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Céline Weyermann,^{1*} Ph.D.; Claude Roux,² Ph.D.; Christophe Champod,¹Ph.D.

Initial Results on the Composition of Fingerprints and its Evolution as a Function of Time by GC/MS Analysis

¹Institut de Police Scientifique, Université de Lausanne, Bâtiment Batochime, CH-1015 Lausanne, Switzerland.

²Centre for Forensic Science, Department of Chemistry, University of Technology, PO Box 123, Broadway, AUS - 2007 Sydney, Australia.

* Corresponding author: celine.weyermann@unil.ch

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ABSTRACT: Determining the time since deposition of fingermarks may prove necessary in order to assess their relevance to criminal investigations. This dating issue was previously addressed in specific forensic cases, but was never resolved due to the lack of fundamental data about fingerprints composition. The crucial factor is the initial composition of fingermarks because it represents the starting point of any ageing model. However, systematic studies on the subject are scarce and quantitative data is particularly lacking. This study mainly aims to characterize the initial composition of fingerprints, which show a high variability between donors (inter-variability), but also to investigate the variations among fingerprints from the same donor (intra-variability). Some solutions to reduce this initial variability using squalene and cholesterol as target compounds are proposed and should be further investigated. The influence of different substrate on the initial composition of fingerprints was also studied and preliminary aging studies over 30 days were carried out on a porous and a non-porous substrate to evaluate the potential for dating of fingermarks.

KEYWORDS: forensic science, fingerprint, aging, dating, gas chromatography mass spectrometry, squalene, cholesterol.

Fingermarks are composed of a complex mixture of substances that are transferred from a person fingers (more particularly from their friction ridges skin) on a surface, generally as latent marks. This means that they have to be detected or enhanced to be further examined as part of a standard forensic procedure. Knowledge of their chemical composition and changes over time proved to be an important factor in the development of new detection techniques [1,2]. A second key issue in the study of fingerprints composition and aging lay in the determination of the time at which a fingermark was placed at a crime scene [3]. For example, the discovery of a fingermark on a crime scene helps establish the presence of a person at the location at some time. However the identified person may declare that the contact took place before or after the crime occurred. The evidence may lose its relevance unless the time of contact can be demonstrated to be within the period when the crime was committed [4]. In order to determine the age of fingermarks, the initial composition is a crucial factor because it is the starting point of the aging curve. This study therefore focused therefore mainly on the initial composition of fingerprints using gas chromatography coupled to mass spectrometry (GC/MS) and aimed at developing solutions to improve the reproducibility of the results for fingerprints initial composition originating from the same donor. Moreover, the influence of different substrate on the initial composition of fingerprints was also studied and preliminary aging studies over several days were carried out on a porous and a non-porous substrate to evaluate the potential for fingerprints dating.

Initial composition of fingerprints

The numerous substances found in fingerprints can have five different sources: eccrine sweat, apocrine sweat, sebum secretions, epidermic substances and external contaminations from the environment. Eccrine secretions are located on the hands and are therefore always

present to some degree in fingerprint residues. While sebaceous secretions are also very common, due to contamination through the touching of the face and hairs; apocrine secretions are less frequent in fingerprints, however generally significant in crimes of a sexual nature [5]. Many studies, generally of a medical nature, investigated into the composition of sweat gland secretions of human skin [6-13]. However, few systematic forensic studies were carried out on the actual composition of fingermarks [5,14,15]. Eccrine and apocrine secretions results in a mixture of inorganic compounds and water-soluble organic compounds (eg. NaCl, urea, amino acids), while sebaceous secretions are fat-soluble compounds (eg. glycerides, fatty acids, wax esters, squalene, sterols, sterols esters) [2]. Proteins are known to be present in fingerprints [10], but have only lately been the subject of forensic research [16]. Gas chromatography was previously used by several authors to determine and study the composition of fingerprints (Table 1).

The influence of the donor's age was studied and fingerprint residues from children were found to have a different composition compared to residues left by adults [20]. This observation is not surprising since the activity of glands varies throughout life [21,8]. Based on this observation, a recent study proposed to predict the age of individuals from the composition of their fingerprint residues [22]. The influence of the gender was also studied and no significant differences were highlighted between male and female donors [23]. It should however be noted that some important variations among different individuals were generally observed (inter-variability) [14,17]. Nevertheless, the following compounds were detected in all residues from fresh fingerprints from adults: fatty acids (palmitoleic acid, palmitic acid, oleic acid, stearic acid), steroid precursors (squalene) and wax esters.

Factors affecting the composition

Several factors affecting the composition of fingerprints were addressed in the literature, however the extent of influence of combination of factors has not been fully elucidated to date [3]. The influence of the substrate on fingerprints transfer was acknowledged, but actually barely studied. The state of knowledge in the fingerprint physics was summarised early by Thomas [24], and much later the physico-chemical processes and surface phenomena were also considered [25]. Factors such as temperature, humidity, structure and electrostatic forces of the receptor surface play significant roles. In general, three types of surfaces are considered to select appropriate detection methods because they influence the composition of fingerprints [2,26]:

- *porous* (eg. paper, cotton, wood): the water soluble compounds are quickly absorbed, while fat soluble compounds stay on the surface for a longer period;
- *semi-porous* (eg. varnished wood; waxed surfaces, plastics): the water soluble compounds are absorbed more slowly, the fat soluble compounds stay longer on the surface.
- *non-porous* (eg. glass, metal, paints, plastics): all fingerprint substances stay on the surface until they degrade or are wiped out.

However no detail about the differences in fingerprint composition as a function of the substrate was published yet, except the recent work of Almog et al. [27] about the correlation of fingerprints penetration depth and quality.

Change in composition after deposition

Generally, studies on the composition changes of fingerprints were carried out in order to improve and adapt detection techniques [14,26,28,29], but not to actually help assess the age

of prints. The influence of environmental factors on the composition / degradation of fingerprints were studied for exposure to high temperature [19], exposure to light [17], immersion into water, humidity, exposure to radiation [30,31], and biological decontaminant [32].

Some authors though inferred the fingerprint age according to its rate of development or quality [33,34]. However, this approach was later demonstrated to be unreliable [35,36,37]. Fluorescence shifts as a function of the age was proposed [38], differences among individual donors and influence of washing hands were found to be crucial uncontrolled factors [39]. Some studies reported the diminution of squalene and other sebaceous compounds as a function of time in fingerprint residues [17,18,14], and confirmed the crucial influence of the initial quality of the trace. One study focused on the degradation products of squalene in latent fingerprint [40].

EXPERIMENTAL

Sample preparation

Six donors, three female donors (F1-F3) and three male donors (M1-M3) between 25 and 35 years old were chosen to prepare fingerprint samples. The donors did not follow a specific diet. They passed their finger onto their forehead before apposing their thumbs onto the substrates. After half an hour, the operation was repeated by the same donor, thus analysis was carried out on the residues obtained from four fingerprints (2 times left thumbs, 2 times right thumbs) for each sample type (specific donor, substrate and aging span) in order to take into account the intra-variability. The measurement standard deviations are represented in the

figures as error bars. The experimentations carried out in this study are summarised in Table 2. The inter-donor variations of fingerprint composition were studied on microfilters and on glass inserts, while fingerprints from one female donor F2 (30 years old, non-smoking, using skin lotions) were deposited on five different substrates to study the inter-substrate variability: microfilter (Whatman[®] GF/F paper, 2,5 cm diameter, 0.42 mm thickness, 0.7 µm pore size, Sigma Aldrich GmbH, Switzerland); glass insert (300µl, Laubscher Labs, Switzerland); glass surface (on the bottom of a volumetric flask, 100ml, Merck, Switzerland); paper (Business White Paper, A4, 89 g/m², Xerox[®], Switzerland); pvdf membrane (Immobilon[™] PSQ polyvinylidene difluoride membrane, Sigma Aldrich GmbH, Switzerland).

Samples from this donor (F2) on microfilter and glass insert were then stored in a cupboard in the laboratory with no light and no airflow for a few hours and up to 30 days. Temperature and humidity were not controlled; but they fell in a normal range of laboratory conditions (i.e., approximately 20-25 degree and 40-80 % RH).

Sample extraction

Fingerprints on substrates were extracted with 2 ml dichloromethane (> 99.9% pure, Fluka, Switzerland). The extraction solution was then evaporated under nitrogen flux. The dry extract was diluted in 20 µl of dichloromethane with 0.01 mg/ml anthracene as internal standard and 2 µl of this solution was injected for GC/MS analysis.

The reference substances squalene (Sigma Aldrich, Switzerland) and cholesterol (Fluka, Switzerland) were prepared at 0.05 mg/ml in dichloromethane with 0.01 mg/ml anthracene in order to confirm their identification in the chromatogram and associated MS data. A calibration curve was prepared for the quantification of squalene at the following concentrations: 0.1, 0.05, 0.01, 0.005, 0.001, 0.0005 mg/ml.

GC/MS analyses

Analyses were carried out on a Gas Chromatograph / Mass Spectrometer (6890N/5973 inert, Agilent Technologies Schweiz AG, Basel, Switzerland). Separation was carried out on a DB-1 / MS capillary column from Varian (Agilent Technologies Schweiz AG, Basel, Switzerland). The column was 30 m long and had an internal diameter of 0.25 mm and film thickness of 0.25 μm . The chromatographic elution was temperature programmed as follows: isothermal at 150°C for 2 min, from 150 to 250°C at a rate of 8°C/min, then from 250°C to 310°C at a rate of 6°C/min and finally isothermal at 310°C for 10 min. The carrier gas was helium with a constant flow of 1 ml / min. To improve sensitivity, the sample was injected in the splitless mode with a solvent delay of 4 min by autosampler. The injector temperature was maintained at 250°C.

For MS detection, ions were formed by electron impact (EI). Masses were scanned in the quadrupole from m/z 10 to 420 u. Peak areas were calculated on target ion basis (i.e., anthracene m/z 178; squalene m/z 69; cholesterol m/z 386). The obtained mass spectra were further evaluated employing the NIST database (MS Search Program Version 5.0, NIST, MSS Ltd. Manchester, England). For squalene and cholesterol, the results were confirmed by GC/MS analysis of reference substances, which allowed the comparison of the relative retention times and mass spectra of the samples and standards. For quantification of squalene, the calibration curve was determined by liquid injection of 1 μl of the solutions.

RESULTS AND DISCUSSION

3.1. Initial composition

Squalene and cholesterol were identified in all fingerprint residues (confirmation was obtained by the analysis of reference substances). Additionally, several squalene derivatives, fatty acids and wax esters were also identified by comparison to the MS library. Among the six donors, the main fatty acid detected was palmitic acid. Squalene was always the largest peak, except for one female donor. The largest peak in her fingerprints was octyl methoxy cinnamate, which is a common ingredient in beauty products. In fact, female fingerprints showed more compounds than male fingerprints in this study. These compounds could generally be associated with the use of skin lotions or perfumes. Excluding contaminations by skin lotions, squalene actually was the largest peak in all analysed fingerprints. When the mean peak area of squalene was compared between donors, important differences were observed in the initial amount of squalene: concentration actually ranged from 1 to 11 μg (absolute weight in fingerprint residues). The relative amount of cholesterol varied comparatively to squalene between the different donors (Figure 1). F1 fingerprints showed the mean lowest amount of squalene and cholesterol, while M2 fingerprints showed the mean largest amount of squalene and cholesterol.

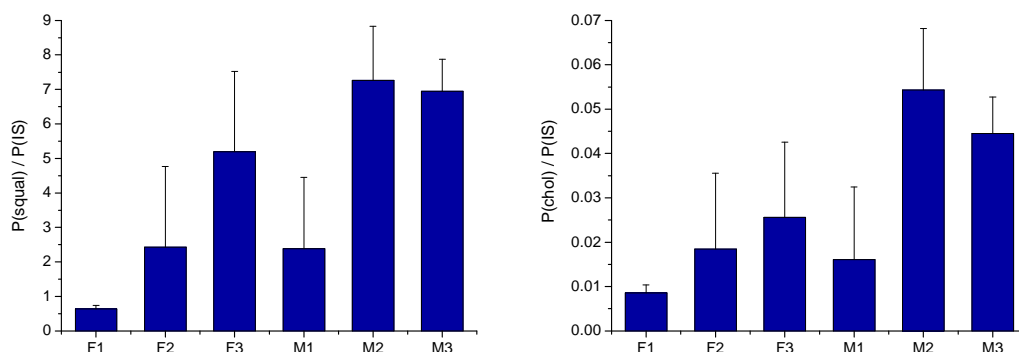


Figure 1 – Differences in the relative peak areas (P) of squalene (squal) and cholesterol (chol) to internal standard (IS) in the fingerprint residues from three female donors (F1-F3) and three male donors (M1-M3) on microfilters.

A main problem is to achieve reproducible quantitative sampling (see large error bars in Figure 1). This is particularly important as it represents the starting point of the aging curve of fingerprint residues. For example, people do not have all the same finger sizes and that may have influence significantly the results. However, no significant correlation could be measured between the fingerprint surface and the amount of squalene and cholesterol detected (i.e., Pearson correlation values obtained were 0.19 and 0.31 respectively). Other crucial factors are the difference in diet, the amount of perspiration or the washing of hands. These are however particularly difficult to evaluate. The repeatability (analyses carried out on the same day) and reproducibility (analyses carried out on different days) were evaluated on fingerprints from one donor (F2) on microfilter. Each analysis was repeated on four fingerprints made under the same conditions. (Figure 2 - left). The relative standard deviations (RSD) were calculated for the compounds squalene and cholesterol and proved to be very high (up to 80%; see Table 3). These values could be substantially reduced using relative peak areas definitions with the addition of an internal standard (eg. anthracene) or the use of a compound inherent to fingerprint composition (eg. cholesterol). By dividing the peak area of squalene by the peak area of cholesterol, the RSD for repetability was always under 20% (Table 3, Figure 2 - right).

RSD / %	peak area P		ratio to internal standard (IS)		ratio of peak areas
	P(squal)	P(chol)	P(squal) / P(IS)	P(chol) / P(IS)	P(squal) / P(chol)
same day	50	50	51	50	10
different days	71	79	50	53	39

Table 3 – The relative standard deviation (RSD) of squalene (squal) and cholesterol (chol) were calculated on measurements carried out on fingerprint of donor F2 on microfilms deposited the same day or different days. Results can be slightly improved by the use of an internal standard, while the best reproducibility was obtained when the peak area of squalene was divided by the peak area of cholesterol.

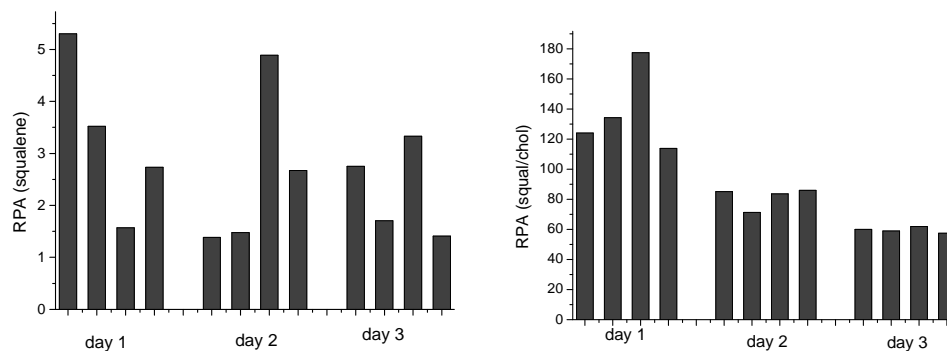


Figure 2 – Representation of the intra-donor variability. Each bar represents one analysis of a fingerprint from donor F2. The $RPA_{\text{squalene/IS}}$ showed large differences between measurements (right). While $RPA_{\text{squalene/cholesterol}}$ allow to reduce the difference for the samples taken the same day, large differences can still be observed between different days (left) .

The influence of donors could be slightly reduced by this approach (Figure 5 - left), however the observed variations are still significant and must be considered when studying the aging of fingerprints, i.e. the initial composition is essentially donor dependent.

3.2. Influence of the substrate

The initial composition of fingerprint residues was also compared on different porous to non-porous substrates (Figure 3). For fingerprint residues from donor F2, best results were obtained on pvdf for which all peaks were generally slightly larger than for microfilter and paper, while fingerprints on glass gave the lowest results (ca. 50% of the quantity found on pvdf). Residues were found in higher amount on porous surface than non-porous surfaces.

The tendency was confirmed with fingerprints residues from other donors (Figure 4). A clear influence of the substrate was thus demonstrated.

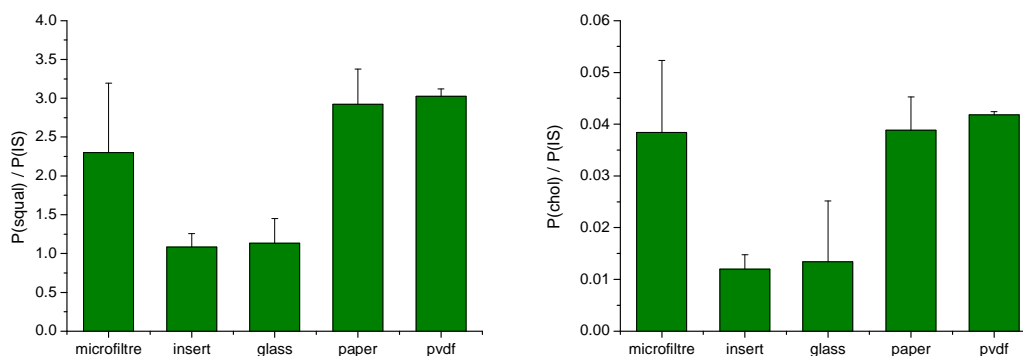


Figure 3 – Differences in the relative peak areas (P) of squalene (squal) and cholesterol (chol) to internal standard (IS) in the fingerprint residues from donor F2 on five different surfaces: microfiltre, glass insert, glass, paper and pvdf. The amount of recovered substances is higher on porous surfaces.

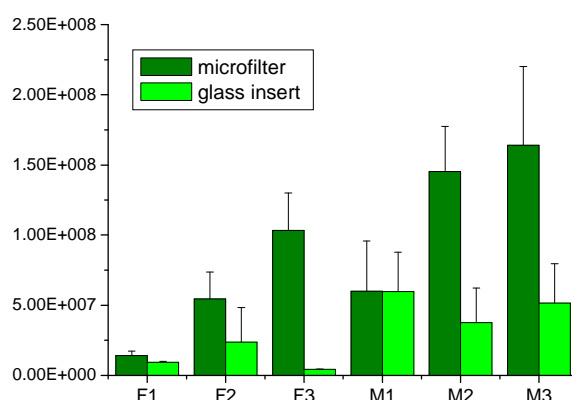


Figure 4 – Differences in the peak areas (P) of squalene (squal) in the fingerprint residues from six donors deposited on microfiltre and glass insert. The amount of recovered substance is higher on porous surfaces.

The influence of the substrates is also slightly reduced by the relative peak area approach (Figure 5 - right), however the observed differences are still significant and the influence of the substrate must therefore be considered when studying the aging of fingerprint residues.

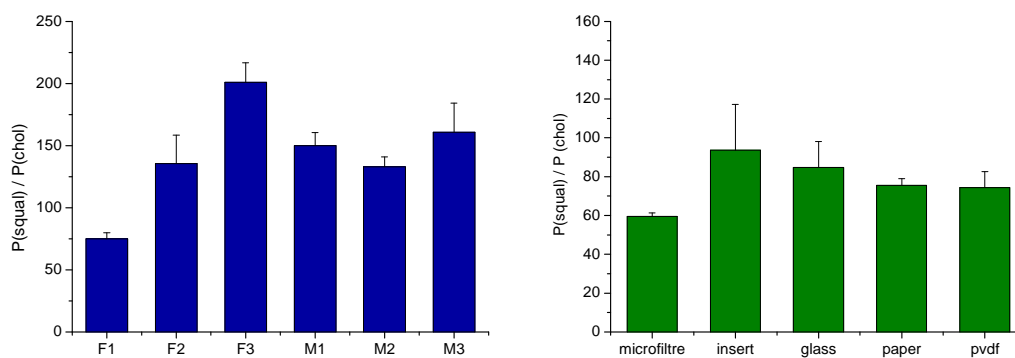


Figure 5 – Relative peak areas (P) of squalene (squal) to cholesterol (chol) in the fingerprint residues from six different donors (left) and five different substrates (right).

3.3. Aging kinetics

The evolution of identified compounds was studied as a function of the time for one donor (F2) on microfibre and glass substrates. The majority of the substances, such as fatty acids, did not show any significant change as a function of time. Squalene peak area decreased, while some squalene derivatives did initially increase (i.e. these are probably products of the degradation of squalene [40]). Interestingly, squalene was observed to diminish extremely quickly on glass and could barely be detected anymore after a few days (Figure 6 - left). Whereas the decrease on microfibre substrate followed a much slower rate and could still be detected in relatively high amount after 30 days (Figure 7 - left). Cholesterol diminished at a much slower rate on glass support (no decrease after one day) and did not show any change on microfibres over the time frame studied. When the peak area of squalene was divided by the peak area of cholesterol, lower error rates particularly for the initial hours and better fits were obtained for the aging curves (Figure 6 and 7; Table 4), particularly on microfibres.

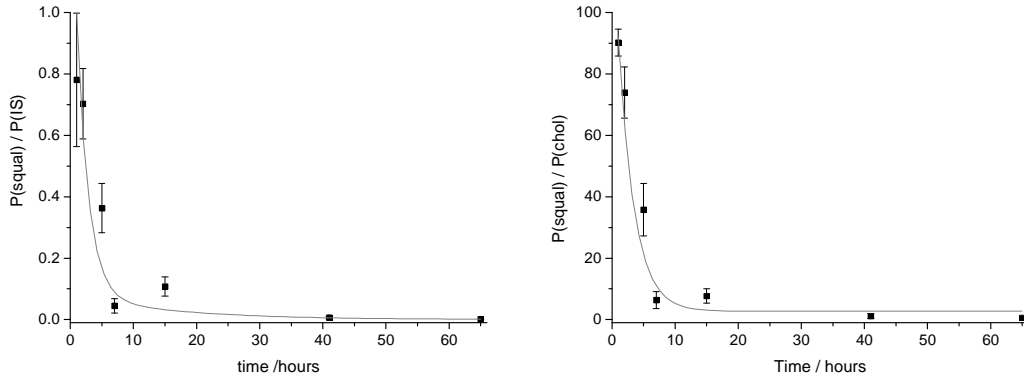


Figure 5 - GC/MS relative peak areas (P) of squalene (squal) divided by the peak areas of an internal standard (IS - left) or cholesterol (chol - right) as a function of time after deposition of fingerprints on glass inserts: 1, 2, 5, 7, 15, 45 and 65 hours.

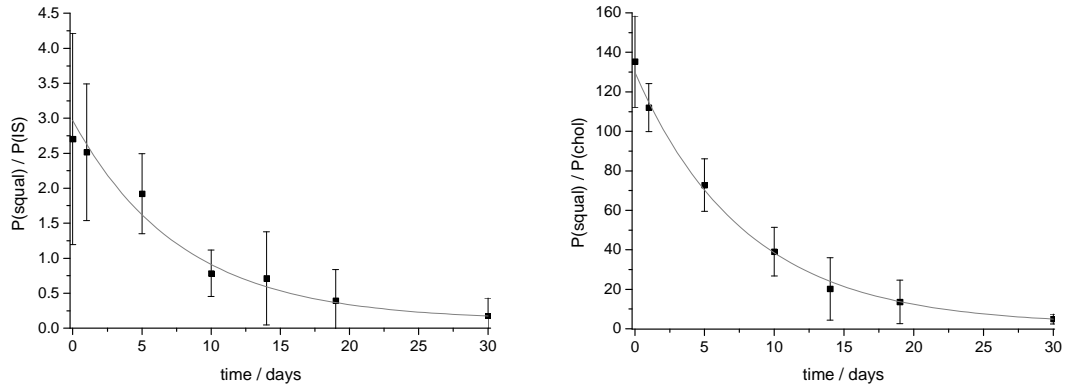


Figure 6 - GC/MS relative peak areas (P) of squalene (squal) divided by the peak areas of an internal standard (IS - left) or cholesterol (chol - right) as a function of time after deposition of fingerprints on microfilters: 0, 1, 5, 10, 14, 19 and 30 days.

$y = a \cdot e^{(-x/t)}$	glass insert		microfilters	
	P(squal)/P(IS)	P(squal)/P(chol)	P(squal)/P(IS)	P(chol)
a	1.48	131.35	2.84	127.56
t	2.37	2.80	7.27	8.79
R²	0.724	0.964	0.962	0.997

Table 4 - Exponential decay fitting of the aging curves represented in Figure 6 and 7 for the decay of squalene in fingerprint residues. The fits are better for the relative peak area (P) of squalene (squal) divided by the peak area cholesterol (chol).

These results demonstrate the potential of GC/MS to follow the aging of fingerprint in a more reproducible way than was suggested previously [17,18], when ratios of internal products are used.

4. CONCLUSION

Previous research have shown that the composition of fingerprints residues are too variable to allow any prediction about the age of fingermarks within reasonable boundaries [17]. This paper showed that a study into the relative amounts of intrinsic residue components may help towards developing a reliable method. The composition and aging of fingerprint residues were studied by GC/MS in order to assess the potential of the method for the purpose of dating fingermarks. The initial composition of fingerprint residues was therefore studied among six donors and on five different substrates. Squalene and cholesterol were identified in fingerprints of all the donors, which additionally contained several squalene derivates, fatty acids and wax esters. The reproducibility of the results represented the main challenge, because composition varied greatly from one deposition to another among fingerprints from

different donors (inter-variability), but also among fingerprints from the same donor (intra-variability). The initial composition is a crucial factor because it is the starting point of fingerprints aging curves. Therefore, a solution to address this variability issue was proposed using relative peak area definitions between compounds inherent to fingerprints. For example, using the relative peak area of squalene to cholesterol, the relative standard deviation of the relative amounts could be reduced substantially. This variation was however still significant for fingerprints from the same donor deposited on different days.

The influence of substrates was also evaluated and the initial composition was observed to be larger on porous surface than non-porous surfaces. The relative peak area definition allowed to diminish slightly this variability, because the amount of squalene and cholesterol transferred on the surfaces was comparable.

The aging of fingerprints was then studied on glass and microfilter over several days. On glass, squalene decreased very quickly the first day and could not be detected anymore over one week, while the decrease occurred in a much slower pace on microfilters. It could still be detected after 30 days. Other compounds did not show any significant changes over the time frame studied and the relative peak area of squalene to cholesterol yielded more reproducible aging curves.

The intra-donor variability for the ratio squalene/cholesterol at the time of deposition is currently the main limiting factor towards robust time since deposition estimation. Further studies, including alternative analytical methods such as desorption electrospray ionization mass spectrometry (DESI-MS), should aim at identifying other potential intrinsic target compounds that, in combination, will show limited intra-donor variability at $t=0$ while still showing detectable changes over time.

5. ACKNOWLEDGEMENTS

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