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**PAPER**

**CRIMINALISTICS**

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# Laser Desorption/Ionization Time-of-Flight Mass Spectrometry of Triacylglycerols and Other Components in Fingermark Samples*

**ABSTRACT:** The chemical composition of fingermarks could potentially be important for determining investigative leads, placing individuals at the time of a crime, and has applications as biomarkers of disease. Fingermark samples containing triacylglycerols (TAGs) and other components were analyzed using laser desorption/ionization (LDI) time-of-flight mass spectrometry (TOF MS). Only LDI appeared to be useful for this application while conventional matrix-assisted LDI-TOF MS was not. Tandem MS was used to identify/confirm selected TAGs. A limited gender comparison, based on a simple t-distribution and peaks intensities, indicated that two TAGs showed gender specificity at the 95% confidence level and two others at 97.5% confidence. Because gender-related TAGs differences were most often close to the standard deviation of the measurements, the majority of the TAGs showed no gender specificity. Thus, LDI-TOF MS is not a reliable indicator of gender based on fingermark analysis. Cosmetic ingredients present in some samples were identified.

**KEYWORDS:** forensic science, latent fingermarks, triacylglycerols, lipids, laser desorption/ionization, time-of-flight mass spectrometry

Fingerprints found at crime scenes are valuable pieces of evidence in the investigation of criminal suspects. The evidentiary value of fingerprints lies in the fact that no two people, even identical twins, have the same fingerprint pattern. These individual characteristics often make fingerprints the "smoking gun" in criminal cases. To date, with millions of individual fingerprints classified, no two prints have ever been found to be alike (1). Fingerprint identification is made using ridge characteristics or minutiae of the print. Pattern recognition is an established method because of print variability from one person to another and the consistency of the pattern over an individual's lifetime. At the present time, the chemical composition of a fingermark has not been shown to be of great forensic value. At this point, it is important to make a distinction between the terms fingerprints and fingermarks. Fingerprints refer to the actual friction ridges on the skin and the transferred images of these patterns to another surface. Fingermarks, on the other hand, are the recovered traces of material transferred to other surfaces upon contact. Knowledge of the fingermark composition is potentially advantageous for possible investigative leads (gender, use of cosmetics, or presence of explosives or illicit drugs) and as a potential dating method to place an individual identified through a fingerprint at the time of the crime. Chemical analysis and the development of statistically significant databases may also lead to the discovery of disease indicators. The small amount of chemically significant components in a single fingermark, and the analytical challenges thus presented, may be the reason for the lack of interest in this area. Advances in analytical instrumentation and methods development, however, suggest that a reevaluation of the chemical composition of fingermarks as a forensic tool may be productive.

The chemical composition of fingermarks is mainly related to the secretions of the eccrine and sebaceous glands. Eccrine glands, located on the palm and fingertips, are the origin of most of the deposited material left in a fingermark (2). These glands produce inorganic (chlorides, metal ions, phosphates, sulfates) and organic constituents (amino acids, lactic acids, sugars; [3,4]). In addition to these glands, secretions from the sebaceous gland may also be transferred to the fingertips through occasional contact with sebaceous-rich regions, such as the face and hair (4,5). Sebaceous glands secrete a clear lipid mixture, known as sebum, onto the skin surface. Sebum collected from the skin surface contains triacylglycerols (TAGs; 25%), free fatty acids (25%), wax esters (22%), di- and monoglycerides (10%), squalene (10%), sterol esters (2.5%), and sterols (1.5%; [6]).

The fatty acid content of skin surface lipids has been examined using gas chromatography/mass spectrometry (GC-MS; [7–10]). In these investigations, lengthy sample preparation, de-esterification of the TAGs and derivatization of the resulting fatty acids (into volatile components), is necessary to produce a sample suitable for analysis. Although the specific fatty acids that constitute surface lipids are known, it is not known how those fatty acids combine to yield a distinct TAG. The study of TAGs is therefore significant for understanding these relationships. Additionally, the identity of the TAGs may be of importance for their use as distinguishing markers. It has been suggested that it is through the complexity and number of fatty acids that are contained in surface lipids that individuals acquire a unique "chemical signature" (6). Differences in the pattern of fatty acids contained in the TAGs could therefore

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be indicative of gender, race/ethnicity, or some other distinguishing characteristic. While this topic has been the subject of much debate, one report suggests that the concentration of free fatty acids in skin surface lipids differs widely between individuals. Little variation is observed, however, between samples collected successively from the same individual over long periods of time (11).

The focus of this report is the use of time-of-flight mass spectrometry (TOF MS) to examine the chemical composition of surface lipids, namely the TAGs, of a fingermark. Using this data, differences in composition between samples from male and female fingermarks have been examined. In addition to gender analysis, the fatty acids composing the TAGs, along with the cosmetic profile of a print, have been investigated. To the authors' knowledge, this is the first study of the TAG structure in fingermarks and thus any indication of gender significance from the subsequent TAG analysis. This investigation was undertaken in light of a similar study performed at the Oak Ridge National Laboratory where the fatty acids, rather than TAGs, from fingermarks were examined for gender differences (7). No gender significance from fatty acids was observed in the Oak Ridge investigation.

## Experimental Methods

### *Sample Collection*

Fingermark samples were collected from individuals by having them undergo a typical "grooming procedure." In this procedure, the index finger was wiped once across the forehead, over the bridge of the nose from cheek to cheek, and across the chin area. This procedure was carried out to ensure that the finger was loaded with sebaceous secretions. This sample collection procedure was adapted from groups who have previously studied the composition of fingermarks (7,8,10).

A stainless steel MTP Multiprobe Adapter matrix-assisted laser desorption/ionization (MALDI; Bruker Daltonics, Bremen, Germany) target was used for sample analysis. This target has up to 12 removable 23-mm diameter disks with 10 wells on each disk. No solvent or matrix, beyond the basic stainless steel target, was used. After undergoing the "grooming procedure," the individual was instructed to press the index finger onto one of the disks on the MALDI target twice over the same area. This was performed to ensure privacy, as the fingerprint was now smeared, and to create homogeneity between samples. Volunteers were asked to press their index finger on the disk/target in such a manner that their fingermark covered at least three wells of the 10 on the disk. This sampling procedure was repeated with another volunteer using another three wells of the other side of the disk/target. Using this approach, 24 fingermarks could be collected on the 12 disks that can be attached to a single target plate for analysis. A peptide mixture standard was spotted on the each target plate using empty well between the two individual fingermarks samples for mass calibration. To further ensure privacy and method validity, each fingermark sample was blind coded with a number prior to analysis. All samples were run within 30 min of sample collection.

To group the samples, additional information was acquired from the volunteers upon sample collection. Volunteers were asked their age and whether they were wearing any cosmetics. Samples were collected for gender comparison from eight white men ranging in age from 20 to 27 years and from eight white women ranging in age from 20 to 31 years. On different dates, replicate samples were collected from a white male and female volunteer for reproducibility studies. Samples were also accessed for transferability from another source to the MALDI target. Other sources included using a glass slide and a cotton swab to determine whether the fingermark secretions could be adequately transferred.

Care was taken to ensure that the MALDI disks of the target were not contaminated with additional fingermarks during handling and analysis. Nitrile gloves were worn during sample collection, and the disks of the target were handled with tweezers. Before sample collection, the target disks were submersed in a mixture of deionized water and methanol and then a mixture of deionized water and acetone (*c.* 50:50 v/v mixtures). In between each cleaning mixture, the target disks were wiped with a paper wipe. Target disks were rinsed with water and then acetone and allowed to air dry. Using tweezers, the disks were replaced onto the MALDI target for sample collection. During collection, the MALDI target was transported in a small cardboard box. MS analysis of the cleaned target confirmed that the cleaning procedure adequately removed all interfering fingermark material from the target disks before their use.

### *Sample Analysis*

Mass spectra were obtained on a Bruker Ultraflex II (Bruker Daltonics) TOF mass spectrometer operated in the positive-ion reflectron mode. Spectra were acquired in the *m/z* range 400–1200 with 1500 laser shots for each spectrum. Three spectra were summed (over the entire fingermark region on the three adjacent sample wells) for a total of 4500 laser shots. MS/MS spectra were obtained by operating the instrument in LIFT mode® (Bruker Daltonics) (12).

### *Data Processing*

Forty-three observed TAGs were selected for analysis in the *m/z* range of 770–900. This region contained the TAGs of highest intensity. Using the eight most prominent fatty acids in skin surface lipids (C14:0, 15:0, 16:0, 16:1, 17:1, 18:0, 18:1, 18:2, where CN:DB represents the number of carbon atoms and number of double bonds in the fatty acid; [6,13]), expected masses for *m/z* values of TAGs from the selected *m/z* region were derived. Reported values (6,13) for fatty acids isolated from the skin surface are listed in abundance as follows: C14:0 (6.8–7.0%), C15:0 (5.0–13.0%), C16:0 (22.2–25.3%), C18:0 (2.3–5.1%), C16:1 (21.1–25.7%), C17:1 (2.9%), C18:1 (13.3–19.7%), and C18:2 (1.7%). These calculated *m/z* values were used for the initial identification of the various suspected TAGs contained in the fingermark spectra.

Interferences were present in some samples with additional peaks observed in the selected mass region of the fingermark spectra. Some of the calculated TAGs masses coincided with peaks present from derivatives of polyethylene glycol (PEG) and polypropylene glycol (PPG). PEGs/PPGs were present in some samples, particularly from female volunteers, presumably because of cosmetic and/or personal hygiene products. Possible TAGs, which coincided with peaks present from these polymers species, were not included in the data analysis. TAGs with low intensities, and thus poor signal-to-noise ratios (<4:1), were also not included in analysis.

Using the Bruker Flex Analysis program (version 2.4), the *m/z* value and intensity of each TAG (monoisotopic peak) was calculated. The *m/z* value of each peak was used in the assignment of the TAG and its fatty acid composition. The intensities were used in comparative studies between samples from men and women. Fingermark spectra between men and women were compared using two different data evaluation methods. These two data evaluation methods were also used in comparison of the spectra from the reproducibility studies.

In Method 1, the peak intensity of a given TAG was calculated as a percentage of the total intensities of all the selected TAGs. TAGs that had the same *m/z* value as PEG or PPG derivatives were removed from all calculations to ensure that peak intensities were not skewed. A total of 13 of 43 peaks in the mass spectra were ignored as a result.

In Method 2, the peak intensity of an individual TAG was calculated as a percentage of the ion at *m/z* 827.6. This peak was chosen as the reference peak for all samples because it was the most abundant peak in 77% of the samples. TAGs that coincided with PEGs or PPGs were not included in these calculations.

The intensities of peaks with the same *m/z* obtained with each method were pooled according to gender and the average intensity and the standard deviation of the intensity calculated. The average intensities and standard deviations were then used to determine whether or not there was a difference between fingermarks obtained from male and female volunteers through application of a *t*-test at various confidence levels.

## Results and Discussion

### *Methodology*

Application of fingermarks directly onto the stainless steel MALDI target produced intense peaks in the mass spectra using laser desorption/ionization (LDI) but not MALDI. The method is rapid because little sample preparation is involved; no solvent or matrix is applied to the target. Good mass spectra were also obtained when samples were mechanically transferred from other surfaces (discussed further in Sample Transferability section). Unlike food oils and other applications involving larger amounts of analyte, incorporation of a solvent and/or matrix into the sample preparation scheme for the fingermark samples produced spectra with no discernable peaks of interest in the TAG region. A previously reported study by Wolstenholme et al. (14), in which fingermarks were placed on aluminum sheets and sprayed with matrix, also showed no TAGs when analyzed by MALDI MS. Herein, we report an alternative rapid LDI-TOF MS method for studying the TAG constituents present in a fingermark sample.

### *TAG Assignments*

MS experiments performed on fingermarks produced unique and characteristic profiles in the resulting mass spectra. The spectrum of a fingermark taken from a white men is shown in Fig. 1*a* with an enlarged view of the characteristic distribution pattern of the TAGs in Fig. 1*b*. The frequent mass difference between the groups of 14 Da is expected for the $CH_2$ repeat unit that varies in number among the fatty acids. The breadth of each group is a result of various contributions that include the distribution of naturally occurring isotopes, the presence of one or more double bonds in some of the fatty acids, and the presence of either sodium or potassium as the charge carrying species in the ionized TAGs. In addition to the TAGs, other components are also present, some of which will be discussed in a later section.

As noted earlier, the general *m/z* values observed in the mass spectra of fingermarks indicate that TAGs are consistent with sodium or potassium adducts, in agreement with similar studies of TAGs from edible oil samples (15–17). Ions are seen as sodium or potassium adduct ions, rather than protonated molecules, because of the presence of these salt constituents in fingermarks (2) and as impurities on the target plate (15), and also because protonated molecules of TAGs are not stable (16). Because of sodium's greater abundance in the laboratory and in biological samples (compared to potassium), most TAGs were expected to be sodiated. In eccrine sweat alone, the concentration of sodium is reported to be approximately 100 mM compared to a concentration of approximately 5 mM for potassium (2).

Because isomeric TAGs have the same mass, the exact identity of a given TAG cannot be determined based solely on the *m/z* value. For example, a *m/z* value of 797.7 could be assigned to a sodiated TAG ion with the fatty acids (14:0, 14:0, 18:2) or (14:0, 16:1, 16:1). Tentative assignments of the TAGs can be made, however, based on the known natural abundance of the fatty acids contained in skin surface lipids. Thus, for isomeric TAGs, the most likely composition is the one corresponding to the composition comprised of abundant rather than rare fatty acids. Subsequent identification of some TAGs assignments were confirmed through MS/MS experiments by fragmentation.

### *MS/MS*

MS/MS experiments were performed to characterize TAGs in the fingermarks. The analysis of the male fingermark giving rise to the spectrum in Fig. 1 is used to illustrate our approach. In this experiment, the instrument was operated in the LIFT mode® (17). Prominent ions in Fig. 1 corresponding to likely TAGs were selected for MS/MS analysis. In each MS/MS spectrum, parent ions were selected with a nominal mass tolerance of ±4 Da. Thus, a selected ion with a *m/z* value of 853 includes potential TAG parent ions for *m/z* values of 849, 851, 853, 855, and 857 (*c*. 9 Da window). Ten LIFT experiments for different *m/z* values were performed to obtain fragmentation patterns for all 43 TAGs present in the fingermark samples. In addition to up to five possible TAG peaks, the $^{13}C$ isotopes would also be included in this mass window in each spectrum. However, as shown in the MS/MS spectrum (centered on *m/z* 853, Fig. 2), this merely results in $^{13}C$ isotopes for the major fragment ions and does not interfere with interpretation of the data.

Interpretation of the MS/MS spectra is simplified somewhat because the expected fragmentation for TAGs is well known. The MALDI spectra of TAGs invariably show salt (sodium or potassium) adduct ions rather than protonated molecules. These salt cationized TAGs fragment via loss of a fatty acid moiety to give a diacylglycerol (DAG)-like ion (18). This neutral fatty acid loss can include either a proton (RCOOH) or another salt-cation (RCOONa or RCOOK) as the counter ion to the anionic fatty acid moiety. Thus, two prominent DAG-like fragments are generally observed from each cationized TAG parent. Their mass difference will either be 22 (Na) or 38 (K) Da depending on the specific salt. This same fragmentation has also been observed in many kinds of edible oil samples (15,18). For oils that typically differ by multiples of C2 units (i.e., C18 and C16), the usual mass difference corresponds to a multiple of 28 Da or n*($C_2H_4$). On the other hand, the mass difference between loss of RCOOH and RCOONa for a given fatty acid is 22 Da. Thus, the mass differences in the MS/MS spectra typically allow identification of fragments corresponding to different fatty acids. The only complication is the presence of double bonds. The mass differences characteristic of fatty acids (and their losses) having a specific carbon number changes by 2 Da for each double bond change. If the difference in the number of double bonds in a given set of TAGs (having the same carbon number) exceeds three, the interpretation of the MS/MS spectra is much more difficult. This is because the mass window selected for MS/MS experiments includes these multiple TAGs that differ only by their number of double bonds.

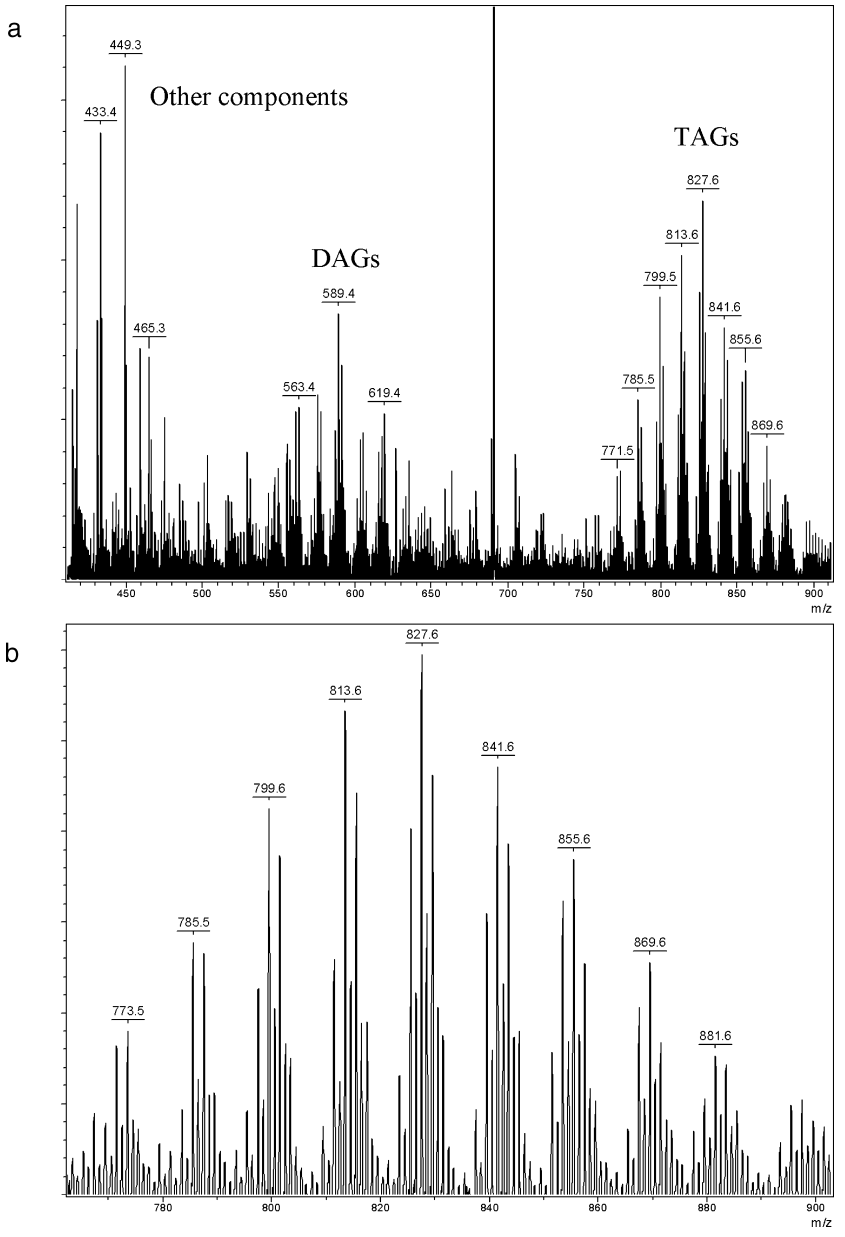


FIG. 1—*(a) LDI-TOF mass spectrum of a fingermark sample taken from a white man and (b) enlarged view of the triacylglycerol region of the fingermark. LDI, laser desorption/ionization; TOF, time-of-flight.*

The MS/MS spectrum of *m/z* 853 (which includes TAGs containing C50:0–50:3) is shown in Fig. 2. An enlarged view of the region showing prominent fragments is shown in Fig. 2*b*. The series of peaks observed in the spectrum at *m/z* 595–601 most likely corresponds to loss of a C16:*n* fatty acid residue ($R_1COOH$) from the C50 TAGs. The number of double bonds (*n*) in this C16 fragment may or may not be the same. To some extent, this can sorted out by inspection of the fragment ion intensity ratios. It can be assumed that two of the three TAG fatty acids in these precursors have the same composition (including double bonds), and when either of these two fatty acids is lost, the precursor and fragment ion intensity profiles should be similar. On the other hand, for the cleavage of the single fatty acid having a different number of double bonds (*n*), the resulting fragments would all have the same mass as the only moiety differentiating these TAGs is lost as a neutral fragment. Likewise, peaks at *m/z* 573–579 correspond to loss of the same C16 fatty acid sodium salt residues ($R_1COONa$). The additional peaks observed at *m/z* 569–573 and 547–551 most likely result from loss of a C18 fatty acid ($R_2COOH$) along with the corresponding sodium salt ($R_2COONa$). The ion at *m/z* 573 is difficult to identify because it could correspond to the loss of a C16 $Na^+$ salt or a C18 fatty acid. No peaks were observed as resulting from loss of a fatty acid potassium salt residue (RCOOK), indicating that all TAGs in the isolated region were sodiated.

Prospective parent/fragment ion relationships may be considered using a strategy based on the fragment ion masses and the masses of the fatty acid losses as described earlier. However, the known natural abundances of the constituent fatty acids, coupled with the specific fragment ion masses, can also be very helpful, as noted below. To begin interpretation of the fragmentation, it must be determined whether the fragment contains a proton, sodium ion, or potassium as the charge-bearing species. Usually, the masses of the selected parent ions and the neutral losses can rule out one or more alternatives when coupled with the masses of the expected TAG

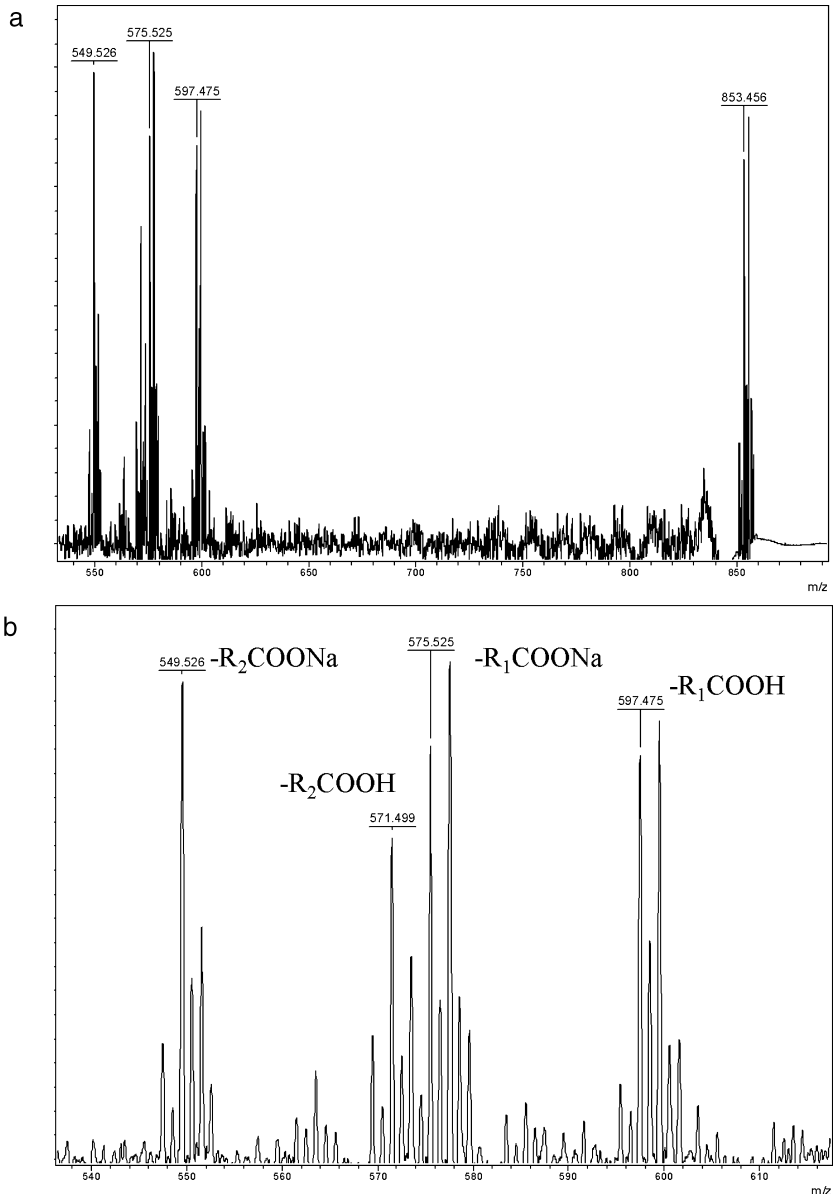


FIG. 2—*(a) MS/MS mass spectrum operated in LIFT mode® of* m/z *853 of a white male fingermark sample and (b) enlarged view of the fragmentation region.*

fatty acids. Then, the expected natural abundance of the fatty acids (and double bonds) is used as a guide to prioritize assignment of the fragment ion identities. Because DAG-like fragments only contain two fatty acid moieties, the number of reasonable fatty acid assignments for a given number of C and H atoms in any fragment is limited. Working backward from a DAG composition, potential parent ions can be selected based on reasonable fragments. The DAG-like fragment must arise from the mass-selected parent by loss of a fatty acid of reasonable abundance rather than a rare one, especially for significant peaks in the MS and MS/MS spectra. In other words, it is determined which specific parent/fragment relationships are reasonable. The relative abundances of a series of related TAGs differing only in the number of double bonds (the most likely difference in any 9 Da mass window) should change very little in the fragments when they lose a common fatty acid.

This approach is illustrated from the data in Fig. 2 and Table 1 for the TAGs around *m/z* 853. The parent mass corresponds to a TAG containing three fatty acids with the combined carbon number C50. Considering the fragments around *m/z* 597, the likely fatty acid composition must arise from a DAG-like ion with two fatty acids having a combined carbon number of C34. The specific fragment at *m/z* 597 is indicative of fatty acids having two double bonds (C34:2). A composition of C34:2 is "reasonable" because both C16:1 and C18:1 fatty acids are known to be very abundant in sebum. Assuming that this abundant ion at *m/z* 597 is the fragment from the similarly abundant *m/z* 853, it most likely arose from the loss of a 16:0 fatty acid from a precursor TAG ion corresponding to C50:2. The fatty acid C16:0 is also biologically reasonable and abundant. In this instance, the parent TAGs (C50:2) could reasonably be composed of three fatty acids with carbon numbers (16:0, 16:1, and 18:1). The combinations of (16:0, 16:0, 18:2) and (16:0, 17:1, 17:1) are also possible but less likely because of the expected lower abundance of the 18:2 and 17:1 fatty acids in sebum. For example, the expected fatty acid abundances of C16:0, C16:1, and C18:1 are all between 10–25%, whereas those of C17:1 and C18:2 are only about 2%. Thus, the most likely relationship is

TABLE 1—*Possible and eliminated triacylglycerols (TAGs) for the* m/z *851–857 series from MS/MS fragmentation (carbon numbers of the fatty acids indicate carbon number:double bond).*

| RCOOH Mass | $Na^+$ DAG Combinations | Possible TAGs | Eliminated TAGs |
|---|---|---|---|
| 595.5 (851) | 16:1, 18:2 | 16:0, 16:1, 18:2 | 14:0, 18:1, 18:2 |
| | | | 15:0, 17:1, 18:2 |
| | | | 16:1, 16:1, 18:1 |
| | | | 16:1, 17:1, 17:1 |
| 597.5 (853) | 16:0, 18:2 | 16:0, 16:0, 18:2 | 14:0, 18:0, 18:2 |
| | 16:1, 18:1 | 16:0, 16:1, 18:1 | 14:0, 18:1, 18:1 |
| | 17:1, 17:1 | 16:0, 17:1, 17:1 | 15:0, 17:1, 18:1 |
| | | | 18:0, 16:1, 16:1 |
| 599.5 (855) | 16:0, 18:1 | 16:0, 16:0, 18:1 | 14:0, 18:0, 18:1 |
| | 18:0, 16:1 | 16:0, 18:0, 16:1 | 15:0, 18:0, 17:1 |
| 601.5 (857) | 16:0, 18:0 | 16:0, 16:0, 18:0 | 14:0, 18:0, 18:0 |

DAG, diacylglycerol.

*m/z* 853 (C16:0, C16:1, C18:1) fragmenting by loss of C16:0 to give *m/z* 597 (C16:1, C18:1). Using this approach, if the number of double bonds in the fatty acid neutral loss is uncertain, or a conservative approach is taken to data interpretation, the carbon numbers alone can also provide some specificity. In this application, precursors containing only C16 and/or C18 fatty acids will be more likely than those containing one or more C17. A similar procedure was performed on the rest of the DAG fragments in the C50 series to obtain a list of assignments of the remaining TAGs associated with loss of a C16 in Fig. 2 (see Table 1).

Following the same process for the rest of the TAGs in the fingermark sample, the composition of 25 of the 43 TAGs was identified (Table 2). In some cases, a single TAG was identified for a given *m/z* value based on the fragmentation pattern. In other cases, two or three possible TAGs are listed because of an inability to determine assignment based on the fragmentation. However, based on the known fatty acid composition of skin oils, some of the assignments are much more likely than others. Of the TAGs identified, all contained sodium as the cation rather than potassium. In addition to the TAG assignments, each *m/z* value is designated by CN:DB.

The identities of some ions in the TAG region of the mass spectrum were not able to be confirmed through MS/MS experiments. These ions were very low in abundance giving either no fragmentation or poor signal-to-noise ratios in MS/MS experiments. In most of these cases, the *m/z* value of the TAG could correspond to a low abundance potassiated ion species given the observation of abundant sodium-containing species 16 Da lower in mass and the common fatty acids used for their assignments (marked with an †, Table 2). No biologically plausible sodiated TAG combination could be derived corresponding to these *m/z* values, but as noted earlier, in each case, a significant sodium-containing species was observed 16 Da lower in mass. The observation of small peaks containing potassium in association with more prominent sodium-containing species would not be unusual in a biological sample.

Alternatively, these peaks could be attributed to oxidation of unsaturated fatty acids rather than the difference in mass between Na and K (marked with an †, Table 2). While oxidation of TAGs eventually leads to final decomposition products, such as alcohols, aldehydes, and ketones (19,20), mass spectra of oxidized TAGs have shown peaks at higher *m/z*, differing by 16 Da, that correspond to the addition of multiple oxygen atoms (21–23). Oxidation of fatty acids in edible oils is influenced by different parameters including light, heat, and minor compounds, such as metals,

TABLE 2—*Mass values and assignments for the triacylglycerols (TAGs) observed in a male fingermark sample.*

| | m/z (Observed) | CN:DB | $Na^+$ TAGs |
|---|---|---|---|
| | 771.6 | 44:1 | 14:0, 14:0, 16:1 |
| | 773.6 | 44:0 | 14:0, 14:0, 16:0 |
| * | 775.6 | | |
| | 785.6 | 45:1 | 14:0, 15:0, 16:1 |
| | 787.6 | 45:0 | 14:0, 15:0, 16:0 |
| * | 789.6 | | |
| † | 797.6 | 46:2 | |
| | 799.6 | 46:1 | 14:0, 16:0, 16:1 |
| | 801.6 | 46:0 | 14:0, 16:0, 16:0 |
| | | | 16:0, 15:0, 15:0 |
| * | 803.6 | | |
| | 811.6 | | |
| | 813.6 | 47:1 | 14:0, 16:0, 17:1 |
| | | | 15:0, 16:0, 16:1 |
| | 815.6 | 47:0 | 15:0, 16:0, 16:0 |
| * | 817.6 | | |
| | 823.6 | | |
| † | 825.6 | 48:2 | 14:0, 16:0, 18:2 |
| | | | 16:0, 16:1, 16:1 |
| | 827.6 | 48:1 | 14:0, 16:0, 18:1 |
| | | | 15:0, 16:0, 17:1 |
| | 829.7 | 48:0 | 14:0, 16:0, 18:0 |
| * | 831.6 | | |
| † | 837.6 | 49:3 | |
| | 839.6 | 49:2 | 15:0, 16:0, 18:2 |
| | | | 16:0, 16:1, 17:1 |
| † | 841.7 | 49:1 | 15:0, 16:0, 18:1 |
| | | | 16:0, 16:0, 17:1 |
| | 843.6 | 49:0 | 15:0, 16:0, 18:0 |
| * | 845.6 | | |
| † | 851.6 | 50:3 | 16:0, 16:1, 18:2 |
| † | 853.7 | 50:2 | 16:0, 16:0, 18:2 |
| | | | 16:0, 16:1, 18:1 |
| | | | 16:0, 17:1, 17:1 |
| | 855.7 | 50:1 | 16:0, 16:0, 18:1 |
| | | | 16:0, 18:0, 16:1 |
| | 857.6 | 50:0 | 16:0, 16:0, 18:0 |
| * | 859.6 | 49:0 | |
| † | 865.7 | 51:3 | 16:0, 17:1, 18:2 |
| † | 867.7 | 51:2 | 16:0, 17:1, 18:1 |
| † | 869.7 | 51:1 | 16:0, 18:0, 17:1 |
| * | 871.6 | | |
| * | 873.6 | | |
| | 877.7 | 52:4 | 16:0, 18:2, 18:2 |
| † | 879.7 | 52:3 | 16:0, 18:1, 18:2 |
| † | 881.7 | 52:2 | 16:0, 18:0, 18:2 |
| | | | 16:0, 18:1, 18:1 |
| | 883.7 | 52:1 | 16:0, 18:0, 18:1 |
| * | 885.7 | | |
| * | 887.7 | | |
| * | 893.7 | | |
| *† | 895.7 | | |
| *† | 897.7 | | |

CN:DB, carbon number:double bond.

*TAGs that corresponded to oxidized $Na^+$ TAGs or $K^+$ TAGs.

†TAGs that coincided with polyethylene glycol or polypropylene glycol peaks in some samples.

pigments, phospholipids, free fatty acids, mono and DAGs, thermally oxidized compounds, and antioxidants (20). Although some of these factors could influence oxidation in sebaceous secretions, it is less likely under the experimental conditions used in this study. Because samples were run within 30 min of collection, the TAGs had very little time for oxidation to occur. A longer time period, along with exposure of the fingermark to light and heat, could have resulted in oxidized species. It is also unlikely that the TAGs have any time to undergo oxidation while being present on the skin surface. Throughout the day, sebum is depleted getting spread to other

areas of the body and to other materials through touch. It is constantly secreted onto the skin surface. It has been reported that sebum is secreted at a rate of 25 μg/sq cm/h on the forehead (21). However, TAGs and fatty acids are oxidized within the body and could be secreted as such.

### *DAGs and Other Components*

In addition to the TAGs found in the fingermark samples, other components were also analyzed. The cluster of peaks in the *m/z* region of 540–650 (see Fig. 1*a*) is consistent with sodium adducts of DAGs. It is important to note that the DAGs described here are not the same as the DAG-like fragments observed in the MS/MS spectra. In this case, the DAGs are "true" DAGs in that they are comprised of a glycerol backbone with two fatty acid chains and an OH terminus. The DAG-like fragments do not have an OH terminus as they result from the simple cleavage of the third fatty acid and accompanying rearrangements of the glycerol backbone (18).

DAGs present in fingermark samples result from action on the TAGs as they leave the sebaceous gland. TAGs are broken down into DAGs, then monoglycerides, and finally free glycerol by lipolytic activity as they pass through the sebaceous duct, and by bacterial lipases present on the skin surface (24). As seen in Fig. 1*a*, the DAGs region of the mass spectrum has a similar distribution pattern as the TAGs region but a smaller overall abundance. Because DAGs arise by degradation of TAGs, the DAGs in a sample can be used to tentatively identify TAGs using the same logic employed in the MS/MS experiments. A tentative assignment of the TAG at *m/z* 827.6 can be made based on the probability of losing a C16:0 fatty acid (a difference of 256 Da) to give the resulting DAG at *m/z* 589.4. A DAG-like fragment, a peak 18 Da lower at *m/z* 571.4, is not observed for this TAG.

Prominent peaks at *m/z* values of 433.4, 449.3, and 465.3 were also observed in the mass spectrum. These *m/z* values were searched using the Lipids Maps Structure Database (LMSD) [25]. Possible assignments of these peaks at *m/z* 433.4 and 449.3 are sodiated and potassiated adduct ions of squalene. Squalene is a hydrocarbon precursor to cholesterol and has been found in skin surface lipids (6,26). The peak at *m/z* 433.4, along with the peak at *m/z* 449.3, could also be observed because of sodiated compounds present in the biosynthetic pathway of cholesterol (27). An alternative assignment for the peak at *m/z* 449.3 is a sodiated ion of squalene monohydroperoxide that has previously been identified in skin surface lipids (28,29).

### *Cosmetic Components*

PEGs are polymers of ethylene oxide with the general formula $HO–(CH_2CH_2O)_n–H$, where "*n*" represents the number of oxyethylene groups comprising the structure (30). In cosmetic and personal hygiene products, derivatives of PEG can be used. They are typically fatty acid esters (e.g., laureates, dilaurates, stearates, distearates) and ethers (e.g., laureths, ceteths, steareths, glyceryl cocoates) depending on the product and its desired effects (30,31).

These derivatives of PEG compounds were found in some of the fingermark samples as sodiated or potassiated ions and are listed in Table 3. The listing shows possible identifications based on the *m/z* values, although the exact identity of compounds cannot be determined based solely on these values. Some fingermark samples were found to contain more than one PEG derivative with some samples containing up to three different PEG derivatives. In addition, a PPG derivative was also present in one male sample. A possible identification of this compound would be $K^+$ $[PPG]_n$ myristal ether based on the *m/z* and spacing between peaks. PEG and PPG have different monomer units and can thus be easily differentiated. PEG distributions are separated by 44 Da and PPG by 58 Da. This analysis procedure suggests the determination of which PEG or PPG derivatives present in a test subject can easily be determined by LDI-TOF MS.

TABLE 3—*Possible derivatives of polyethylene glycol (PEG) observed in fingermark samples.*

| Sample 1 | PEG Derivative |
|---|---|
| FM 1, 2, 3, 4, 6 | $[Na]^+$ Steareth |
| FM 1, 2, 3, 4, 5, 6 | $[Na]^+$ Ceteth |
| | $[Na]^+$ Distearate |
| | $[K]^+$ Steareth |
| FM 1, 5, 6, 8 | $[K]^+$ Distearate |
| M 1, 2 | $[K]^+$ Ceteth |
| FM 7 | $[Na]^+$ Ceteth |
| | $[K]^+$ Stearate |
| FM 7, 8 | $[Na]^+$ Stearate |
| M 1 | $[K]^+$ Oleth |
| | $[K]^+$ Glyceryl Oleate |
| M 1 | $[Na]^+$ Oleth |
| | $[Na]^+$ Glyceryl Oleate |
| M 3 | $[K]^+$ Laureth |
| | $[K]^+$ Glyceryl Laurate |
| M 2, 3 | $[Na]^+$ Laureth |
| | $[Na]^+$ Glyceryl Laurate |

FM, female sample; M, male sample.

### *Reproducibility*

The reproducibility of a fingermark was examined by collecting samples over time and comparing the absolute and relative intensities of the TAGs. Seven samples were collected from a man over a 7.5-month period on days 1, 10, 70, 71, 216, 225, and 231. Four samples were collected from a woman over a 6-month period on days 1, 38, 158, and 177. On each collection day, only one fingermark sample was obtained from the volunteers. Relative standard deviation (RSD) percentages were calculated for the TAGs using the same methods as used for the previous samples. Using Method 1, the average %RSD values were 21% for the sample from the male volunteer and 12% for the sample from the female volunteer. Average %RSD values for Method 2 were higher with 34% for the male volunteer sample and 15% for the female volunteer sample. On average, the reproducibility of the samples from female volunteers was lower in both methods.

The reproducibility of placing a male fingermark onto the MALDI target was also measured. A fingermark was first collected from a male volunteer. Then after repeating the "grooming procedure," another fingermark from the same man was collected onto the same target. This process was repeated to give four samples from the same man on the target. The average %RSD values were 10% for Method 1 and 16% for Method 2. The reproducibility of laying down the print indicates that there is some variation with subsequent collection. This variation may be attributed to the fingertip not being adequately loaded with sebaceous secretions each time the sample was collected. Although there is a larger variation in the values for the fingermark collected over time, some of these differences may be attributed to collection and laying down the print onto the target.

### *Sample Transferability*

To access the adaptability of the method for use in a setting other than having the fingermark pressed directly onto the MALDI target,

additional tests were performed. Prior research (data not shown) showed that no detectable TAG signal could be obtained through a solvent-based transfer of a fingermark placed on a glass slide. Subsequent evaporation of the solvent from the dissolved fingermark sample to a small volume (*c*. 10 μL) and spotting with 2,5-Dihydroxybenzoic acid (DHB) matrix onto the MALDI target did not produce a signal. A spectrum could be obtained, however, by rubbing a cotton swab moistened with distilled water over the sample placed on a glass slide and applying the sample onto the MALDI target (for direct analysis with no matrix). This method was compared to the conventional method of having the sample placed directly onto the target. A volunteer was instructed to follow the ''grooming procedure'' and touch the MALDI target. The volunteer was then asked to again follow the ''grooming procedure,'' re-load the finger with sebaceous secretions, and touch a glass slide. A moistened cotton swab was rubbed across the area and then onto the MALDI target. The two samples were compared as mentioned in the Data Processing section using Methods 1 and 2. An average %RSD value of 9.0% was calculated between the sample applied directly to the target and the transferred sample for Methods 1 and 6.7% for Method 2. This method was repeated for another fingermark sample, and the %RSD was calculated as 5.6% for Method 1 and 10.2% for Method 2. This approach would allow a fingermark to be sampled, which was previously deposited onto a surface.

### *Statistical Analysis—Gender Differences*

The comparison of TAGs between samples from male and female volunteers was performed using a standard t-distribution (32) for each of the two methods listed in the Data Processing section. Two methods for calculating peak intensities were used to determine whether a significant difference would result depending on how the peak areas were calculated. This test was used as a basis for examining the difference between two population means for small and independent samples. For significance testing, degrees of freedom were taken as 2n-2, where *n* is the number of volunteers in each group. Calculated values of t were compared to a t-distribution table using the corresponding degrees of freedom. Conclusions of significance were made based on confidence levels of 90% or greater (32). Table 4 shows the *m/z* values and the corresponding intensities for both male and female volunteer samples that were found to be significant at the 95% or greater significance level. For Method 1, one TAG was found to be significant at 97.5% significance. In Method 2, three significant TAGs were found with the most significant TAG having a *m/z* value of 771.6 and 97.5% significance. Nine additional TAGs were found to be significant at 90%. Among the TAGs showing some gender differences, the two at *m/z* 803.6 and 823.6 could not be identified. The TAGs at *m/z* 771.6 and 853.7 are identified in Table 2. Average values for women as a group were higher for the four TAGs listed.

TABLE 4—*Significant triacylglycerols for two methods of comparison between white male and female fingermark samples.*

| m/z | Males* | Females* | % Significance |
|---|---|---|---|
| Method 1 | | | |
| 803.6 | 2.11 ± 0.23 | 2.53 ± 0.46 | 97.5 |
| Method 2 | | | |
| 771.6 | 34.13 ± 3.45 | 38.59 ± 3.63 | 97.5 |
| 823.6 | 27.25 ± 5.06 | 32.30 ± 5.78 | 95 |
| 853.7 | 64.43 ± 9.30 | 73.91 ± 5.05 | 95 |

*Calculated intensities and standard deviations as explained in the Data Processing section.

Because gender differences were close to the standard deviation of the measurements, and the number of test subjects was limited, we conclude that application of this approach in a real world setting is not reliable. Any given sample would, therefore, not be able to be grouped into a gender category based on our current data. The same conclusion was reached in the Oak Ridge study where gender significance was not observed for fatty acids (7).

## Conclusions

The chemical composition of a fingerprint reflects an intricate mixture composed of many different compounds and even different classes of compounds. Among the classes of compounds contained in a fingermark are TAGs. However, these TAGs have not been previously characterized directly; rather, their presence has most often been inferred from the detection of their constituent fatty acids. LDI-TOF MS of the TAGs from fingermarks gave characteristic spectra containing numerous strong peaks whose *m/z* values could be fit to expected TAGs. Of significant note is the observation that these TAGs could only be detected by LDI. Attempts to obtain MALDI spectra resulted in no signals in the TAGs region of the spectrum. Additional MS/MS experiments were performed for confirmation of the TAGs structures. To the authors' knowledge, this is the first time that the TAGs contained in a fingermark have been identified. Gender differences in the TAGs composition were assessed, and some TAGs were found to be significant at the 95% and 97.5% confidence levels. Depending on the approach used for data treatment, either one TAG at *m/z* 803.6 was found to be significant at 97.5% or TAGs at *m/z* 823.6 (C48:3), 853.7 (C50:2), and *m/z* 771.6 (C44:1) were found to be significant at 95% confidence. Because differences were close to the standard deviation of the measurements, and because there is no biological basis for predicting it, the analysis of gender from the measurement of specific TAGs in fingermarks by LDI-TOF MS is not reliable. Because this study was not designed to address gender specificity based on multiple TAGs, additional work with a larger population using multivariate analysis is warranted. Although TAGs were the focus of this investigation, additional components were also present in fingermark samples. Cosmetic components were identified as PPG and PEG derivatives.

Currently, the authors are examining fingermark samples exposed to different experimental conditions over time. Preliminary data have been collected for degradation effects on TAGs to determine whether dating techniques could be developed. This approach has the potential to place an individual at the time of a crime based on the collected fingermark. The authors are also considering additional studies using a larger sample population, blind coded samples, and multivariate analysis to probe potential correlations between TAGs and specific health-related endpoints. Such a study could also be used to revisit gender specificity using a larger data set and a statistical design tailored to the magnitude of differences observed in this first study of fingermark TAGs.

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