An untargeted lipidomic approach for qualitative determination of latent fingermark glycerides using UPLC-IMS-QToF-MS^E

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Abstract

More detailed fundamental information is required about latent fingermark composition in order to better understand fingermark properties and their impact on detection efficiency, and the physical and chemical changes that occur with time following deposition. The composition of the glyceride fraction of latent fingermark lipids in particular is relatively under-investigated due in part to their high structural variability and the limitations of the analytical methods most frequently utilised to investigate fingermark composition. Here, we present an ultra performance liquid chromatography-ion mobility spectroscopy-quadrupole time-of-flight mass spectrometry (UPLC-IMS-QToF-MS^E) method to characterise glycerides in charged latent fingermarks using data-independent acquisition. Di- and triglycerides were identified in fingermark samples from a population of 10 donors, through a combination of *in silico* fragmentation and monitoring for fatty acid neutral losses. 23 diglycerides and 85 families of triglycerides were identified, with significant diversity in chain length and unsaturation. 21 of the most abundant triglyceride families were found to be common to most or all donors, presenting potential targets for further studies to monitor chemical and physical changes in latent fingermarks over time. Differences in relative peak intensities may be indicative of inter- and intra-donor variability. While this study represents a promising step to obtaining more in-depth information about fingermark composition, it also highlights the complex nature of these traces.

Introduction

In recent decades, analysis of the chemical composition of latent fingermarks has seen increasing focus in forensic research, in an effort to better understand and overcome the issues facing fingermark detection.[1-6] It is recognised that there is a need to identify not only the processes by which current detection techniques work, but also the factors that may cause them to fail, such as time since deposition (fingermark age) or lack of sensitivity.[7-9] Other investigations have been conducted into the variability of fingermark residue to differentiate between individuals based on characteristics such as sex or age,[6, 10-13] or to estimate the age of a latent fingermark based on changes in its properties since deposition.[2, 14, 15]

The lipid fraction of fingermark residue is of particular interest, as these non-volatile, hydrophobic compounds enable fingermark detection on wetted substrates. This fraction has been shown to exhibit significant physical and chemical changes over time following deposition, as well as inter-donor variation.[1, 4, 9, 16, 17] The lipid classes identified in fingermark residue include squalene, cholesterol, wax esters, glycerides and free fatty acids.[4, 9, 18] These skin surface lipids are largely derived from the secretions of the sebaceous glands, which are found in greatest density on the face and scalp, with other compounds contributed by the epidermis.[19] Sebaceous lipids become incorporated into fingermark residue through incidental touching of the face and hair, and so the relative abundance of the lipid fraction can vary considerably. Significant differences may also be observed in latent

fingermarks deposited by adults and children, as the sebaceous glands do not become active until shortly before the onset of puberty.[13, 18]

The glycerides are one of the largest and most structurally diverse fingermark lipid classes, comprising approximately 30 % of total skin surface lipids.[20, 21] Mono- and diglycerides, as well as free fatty acids, are the products of bacterial lipolysis of sebaceous triglycerides (TGs) on the skin surface.[22, 23] The relative amounts of free fatty acids and TGs are therefore inversely proportional, and show greater inter-donor variation than other skin surface lipid components.[24-26] The total profile of the constituent fatty acids exhibits similar high variability.[19, 27, 28] In the weeks following fingermark deposition, TGs may be prone to degradation to free fatty acids,[4] ozonides,[29] and eventually shorter chain organic compounds [30, 31], depending on environmental conditions. Such processes contribute to the changes in chemical and physical properties of the latent residue which can affect detectability, and may provide a basis for the estimation of the age of a latent fingermark.[17, 29]

The exact glyceride species present in human sebum or latent fingermarks remain relatively under-investigated in comparison to the smaller, more volatile sebaceous compounds which are amenable to gas chromatography (GC) separation.[1, 4, 18, 32-34] Despite this, TGs in particular are considered an important class in fingermark residue, as their presence provides a target for lipid-sensitive detection methods such as lipophilic dyes,[35-37] and may assist in the successful application of metal deposition techniques such as physical developer and single metal deposition.[38, 39] Saponification is traditionally utilised in the preparation of glyceride samples,[40-44] so while much is known about the variations in sebaceous fatty acid chain length, unsaturation and branching,[19, 20] their arrangement on the glycerol backbone is less clear.[10, 45]

Intact latent fingermark glycerides were first described in 2011 by Emerson *et al*., who utilised laser desorption/ionisation tandem mass spectrometry (LDI-MS/MS) to tentatively identify 35 TG species from 43 CN:DB families (where CN denotes the total number of carbon atoms in the fatty acyl groups and DB the total number of double bonds).[10] The presence of numerous diglyceride (DG) species was also reported, though no structural data were presented. Numerous studies into fingermark composition have reported the presence of di- and triglycerides in samples,[46-48] but often limited data has been provided regarding their structures; usually only the molecular ion *m/z* or a generic CN:DB label is provided. Lauzon *et al*. reported a number of TG and DG structures in fingermarks using matrix-assisted laser desorption/ionisation mass spectrometry (MALDI-MS), but only 7 TGs were subjected to MS/MS.[32] Most recently, Pleik *et al*. examined the degradation process of unsaturated TGs by ozonolysis, focusing on a single TG.[29]

As described above, the fatty acid profile of sebaceous triglycerides has been researched extensively in the field of dermatology, but limited data is available in the literature regarding intact glycerides, due to their complexity.[49, 50] Michael-Jubeli *et al*. utilised high-temperature GC to separate skin surface lipids following trimethylsilylation, which enabled the characterisation of 5 monoglycerides (MGs), 7 families of DGs and 35 families of TGs.[27] However, this approach can lead to pyrolytic decomposition of the analytes, particularly highly unsaturated species.[40, 51-54]

Liquid chromatography presents a more suitable approach for the characterisation of these lipid classes. In the context of latent fingermark analysis, it has thus far seen very limited use, with focus constrained only to a narrow range of targeted compounds.[16, 29, 55] Conversely, numerous methods have been developed for lipidomics analyses targeting TGs in a variety of biological samples,[52] including plasma and tissues,[56, 57] animal fats[41, 58] and vegetable oils. [25, 40, 42, 58, 59] Camera *et al*. reported a high performance liquid chromatography (HPLC)-MS method to simultaneously analyse all classes

comprising the 'sebum lipidome', including over 100 glycerides, demonstrating the potential of such an approach to be applied to latent fingermarks.[49, 50]

We present a method for the untargeted separation and characterisation of latent fingermark glycerides using ultra performance liquid chromatography-ion mobility spectroscopy-quadrupole time-of-flight mass spectrometry (UPLC-IMS-QToF-MS^E). Samples were collected from a small population of donors to explore the range of variation in glyceride chain lengths and unsaturation, and to identify common, abundant species as potential targets for further studies into fingermark composition and degradation over time. Several approaches were taken to identify glyceride structures, such as *in silico* fragmentation of molecular structures previously reported in latent fingermarks and human skin surface lipids, and monitoring for neutral losses of common sebaceous fatty acids from MS^E acquisition.

Materials and methods

Chemicals

Dichloromethane (GC grade; Sigma-Aldrich, Switzerland), acetonitrile (ULC/MS grade; Biosolve, France), water (LC-MS Ultra grade; Honeywell, Germany), 2-propanol (ULC/MS grade; Biosolve, France), formic acid (ULC/MS grade; Biosolve, France), ammonium formate (LC-MS Ultra grade; Fluka, Switzerland), monomyristin (MG 14:0), monopalmitolein (MG 16:1), monoolein (MG 18:1), monostearin (MG 18:0), dimyristin (DG 28:0), dipalmitolein (DG 32:2), dipalmitin (DG 32:0), diolein (DG 36:2) and distearin (DG 36:0) (all >99 %; Nu-Chek Prep, Inc, USA), tricaprilin (TG 24:0), tricaprin (TG 30:0), trilaurin (TG 36:0), trimyristin (TG 42:0) and tripalmitin (48:0) (all 99.9 %; Sigma-Aldrich, USA) were used as received.

Stock solutions of glycerides were prepared in dichloromethane. To determine the ability of the UPLC method to separate glycerides and the elution profiles of each glyceride class, mixed standard solutions were prepared for each class (MGs, DGs and TGs) by serial dilutions in 2:1:1 isopropanol/acetonitrile/water. Mixed standard solutions were run at concentrations of 0.2 µM and 1 µM, except for the MGs, which were prepared at 0.6 µM and 3 µM. Each mixed standard solution was run in triplicate, to give a maximum of 6 measurements (retention time, *m/z* and collisional cross section (CCS)) per standard compound. All standard solutions were stored at -20 °C before and after analysis to prevent degradation and solvent evaporation.

Sample collection and storage

Latent fingermark samples were collected from 10 adult donors (21 – 37 years old) on 25 mm filter paper circles (Grade 1 qualitative filter paper; Whatman, UK). Donors were asked to refrain from handling food or chemicals, or washing hands 30 minutes prior to sampling, but were otherwise free to carry out normal activities and use of skin products. Donors were asked to provide charged fingermarks by briefly rubbing the middle three fingertips of both hands on their forehead and nose, then rubbing the fingertips of each hand together to homogenise the secretions. Three fingertips from one hand were pressed gently to individual filter paper circles for approximately ten seconds, providing a total of 30 samples (3 per donor). Samples from each donor were immediately wrapped in aluminium foil and stored in an office cupboard at ambient conditions for 24 hours prior to extraction, following the recommendations of the International Fingerprint Research Group.[60] A set of clean filter papers were also wrapped in aluminium foil and stored with the samples to provide analytical blanks.

Fingermark extraction

The filter papers were individually placed in 1.75 mL glass screw-top vials (Thermo Fisher Scientific) that had been cleaned by rinsing with dichloromethane and left to air-dry. Samples were immersed in 750 µL dichloromethane for 2 minutes, with gentle manual agitation to ensure that the filter papers were completely submerged in the solvent. After 2 minutes, the extract was transferred to a second vial. Sample cleanup was performed by adding 750 µL water and vortex mixing before allowing phase separation. The aqueous top layer was discarded, together with a small amount of the organic layer to ensure the complete removal of the water. The organic layer was evaporated under nitrogen gas until approximately 150 µL remained. This residue was transferred to an amber glass vial containing a 250 µL glass insert, and further evaporated to dryness. The remaining residue was dissolved in 200 µL of 2:1:1 2-propanol/acetonitrile/water with 20mM ammonium formate.

Chemical analysis

Chromatographic separation was performed using a Waters Acquity UPLC I-Class system, coupled to a Waters Vion IMS-QToF mass spectrometer (mass resolving power >40 000 FWHM) equipped with an electrospray ionisation (ESI) source. The UPLC system was equipped with a binary pump, a 96 well autosampler (maintained at 8 °C), and a temperature-controlled column compartment. Separation was performed using an Acquity UPLC CSH C_{18} column (2.1 x 100 mm, 1.7 µm), connected to an Acquity UPLC in-line filter to protect the column (both from Waters). The mobile phases were A) 60:40 acetonitrile/water with 10 mM ammonium formate and 0.1 % formic acid and B) 90:10 isopropanol/acetonitrile with 10 mM ammonium formate and 0.1 % formic acid. Gradient parameters are described in Table 1. The flow rate was maintained at 0.4 mL/min with a column temperature of 55 °C. The injection volume was 1 µL. Injections of the blank filter paper extracts were run before and after sample sets from each donor to monitor for carryover and contamination.

Time (min)	%A	%B
0.0	60	40
2.0	57	43
2.1	50	50
12.0	46	54
12.1	30	70
18.0	1	99
19.0	1	99
19.1	60	40
21.0	60	40

Table 1: UPLC mobile phase gradient parameters.

The ESI source was operated in positive mode, using the following parameters: the source temperature was set to 120 °C, the desolvation temperature to 600 °C, the cone gas flow to 50 L/hr, the desolvation gas flow to 1000 L/hr, and the capillary voltage to 2 kV. Data were acquired over the *m/z* range of 50 – 1000 with a scan time of 0.2 seconds. A 200 ng/mL solution of leucine enkephalin (*m/z* 556.2766) was used as the lock mass reference and infused into the ion source at 5 minute intervals. Data acquisition was performed using high definition MS^E. The low collision energy was set at 6 eV, and the high collision energy ramp at 30 – 60 eV. Nitrogen was used as the drift gas in the IMS and as the collision gas. Ion mobility and mass calibration were performed using a Major Mix IMS/Tof Calibration Kit (Waters).

Data processing

Data were processed using UNIFI (Waters MS Technologies, Manchester, United Kingdom). Deconvolution and peak picking were performed with 4D peak detection, with a low energy intensity threshold of 250 counts and a high energy intensity threshold of 100 counts. The fraction of the chromatographic peak width applied during isotope cluster creation and high-to low energy association was 0.5. The fraction of the drift peak width applied during cluster creation and high-to low energy association was 0.5, except for critical pairs that appeared together in the low energy mass spectra. To resolve these compounds, the fractions of the drift peak width applied during cluster creation and high-to low energy association were 0.1 and 0.15, respectively.

A molecular database was created by uploading .mol files obtained from ChemSpider (www.chemspider.com) to UNIFI. UNIFI's MassFragment algorithm was used to match expected compound structures to deconvoluted peaks, and explain collision-induced fragmentation based on bond breakage.[61, 62] A total of 104 MG (8:0 – 20:0), DG (16:0 – 40:0) and TG (24:0 – 60:0) structures were selected, comprised of the glyceride standards described above, additional species reported in latent fingermarks and skin surface lipids (where constituent fatty acids were indicated),[10, 49] as well as monoacid species representing fatty acyl groups reported as abundant free fatty acids in latent fingermarks.[9] A number of isomeric DG and TG structures were included. $[M+NH_4]^+$ and $[M+Na]^+$ were defined as precursor ions for both TGs and DGs. $[M-OH]^+$ was defined as an additional target precursor for DGs due to in-source fragmentation.

To assist in reducing the number of false positives, peaks identified based on *in silico* fragmentation were filtered based on the following criteria: ≥ 1 theoretical fragment observed, retention time ≤ 18 min, mass error ±2.5 ppm (based on expected precursor). Unidentified candidate peaks were filtered based on the following criteria: ≥ 1 fatty acid neutral loss (RCOOH + NH_3) observed, response > 1000, retention time 2 – 18 min, CCS ≥ 200 (based on observed retention times and CCS measurements of glyceride standards). Further examination of filtered candidate peaks was performed manually due to the coelution of isomeric TGs. Identification was performed based on known fragmentation pathways, i.e. the neutral loss of fatty acyl groups with ammonia from the *sn*-1, -2 or-3 positions. Candidate *m/z* were compared against calculated values for $[M+NH_4]^+$ of TGs to ascertain CN:DB, and high energy mass spectra were examined to identify predominant 'DG-like' ions corresponding to fatty acid neutral losses. Finally, all potential glycerides were required to have a peak response at least 2.5 times higher than that of any corresponding peak in a preceding blank filter paper extract.

Results and discussion

Nomenclature

Annotation of glyceride structures in this paper follows the recommendations of Liebisch *et al*.[63] Glyceride classes (mono-, di-, and triglycerides) are denoted as MG, DG, and TG, respectively, followed by the CN:DB family as a generic identifier (where CN denotes the total number of carbon atoms in the fatty acyl groups and DB the total number of double bonds). Where structural information is provided, the separator '_' is used to indicate that the *sn*-positions of the fatty acids are unknown (e.g. DG 14:0_16:0 indicates that myristic acid and palmitic acid are present in any possible 1,2- or 1,3-DG structure), otherwise the separator '/' is used (e.g. tripalmitin may be expressed as TG 48:0 or TG 16:0/16:0/16:0).

It should be noted that while alkyl branching has been observed in a number of sebaceous fatty acids, and several unsaturated fatty acids exist as positional isomers, the location of branches or double

bonds is beyond the aims of this study, and so fatty acyl groups are referred to only by their generic CN:DB.

Preliminary considerations

Preliminary experiments showed that a limited number of sebaceous compounds other than glycerides could be readily detected using this method, primarily monounsaturated wax esters (data not shown). Other non-polar fingermark constituents such as cholesterol, squalene and saturated wax esters exhibited very poor or no signal, likely due to the incompatibility of such compounds with ESI.[64] As these compounds may instead be easily analysed using GC-MS,[1, 4, 12, 15, 33, 34] the focus of this work was constrained only to the glyceride fraction.

Figure 1 shows a total ion chromatogram of a latent fingermark, with highlighted regions indicating where DGs and TGs were eluted. Using this UPLC method, glycerides are separated based on the chain length and degree of unsaturation of their fatty acyl groups, rather than by lipid class. This is often expressed as the equivalent carbon number (ECN = CN – 2DB), whereby mono- and polyunsaturated glycerides can be shown to have similar retention behaviour to a smaller, more saturated counterpart.[41, 65, 66] Complete separation can be extremely difficult to achieve for neutral lipid extracts.[52, 56, 67, 68] Under optimised separation conditions, resolution of TGs within an ECN group (known as 'critical pairs') can be achieved, but this comes at the cost of substantially longer run times than the method presented here.[42, 69-71] Complete separation of some positional isomers can require as long as several hours. The aim of this study to identify potential targets for future investigations, based on CN:DB families which are common between donors and present in high abundances. A shorter run time was thus preferred in order to acquire untargeted data from several fingermark samples.

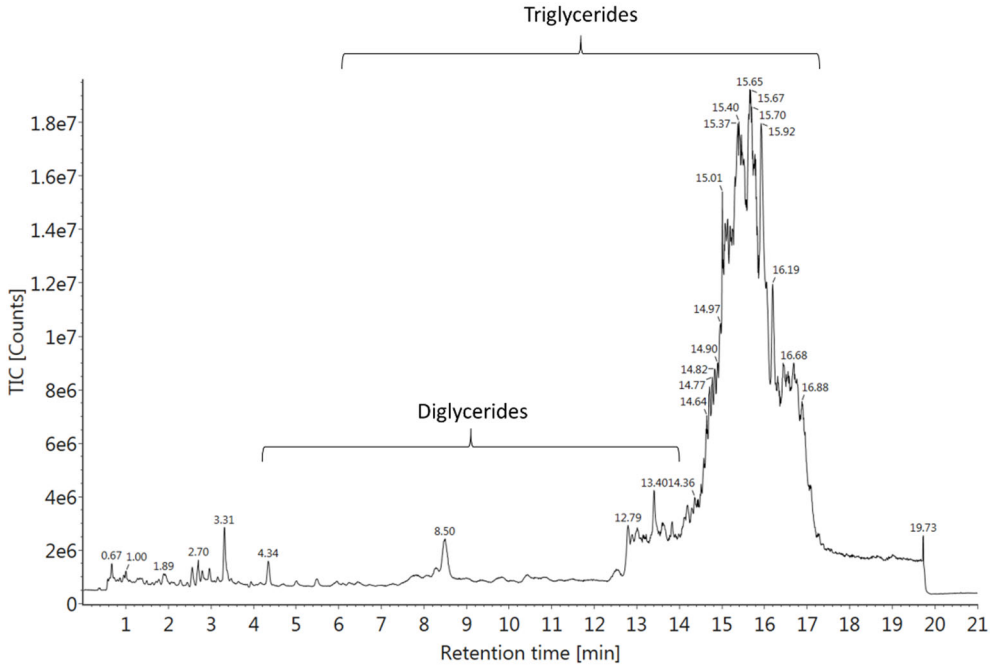

Figure 1: Total ion chromatogram of UPLC separation of latent fingermark residue, showing elution ranges of identified di- and triglycerides.

MS^E mass spectra of MG and DG standards were complicated by a significant peak appearing in both the low and high collision energy mass spectra, which corresponded to a loss of a hydroxyl group from the glycerol backbone ($[M+H-H_2O]^+$). In-source fragmentation in this manner is a known occurrence in the analysis of DGs.[49, 52, 59, 66, 72, 73] Resultant signal loss was subsequently overcome by utilising this peak as an expected 'adduct'. Due to excessive fragmentation, MGs could not be conclusively identified in standard solutions or fingermark samples, as no theoretical fragments were found in the high collision energy mass spectra. The collision energies utilised in MS^E are applied to all sample ions, and different energies cannot be selected within a method to target select fractions. It was therefore decided not to lower the collision energy settings, as this could compromise the collisional dissociation of the TGs.[43]

The use of *in silico* fragmentation to identify glycerides, rather than the use of standard compounds, was chosen as an alternative approach when no standard was available, as many of the glycerides previously identified in skin surface lipids are unavailable commercially. Furthermore, the use of physical reference substances can create carryover issues in the UPLC system.[61] While an *in silico* approach confers several advantages in these regards, other issues arise from a lack of information of a compound's chromatographic behaviour, namely the retention time. Without this reference information, several peaks were identified as a single glyceride species, requiring manual examination of the data to exclude any false positive results. By doing so, it was often found that MassFragment was unable to conclusively identify some glycerides, due to the presence of isomers for which .mol files had not been obtained. This was easily seen in the high energy mass spectra, due to the presence of fragment ions corresponding to the losses of fatty acids in addition to, or instead of, those of the proposed structure. Where a glyceride contained fatty acyls groups different to those expected from the .mol file, MassFragment would explain observed losses as part of a fatty acid chain (where the loss was smaller than expected) or as the loss of a fatty acid and a small fragment of another (where the loss was larger than expected).

Diglyceride identification

The 5 DG standards (DG 28:0 – 36:0) eluted between 7.3 and 14 minutes, and were detected in the low energy mass spectra as predominantly $[M+H-H_2O]^+$ ions, due to in-source fragmentation as described above (Table S1). All five standard DG compounds were detected in latent fingermark samples as primarily sodiated adducts. The higher proportion of sodiated adducts in sample extracts compared to the standards can be explained by the presence of sodium originating from the eccrine (sweat) constituents of the samples,[9, 10] as well as from potential sources within the laboratory such as the filter paper substrates. A further 18 DGs were identified based on *in silico* fragmentation, including 16 structures previously reported in human sebum.[49] In total, 23 DG structures were identified, comprising 20 CN:DB families, which eluted between 4 – 14 minutes (Table 2). The observed structures ranged from 24:0 – 36:0 with 0 – 4 double bonds. Manual examination of the high energy mass spectra of proposed DGs was required to confirm molecular structure, as MassFragment was not consistently able to differentiate between isomers. Isomeric DGs eluted closely together, but exhibited sufficient chromatographic resolution for differentiation (Figure 2). While positional isomers (i.e. 1,2-DGs and 1,3-DGs) may be resolved by HPLC,[42] this was not observed here.

Diglyceride species	CN:DB	Average retention time (min)	Average experimental *m/z* ($[M+H-H_2O]^+$)	Average CCS (Å²) ($[M+H-H_2O]^+$)	Average experimental *m/z* ($[M+Na]^+$)	Average CCS (Å²) ($[M+Na]^+$)	Number of fingermarks [n = 30] (number of donors [n = 10])
DG 12:0_12:0	24:0	4.18	439.3780	233.6	479.3707	230.8	7 (3)
DG 12:0_14:0	26:0	5.46	467.4091	242.0	506.6519	237.5	8 (4)

DG 14:0_14:1	28:1	5.86	493.4246	245.6	533.4177	243.2	6 (2)
DG 14:1_16:1	30:2	6.29	n/a	n/a	559.4332	246.6	2 (1)
DG 14:0_14:0	*28:0*	*7.28*	*495.4408*	*249.5*	*535.4334*	*244.6*	*9 (4)*
DG 12:0_18:1	30:1	7.52	521.4554	252.8	561.4493	250.6	3 (2)
DG 14:0_16:1	30:1	7.78	521.4565	253.6	561.4492	250.7	11 (5)
DG 16:1_16:1	*32:2*	*8.32*	*547.4713*	*256.6*	*587.4649*	*255.4*	*18 (7)*
DG 18:2_18:2	36:4	8.68	599.5038	263.8	639.4959	262.8	5 (2)
DG 15:0_16:1	31:1	9.00	535.4717	257.2	575.4649	254.8	10 (5)
DG 14:0_16:0	30:0	9.70	523.4718	257.7	563.4646	252.1	20 (7)
DG 14:0_18:1	32:1	10.03	549.4876	260.7	589.4802	258.9	6 (3)
DG 16:0_16:1	32:1	10.36	549.4880	261.0	589.4801	258.5	25 (9)
DG 16:0_18:2	34:2	10.56	575.5028	264.0	615.4956	263.1	3 (1)
DG 16:1_18:1	34:2	10.75	575.5029	263.9	615.4959	263.5	13 (5)
DG 18:1_18:2	36:3	10.85	601.5190	266.8	641.5113	266.5	3 (1)
DG 15:0_16:0	31:0	11.17	536.3765	261.6	577.4799	255.4	9 (5)
DG 15:0_18:1	33:1	11.56	563.5036	264.1	603.4956	262.3	2 (1)
DG 16:0_16:0	*32:0*	*12.76*	*551.5033*	*265.4*	*591.4956*	*259.7*	*30 (10)*
DG 16:0_18:1	34:1	12.86	577.5193	268.0	617.5115	267.2	12 (5)
DG 18:1_18:1	*36:2*	*12.92*	*603.5343*	*271.1*	*643.5271*	*270.0*	*9 (3)*
DG 16:0_18:0	34:0	13.47	579.5318	271.9	619.5270	267.1	12 (5)
DG 18:0_18:0	*36:0*	*14.01*	*607.5659*	*279.2*	*647.5586*	*274.6*	*6 (2)*

Table 2: Diglycerides detected in latent fingermarks from 10 donors. Compounds identified using standards are indicated in italics.

The peak intensities of the DGs were usually very low compared to those of the TGs (total peak response of the DG fraction in a sample was typically <5 % of the total identified glycerides). Often, a DG species could not be identified in all samples from a single donor, as diagnostic fragment ions could not be detected at low signal-to-noise ratios. This is consistent with literature stating that DGs constitute a very minor proportion of the glyceride fraction of skin surface lipids, as a hydrolysis product of TGs by skin surface bacteria.[20, 24, 74] Only four species (DG 16:0_16:0 (dipalmitin), DG 16:0_16:1, DG 16:0_14:0 and DG 16:1_16:1 (dipalmitolein)) were found to be common to over half of the donor population, with only dipalmitin found in all 30 samples.

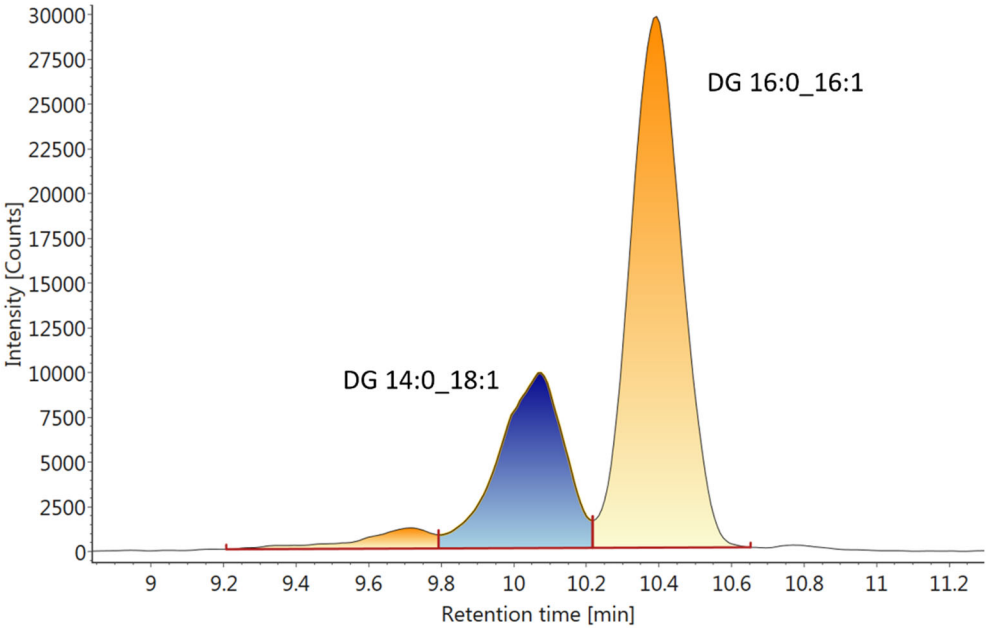

Figure 2: Extracted ion chromatogram *m/z* 589.4800 (DG 32:1), showing chromatographic separation of two isomers.

That fewer DGs were detected in comparison to the study of Camera *et al*. (where a total of 52 DGs were reported),[49] can in part be explained by the differences in the samples collected (i.e. skin surface lipids and latent fingermarks). Skin surface lipids are typically collected by affixing an absorbent material directly to the sampling site for an extended period of time (e.g. 30 minutes), which would enable a larger amount of secretions to be collected as opposed to the residual amount of material that would be collected through briefly contacting a fingertip to a porous substrate.

To the best of the authors' knowledge, no information has been previously reported regarding the specific DG species in latent fingermarks. While this lipid class may not comprise a significant proportion of recently deposited latent fingermarks, they may still be relevant in composition studies. As with other sebaceous lipids, a correlation has been noted between the age and sex of an individual, and the proportions of TGs and DGs in skin lipid samples.[75] Over time following fingermark deposition, degradation processes may further alter this ratio.[4] Therefore, knowledge of the full glyceride content of latent fingermarks will be of greater value towards understanding fingermark detection and degradation, by identifying reactants and intermediates in degradation mechanisms.

Triglyceride identification

A great challenge to the separation and identification of TGs within a natural lipid sample is the diverse range of structures present, including the presence of isomers within a single CN:DB family.[59, 76, 77] The number of potential isomers for a given CN:DB increases with molecular weight, resulting in thousands of total structures that could be theoretically be present in a single sample.[51, 52, 78, 79] For example, not including positional isomers, 18 fatty acids have been reported in the literature as components of human sebum and/or latent fingermarks. The resulting theoretical number of potential TG species in a latent fingermark, disregarding isomers, exceeds 1000, and if all isomers are considered, near 6000.[79] The differing relative abundances of the constituent fatty acids presents some indication as to the

likely higher concentration glyceride structures.[80] Reported TGs in human skin surface lipids range from 27 – 94 families, comprising a wide variety of total fatty acyl chain lengths (34:0 – 60:0) and degrees of unsaturation.[27, 49, 75, 81] The extremely diverse range of TG species present in human skin presents many challenges to determining a complete catalogue in latent fingermarks. Emerson *et al.* noted problems due to the number of TGs species (differing by numbers of double bonds) that could occupy a single mass window specified for MS/MS experiments.[10]

The 5 TG standards (TG 24:0 – 48:0) eluted between 3.5 and 15.9 minutes, and were detected in the low energy mass spectra as predominantly $[M+NH_4]^+$ (Table S2). Dissociation fragments produced in the high energy mass spectra were consistent with known fragmentation patterns of TGs. Upon collisional activation, ammonium adducts of TGs fragment to produce 'DG-like' ions, resulting from the neutral loss of each unique fatty acyl group from the *sn*-1, -2, or -3 position, plus ammonia.[44, 77] This pattern of fragmentation provides a basis for the untargeted identification of TGs by monitoring datasets for fatty acid neutral losses, when the identities of the constituent TG fatty acids are known. While MS^E and MS/MS are not sufficient to conclusively identify a TG species, due to the ambiguity of the remaining two fatty acids, some structures may be assumed based on the most prevalent fatty acids.[10] MS^3 is required to acquire more comprehensive structural information by fragmentation of the resultant DG-like ion.[44, 51, 52, 82]

The fatty acid constituents of human skin lipids are unusual in the wide range of chain lengths and structural variations that are present, including methyl branching (iso-, anteiso and other isomers) and double bond positional isomers.[20] However, it should be noted that collision-induced dissociation (CID) is not sufficient to identify structural characteristics such as double bond position or alkyl branching, nor the *sn*-position of a fatty acid.[83] Table 3 lists the calculated mass losses of fatty acids that have been identified as major components of human sebum and latent fingermarks, as both free acids and components of glycerides.[19, 20, 30, 34] Up to 36 fatty acid structures originating from skin surface TGs have been described in dermatological studies.[19, 20, 84] Additionally, Nicolaides reports that over 200 other acids are present in trace amounts, including unusually long and short fatty acids which can be present in trace amounts in skin surface lipids.[20] Expected mass losses for 56 fatty acids ranging from 2:0 – 25:2 were incorporated into the analysis method to account for fatty acid neutral losses beyond the minimum and maximum chain lengths described above (Table S3).

Fatty acid(s)	**CN:DB**	**$RCOOH+NH_3$ neutral loss**	
		Formula	**Mass (Da)**
Octanoic acid (caprylic acid)	8:0	$C_7H_{15}COOHNH_3$	161.14158
Nonanoic acid (pelargonic acid)	9:0	$C_8H_{17}COOHNH_3$	175.15723
Decanoic acid (capric acid)	10:0	$C_9H_{19}COOHNH_3$	189.17288
Dodecanoic acid (lauric acid)	12:0	$C_{11}H_{23}COOHNH_3$	217.20418
Tridecanoic acid	13:0	$C_{12}H_{25}COOHNH_3$	231.21983
Tetradecanoic acid (myristic acid)	14:0	$C_{13}H_{27}COOHNH_3$	245.23548
Δ6-tetradecenoic acid	14:1	$C_{13}H_{25}COOHNH_3$	243.21983
Pentadecanoic acid	15:0	$C_{14}H_{29}COOHNH_3$	259.25113
Pentadecenoic acid	15:1	$C_{14}H_{27}COOHNH_3$	257.23548
Hexadecanoic acid (palmitic acid)	16:0	$C_{15}H_{31}COOHNH_3$	273.26678
Hexadecenoic acid (sapienic acid)	16:1	$C_{15}H_{29}COOHNH_3$	271.25113
Heptadecanoic acid	17:0	$C_{16}H_{33}COOHNH_3$	287.28243
Heptadecenoic acid (Δ6- and Δ8-heptadecenoic acids)	17:1	$C_{16}H_{31}COOHNH_3$	285.26678
Octadecanoic acid (stearic acid)	18:0	$C_{17}H_{35}COOHNH_3$	301.29808

Octadecenoic acid (Δ8-octadecenoic acid, oleic and petroselenic acids)	18:1	$C_{17}H_{33}COOHNH_3$	299.28243
Octadecadienoic acid (sebaleic and linoleic acids)	18:2	$C_{17}H_{31}COOHNH_3$	297.26678
Eicosanoic acid (arachidic acid)	20:0	$C_{19}H_{39}COOHNH_3$	329.32938
Δ10-eicosenoic acid	20:1	$C_{19}H_{37}COOHNH_3$	327.31373
Δ7,10-eicosadienoic acid	20:2	$C_{19}H_{35}COOHNH_3$	325.29808

Table 3: Neutral losses of major fatty acids identified in human sebum and latent fingermarks.[19, 20, 30, 34]

Fingermark TGs were detected primarily as $[M+NH_4]^+$, with sodiated adducts comprising a minor part of the low collision energy mass spectra. TGs consistent with $[M+NH_4]^+$ ions of the TG standards were identified within fingermark samples, with the exception of tricaprilin (TG 24:0). Close to 100 peaks with *m/z* corresponding to ammoniated TGs eluted over a wide range of 6 – 17 minutes, with the greatest numbers (*ca*. 70 %) eluting between 15 – 17 minutes. Based on *in silico* fragmentation, 28 families of TGs (including 21 that had previously been reported in skin surface lipids and latent fingermarks) were identified by UNIFI in 30 fingermark samples. Manual examination of the mass spectra of identified peaks was required to identify any false positives, as identification was based only on high definition mass spectrometry measurements in lieu of reference data (i.e. retention times or CCS measurements). From these data, it could be seen that in many instances that relying only on *in silico* fragmentation for compound identification was insufficient, as the interpretation of MS^E data was complicated by the presence of multiple fatty acid neutral losses, indicating coelution of isomeric TGs.

In total, 85 families of TGs were identified from the total 30 fingermark samples (Table S4), ranging in structure from 28:0 – 60:2 and comprising 27 ECNs. The majority consisted of saturated, mono- and diunsaturated TGs, with a smaller number of polyunsaturated TGs containing up to 6 double bonds. Among the TGs showing the highest response, 21 families were identified as being common to samples from all or most of the 10 donors (Table 4). These compounds are predominantly unsaturated, and so may therefore be useful targets in the monitoring of chemical changes in fingermark residue over time.[29] As a comparison, 27 TG families have been previously identified in latent fingermarks, with possible structures assigned to all but 2,[10] and 94 families and 142 species separately in human sebum.[27, 49]

Major observed neutral losses for all glycerides for the most part corresponded predominantly to the free fatty acid species previously reported in the literature. This is consistent with the results obtained by Emerson *et al*.,[10] who focused on TG species that could be elucidated from the 8 most abundant free fatty acids as reported by Nicolaides.[20] Up to 5 fatty acid neutral losses were observed for each TG family in a single sample, indicating isomeric species. In some cases, possible structures can be tentatively assigned, where the high energy mass spectra are relatively simple. For example, the only neutral loss observed across all samples for TG 54:6 was 18:2, indicating that the structure is most likely simply 18:2/18:2/18:2. In other instances, the observed neutral losses did not appear to correspond to the parent CN:DB; e.g. some TGs with an odd CN did not exhibit any neutral losses of odd-chain fatty acids. An example is shown in Figure 3, where the base peak in the low energy spectrum (*m/z* 860.7691) is consistent with the ammoniated adduct of TG 51:3. In the high energy mass spectrum, the two most abundant fragments (*m/*z 561.4872 and 589.5184) correspond to neutral losses of 18:1 and 16:1, respectively. The presence of an odd-chain fatty acid (possibly 17:1) may be inferred based on the CN:DB of the parent TG, but no corresponding neutral loss is observed. The loss of a fatty acid at the *sn*-1 or -3 position is demonstrably more favourable than a loss at the

sn-2 position [85]. It is possible that the 'missing' fatty acids are located at this position,[45] and are therefore less likely to be observed in CID experiments.

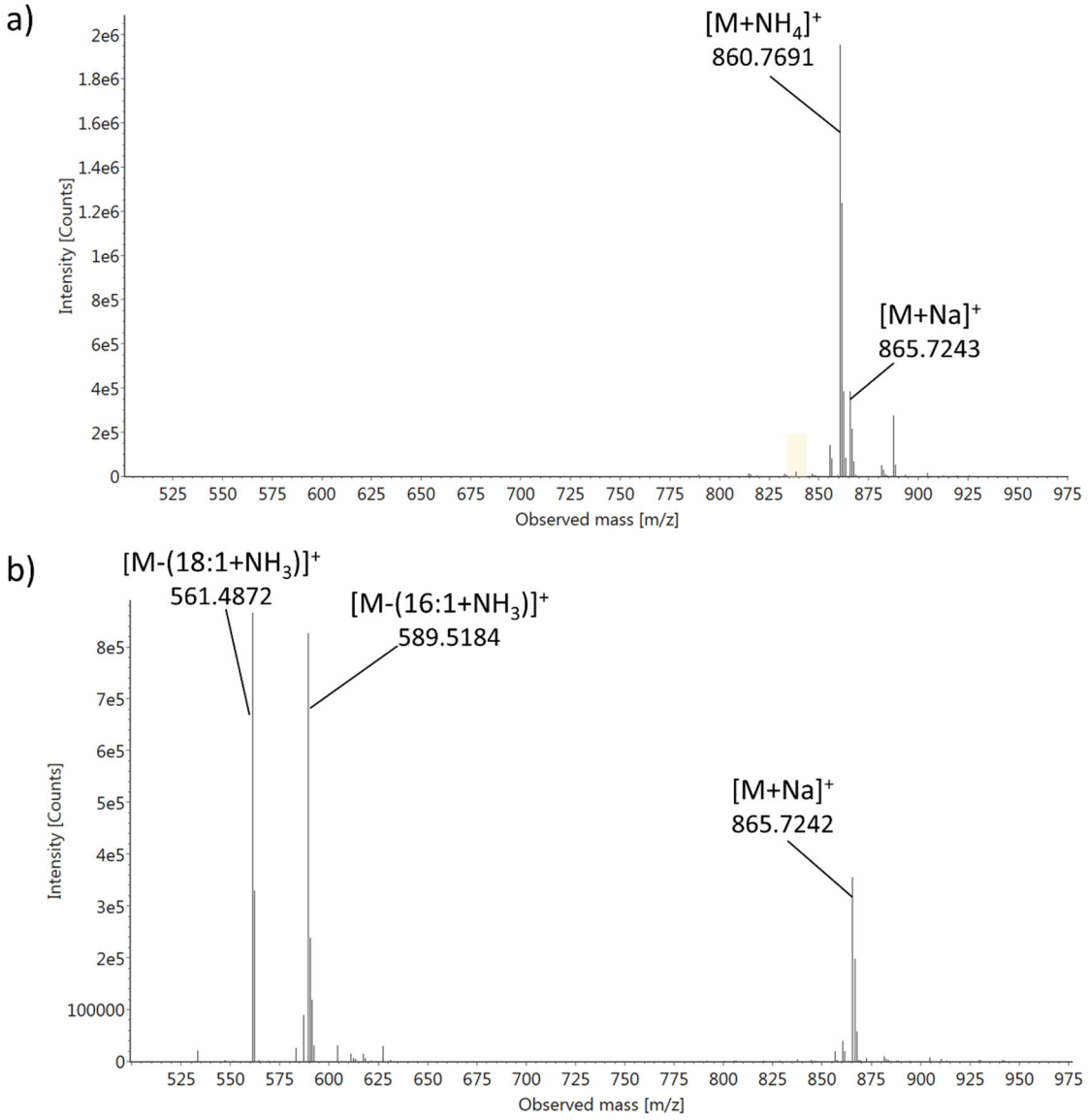

Figure 3: MS^E spectra of *m/z* 860.7691 (TG 51:3), showing (a) the low energy mass spectrum containing ammoniated and sodiated adducts; and (b) the high energy mass spectrum containing DG-like fragments corresponding to fatty acyl neutral losses.

Examination of low energy spectra where several TGs were present confirmed the coelution of TG critical pairs, as described above.[40, 78, 80] The use of IMS confers an advantage in this regard, as coeluting TG species may be resolved on the basis of drift time, which is a reflection of differences in molecular structure and *m/z*.[68, 71, 86] Due to the close structural similarities between critical pairs, and therefore close drift times, it was necessary to reduce the fractions of the drift peak width for isotope clustering and high-to low energy association from the default value of 0.5. This process enabled the removal of critical pairs and their assigned fragments from the mass spectral data, facilitating the identification of fatty acyl losses for all but 6 of the 85 identified TG families.

While no quantification approach was used in this study, it can be inferred from the relative variations in peak areas between samples from different donors that inter- and intra-donor variation can be expected in glyceride profiles of latent fingermarks. Emerson *et al*. attempted to discriminate donors by sex based on TG peak intensities, but were unable to develop a reliable method despite observing statistically significant differences between male and female donors.[10] Pleik *et al*. made the observation that monounsaturated TGs showed higher intensity than other TG components in recently deposited fingermarks.[29] Similar results were reported by Camera *et al.* for human sebum samples.[49] It should be noted however that the ionisation efficiency of glycerides is significantly affected by chain length and degree of unsaturation, so that relative peak response is not necessarily representative of concentration.[42, 83]

ECN	CN:DB	Average retention time (min)	Average experimental *m/z* ($[M+NH_4]^+$)	Average CCS (Å^2) ($[M+NH_4]^+$)	Observed fatty acid neutral losses	Number of fingermarks [n = 30] (number of donors [n = 10])
42	TG 44:1	15.03	766.6915	313.1	12:0, 14:0, 14:1, 15:0, 16:0, 16:1, 18:0	30 (10)
	TG 46:2	15.09	792.7073	318.4	14:1, 15:0, 16:0, 16:1	30 (10)
	TG 48:3	15.14	818.7228	323.3	16:1, 14:1	30 (10)
43	TG 45:1	15.18	780.7072	316.7	12:0, 14:0, 14:1, 15:0, 16:0, 16:1, 17:1, 18:0	26 (10)
	TG 47:2	15.25	806.7228	322.2	14:1, 15:0, 16:1, 17:1, 18:1	30 (10)
44	TG 46:1	15.38	794.7228	320.7	12:0, 14:1, 15:0, 16:0, 16:1, 18:0, 18:1	30 (10)
	TG 48:2	15.42	820.7385	325.8	14:0, 14:1, 16:0, 16.1, 18:0, 18:1	30 (10)
	TG 50:3	15.45	846.7541	330.7	16:1, 18:0, 18:1	30 (10)
45	TG 47:1	15.52	808.7385	324.3	14:0, 14:1, 15:0, 16:0, 16:1, 18:0, 20:1	29 (10)
	TG 49:2	15.56	834.7541	329.7	15:0, 16:0, 16:1, 17:1, 18:0, 18:1, 18:2	30 (10)

46	TG 46:0	15.65	796.7385	322.4	14:0, 15:0, 16:0, 18:0	20 (8)
	TG 48:1	15.69	822.7542	327.8	14:0, 16:0, 16:1, 18:0, 18:1	30 (10)
	TG 50:2	15.72	848.7698	333.2	14:0, 16, 16:1	30 (10)
	TG 52:3	15.72	874.7854	337.5	16:0, 16:1, 18:1, 18:2	30 (10)
47	TG 47:0	15.77	810.7541	325.8	16:0, 18:0	29 (10)
	TG 49:1	15.81	836.7698	331.5	14:0, 15:0, 16:0, 16:1, 17:1, 18:1	30 (10)
	TG 51:2	15.84	862.7854	336.8	14:0 15:0, 16:0, 16:1, 17:1, 18:1, 18:2	30 (10)
48	TG 48:0	15.95	824.7698	329.8	16:0, 18:0, 20:0	23 (8)
	TG 50:1	15.97	850.7854	335.5	14:0, 16:0, 16:1, 18:0, 18:1	30 (10)
	TG 52:2	15.97	876.8009	339.9	16:0, 16:1, 18:0, 18:1	30 (10)
49	TG 51:1	16.08	864.8010	338.7	16:0, 16:1, 18:0, 18:1	30 (10)

Table 4: Triglycerides identified as major and common components in fingermarks from 10 donors.

Conclusions

The glyceride fraction of human skin surface lipids exhibits a high degree of complexity, presenting challenges to the complete characterisation of latent fingermark residue. Using UPLC-IMS-QToF-MS^E in a lipidomics-based approach, over 100 intact glycerides (DG 24:0 – 36:0 and TG 28:0 – 60:2), were detected in fingermarks from a population of 10 donors. This represents a more comprehensive list of the glyceride species present in fingermark samples than have been previously reported. To the authors' knowledge, specific diglyceride species in latent fingermarks have been identified for the first time, as a minor component of the total glyceride fraction. IMS enabled the use of a high-throughput UPLC method, as mass spectra of chromatographically coeluting TGs could be further resolved based on drift times.

This study represents a first step to better understanding the glyceride fraction of latent fingermark residue, and its potential impact on fingermark detection. Monitoring the chemical and physical changes in the lipid fraction of latent fingermarks following deposition might significantly contribute to understanding the mechanisms behind lipid-sensitive fingermark enhancement. Further studies are currently underway to examine the effect of increasing fingermark age on the glyceride composition of older samples (up to several weeks old), with a primary focus on the most common and abundant triglycerides.

Conflicts of interest

There are no conflicts to declare.

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