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Identification of wax esters in fingermark residues by GC/MS and their potential use as aging parameters

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Abstract

Recent studies highlighted that the initial fingermark residues composition significantly varies within residues from the same donor as well as between donors. This variability is a major drawback in fingermarks dating issues. This study aimed therefore at the definition of a parameter that is less variable from mark to mark, using a ratio of the area of a target compound degrading over time divided by the summed area of more stable compounds also found in fingermark residues.

Gas chromatography - mass spectrometry (GC/MS) analysis of the lipid initial composition of fingermarks allowed the identification of four main classes of compounds that can be used in the definition of an aging parameter: fatty acids, sterols, sterol precursors and wax esters (WEs). Although the entities composing the first three groups are quite well known, those composing WEs were so far poorly reported. Therefore the first step of the present work was to identify WE compounds present in fingermark residues deposited by different donors. Out of 29 WEs recorded in the chromatograms, seven were observed in the majority of samples.

The identified wax ester compounds were subsequently used in the definition of ratios in combination with squalene and cholesterol, in order to reduce the variability of the initial composition between fingermark residues from different persons, and more particularly from the same person. Finally, the influence of the enhancement process on the initial composition was studied by analyzing traces after treatment with magnetic powder, 1,3-indanediones and cyanoacrylate. The ratio proposed seemed promising and should further be tested to follow reproducibly the aging of fingermark.

Keywords: fingermark, fingerprint, ageing, wax esters, composition, enhancement, GC/MS, lipid, intra-variability

Introduction

Analysis of the chemical composition of fingermarks residues began years ago in forensic sciences, principally aiming at the resolution of two different issues: development and improvement of enhancement techniques [1-13]; and estimation of the time of deposition of fingermarks [4,12,14-19]. All those studies showed that the initial composition of fingermarks residues varied qualitatively and quantitatively between people (intervariability) as well as among residues from the same person (intravariability). Moreover the residues were also subject to ageing: the components underwent different modifications as a function of time [4-5,7-8,12,14-19] and other influence factors such as storage conditions [4,8,10,12,19]. Those variations and modifications might represent major drawbacks for both issues mentioned above.

Concerning the dating issue, a recent paper proposed a way of reducing lipids variability using an aging parameter less variable from fingermark to fingermark. This parameter has the form of a ratio of the peak area of squalene (compound degrading over time) divided by the peak area of cholesterol (compound more stable over time) and showed promising results that should be further investigated [19].

Thus, the present work aimed at developing this new approach particularly useful for dating purposes. The objective was to study alternative ratios including other fingermarks intrinsic compounds analysed by GC/MS in order to improve the reproducibility of the results for fingermarks initial composition, principally focusing on the residues originating from the same donor. Recent studies conducted on the lipid initial composition of fingermarks by GC/MS allowed the identification of four main classes of compounds that can be used in the definition of this ratio [4-5,7-8,19]: the fatty acids, the sterols, the precursors of sterol and the wax esters (WEs). Although the entities composing the first three groups are quite well known, those composing the WEs were so far poorly detailed. Therefore the first step of this work was to study the lipid components of fingermarks from seven donors using GC/MS and giving a particular attention to the identification of wax esters. In a second step, the identified wax esters as well as other identified compounds were used in the definition of new ratios in order to find the better to minimize the lipids variability. Finally, the influence of common enhancement techniques (magnetic powder, cyanoacrylate and 1,3-indanediones) on the initial composition of fingermarks residues through the calculation of the proposed ratio was also studied. Those tests were carried out keeping in mind the fact that in real cases fingermarks were always first enhanced for identification purposes and then chemically analysed for others issues (for example, dating purposes). This is the reason why the proposed ratio should remain stable even after the use of enhancement techniques.

Material and methods

Products and material

The solvents used (methanol 99.9%, dichloromethane 99.9%) were purchased from Merck (Geneva, Switzerland). Nonanoic acid (C9:0), myristic acid (C14:0), pentadecanoic acid (C15:0), palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), squalene (SQA) and cholesterol (CHO) used as reference material as well as squalane, decane, 2-octanol, dodecane used as internal standards (IS) were purchased from Sigma-Aldrich (Buchs, SG, Switzerland). Finally, wax esters (WE) used also as references (palmityl palmitate (pP), palmityl palmitoleate(pPo), myristyl myristate (mM), myristyl palmitoleate (mPo) and stearyl palmitoleate (sPo)) were purchased from Nu-Chek Prep (elysian, MN, USA).
Samples were deposited on Glass microfiber filters (diam. 25mm) purchased from Whatman® (Bottmingen, Switzerland) or on glass slides for microscopy from Glasbearbeitung GmbH (Braunschweig, Germany). The analyses were performed in cap vials (32 x 11.6 mm) containing inserts of 50µl carried by steel springs. This material was provided by Laubscher (Geneva, Switzerland). The enhancement products were obtained from BVDA (Haarlem, Holland). Syringe for filtration with a head filter of PFTE 0.45µm were provided by Laubscher (Geneva, Switzerland). Cyanoacrylate glue (Distrelec, Switzerland) and an hermetic tank Projectina (Heerbrugg, Switzerland) were moreover used.

Sampling

Traces were collected from seven different donors, two males and four females, all Caucasian between 23 and 25 years old and not following a special diet. Their traces were collected according to the following steps:

1. Both thumbs were rubbed on the forehead and the edge of the nose to increase their amount of greasy substances.
2. To homogenise the compounds between both fingers, thumbs were rubbed together.
3. Each fingermark was deposited on glass microfiber filters or glass slides for microscopy with a controlled pressure (500 gr.) and time of deposition (5s.).

For each experiment, 3 traces were deposited sequentially and analysed on the same day (see Table 1).

Extraction

The microfilters on which fingermarks were deposited were immersed during 120s in 1.5 ml of dichloromethane before removing the substrate from the glass vial. The traces deposited on *glass slides* were rinsed with 2 ml of dichloromethane. The liquid was recovered in a becher and transferred in a vial.

For both substrates, a step of concentration of the residues was then performed. Dichloromethane was evaporated under a stream of nitrogen until only a few microliters were left. The remaining liquid was transferred into an insert of 50µl installed on a spring and evaporated to dryness under a stream of nitrogen. The residue was finally dissolved in 20µl of dichloromethane containing an internal standard (0.05mg/ml of decanol).

Blank samples were extracted and analysed before each sets of data

GC-MS analysis

Samples were analysed on a gas chromatograph/mass spectrometer 5795 C inert MSD from Agilent equipped with an autosampler Agilent GC 7890. Separation was carried out on a HP5-MS column (30m x 0.25mm x 0.25µm). The carrier gas was Helium with a constant flow of 1ml/min. 2µl of sample were injected in splitless mode in an injector maintained at 250 °C with a purge time of 1.5min. The program of temperature was developed based on the results of three previous studies [4-5,8] in order to obtain an optimal resolution for the whole range of compounds. The program was isothermal at 80 °C for 1 min, then 80 to 230 °C at a rate of 10 °C/min, isothermal at this temperature for 2 min. A new rate was performed from 230 to 310 °C at 4 °C/min and finally isothermal for 8 min. A solvent delay of 3.6 min was chosen. The interface line was maintained at 280 °C. The detector was a mass spectrometer in Electron Impact (EI) mode at 230 °C. Masses were measured in scan mode between 30m/z and 650 m/z by a quadrupole at 150 °C. In order to ensure the quality of the MS response, a standard solution containing squalene at 0.03mg/ml, cholesterol at 0.02 mg/ml and the internal standard were injected every week. The mass spectra obtained where compared with stored spectra in the NIST05 library© (Gaithersburg, USA). The relative quantification was made using target ions peak areas; one target ion for each compound was selected according to its specificity and abundance in the mass spectrum (Table 2).

Experiments (see Table 1)

• *Composition of the fingermark residues*

In a first step, the composition of each fingermark residues was determined qualitatively. The presence of a compound in the fingermarks from one donor was established if it was identified in at least half of traces. Intra-variability was studied on the traces from donor 1 using traces collected on the same day, and also traces deposited over a period of two months. Inter-variability was studied using the traces from 7 donors.

Relative quantification of the detected compounds was measured using the Relative Peak Area (RPA_1):

$$RPA_1 = \frac{PA(X)}{PA(IS)} \qquad \text{Eq. 1}$$

It is a ratio between the peak area (PA) of a selected compound X and the internal standard (IS). It allows the evaluation of the relative quantity of each identified compound in the fingermark residues. In a second step, the RPAs of one compound X to one or several other compounds Y, Z inherent to the fingermark residues were investigated in order to define a parameter (RPA_2) with minimal intra and inter-variability between traces:

$$RPA_2 = \frac{PA(X)}{PA(Y) + PA(Z)} \qquad \text{Eq. (2)}$$

• *Enhancements:*

Three enhancement techniques were carried out on traces from donor 1. Results were compared in relation to non-treated traces. Thus, for each experiment, 3 enhanced traces and 3 non treated traces were collected at the same time. In addition, contaminations brought by the enhancement steps were determined through the analysis of blank samples.

a) Powder

The traces were deposited on glass slides and powdered using the magnetic powder (Lightening, USA). During the extraction, a step of filtration with a syringe was added to avoid contamination.

b) 1,2-Indanediones

Fingermark residues on filters were immersed a few seconds in the enhancement solution and then warmed 10s in a press at 165°C [20]. Three different formulations available in our laboratory were tested using different solvents: dichloromethane, methanol or hydrofluoroether-7100 (HFE-7100).

c) Cyanoacrylate

The cyanoacrylate fumigation was performed with cyanoacrylate glue in a hermetic tank at 80% humidity [21]. Traces were extracted 1 hour after the treatment to allow the drying of the glue.

Insert Table 1

Results and discussion

Qualitative characterization

The composition of fingermark residues was examined for seven donors. A large number of compounds were identified, squalene being the major compound in every donor's traces. Cholesterol was also present in all traces in lower amount. Additionally several saturated fatty acids were observed in most traces: dodecanoic acid (C12:0), tridecanoic acid (C13:0), tetradecanoic acid (C14:0), pentadecanoic acid (C15:0), hexadecanoic acid (C16:0) and octadecanoic acid (C18:0). The mono-unsaturated acids 11-tetradecenoic acid (C14:1), 14-pentadecenoic acid (C15:1), 9-hexadecenoic acid (C16:1) and 9-octadecenoic acid (C18:1) were also identified frequently (see Table 2).

In addition, some molecules with a high retention time were found in the majority of the traces. The library assigned them to the group of wax esters (WEs) without accurately identifying them. Thus, it was necessary to elucidate their mass spectra to determine their structure [22-23]. This was carried out using a method based on the fact that WEs, which are the result of the condensation between a fatty acid and a fatty alcohol, fragment specifically with electron impact (EI) ionization. By analysing those fragments, it is possible to determine the general structure of the molecule, especially the number of

carbons and double bonds of the alcohol and acid, and thus also those of a specific WE. Some limitations are however reported, such as the impossibility to determine the exact position of potential double bonds and branched carbons. It means that this method does not allow the differentiation between WEs isomers. A step of confirmation through analysis of reference substances is therefore needed for all WEs with double bonds and/or branched carbons in order to differentiate them through their retention time.

Insert Figure 1

The analysis of the mass spectrum of a specific WE present in all donor fingermark residues at a retention time (RT) of 32.32 min (Figure 1a) will serve as an example to highlight the key points of the identification procedure [22]:

1. The molecular ion (M^+) is always found at the end of the mass spectrum (major peak at the end of spectra). This ion allows the determination of the number of carbon atoms and the number of unsaturated moieties composing the wax ester. The reference tables in Fitzgerald & Murphy [2007] [22] represent a quick help for this step.

   In the example (Figure 1), this ion was identified to be the 450 m/z corresponding to a WE of 30 carbons and 1 double bond.

2. The number of carbon and double bonds composing the acid can be determined in observing the base peak of the mass spectrum. The ionisation of the acid composing the wax ester creates two specific fragments, $[R_nCO_2H_2]^+$ and $[R_nCO]^+$ (Figure 1B). If the acid is saturated, the ion $[R_nCO_2H_2]^+$ is the base peak, otherwise it is the ion $[R_nCO]^+$. If the base peak does not correspond to one of those ions, it means that the alcohol supports the unsaturated moiety. In this situation, the base peak would correspond to the ion $[CH_2=CH(CH_2)_nCH_3]^+$.

   The base peak in figure 2 at 236 m/z corresponds to an acid with 16 carbons and one unsaturated moiety (fragment $[R_{15}CO]^+$ of C16:1, for example palmitoleic acid). The second characteristic ion corresponding to that particular acid is also present (255 m/z = $[R_{15}CO_2H_2]^+$).

3. The last step is to determine the number of carbon and unsaturated moiety of the alcohol part (or acid) which is extrapolated from the two previous steps and finally verified with the two characteristic ions for the alcohol part of the WE: $[CH_2=CH(CH_2)_nCH_3]^+$ and $[R'_nOCO]^+$.

   In figure 2 the expected alcohol should have 14 carbons and no unsaturated moiety, which is confirmed by the presence of the fragments $[CH_2=CH(CH_2)_{11}CH_3]^+$ = 196 m/z and $[R'_{13}OCO]^+$ = 241 m/z.

To summarize, the wax ester at 32.32 min possesses 30 carbons and one unsaturated moiety located on the acid. The acid possesses 16 carbons and the alcohol 14 which corresponds to a wax ester of the family of myristyl palmitoleate (Figure 1B).

29 WEs were thus observed in the chromatograms (Table 2). In order to validate the identifications obtained by analysis of the mass spectra, reference compounds corresponding to the following five WEs (among the seven major WEs peaks recovered in the majority of traces) were purchased and analyzed by GC/MS: myristyl myristate (mM) myristyl palmitoleate (mPo), palmityl palmitate (pP), palmityl palmitoleate (pPo) and stearyl palmitoleate (sPo). Their retention times and mass spectra were compared to those found in the fingermark residues and the identification of these WEs was confirmed. It was however not verified that isomers did not yield the same retention time.

A list of all compounds identified and considered for further relative quantification as well as their distribution for each donor fingermarks is showed in Table 2.

Insert Table 2

Concerning the intra-variability in the traces from one donor collected over a period of two months, all the major compounds such as fatty acids, squalene and cholesterol were observed in every analysis, and in approximatively the same relative amount: squalene was the major peak, followed by the saturated fatty acids C16:0, C15:0 and C14:0 and finally cholesterol as the lowest peak. Concerning WEs, all those compounds were found in traces of donor 1 (Table 2) over the period of two months. If some of them were present in all traces such as mMo, mM, mPo, mP, pPo, pP and sPo, others were present only in traces containing a larger total amount of compounds. This indicated that these latter compounds probably fell under the limit of detection when the fingermark residues were poorer.

In the study of the intervariability, large qualitative variations were observed. In fact, among the compounds identified, only squalene, cholesterol, and one wax esters (mPo) were observed in the traces from all donors, but their relative amount varied largely. All other molecules were recovered in the traces from six to only one donor (Table 2). Among the major WEs recovered in traces from donor 1, their were frequently identified in the traces from other donors, from 4 (for example sPo) up to 6 donors (for example pPo). While some of the remaining WEs were present in the traces of up to five donors, some were identified only in the traces of one donor. The group showing the larger variability between donors was fatty acids, which were not present in traces from donor 2 and barely in traces from donor 5, while they were identified in relative large amount in traces for donor 1 and 7. According to these results, donors were classified in three groups of fingermark residues composition (see Figure 2). Group 1 with residues from donors 1 and 7 possessed the larger number of compounds including WEs. The relative amount of WEs was high particularly regarding fatty acids. Donor 7 presented some extra compounds not classified in any of the four groups mentioned before, methyl parabene and propyl myristate. Those compounds are commonly used in cosmetics products and were probably present on fingers by contamination; those results are coherent with the use of gel self-reported by this donor and are consistent with previous literature [19]. The fingermarks from donor 3, 4 and 6 (group 2), presented all the fatty acids identified but fewer WEs. The general amount of compounds was weaker than for group 1. Finally, the fingermarks from group 3 was poor in fatty acids of all kind and in WEs.

Insert Figure 2

This large intervariability may be significantly influenced by diet, disease, and physiology of the different donors [24]. Among the chosen population only the poor composition of donor 2 could be explained. This donor was using a cream against acne. In order to estimate its impact, this donor gave additional traces. Three were collected three days after a regular application of the cream, while three traces were collected three days after stopping the application. Results showed that regular use of cream significantly decreased the amount and variety of compounds present in the fingermark residues (Figure 3a). In spite of that phenomenon, squalene, cholesterol and some wax esters, such as mM, mMo, mPo and mP were still recordable (Table 2). In contrast, the group of fatty acids was absent. When the cream was not applied for several days, traces presented in general a greater amount of compounds. However, the more marked change was the fatty acids which appeared in the residues (Figure 3b), and this would place donor 2 in group 2 (without cream) instead of group 3 (with cream). The wax esters did not seem to be influenced by the use of the cream since they were comparable in both sets of experiments. Therefore, it was demonstrated that the use of the cream influenced directly the amount of compounds recovered in traces but also the initial composition of fingermarks. The group of fatty acid was the most sensitive group to the application of cream. This phenomena could be explained by the nature of acne, which is a particular illness increasing the production of sebum. The cream used to treat it contains active molecules which clean the skin and diminish the quantity of sebum on the face and, as a consequence, the quantity of compounds transferred on the fingers. The cream could not be detected in the chromatograms; therefore its use could not be demonstrated by through analysis of the fingermark residues.

Insert Figure 3

Relative Quantification:

Since important variations in the relative amounts of compounds were observed, the Relative Peak Areas (RPA_1) of the identified compounds was calculated to quantify them relatively. The reference compound used in the calculation from equation (1) was in a first step the internal standard (IS). Relative standard deviations (RSDs) were calculated using peak areas from the replicate analyses of the three collected traces (n=3) for each experiment. Concerning intravariability, large variations were observed for relative quantitative measurements on traces collected from donor 1. RSD calculated on traces collected over one day reached 47% for squalene and 35% for cholesterol. They were greater for fatty acids (up to 121%) and WEs (up to 90%). These variations increased for traces collected over a period of two months, to reach 48% for squalene and 52% for cholesterol and up to 114% for WEs (Table 3), while fatty acids were more reproducible over a period of two months than on one day.

Insert Table 3

Concerning the intervariability (different donors; n=7) all RSD increased in comparison to the intravariability (one donor; n=3): 95% for squalene, 75% for cholesterol, up to 160% for fatty acids and up to 120% for WEs (Table 3). For both intra and intervariability, squalene and cholesterol were the more stable compounds. Their RSD values were similar to those reported previously in the literature [8,19]. In practice, the quantity of initial secretion on fingers at the moment of deposition cannot be controlled and will influence significantly the quantity of recovered compounds. In order to reduce the influence of that factor, different ratios on the form of relative peak areas (RPA_2, eq. 2) between compounds inherent to fingermark residues were studied. A RPA_2 previously proposed was a ratio of squalene to cholesterol [19] and while it reduced the intra and intervariability significantly, other RPA_2 may be more adequate and were therefore investigated here.

Different RPA_2 were tested according to their RSD to find a more stable parameter in fingermarks residues initial composition. Moreover in order to be adequate to follow the aging of fingermarks over time, the following criteria had to be taken into account: the target compound X (quotient in the RPA_2), must decrease or increase as a function of time; the reference compounds Y, Z (denominator in the RPA_2), should be more stable over time than the target compound. This will allow the use of the defined RPA_2 as aging parameters to study the composition of fingermarks residues as a function of time after deposition. These selected compounds X, Y, Z, etc. should in addition be present in a majority of the residues in order for the ratio to be as universal as possible.

According to those criteria, two types of compounds had the potential to be target compounds: squalene and unsaturated fatty acids, since they degrade over time [4-5,8,19]. Squalene was in this study the major and less variable compound in each trace, it was therefore considered as a good target compound. In opposition, the amount of unsaturated fatty acids was considered to be too variable and they were therefore excluded. Cholesterol was selected because it was also quantitatively very reproducible in fingermarks residues, was also present in all analyzed traces and is known to degrade in a slower rate over time [19]. In addition, the seven major WEs (mMo, mM, mPo, mP, pP, pPo and sPo) were considered. They were indeed identified in the majority of traces and were the most abundant compounds of their group. Besides, Mong [5] previously suggested they have a more stable behavior over time than squalene or unsaturated fatty acids. Therefore, they may be good reference compounds for our purpose (Y, Z).

Among the combinations of the RPA_2 tested, those dividing squalene to one of WE were not an improvement in regard to the RPA(squalene/cholesterol). Therefore, an improvement was investigated by summing the cholesterol with one or several WEs as denominator. The intravariability and intervariability of each combination are illustrated in Figure 4A. The results are presented in function of the number of WE used in the sum. While intervariability (between different donors residues) increased sharply as a function of the number of wax esters used in the denominator of the ratio, intravariability (among residues from the same donor) only increased slightly and was never above the RSD of RPA(squalene/cholesterol). The RPA_2 using more than two wax ester in their definition

were less optimal in term of RSD. The RPA_2 with the lower RSDs using 0, 1, 2, 3, 4, 5, 6 and 7 WEs respectively are represented in Figure 4B: SQA/(cho+mMo), SQA/(cho+mM) and SQA/(cho+mMo+mM) presented the better reproducibility as well for the intravariability (< 20%) than for the intervariability (~ 40%) (Table 3).

Insert Figure 4

The results, particularly the RPA(SQA/(CHO+mM)), allow to reduce significantly the intravariability but also intervariability in the initial fingermark residues (Table 3). However the RPA_2 did not help much when the same donor stopped using anti-acne cream (donor 2, Table 4). In fact the RPA increased consequently when the cream was not used and the relative error decrease only slightly down to 38% for RPA(SQA/(CHO+mM)). This RSD value corresponds thus more to inter-variability (between donors) than intra-variability (same donor). Therefore the use of such a cream may influence importantly the results. The proposed RPAs should now be tested over time in order to evaluate if it can be used to reproducibly follow the aging of fingermark residues over time.

Insert Table 4

Enhancement techniques

In real cases, fingermarks need to be enhanced before being chemically analyzed. In order to evaluate if proposed RPAs could be implemented in practice, enhanced traces were analyzed. Thus, the influence of enhancement techniques was assessed on the ratio SQA/(cho +mM), which showed the best reproducibility. The RPAs of squalene, cholesterol and myristyl myristate to the internal standard were calculated.

Magnetic powder

This enhancement method yielded a large amount of contaminations in analyses which widely interfered with target compounds. Even with an additional step of filtration, compounds such as alcanes (eicosane - dotriacontane), phthalates and benzophenone were present in chromatograms. In the extract ion chromatograms (EIC), foreign compounds co-eluated with fatty acids, but not with squalene, cholesterol and WEs. The RPA could therefore be calculated. Concerning the influence of powder on the initial composition of traces, no significant differences were observed between treated and non treated traces as well for each compound than for the RPA_2 (Table 5 and figure 5). Therefore the only disadvantage of this method of enhancement is the contamination it brings in the analysis and its tendency to dirty the column.

1,2-Inadanediones

Regarding contaminations, this technique of enhancement brought some additional compounds which appeared in analyses such as naphtalenone and benzenamine. However they did not interfere with the target compounds use in the RPA_2 definitions. Since contaminations can result from the solvents or glassworks used for the treatment, specific recommendations should be made when chemical analysis may be performed after the enhancement process. Besides, since this enhancement technique is a bath of solution, cross contamination can occurred between samples treated in the same solution. The compounds dissolved in the solution from the previous samples may interfere with the following samples. Thus, it is suggested to treat samples in different solutions to avoid it. However, this does not prevent cross contaminations which could occur between traces on a same sample.

Concerning the results obtained for the three formulations of indanedione used in our laboratory, influence of the solvent on the different fingermarks compounds was observed (Table 5). Thus, the formulation using dichloromethane presented larger impoverishment of compounds, since only half the amount of squalene and cholesterol present in non treated traces were recovered in treated traces. In contrary, the preparation with methanol allowed the recovery of a similar amount of compound than for non treated traces. Regarding the RPA_2, the results showed no significant differences for the formulations with HFE-7100 in comparison to non-treated traces, while dichloromethane and methanol presented slightly larger differences; they cannot be interpreted as significant at this stage of research (Figure 5). Further investigations are actually needed to evaluate if these differences are reproducible.

The amount of compounds recovered between the solvents can be explained by their power to extract lipids. The step of immersion acts as an extraction step which is more or less efficient depending on the solvent. The loss of compounds it produces could be a limitation especially if traces contain low amount of residues. Therefore, the solvent allowing the recovery of the higher amount of compounds after treatment (methanol) should be chosen to enhance traces. However HFE-7100 can also be considered as it does not influence significantly the RPA_2.

Cyanoacrylate.

No contamination coming from the enhancement method was detected during the analysis and all the components of fingermark were recovered after treatment. Concerning the effect of the technique on the recovered amount of fingermark residues and on the RPA, results tend to show that the amount of squalene, cholesterol and mM of treated traces were not significantly different from those non-treated (Table 5). The ratios SQA/(CHO+mM) were also comparable (Figure 5).

Insert Table 5 and Figure 5

Conclusion

This study allowed identifying numerous lipid components in fingermarks residues. The wax esters were most particularly studied, as they were not specifically identified in traces so far. The results also confirmed the initial variability of those residues between fingermarks from different persons as well as among traces from the same person. This variability represents a major drawback for the development of fingermarks datation techniques. Thus, this study proposes a new approach reducing the relative lipid variability using parameters more reproducible from mark to mark: ratios between compounds intrinsic to fingermark residues.

In order to select the target compounds that could be used in the definition of these ratios, the fingermarks residues of seven donors were studied by GC/MS. It was observed that squalene and cholesterol were present in all fingermark residues, with squalene being always the largest peak. These observations confirmed the results obtained in previous publications [5,8,12,19]. The following 10 fatty acids were additionally identified in most residues: dodecanoic (C12:0), tridecanoic (C13:0), tetradecanoic (C14:0), pentadecanoic (C15:0), hexadecanoic (C16:0), octadecanoic (C18:0), 11-tetradecenoic (C14:1), 14-pentadecenoic (C15:1), 9-hexadecenoic (C16:1) and 9-octadecenoic (C18:1). Moreover 29 peaks could be attributed to WEs. Among them, seven were recorded in the majority of the fingermark residues: myristyl myristoleate (mMo), myristyl myristate (mM) myristyl palmitoleate (mPo), myristyl palmitate (mP), palmityl palmitate (pP), palmityl palmitoleate (pPo) and stearyl palmitoleate (sPo). In addition to fingermarks intrinsic compounds, cosmetics were identified in the residues of one donor. However the reported use of a cream against acne could not be detected in the chromatograms, while important effects on the lipid composition were observed. When the donor using this cream applied it regularly, a significant decrease in the amount and variety of compounds was noticed: the fatty acids were totally absent, while squalene, cholesterol and wax esters were recorded in lower amount than when the cream was not applied for several days.

The relative quantification of the compounds showed that squalene and cholesterol were more reproducible in the initial composition. However, in general, large variations in intra- and intervariability were observed for all the identified compounds. The identified compounds were then used for the definition of ratios, where the numerator was the peak area of squalene (compound decreasing as a function of time) and the denominator was the peak areas of several compounds more stable over time. Those definitions of new RPAs allowed reducing considerably the inter and intra-variability. PA(squalene) / PA(cholesterol) + PA(myristyl myristate) being the RPA showing the best reproducibility with a relative standard deviation under 20% for the intra-variability.

The influence of fingermarks enhancement techniques on the proposed ratio was also tested in this work. No significant differences between treated and non-treated traces were noticed after powdering or cyanoacrylate fuming. However, while no additional peaks were observed with cyanoacrylate, magnetic powder brought a lot of contaminations on the GC/MS column even after a filtration step. Enhancement with 1,2-indanediones also yielded some contaminations in the chromatograms and it was observed that the formulation using dichloromethane reduced the recovered amount of cholesterol and squalene by half in the fingermarks residues. With the formulations using methanol and HFE-7100, no significant differences were observed between the amounts

recovered in non-treated and treated traces. The RPAs was not influenced significantly by the enhancement techniques.

In conclusion the present study allowed finding a parameter that diminished significantly the lipid relative variability from mark to mark just after deposition and that also remained stable after applying enhancement techniques. The suitability of such a parameter must now be tested for the development of fingermarks residues dating methods. It should therefore be studied as a function of time in order to evaluate if it can be used to reproducibly follow the aging of fingermarks residues.

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Table 1 : Summary of parameters related to the sampling of traces for the different experiments carried out.

Aims	Experiment	Donor	Number of traces	support
Qualification and semi-quantification	Intervariability	1-7	3 per donor	Filter
	Intravariability	1	3 on morning 3 at midday 3 on afternoon	Filter
		1	3 x12 days (over two months)	Filter
		2	3 traces using anti-acne cream 3 traces not using anti-acne cream	Filter
Enhancement Methods	Powder	1	3 traces powdered 3 non treated traces	Glass
	1,2-Indanediones	1	3 formulation methanol, 3 formulation dichloromethane 3 formulation HFE-7100 3 non-treated	Filter
	Cyanoacrylate	1	3 traces enhanced 3 trace non treated	Glass

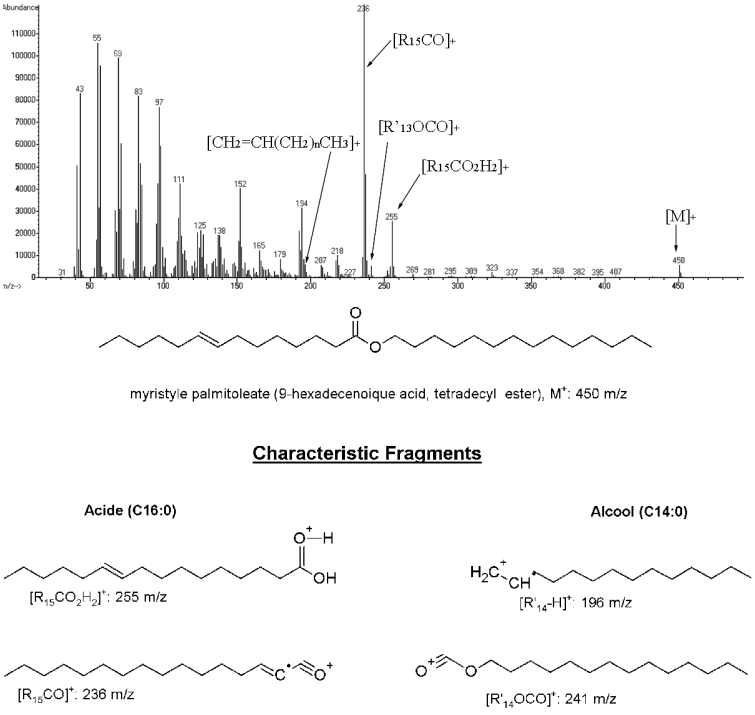

Figure 1 - (Above): Mass spectrum of peak of myristyl palmitoleate at 32.32 minutes in the chromatogram. The characteristic ions are labeled, including the molecular ion (M+) at 450 m/z and the base peak at 236 m/z. (Down): structure of the myristyl palmitoleate and its characteristics electron impact fragments for the acid and alcohol.

Table 2 : List of the identified compounds with their retention time (Rt), the methods of identification (DB= only through library comparison; MS = only through analysis of mass spectra, STD= identification confirmed by comparison with a reference compound), their group of molecule (FA = Fatty Acid; SP = sterol precursor; WE = Wax esters; S = Sterol), their target ion (tgt ion) and their absence/presence in each donor's traces (X= present; -= absent). In grey, three compounds present in the traces from all donors (squalene, cholesterol and myristyl palmitoleate).

Rt (min)	Compounds		Confir-mation	Type	Tgt ion	Donor 1	Donor (3 traces)						
						36 traces	1	2	3	4	5	6	7
10.97	Dodecanoic acid	(C12:0)	DB	FA	73	x	x	-	x	x	x	x	x
12.11	Tridecanoic acid	(C13:0)	DB	FA	73	x	x	-	x	-	-	x	x
13.08	11-tetradecenoic acid	(C14:1)	DB	FA	55	x	x	-	x	x	-	x	x
13.28	Tetradecanoic acid	(C14:0)	STD	FA	129	x	x	-	x	x	x	x	x
14.13	14-Pentadecenoic acid	(C15:1)	DB	FA	55	x	x	-	x	x	x	x	x
14.29	Pentadecanoic acid	(C15:0)	STD	FA	129	x	x	-	x	x	x	x	x
15.25	9-hexadecenoic acid	(C16:1)	STD	FA	236	x	x	-	x	x	-	x	x
15.31	hexadecanoic acid	(C16:0)	STD	FA	129	x	x	-	x	x	x	x	x
17.09	9-octadecenoic acid	(C18:1)	STD	FA	55	x	x	-	x	x	-	x	x
17.33	Octadecanoic acid	(C18:0)	STD	FA	129	x	x	-	x	-	-	x	x
27.46	Squalene	(SQA)	STD	SP	69	x	x	x	x	x	x	x	x
29.23	Al (C12:0); Ac (C16:1)		MS	WE	236	x	x	-	x	x	-	-	x
29.32	Al (C14:0), Ac (C14:1)	(mMo)	MS	WE	208	x	x	x	x	x	-	x	x
29.59	Myristyl Myristate	(mM)	STD	WE	229	x	x	x	x	x	-	x	x
31.13	Al (C14:0); Ac (C15:0)		MS	WE	243	x	x	-	x	-	-	-	x
31.74	Al (C14:0); Ac (C16:1)		MS	WE	236	x	x	-	x	-	-	-	-
31.93	Cholesterol	(CHO)	STD	S	386	x	x	x	x	x	x	x	x
32.31	Myristyl palmitoleate	(mPo)	STD	WE	236	x	x	x	x	x	x	x	x
32.41	Al (C16:0); Ac (C14:1)		MS	WE	208	x	x	x	x	x	-	-	x
32.64	Al (C14:0); Ac (C16:0)	(mP)	MS	WE	257	x	x	x	x	x	-	-	x
33.29	Al (C16:0); Ac (C15:1)		MS	WE	222	x	x	-	x	-	-	-	x
33.39	Al (C14:0); Ac (C17:1)		MS	WE	250	x	-	-	x	-	-	-	-
33.53	Al (C17:0); Ac (C14:1)		MS	WE	208	x	x	-	x	-	-	-	x
33.8	Al (C15:0); Ac (C16:1)		MS	WE	236	x	x	x	x	x	-	-	x
34.12	Al (C16:0); Ac (C15:1)		MS	WE	243	x	x	-	x	-	-	-	x

34.72	Al (C16:0); Ac (C16:1)		MS	WE	236	x	x	-	x	x	-	-	-
35.28	Palmityl palmitoleate	(pPo)	STD	WE	236	x	x	x	x	x	-	x	x
35.37	Al (C18:0); Ac (C14:0)		MS	WE	208	x	x	x	x	-	-	-	x
35.54	Palmityl palmitate	(pP)	STD	WE	257	x	x	x	x	x	-	-	x
35.54	Al (C18:0); Ac (C14:0)		MS	WE	229	x	x	x	x	-	-	-	-
35.81	Al (C17:0); Ac (C16:1)		MS	WE	236	x	x	x	x	x	-	-	x
36.34	Al (C17:0); Ac (C16:1)		MS	WE	236	x	x	x	x	x	-	-	-
37.57	Al (C18:0); Ac (C16:1)		STD	WE	236	x	x	x	x	x	-	-	x
38.1	Stearyl palmitoleate	(sPo)	MS	WE	236	x	x	-	x	x	-	-	x
38.37	Al (C18:0); Ac (C16:0)		MS	WE	257	x	x	-	x	-	-	-	-
39.5	Al (C20:0); Ac (C15:0)		MS	WE	257	x	-	-	x	-	-	-	-
40.19	WE unknown		MS	WE	69	x	-	-	x	-	-	-	-
40.65	Al (C20:0); Ac (C16:1)		MS	WE	236	x	x	-	x	-	-	-	-
40.94	WE unknown		MS	WE	237	x	x	-	x	-	-	-	-
41.31	Al (C20:0); Ac (C16:1)		MS	WE	236	x	x	-	x	-	-	-	-
43.64	WE unknown		MS	WE	236	x	x	-	-	-	-	-	-

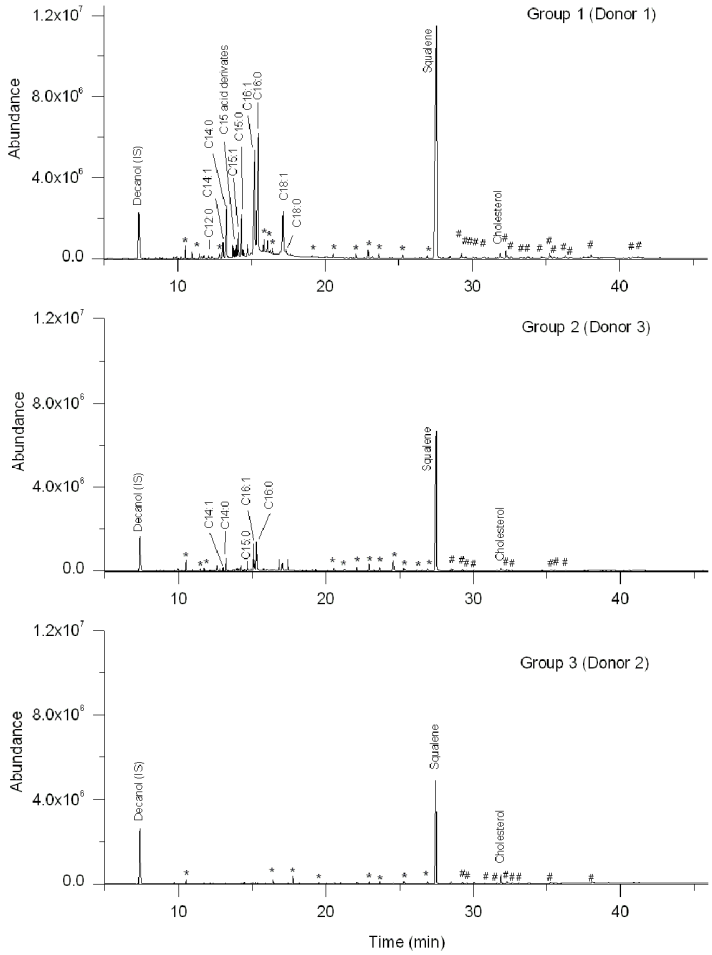

Figure 2 : Representative chromatograms of the three groups of fingermark residues composition observed: above group 1 (donor 1 and 7), middle group 2 (donor 3, 4 and 6), below group 3 (donor 2 and 5). Contaminations brought by the method of extraction are represented by *, while the wax esters are labeled with #.

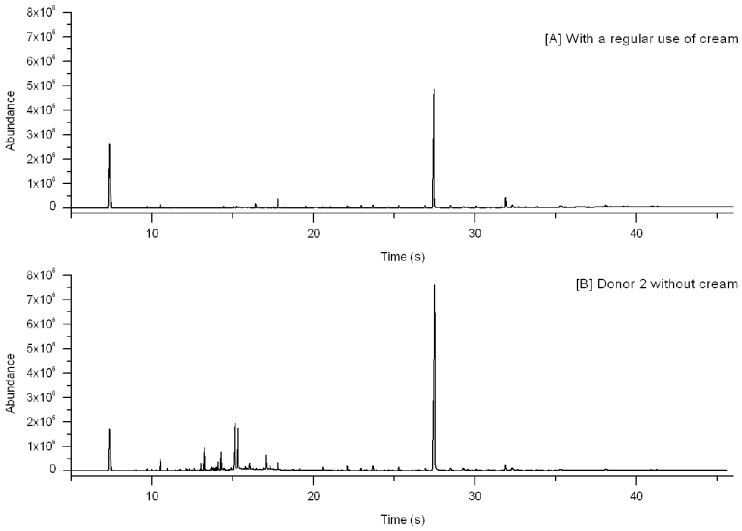

Figure 3 : Chromatograms of traces from donor 2 when he applied cream regularly [A] and when he did not apply cream during 3 days [B]

Table 3: Relative Standard Deviation (RSD) calculated on the relative peak areas (RPAs) of the major compounds among those identified frequently in the traces. The intra-variability was calculated for donor 1 on 1 day and over 2 months, while the inter-variability was calculated from traces from the seven donors. n is the number of traces analyzed for the given calculations.

RSD % (RPA)	Donor 1, one day (n=3)	Donor 1, traces over a period of 2 months (n=12x3)	Donor 1-7, (n=7x3)
11-Tetradecenoic acid/IS	111	70	154
Tetradecanoic acid/IS	110	79	158
Pentadecenoic acid/IS	121	70	157
Pentadecanoic acid/IS	114	71	153
Palmitoleic acid /IS	115	67	158
Palmitic acid/IS	114	68	160
SQA/IS	47	48	95
CHO /IS	35	52	75
mMo/IS	76	74	116
mM/IS	74	68	104
mPo/IS	74	75	113
mP/IS	76	73	108
pPo/IS	83	79	110
pP/IS	90	74	113
sPo/IS	85	114	120
SQA/CHO	14	36	54
SQA/(CHO+mMo)	7	19	38
SQA/(CHO+mM)	6	18	41
SQA/(CHO+mMo+mM)	11	19	40

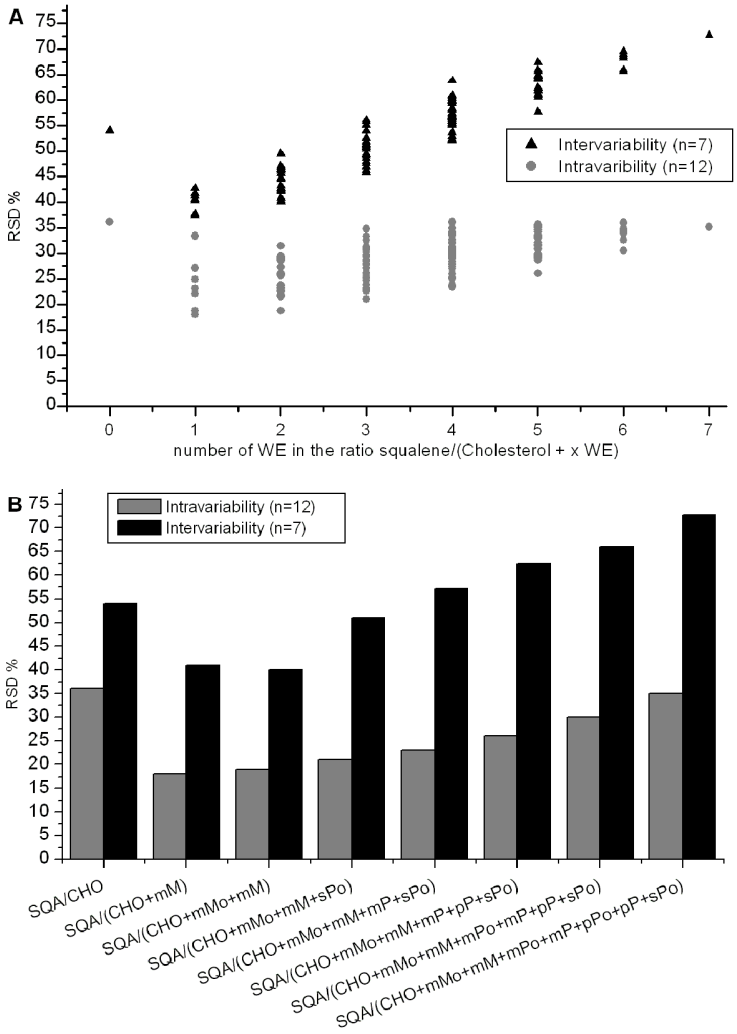

Figure 4 : A: Relative standard deviations (RSDs) of tested relative peak areas (RPAs= SQA/(cho+ x WE) tested as a function of the number (x) of wax ester (WEs) used in the denominator. The intervariability was calculated from the fingermark residues of one donor, while the intervariability was calculated from the residues of seven donors. B: Details of the lowest RSDs of each class (x) illustrated in figure A.

Table 4: Mean RPAs and their RSD for donor 2 with and without anti-acne cream. The relative error between the two sets of mean measurements was calculated.

RPA	Cream (n=3)		No Cream (n=3)		Relative Error
	mean	RSD %	mean	RSD %	%
Squalene	10.19	40	124.04	29	85
Cholesterol	0.17	47	0.61	32	56
Myristyl myristate	0.05	42	0.55	36	83
SQA/CHO	62.75	13	204.36	6	53
SQA/(CHO+mM)	48.06	13	108.04	6	38

Table 5 : RPA and RSD of the three compounds used in the ratio and the ratio itself for the three enhancement techniques studied. The RSD were calculated on the three replicates.

Enhancement technique		RPA Squalene		RPA cholesterol		RPA mM		SQA/(CHO+ mM)	
		mean	RSD %	mean	RSD %	mean	RSD %	mean	RSD %
Powder	non-treated	78.45	31.49	0.24	26.52	0.14	37.86	209.39	3.22
	treated	109.82	18.15	0.30	11.89	0.21	18.04	214.09	4.82
Indanediones	non-treated	94.69	26.10	0.37	20.19	0.18	27.59	170.71	7.51
	Dichloromethane	41.27	36.94	0.15	38.38	0.07	43.77	184.22	3.86
	HFE-7100	76.81	71.97	0.34	70.23	0.13	106.65	176.71	19.65
	Methanol	113.46	31.08	0.52	31.51	0.22	33.54	153.67	1.22
Cyanoacrylate	non-treated	78.45	31.49	0.24	26.52	0.14	37.86	229.61	6.70
	treated	109.82	18.15	0.30	11.89	0.21	18.04	214.09	4.82

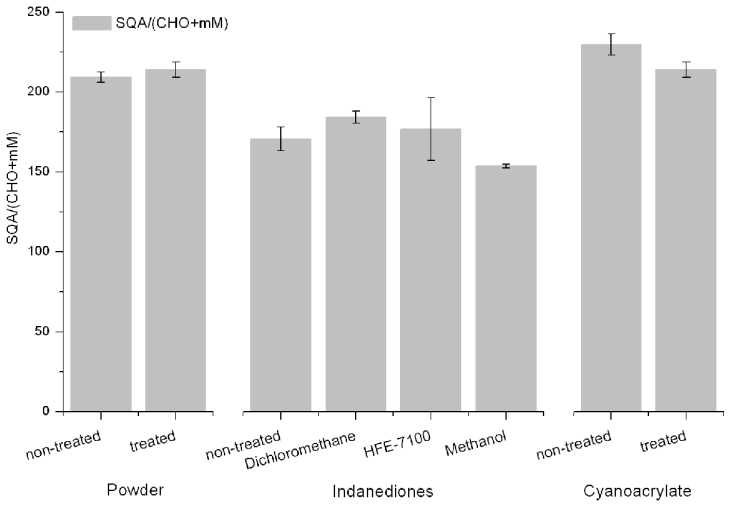

Figure 5 : Relative Peak Area (RPA) of squalene to cholesterol and myristyl myristate (SQA/(CHO+mM)) as a function of the applied enhancement technique.