid composition between female and male donors was comparable, although for serine there was a distinct difference with much higher levels in males. The study by [11] using GS-MC analysis only mentions that the quantity of urea is sex-dependent but provides no values or number of donors in their study as this was a report on method development for fingerprint extraction using GC–MS analysis and not a study of the chemical components themselves.

Michalski et al. [38] likewise used GC–MS for the analysis of the fatty acids of fingermark residue of 22 male and 15 female donors, all within an age range of 18–21 years. They found higher levels in males of (*Z*)-6-octadecenoic acid while higher levels of octadecanoic acid were found for females. They also found higher levels of fatty acids from females with chain lengths intermediate between C_{21} and C_{22}, C_{18} and C_{19}, and higher levels in males of acids with chain length intermediate between C_{16} and C_{17}. Since they used a GC column (Rxi-5Sil MS) of similar polarity with similar elution characteristics to that used in this investigation, it is likely that these acids were the branched *i*-C_{22}, *a*-C_{19}, and *a*-C_{17} homologs, respectively. However, the statistical significance between the sexes for all these observations was not reported. They analyzed the fatty acids as methyl esters after chemical derivatization. However, they did not quantify their data in the same way as used in our study, that is, based on a fixed amount of internal standard added to each sample. Instead, they quantified each compound based on the level of endogenous hexadecanoic acid ($C_{16:0}$), in each of the individual samples, effectively an internal-internal standard. When the same approach was used on the mean values for males and females in our dataset (Table 2, M/F ratio, values in italic), the overall result was the same in terms of inter-sex differences as found when based on a non-sample related internal standard. Although not statistically significant, mean levels of most fatty acids, but in particular *n*-$C_{18:1}$, *n*-$C_{15:0}$, *n*-$C_{17:0}$, and *a*-$C_{17:0}$ were greater for males, whereas those for *n*-$C_{18:2}$ and *i*-$C_{16:0}$ were greater for females. Additionally, mean abundances of several of the saturated even carbon number wax esters (C_{28}-C_{32}), *i*-branched unsaturated esters ($C_{32:1}$ – $C_{34:1}$) and squalene were also greater for females. There are therefore similarities between our findings, and those of [38], particularly in finding higher levels of (*Z*)-6-octadecenoic acid (*n*-$C_{18:1}$) and *a*-$C_{17:0}$ from males and higher levels of *i*-even C acids from females (*i*-C_{22}, *i*-C_{16}).

The potential for differentiation between sex in the distribution of *i*- and *a*- branched free fatty acids and *i*-branched wax esters, as suggested by our results and [38] is worthy of further investigation. Although rare in humans and apparently restricted to the sebaceous gland, it is likely that formation of the *br*- acids arises from parallel but separate elongation systems utilizing branched primer units (isobutyryl-CoA for the *i*-even C series, 3-methylbutyryl-CoA for the *i*-odd C series, 2-methylbutyryl-CoA for the *a*-odd C series), as is the case in plant cuticular waxes [58, 59]. Unbranched *n*-fatty acids arise from elongation of acetyl-CoA (even C acids) and propionyl CoA (odd C acids), possibly also from separate but parallel elongation systems. The C_2 elongating units are primarily malonyl-CoA derived from acetyl-CoA. A branched elongating unit, methylmalonyl-CoA, can be incorporated either to give *a*-odd C compounds or internally branched compounds. Acetyl-CoA used in sebaceous glands for fatty acid synthesis, particularly in wax ester formation, is formed primarily by β-oxidation of linoleic acid extracted from circulating lipids [43]. There is therefore the possibility for differential expression of products from

these pathways influenced by various genetic and environmental factors, which might affect the structural and chain length distributions between and within the *n*- and *br*- classes. This is similar to the ways in which cuticular wax formation in plants is strongly influenced by genetics and interaction with environmental factors such as exposure to pollutants and mutagens, irradiation levels, temperature, and humidity [58, 59].

Although our study was performed on a similar limited number of donors, our study is also in agreement with some of the other results by indicating the value of chemical analysis of fingermark residue for donor information such as sex.

5 | CONCLUSION

Within the 44 compounds identified in this study, two alcohols, octadecanol C_{18} and eicosanol C_{20}, were found to show a difference that was statistically significant between male and female donors. This result indicates that these two compounds could potentially be analyzed to provide information about the sex of the donor in cases when unknown latent fingermarks cannot be used for identification from ridge pattern comparison. There is also some evidence for the possibility of distinguishing sex from a latent fingermark based on the distribution of branched chain fatty acids, as free compounds or esterified in wax esters.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict for the publication of this manuscript and no funding was involved for this study.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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