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Latent Fingermark Chemical Profiling

Identification of Time-dependent Metabolic Markers

Ana Damião dos Santos

Dissertation thesis for the degree of Master of Forensic Sciences and Laboratory Techniques

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Submitted to the University Institute of Health Sciences

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To my parents,
Adélia and José

"Live as if you were to die tomorrow. Learn as if you were to live forever."

Mahatma Gandhi

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ABSTRACT

Latent fingermarks are one of the most commonly used evidence in forensic investigation. Between other constituents, residues of latent fingermarks are predominantly characterized by lipids, which are important compounds in their dating studies, due to their degradability. Thus, this work consisted on studying the chemical profile of fingermarks, generating knowledge about the composition of latent fingermarks at precise moments, in order to perceive chemical changes that occur over time.

Fingermarks were aged and collected in a controlled manner and the chemical profiles were obtained by chromatographic techniques, namely high-performance liquid chromatography with diode-array detection (HPLC-DAD), gas chromatography - flame ionization detector (GC-FID) and gas chromatography - mass spectrometry (GC-MS).

Squalene was the compound that appeared in greater abundance, followed by palmitic and palmitoleic acids. Stearic acid, myristic acid, tetradecanoic acid and pentadecanoic acid were also determined in considerable concentrations. In addition to these results, and to the best of our knowledge, the qualitative analysis of residues obtained from fresh fingermarks allowed the identification of compounds so far unreported, namely polyunsaturated fatty acid *cis*-11,14-eicosadienoic, butanoic acid, dodecanol, methyl-6-palmitoyl-α-D-glucopyranoside, glycerol palmitate, tetradecanol, hexanedioic acid dibutyl ester, hexadecanol, 13-methyltetradecanoic acid, oleic acid methyl ester, heptadecanoic acid, octadecanol, tetramethylhexadecanol, 9-octadecenamide, docosane, nonacosane, cholesta-3,5-diene, docosanyl docosanoate, *cis*-9-tetradecenoic acid heptyl ester, arachidyl palmitoleate and epicholesterol. Cholesterol, docosane, palmitic acid and palmitoleic acid are assumed as potential temporal markers.

With this work, the chemical knowledge on the endogenous compounds of latent fingermarks was broadened, further studies being needed to strengthen the results herein obtained, allowing the establishment of suitable protocols for dating latent fingermarks.

Keywords: Latent fingermarks; chemical profile; endogenous and exogenous compounds; lipid compounds; mass spectrometry

RESUMO

As impressões digitais latentes são um dos principais vestígios analisados em investigação forense. Entre outros constituintes, os resíduos das impressões digitais latentes possuem maioritariamente lípidos, sendo estes compostos importantes em estudos de datação das mesmas, devido à sua degradação. Assim, este trabalho consistiu no estudo do perfil químico de impressões digitais, produzindo conhecimento acerca da composição das impressões digitais latentes em momentos específicos, permitindo perceber as alterações químicas que ocorrem ao longo do tempo.

As impressões digitais foram envelhecidas e coletadas de forma controlada e os perfis químicos foram obtidos através de técnicas cromatográficas, nomeadamente cromatografia líquida de alta eficiência com detetor de díodos (CLAE-DAD), cromatografia gasosa acoplada ao detetor de ionização de chama (CG-DIC) e cromatografia gasosa acoplada a espectrometria de massa (CG-EM).

O esqualeno foi o composto que apareceu em maior abundância, seguido pelos ácidos palmítico e palmitoleico. O ácido esteárico, o ácido mirístico, o ácido tetradecanoico e o ácido pentadecanoico também foram determinados em concentrações consideráveis. Para além destes resultados, e segundo o que conhecemos, a análise qualitativa de resíduos obtidos através de impressões digitais frescas permitiu a identificação de compostos ainda não reportados, nomeadamente ácido gordo polinsaturado *cis*-11,14-eicosadienoico, ácido butanoico, dodecanol, metil-6-palmitoil-α-D-glucopiranósido, palmitato de glicerol, tetradecanol, éster dibutílico de ácido hexanodioico, hexadecanol, ácido 13-metiltetradecanoico, éster metílico de ácido oleico, ácido heptadecanoico, octadecanol, tetrametil-hexadecanol, 9-octadecenamida, docosano, nonacosano, colesta-3,5-dieno, docosanil docosanoato, éster heptílico de ácido *cis*-9-tetradecenoico, palmitoleato de araquidil e epicolesterol. O colesterol, o docosano e os ácidos palmítico e palmitoleico são assumidos como potenciais marcadores temporais.

Com este trabalho, o conhecimento químico dos compostos endógenos das impressões digitais latentes foi alargado, sendo ainda necessários mais estudos para fortalecer os resultados obtidos, permitindo o estabelecimento de protocolos adequados para a determinação da idade das impressões digitais latentes.

Palavras-chave: Impressões digitais latentes; perfil químico; compostos endógenos e exógenos; compostos lipídicos; espectrometria de massa.

INDEX OF CONTENTS

INDEX OF FIGURES

INDEX OF TABLES

ABBREVIATION LIST

S	Slopes
ACN	Acetonitrile
BSTFA	*N*,*O*-Bis(trimethylsilyl)trifluoroacetamide
CAF	Cyanoacrylate fuming
DAD	Diode array detection
DESI	Desorption electrospray ionization
DFO	1,2-diazafluoren-9-one
FAME	Fatty acid methyl ester
FID	Flame ionization detector
GC	Gas chromatography
HPLC	High performance-liquid chromatography
IND	1,2-indanedione
LC	Liquid chromatography
LOD	Limit of detection
LOQ	Limit of quantification
MALDI	Matrix-assisted laser desorption/ ionization
MeOH	Methanol
MS	Mass spectrometry
MUFA	Monounsaturated fatty acids
PUFA	Polyunsaturated fatty acids
R^2	Coefficients of determination
SPR	Small particle reagent

SFA	Saturated fatty acids
UFA	Unsaturated fatty acids

Part I: Theoretical background

1.1. INTRODUCTION

Fingermarks can be classified as one of the most affirmative sources of personal identification, being an ubiquitous evidence found at crime scenes **(Weyermann et al., 2011; Peralta et al., 2017)**. Being used for over a century, their analysis still remains as one of the most valuable, trusted and widely used evidence in law enforcement and criminal investigations **(Dhall & Kapoor, 2016; Girod et al., 2016; Lauzon & Chaurand, 2018)**.

Each human ridge pattern is unique and persists unchanged over time for each individual **(Leśniewski, 2016; Wei et al., 2016)**, except in case of a severe skin damage **(van Dam et al., 2016)**. Consequently, due to the uniqueness of a human ridge pattern, fingermarks allow the individual identification and the confirmation or elimination of a suspect of a crime **(Girod et al., 2016; Bumbrah, 2017; Thandauthapani et al., 2018)**.

Normally, a latent fingermark emerges when the mixture of natural secretions and contaminants, present in finger skin ridges, is deposited directly and accidentally over a surface, leading to the development of a ridge pattern image like a copy **(Weyermann et al., 2011; Zhang et al., 2015; Bécue, 2016; Thandauthapani et al., 2018).** They are invisible to the naked eye, having less than 1 mg of biological material which contains hundreds of chemical species **(Francese et al., 2013; Bumbrah, 2016)**. As evidenced in recent years, fingermarks can offer much more than physical information **(Francese et al., 2017)**, namely profiting from their chemical information **(Lauzon et al., 2017)**, several studies being directed to the study of their composition **(Girod & Weyermann, 2014).** In fact, chemical profiling can be portrayed as the most exciting development on the investigation of components from fingermarks **(Bradshaw et al., 2013)**. There are two main types of information being obtained from fingermarks: an image reproducible from a fingermark deposited over a certain surface, dependent on the substrate as well as on environmental and deposition conditions; a group of secretions that is unique from one donor, whose composition can vary between individuals **(Bécue, 2016)**.

Fingermarks contain a wide range of chemical constituents that can be classified as endogenous, exogenous and semi-exogenous compounds **(Wei et al., 2016; Lauzon et al., 2017)**. The endogenous compounds, water-soluble and water-insoluble, originate in the eccrine and sebaceous glands, respectively **(Dorakumbura et al., 2018;**

Thandauthapani et al., 2018). The eccrine glands release sweat which is a mixture of water (approximately 98%) and proteins, amino acids, urea, lactic acid, uric acid, creatinine, choline and sugars. On the other hand, sebaceous glands release sebum, consisting of triglycerides, fatty acids, wax esters, squalene and sterol esters **(De Paoli et al., 2010; Wei et al., 2016; Bumbrah, 2017; Francese et al., 2017)**. Exogenous components, also called contaminants or contact substances, can be adhered to finger skin ridges by previously established contacts, found on the surfaces where the fingermarks have been deposited, or even may fall on the surface/ fingermarks after their deposition. These components include drugs, cosmetics, blood constituents and explosives, among others **(Bécue, 2016; Francese et al., 2017)**. Semi-exogenous compounds correspond to metabolites of xenobiotics administered by inhalation or ingestion, such as drugs and food, being released by the exogenous glands. In a certain way, exogenous and semi-exogenous compounds can give information about substances ingested or touched by the suspect, previously to the crime. As such, one can perceive both the donor's lifestyle and his relationship with a certain object and/or crime scene **(Francese et al., 2017)**.

The composition of fingermarks can vary, both qualitatively and quantitatively, between individuals (intervariability) or even in the same individual, from day to day or at different times of the day (intravariability) **(Szynkowska et al., 2009; Girod et al., 2012; Cadd et al., 2015)**. This intravariability derives, for example, from the daily contact with different contaminants, as well as from the individual's metabolism that varies over the course of a day **(Girod et al., 2012; Huynh et al., 2015)**. Additionally, notable differences derived from a distinct gender, age, race, health conditions and diet habits, have been also perceived as valuable and auxiliary tools on the discrimination of individuals **(Smijs et al., 2016; Wei et al., 2016)**. Particularly, endogenous substances can provide information about an individual, such as gender, age, race, and pathological state **(Leśniewski, 2016; Francese et al., 2017; Lauzon et al., 2017)**. As so, these constituents enable a discriminatory analysis, mainly due to their quantitative variation between individuals **(Huynh et al., 2015)**. The composition of a fingermark can also translate the individual's pathological state, since it can vary qualitatively and quantitatively in several classes of constituents such as lipids, proteins or other metabolites **(van Dam et al., 2016)**. **Francese and co-workers (2017)** reported that biomarkers of disease states, such

as proteins and peptides, can be used on the individual discrimination based on fingermarks' chemical profiling. For example, quantification of chloride may aid in the diagnosis of cystic fibrosis **(Calderón-Santiago et al., 2015)**.

Relevant, is also the fact that when deposited on a surface, the composition of a fingermark does not remain intact due to exposure to physical, biological and chemical agents, such as light, temperature and humidity. As previously mentioned by several authors, the composition of a fingermark has two phases: initial and aged composition **(Girod & Weyermann, 2014)**. The initial composition is defined by the transfer/deposition of residues from the finger skin ridges to a substrate, forming a fingermark at the first instance, while the aged composition is characterized by changes in the fingermark composition over time, derived from donor characteristics, contamination, oxidation, decomposition, evaporation, metabolism, polymerization and/or interactions with the surface **(Croxton et al., 2010; Girod et al., 2012; Girod & Weyermann, 2014; Cadd et al., 2015)**.

Dating a fingermark is a relevant procedure, allowing to estimate the moment of the fingermark's deposition, being extremely valuable on the identification of false events' claiming **(Cadd et al., 2015; Girod et al., 2016)**. However, additional systematic studies are needed since presently it is only possible to estimate the age of fingermarks **(Girod et al., 2016)**, through their classification as fresh or old **(van Dam et al., 2014)**. In order to determine the approximate age of a fingermark it is necessary to identify the best temporal biochemical indicators. Concerning this purpose, preliminary studies indicate that lipids can be valuable targets to be used in fingermark dating techniques, as evidenced in a study by **Girod & Weyermann, 2014**, demonstrating that the concentration of unsaturated fatty acids (UFAs) decreased dramatically in 30 days **(Mong et al., 1999; Cadd et al., 2015)**. In contrast, amino acids remain stable when exposed to light and temperature, being portrayed as suitable temporal indicators, due to their resistance to photo and thermodegradation **(Girod et al., 2016)**. In general, the deposition of a fingermark undergoes evaporation, unsaturated compounds giving rise to saturated analogues. These changes and the nature of the substrate have repercussions on the degradation of the fingermark residue over time **(Mong et al., 1999; Dorakumbura et al., 2018)**.

There are several methods often used to visualize fingermarks, such as physical, physicochemical and chemical methods. Different techniques are suitable or more appropriate under different conditions, which should be finely considered when choosing the most appropriate method, considering the type of surface, weather conditions, composition of latent fingermarks and compatibility with other techniques **(Jelly et al., 2009; van Dam et al., 2013)**. Small particle reagent (SPR) is one physical method of visualization, characterized by suspending fine particles of molybdenum disulfide or fine particles of titanium dioxide/ zinc carbonate or charcoal powder, which interact with sebaceous components (particularly fatty components) forming a gray, white and black deposit, respectively **(Jasuja et al., 2008; Bumbrah, 2016; Dhall & Kapoor, 2016)**. However, while efficient and easy-to-use, SPR displays low reactivity in aged fingermarks **(Bumbrah, 2016)**. Cyanoacrylate fuming (CAF) is a physicochemical method based on the interaction of vapors with the eccrine components, such as amines, alcohols, amino acids, alkanes and proteins, forming a white polymer **(Ramotowski, 2012; Sundar & Rowell, 2014; Bumbrah, 2017)**. Also routinely used, visualization of fingermarks through iodine fuming is based on the absorption of sublimated iodine crystals by sebaceous residues, namely UFAs, resulting in a yellowish brown colored evidence **(Almog et al., 1979; Jasuja et al., 2012; Olszowska et al., 2017; Zheng et al., 2017)**. Chemical methods of visualizing fingermarks such as ninhydrin, 1,2-diazafluoren-9-one (DFO) and 1,2-indanedione (IND), are usually applied to porous surfaces and react with certain endogenous water-soluble compounds **(Girod et al., 2012; Yang & Lian, 2014; Lauzon et al., 2017)**. Ninhydrin is a chemical reagent that targets amino acids of fingermark residues, resulting in the development of a purple color, named Ruhemann's purple, which arises when an amino group is eliminated generating ammonia, proving to be particularly useful in aged fingermarks (**Fig. 1**) **(Menzel, 2001; Jelly et al., 2009; Brunelle et al., 2016)**.

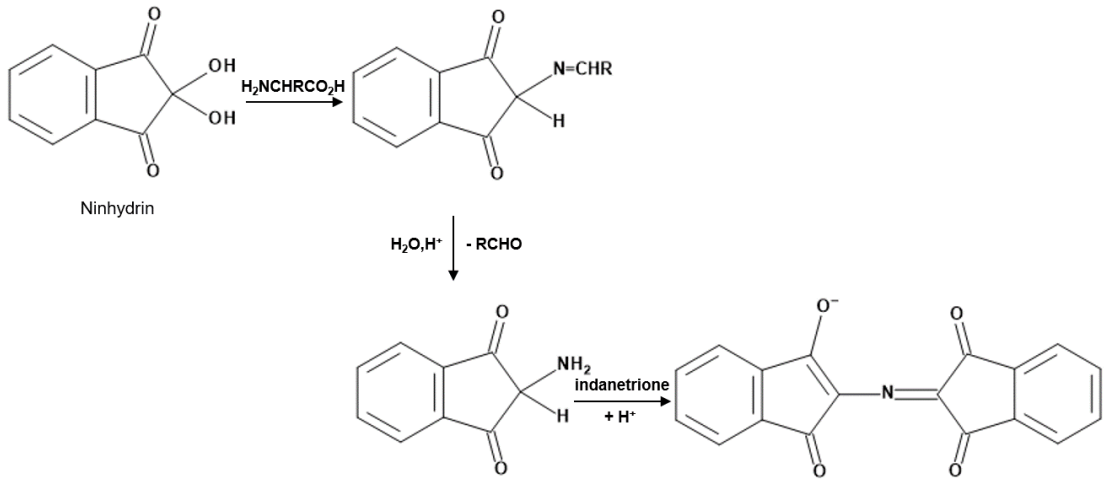

Figure 1. Proposed reaction mechanism between ninhydrin and an amine (such as an amino acid) (Jelly et al., 2009).

Similarly to ninhydrin, DFO also reacts with amino acids, particularly the aromatic amines, to form a red product **(Jelly et al., 2009)**. Another analogue of ninhydrin is IND, also interacting with the amino acids and producing a fluorescent pink (pink Joullié's) **(Levin-Elad et al., 2017).**

In addition to the previously mentioned chemical methods of visualization, the chemical analysis of the residue's compounds of fingermarks has been the object of much study. Mass spectrometry (MS) techniques have been increasingly used in Forensics, namely on the chemical profiling of fingermarks **(Lauzon et al., 2017)**. While visualization techniques only allow the observation of the ridges pattern, MS goes beyond obtaining metabolic profiles, which may assist on the identification of some suspects **(Ifa et al., 2008; Wei et al., 2016)**. The versatility of MS techniques enables a wide overview on the metabolic profile and chemical contaminants present in a certain fingermark, thus being considered an excellent tool in forensic sciences **(Morelato et al., 2013)**.

Part II: Objectives of the thesis

Previous studies, on the qualitative and quantitative chemical profiles of latent fingermarks, already evidenced significant differences on the metabolic patterns between individuals, according to their gender, age and race. However, studies on fingermark aging are still scarce and predominantly focused on lipidic components, the biggest majority of which lacking robustness, thus being unreliable for the development of a new detection and/or identification protocol **(McRoberts et al., 1992; Midkiff, 1993; Greenlees, 1994; Wertheim, 2003; Weyermann, 2011)**. Despite these previous unsuccessful efforts, the determination of specific age-dependent variations on the chemical profiles, constitutes one of the expected developments in forensic science as mentioned by **Cadd and co-workers (2015)**. Although the previous studies highlight the qualitative variation over time of few organic constituents, a specific metabolic marker which time-dependent variation can provide further clues on the age of a fingermark, remains to be identified. Profiting from the results of previous studies on the chemical composition of latent fingermarks, and based on a qualitative and quantitative chemical analysis, the current work primarily aims the identification of specific chemical markers, in order to develop a robust protocol for the determination of the age of a latent fingermark. Consequently, the current work will allow to deepen the knowledge on the chemical composition of aged fingermarks, simultaneously paving the way for the development of new chemical methods for their age dating.

Part III: Materials and methods

3.1. GENERAL CHEMICALS AND MATERIALS

Acetonitrile LiChrosolv®, methanol LiChrosolv®, chloroform and 1-decanol were purchased from Merck (Darmstadt, Germany). Isooctane (2,2,4-trimethylpentane), anhydrous sodium sulphate, boron trifluoride (BF_3)-methanol solution, potassium hydroxide (KOH), pyridine and chlorotrimethylsilane 98% (TMSCl) were from Sigma–Aldrich (St. Louis, MO, US). Dichloromethane was from Fisher Chemical (England, UK). N,O-bis-(trimethylsilyl)trifluoracetamide 98% (BSTFA) was from Acros Organics (Geel, Belgium).

3.2. SAMPLES PREPARATION AND EXTRACTION

The fingermarks were deposited by two female donors aged between 25 and 37 years old, both having signed an informed consent for treatment of lofoscopic data. The donors did not follow any specific wash hands rule but were controlled about cosmetics and both were not using it. The fingermarks were deposited on a glass surface, previously cleaned with ethanol (each donor deposited on an independent glass surface). Each donor passed the right forefinger from the centre to the temples of the forehead and deposited the fingermarks. This process was repeated ten times, i.e. at the end, each glass surface contained ten non-overlapping fingermarks. Temperature and humidity were not controlled, but they were in a normal range of laboratory conditions (≈ 20-21 ºC) being also exposed to light. Fresh and aged fingermark samples were collected with 2x2cm filter paper. Each filter paper collected two fingermark residues. The papers were pooled, cut into pieces and placed in a vial with: a) 6 mL ACN, for 3 hours, for the subsequent analysis of squalene, by HPLC-DAD; b) 6 mL $CHCl_3$:MeOH (2:1), for 3 hours, for the subsequent analysis of fatty acids, by GC-FID and GC-MS. The extraction solutions were evaporated to dryness under a stream of nitrogen.

3.3. HPLC-DAD ANALYSIS

Fingermark residues' extracts were dissolved in ACN (150 µL) and filtered through a 0.2 µm pore size membrane (Millipore, Bedford, MA, US) before injection (20 µL). Samples were analysed in triplicate on an analytical HPLC unit (Gilson Medical Electronics, Villers le Bel, France), using the reverse phase column Hypersil ODS (20 x 0.4 cm i.d., 5 µm, Teknokroma, Barcelona, Spain). Separation was performed through isocratic elution with MeOH/ACN (3:7), delivered at a flow rate of 0.8 mL s^{-1}. Detection was achieved with an Agilent 1260 series DAD (Agilent Technologies, Waldbronn, Germany). Data were processed on Clarity software system, version 5.04.158 (DataApex, Ltd., Prague, Czech Republic). The equation of linear regression (y = 117.51x + 57.486; r^2 = 0.9966) was obtained from the respective calibration curve (concentration *vs* optical absorbance at 205 nm), built with five concentrations of squalene (200; 100; 50; 25; 12.5 µg mL^{-1}), analysed in triplicate. Linearity was determined from the coefficients of determination (r^2) of the calibration curve. The limit of detection (LOD = 0.009 µg mL^{-1}) and the limit of quantification (LOQ = 0.028 µg mL^{-1}) were calculated from the residual standard deviation (σ) of the regression curves and the slopes (S), according to the following equations: LOD = 3.3σ/S and LOQ = 10σ/S **(Lu et al., 2004)**.

3.4. GC-FID QUALITATIVE AND QUANTITATIVE ANALYSIS

A standard solution of fatty acid methyl esters (FAME) (CRM47885), composed by esters of butanoic acid ($C_{4:0}$), hexanoic acid ($C_{6:0}$), octanoic acid ($C_{8:0}$), decanoic acid ($C_{10:0}$), undecanoic acid ($C_{11:0}$), dodecanoic acid ($C_{12:0}$), tridecanoic acid ($C_{13:0}$), tetradecanoic acid ($C_{14:0}$), *cis*-9-tetradecenoic acid ($C_{14:1n\text{-}5c}$), pentadecanoic acid ($C_{15:0}$), *cis*-10-pentadecenoic acid ($C_{15:1n\text{-}5c}$), palmitic acid ($C_{16:0}$), *cis*-9-hexadecenoic acid ($C_{16:1n\text{-}7c}$), heptadecanoic acid ($C_{17:0}$), *cis*-10-heptadecenoic acid ($C_{17:1n\text{-}7c}$), octadecanoic acid ($C_{18:0}$), *cis*-9-octadecenoic acid ($C_{18:1n\text{-}9c}$), *trans*-9-octadecenoic acid ($C_{18:1n\text{-}9t}$), *cis*-9,12-octadecadienoic acid ($C_{18:2n\text{-}6c}$), *trans*-9,12-octadecadienoic acid ($C_{18:2n\text{-}6t}$), octadecatrienoic acid ($C_{18:3n\text{-}6c}$), *cis*-9,12,15-octadecatrienoic acid ($C_{18:3n\text{-}3c}$), eicosanoic acid ($C_{20:0}$), *cis*-11-eicosenoic acid ($C_{20:1n\text{-}11c}$), *cis*-11,14-eicosadienoic acid ($C_{20:2n\text{-}6c}$), *cis*-

8,11,14-eicosatrienoic acid ($C_{20:3n\text{-}6c}$), *cis*-11,14,17-eicosatrienoic acid ($C_{20:3n\text{-}3c}$), *cis*-5,8,11,14-eicosatetraenoic acid ($C_{20:4n\text{-}6c}$), *cis*-5,8,11,14,17-eicosapentaenoic acid ($C_{20:5n\text{-}3c}$), heneicosanoic acid ($C_{21:0}$), docosanoic acid ($C_{22:0}$), *cis*-13-docosenoic acid ($C_{22:1n\text{-}9c}$), *cis*-13,16-docosadienoic acid ($C_{22:2n\text{-}6c}$), *cis*-4,7,10,13,16,19-docosahexaenoic acid ($C_{22:6n\text{-}3c}$), tricosanoic acid ($C_{23:0}$), tetracosanoic acid ($C_{24:0}$), and *cis*-15-tetracosenoic acid ($C_{24:1n\text{-}9c}$), was purchased from Supelco (Bellefonte, PA, US).

Dry extracts obtained from fingermark residues were hydrolysed with 500 µL of a KOH methanolic solution (11 g L^{-1}), at 90 °C, for 10 min. The free fatty acids originally present and those resulting from alkaline hydrolysis were derivatized to their methyl esters (FAMEs) with 500 µL of BF_3-methanol solution (10%), at 90 °C, for 10 min. FAMEs were purified with 2 × 3 mL of isooctane, anhydrous sodium sulphate being added to assure the total absence of water. The resulting extracts were evaporated to dryness under a stream of nitrogen and dissolved in 30 µL of isooctane

Purified FAMEs extracts were analysed in a Finnigan Focus GC apparatus (Thermo Fisher Scientific, Waltham, MA, US), equipped with a flame ionization detector (FID) and a VF-5 ms (30 m × 0.25 mm × 0.25 µm) column (Varian). Injector and detector were maintained at 250 °C, and the oven heating program consisted on a linear increase of column temperature from 40 to 300 °C, at a rate of 5 °C min^{-1}. Derivatized extracts (1 µL) were injected in triplicate. Quantification of each FAME was achieved from the equations of linear regression of the respective standard prepared in isooctane. The linearity range of the method was assessed by building calibration curves (Table 1), using five different concentration levels of the analytes, according to the range of concentrations found in the samples. The limit of detection (LOD) and limit of quantification (LOQ) were determined from calibration curve data, according to the following equations: LOD = $3.3\sigma/S$ and LOQ = $10\sigma/S$, where σ is the residual standard deviation of the linear regression, and S is the slope of the regression line **(Metcalfe and Schmitz, 1961)**.

Table 1. Linear regression equation analysis, LOD and LOQ, for external standards.

Compound	Regression equation (r^2)	Linearity range	LOD	LOQ
Decanoic acid	$y = 8.75 \times 10^4 x - 378460$ (0.997)	(10.2 – 101.8)	5.6	17.0
Dodecanoic acid	$y = 9.10 \times 10^4 x - 86371$ (0.998)	(10.2 – 102.0)	2.8	8.4
Tridecanoic acid	$y = 8.66 \times 10^4 x - 143732$ (0.9938)	(5.1 – 50.9)	0.8	2.5
Tetradecenoic acid	$y = 9.75 \times 10^4 x - 116134$ (0.9933)	(5.1 – 50.9)	1.1	3.2
Tetradecanoic acid	$y = 8.50 \times 10^4 x + 680848$ (0.9914)	(12.7 – 203.3)	9.3	28.2
Pentadecanoic acid	$y = 8.72 \times 10^4 x - 45882$ (0.995)	(6.3 – 101.1)	4.6	13.8
***cis*-9-hexadecenoic acid**	$y = 8.67 \times 10^4 x + 216754$ (0.9909)	(6.3 – 100.8)	2.9	8.9
Palmitic acid	$y = 8.88 \times 10^4 x + 100000$ (0.9881)	(19.1 – 305.0)	26.0	78.7
Heptadecenoic acid	$y = 9.39 \times 10^4 x + 80769$ (0.9804)	(5.1 – 50.8)	0.5	1.6
Heptadecanoic acid	$y = 6.00 \times 10^4 x + 145921$ (0.9852)	(6.0 – 94.5)	23.6	71.6
Octadecatrienoic acid	$y = 7.41 \times 10^4 x + 306627$ (0.9836)	(6.4 – 101.6)	16.7	50.6
***cis*-9-octadecenoic acid**	$y = 1.38 \times 10^5 x + 1000000$ (0.9838)	(12.7 – 203.4)	27.7	84.0
***trans*-9-Octadecenoic acid**	$y = 8.09 \times 10^4 x - 149084$ (0.982)	(5.0 – 50.0)	1.0	3.1
Octadecanoic acid	$y = 1.62 \times 10^5 x + 534150$ (0.984)	(10.2 – 101.7)	3.3	10.0
***cis*-11,14-Eicosadienoic acid**	$y = 6.05 \times 10^4 x - 211116$ (0.9941)	(5.1 – 50.8)	1.3	3.9
Eicosanoic acid	$y = 6.92 \times 10^4 x - 123339$ (0.9903)	(10.2 – 101.8)	3.5	10.5
Heneicosanoic acid	$y = 5.85 \times 10^4 x - 105581$ (0.9922)	(5.1 – 51.0)	1.5	4.5
***trans*-9,12-octadecadienoic acid**	$y = 1.67 \times 10^4 x - 99869$ (0.9928)	(6.3 – 101.1)	5.2	15.9
Docosanoic acid	$y = 4.34 \times 10^4 x - 188520$ (0.9963)	(10.2 – 101.9)	0.6	2.0
Tetracosanoic acid	$y = 2.31 \times 10^4 x - 211450$ (0.998)	(12.7 – 203.6)	46.5	141.0

3.5. GC-MS QUALITATIVE AND QUANTITATIVE ANALYSIS

To increase the volatility of the samples, and thus the sensibility of the GC-MS analysis, three replicates of each fingermark residue were dissolved in 2.8 µL of dichloromethane and 1.2 µL of the internal standard (1-decanol) was added. This mixture was silylated by adding 7.5 µL of pyridine, 7.5 µL of BSTFA, and 3 µL of TMSCl. The mixture was maintained at 70 °C for 30 min and then immediately injected into the GC-MS. The quantity of silylation reagents (BSTFA and TMSCl) used was sufficient to ensure the silylation of all hydroxy groups present in the compounds, including the ones present in the carboxylic group. GC-MS analyses were performed using a GC-MS QP2010 Ultra Shimadzu equipped with a DB-5-J & W capillary column (30 m x 0.25 mm inner diameter and a film thickness of 0.25 µm). Samples were injected with a split ratio of 1:50 and helium was the carrier gas with a flux of 1.13 mL min^{-1}. The chromatographic conditions were as follows: the temperature of the column was maintained at 70 °C for 5 min and then increased, first at 4°C min^{-1} up to 250 °C, followed by 2 °C min^{-1} up to 300 °C which was maintained for 5 min, injector temperature was at 320 °C. The mass spectrometer was operated in electronic impact (EI) mode with energy of 70 eV and data were

collected at a rate of 1 scan s^{-1} over a range of *m/z* 50–1000. The ion source temperature was kept at 200 ºC **(Rahmouni et al., 2018)**.

3.6. STATISTICAL ANALYSIS

Parameters of the exponential model of compound concentrations were calculated relatively to the total concentration (RPA), as a function of time, including residual standard error (RSE), half-lives ($t_{1/2}$) and parameter 95% confidence intervals (CI).

Part IV: Results and discussion

4.1. SQUALENE ANALYSIS BY HPLC-DAD

The study of aged fingermarks has been mainly focused on the degradation of a restricted group of lipidic compounds, their qualitative and quantitative variation over time being previously reported **(Girod & Weyermann, 2014; van Dam et al., 2014)**. The precursor of steroids, the highly unsaturated hydrocarbon squalene, has gained great importance in these studies, as it undergoes significant degradation as a function of time **(Johnston & Rogers, 2018)**. Furthermore, squalene is a major component of fingermark residues, being frequently detected even through low-resolution chromatographic techniques such as TLC **(Matos, 2018)**.

As seen on **Fig. 2**, a marked decrease on squalene content in an extract obtained from fingermark residues deposited on glass is noted over the course of 10 days, its degradation being unequivocally demonstrated on **Table 2**. While the variation on its concentration on the extract obtained from the fresh and 5-day aged fingermark residues remains relatively stable, ranging from 140.71 ± 0.01 to 86.48 ± 0.02 µg mL^{-1}/fingermark, a marked decreased is noted after 8 to 10 days of aging **(Table 2)**.

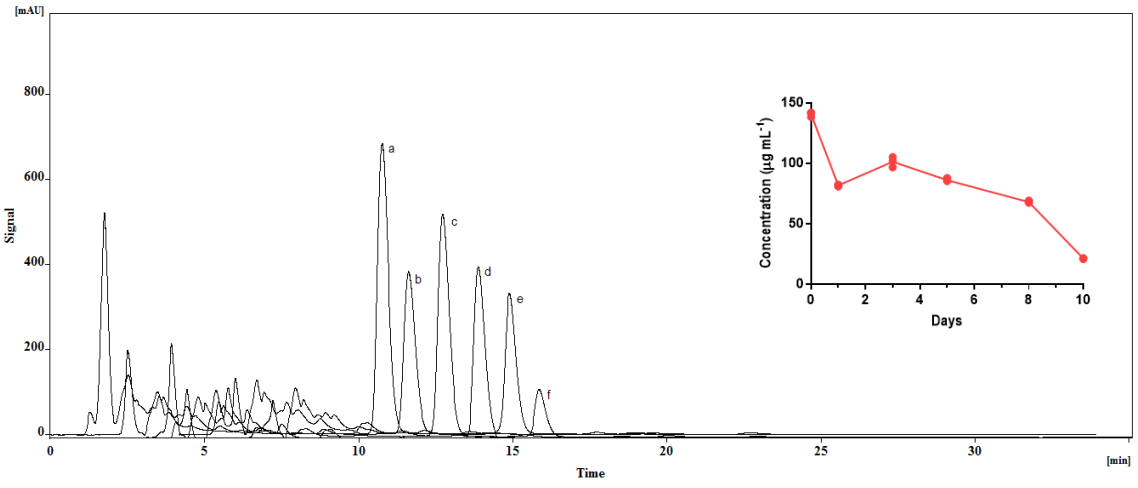

Figure 2. HPLC-UV profile of residues obtained from fresh and aged fingermarks (**a.** fresh, **b.** 1 day, **c.** 3 days, **d.** 5 days, **e.** 8 days, **f.** 10 days).

Table 2. Squalene content as a function of time after deposition of fingermarks on glass surfaces.

Day	Concentration (μg mL^{-1}/ fingermark)
0	140.71 ± 0.01
1	82.04 ± 0.01
3	101.64 ± 0.04
5	86.48 ± 0.02
8	68.30 ± 0.02
10	21.63 ± 0.02

Our results corroborate previous findings on the rapid degradation of squalene in non-porous surfaces, being undetected after nine days on samples aged on non-porous surfaces, stored under light exposition **(Archer et al., 2005; Mountfort et al., 2007; Weyermann et al., 2011; Johnston & Rogers, 2018).** In contrast with non-porous surfaces, degradation of squalene in porous surfaces such as paper, is less pronounced, being detected in 30 days old samples **(Bobev, 1995; Girod et al., 2012).** While the current results are aligned with previous reports on the degradation of squalene in glass, its full degradation after 10 days has not been confirmed **(Fig. 2 and Table 2)**, which may be associated with a distinct extraction procedure and/or detection technique. In a similar study by **Weyermann et al. (2011)**, also with fingermarks deposited in glass surface, squalene was not detected after one week.

From a theoretical point of view, the degradation of squalene over time is expected, mainly due to oxidation and photo-oxidation processes, several degradation products being produced such as epoxides, ketones, alcohols and hydroperoxides, as well as the fully oxidized forms, hexanedioic and pentanedioic acids **(Fig. 3) (Archer et al., 2005; Mountfort et al., 2007; Weyermann et al., 2011)**. In fact, the detection of several oxidation products is also reported, namely the primary oxidation product squalene monohydroperoxide, as well as squalene tetrahydroperoxide and squalene pentahydroperoxide **(Mountfort et al., 2007; Girod et al., 2012)**.

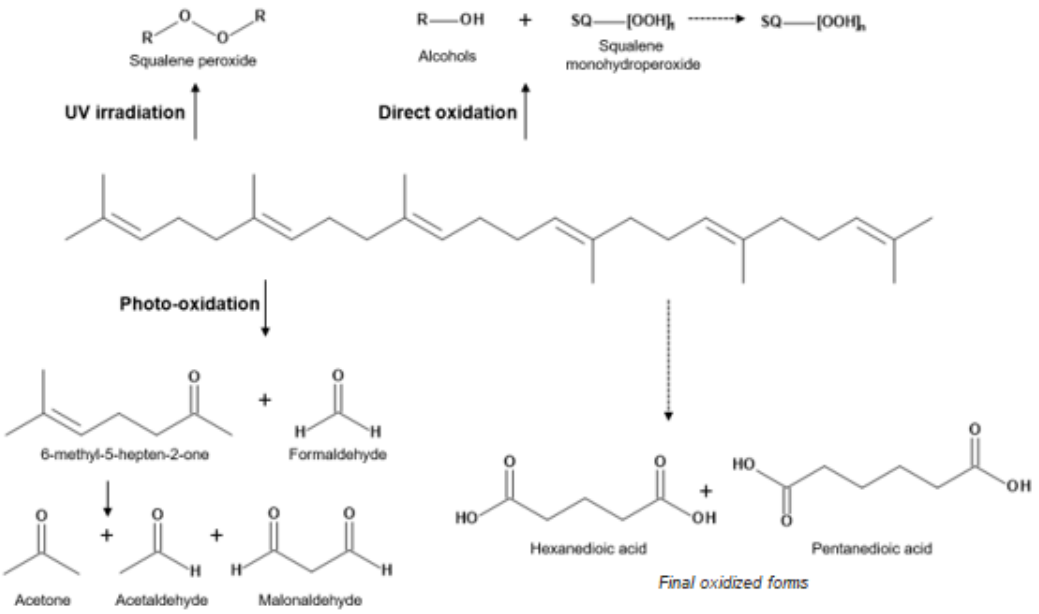

Figure 3. Squalene decomposition through various processes (oxidation, UV and direct oxidation to hydroperoxides) (Cadd et al., 2015).

4.2. FATTY ACIDS ANALYSIS BY GC-FID

4.2.1. Initial composition

Lipidic components, particularly fatty acids, correspond to major constituents of fingermark residues, and represent important targets for enhancement and dating techniques **(Girod & Weyermann, 2014)**. While being well known that the fatty acid profile varies among fingermarks of the same donor and those obtained from different donors, their variation has an immense value in fingermark age dating **(Girod & Weyermann, 2014)**. As such, characterization of the fatty acid profile of fingermark residues was attempted through GC-FID analysis. Characterization of the fresh fingermark residue allowed the identification and quantitation of 20 fatty acid methyl esters, revealing a total fatty acid concentration of 430.45 ± 0.15 µg mL^{-1} dry fingermark residue **(Fig. 4 and Table 3)**.

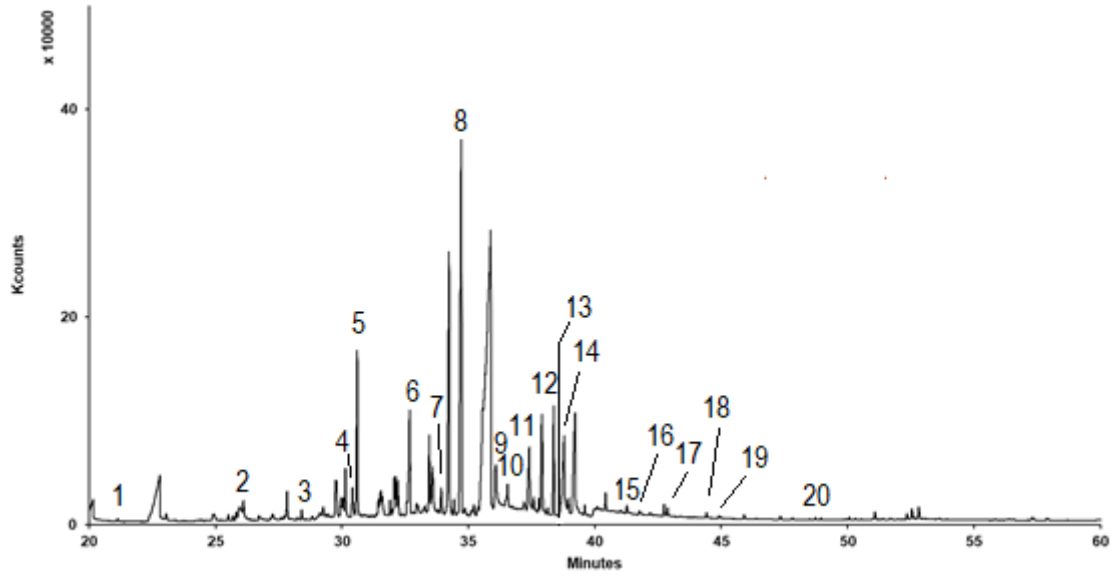

Figure 4. Fatty acid methyl esters content in an extract obtained from fresh fingermark residues deposited on glass.

In agreement with previous records on the fatty acid profile of fingermark residues **(Girod et al., 2012),** the saturated fatty acid (SFA) palmitic acid (**8**) was identified as the main component, corresponding to ca. 31% of the quantifiable total. Relevantly, considerable amounts of the monounsaturated fatty acid (MUFA) palmitoleic acid (**7**) and the saturated tetra (**5**) and pentadecanoic (**6**) acids were also determined **(Table 3)**, corroborating the study by **(Frick et al., 2015)**, reporting the quantitation of **8**, **7** and **6** as main components. Worth to refer also the previous identification of stearic acid (**14**) in considerable amounts in fingermark residues **(Archer et al., 2005; Friesen, 2014)**, herein found also at relevant concentrations (12.95 ± 0.02 μg mL^{-1}), corresponding to ca. 3% of the quantifiable total **(Table 3)**. Similarly to our findings, the major components tetradecanoic (**5**), pentadecanoic (**6**), (*Z*)-9-hexadecenoic (**7**), palmitic (**8**) and octadecanoic (**14**) acids, were also previously reported by **Michalski et al. (2013)**, also through the identification of their methyl ester derivatives. In another study, tridecanoic (**3**), tetradecanoic, pentadecanoic, palmitic, *cis*-9-hexadecenoic (**7**), octadecanoic and eicosanoic acids were identified in a series of fingerprint residues obtained from different donors **(Croxton et al., 2010)**.

Table 3. Fatty acid content of an extract obtained from fresh fingermark residues.[1]

Peak	Compound	Concentration (µg mL-1)
1	**Decanoic acid** (C10:0)	5.16 ± 0.00
2	**Dodecanoic acid** (C12:0)	4.48 ± 0.01
3	**Tridecanoic acid** (C13:0)	4.39 ± 0.01
4	**Tetradecenoic acid** (C14:1*n*-5c)	9.48 ± 0.00
5	**Tetradecanoic acid** (C14:0)	37.43 ± 0.01
6	**Pentadecanoic acid** (C15:0)	33.52 ± 0.00
7	***cis*-9-hexadecenoic acid** (C16:1*n*7-c)	83.39 ± 0.00
8	**Palmitic acid** (C16:0)	133.52 ± 0.00
9	**Heptadecenoic acid** (C17:1*n*-7c)	17.93 ± 0.00
10	**Heptadecanoic acid** (C17:0)	5.12 ± 0.02
11	**Octadecatrienoic acid** (C18:3*n*-6c)	26.68 ± 0.00
12	***cis*-9-octadecenoic acid** (C18:1*n*-9c)	13.77 ± 0.04
13	***trans*-9-Octadecenoic acid** (C18:1*n*-9t)	2.35 ± 0.00
14	**Octadecanoic acid** (C18:0)	12.95 ± 0.02
15	***cis*-11,14-Eicosadienoic acid** (C20:2*n*-6c)	6.54 ± 0.01
16	**Eicosanoic acid** (C20:0)	2.62 ± 0.00
17	**Heneicosanoic acid** (C21:0)	2.34 ± 0.02
18	***trans*-9,12-octadecadienoic acid** (C18:2*n*-6t)	12.51 ± 0.01
19	**Docosanoic acid** (C22:0)	5.55 ± 0.00
20	**Tetracosanoic acid** (C24:0)	10.70 ± 0.00
	TOTAL	430.43 ± 0.15
	SFA/UFA	1.49

[1]Results are expressed as the mean (standard deviation) of a triplicate analysis.

Despite the number of reports on the fatty acid content of fingermark residues, including the identification of additional fatty acids **(Archer et al., 2005; Croxton et al., 2010; Michalski et al., 2013; Friesen, 2014; Frick et al., 2015)**, to the best of our knowledge, there are no records on the identification of the polyunsaturated fatty acid (PUFA) *cis*-11,14-eicosadienoic (**15**). In fact, while **8**, **7**, **5** and **6** have been identified as major fatty acid components, in several studies through matrix-assisted laser desorption/ ionization (MALDI) and desorption electrospray ionization (DESI) **(Girod et al., 2012)**, the qualitative and quantitative profile of minor constituents is variable. Such discrepancies may derive from the use of different profiling techniques and extraction procedures, but also due to the inter and intravariability among donors **(Croxton et al., 2010)**.

4.2.2. Aged composition

Variation of the content of endogenous constituents occur over time through a continuous process involving degradation, metabolism, drying, evaporation, migration,

oxidation or polymerization **(Girod et al., 2012)**. As previously mentioned, lipidic components, particularly fatty acids, have been targeted as potential time-dependent markers of aged fingermarks **(Girod & Weyermann, 2014; Francese et al., 2017; Lauzon et al., 2017)**. Due to their susceptibility to physical and chemical degradation, resulting in the production of new constituents, mostly small oxidized molecules, variation on the content of lipidic constituents has been reported **(Mong et al., 1999; Archer et al., 2005; Weyermann et al., 2011)**.

Quantitative analysis on the fatty acid profile of fingermarks' residues obtained over the course of 20 days **(Table 4)**, denotes an obvious variation on the content of several fatty acid constituents.

As evidenced on **Table 4**, the SFA/UFA ratio tendentiously increases over the time, the fresh fingermark residue displaying a SFA/UFA value of 1.49, while the 20 day-aged residue exhibits a value nearly 4-fold higher. Such variation shows that the concentration of SFA increased over time due to the oxidation of the saturated moieties of UFA **(Fig. 5)**, in analogy with previous findings by **Archer et al. (2005)** and **De Paoli et al. (2010)**, reporting an increase on the content of SFAs up to 20 days.

Table 4. Fatty acid content of extracts obtained from fresh and aged fingermark residues.[1]

		Concentration (µg mL^{-1})				
Peak	**Compound**	**Fresh**	**5**	**10**	**15**	**20**
1	**Decanoic acid**	5.16 ± 0.00	-	-	8.34 ± 0.00	-
2	**Dodecanoic acid**	4.48 ± 0.01	2.24 ± 0.01	1.53 ± 0.01	5.45 ± 0.00	2.70 ± 0.03
3	**Tridecanoic acid**	4.39 ± 0.01	2.49 ± 0.00	2.41 ± 0.02	4.80 ± 0.00	3.50 ± 0.02
4	**Tetradecenoic acid**	9.48 ± 0.00	3.51 ± 0.01	2.59 ± 0.00	8.58 ± 0.01	4.88 ±0.00
5	**Tetradecanoic acid**	37.43 ± 0.01	19.94 ± 0.01	12.87 ±0.01	40.63 ± 0.03	45.69 ± 0.07
6	**Pentadecanoic acid**	33.52 ± 0.00	25.97 ± 0.05	19.38 ± 0.00	40.28 ± 0.03	48.68 ± 0.05
7	***cis*-9-hexadecenoic acid**	83.39 ± 0.00	15.62 ± 0.02	9.83 ± 0.02	7.38 ± 0.01	5.09 ± 0.06
8	**Palmitic acid**	133.52 ± 0.00	109.86 ± 0.00	98.58 ± 0.00	174.86 ± 0.02	274.82 ± 0.05
9	**Heptadecenoic acid**	17.93 ± 0.00	5.75 ± 0.01	4.98 ± 0.02	9.14 ± 0.02	10.70 ± 0.06
10	**Heptadecanoic acid**	5.12 ± 0.02	1.49 ± 0.04	1.80 ± 0.04	6.62 ± 0.03	10.14 ± 0.05
11	**Octadecatrienoic acid**	26.68 ± 0.00	8.96 ± 0.00	2.59 ± 0.03	34.64 ± 0.00	17.14 ± 0.01
12	***cis*-9-octadecenoic acid**	13.77 ± 0.04	-	-	3.27 ± 0.06	19.33 ± 0.06
13	***trans*-9-Octadecenoic acid**	2.35 ± 0.00	2.62 ± 0.01	2.57 ± 0.00	3.67 ± 0.01	3.39 ± 0.02
14	**Octadecanoic acid**	12.95 ± 0.02	14.79 ± 0.02	13.11 ± 0.02	25.16 ± 0.09	38.63 ± 0.02
15	***cis*-11,14-Eicosadienoic acid**	6.54 ± 0.01	5.10 ± 0.00	4.38 ± 0.00	6.43 ± 0.00	6.03 ± 0.00
16	**Eicosanoic acid**	2.62 ± 0.00	2.39 ± 0.00	2.71 ± 0.01	3.32 ± 0.01	5.08 ± 0.03
17	**Heneicosanoic acid**	2.34 ± 0.02	1.94 ± 0.00	-	-	2.56 ± 0.01
18	***trans*-9,12-octadecadienoic acid**	12.51 ± 0.01	11.01 ± 0.00	8.08 ± 0.01	15.84 ± 0.01	15.40 ± 0.02
19	**Docosanoic acid**	5.55 ± 0.00	4.87 ± 0.00	4.90 ± 0.00	6.59 ± 0.00	7.22 ±0.02
20	**Tetracosanoic acid**	10.70 ± 0.00	10.46 ± 0.00	10.09 ± 0.01	12.39 ± 0.01	13.96 ± 0.01
	Total	**430.44 ± 0.01**	**249.03 ± 0.01**	**202.42 ± 0.01**	**417.42 ± 0.02**	**524.96 ± 0.03**
	SFA/UFA	**1.49**	**3.34**	**4.78**	**3.69**	**5.53**

[1] Results are expressed as the mean (standard deviation) of a triplicate analysis. "-" not detected

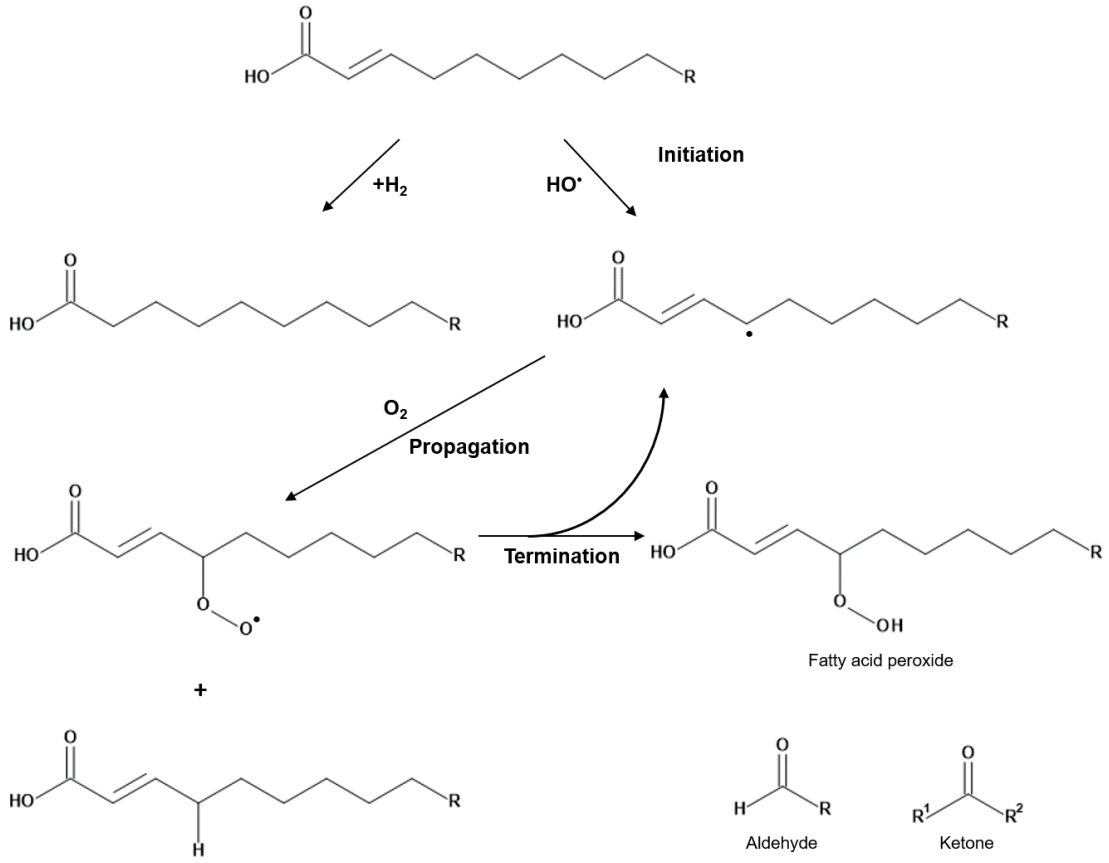

Figure 5. Fatty acids' degradation through aerobic conditions (Cadd et al., 2015).

In order to overcome the quantitative heterogeneity observed in previous studies **(Girod and Weyermann 2014)**, also attempting the use of fatty acids for the age determination of fingermarks, the content variation of each fatty acid was expressed as relative percentage **(Fig. 6)**. In fact, the hardening of fingermarks over time, mainly due to the loss of moisture and to the transformation of unsaturated moieties to saturated molecules, interferes with the extractability of lipidic components. These saturated molecules have a more orderly crystal structure, which leads to a more crystalline surface in older fingermarks, lowering extraction efficiency **(Girod et al., 2012)**.

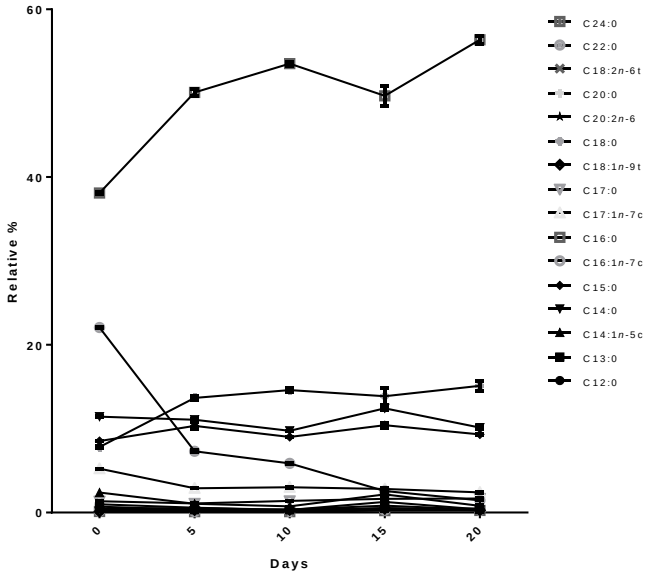

Figure 6. Fatty acids content in residues obtained from fresh and aged fingermarks.

In order to attempt the identification of fatty acids suitable for fingermark dating, only 16 target components were selected, based on their identification in fresh and aged fingermark residues as well as their reasonable content in the samples **(Fig. 6)**. Since the SFAs decanoic acid (**1**), the heneicosanoic acid (**17**), the UFA octadecatrienoic acid **(11)** and the *trans*-9-octadecenoic acid (**13**) did not met such requirements, they were not included. As seen on **Fig. 7A** and **Fig. 7B**, a noticeable increase on the content of palmitic acid (**8**) was observed over 20 days, a rise on the concentration of the SFAs heptadecanoic acid (**10**), eicosanoic acid (**16**), docosanoic acid (**19**) and tetracosanoic acid (**20**) being also evident.

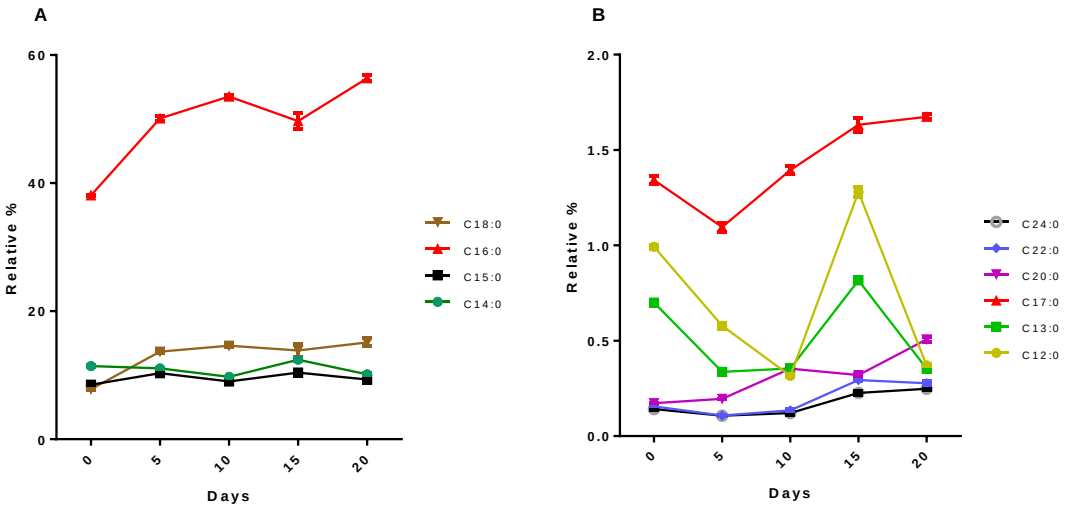

Figure 7. Content in main (**A**) and minor (**B**) fatty acid constituents in residues obtained from fresh and aged fingermarks.

In contrast, the content in UFA decreased over the time, such variation being particularly evident in the concentration of *cis*-9-hexadecenoic acid (**7**) **(Fig. 8)**.

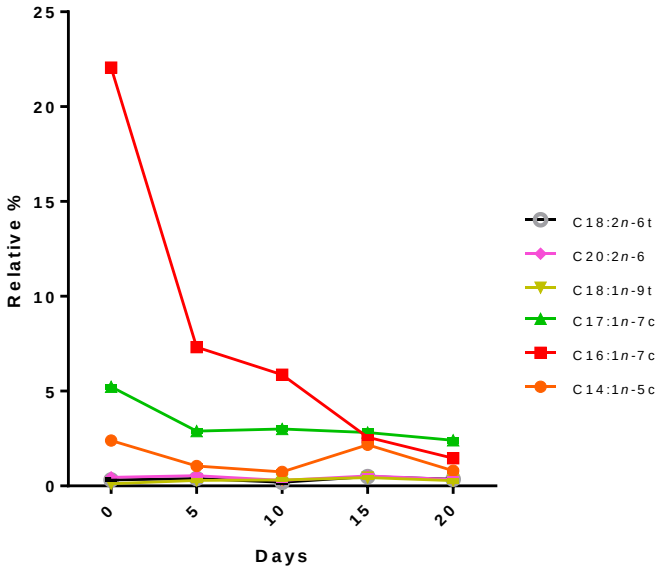

Figure 8. UFAs content in residues obtained from fresh and aged fingermarks.

In fact, it is instructive to observe that the concentration of palmitoleic acid (**7**), relative to the quantifiable total, as a function of time, is well fitted by a simple exponential decay model **(Fig. 9)**. As an exploratory analysis, exponential models of the

concentration of palmitoleic acid (**7**) divided by the concentration of each of the other constituents, were also fitted to the data. Concentration of palmitoleic acid (**7**) divided by *cis*-10-heptadecenoic acid (**9**) revealed model improvements which, if not spurious due to the low number of data samples, should be investigated **(Table 5)**. Further studies on the initial composition variability of intra-donor fingerprint residues are necessary to be able to better assess the possibility of using this type of curve to determine fingerprint age.

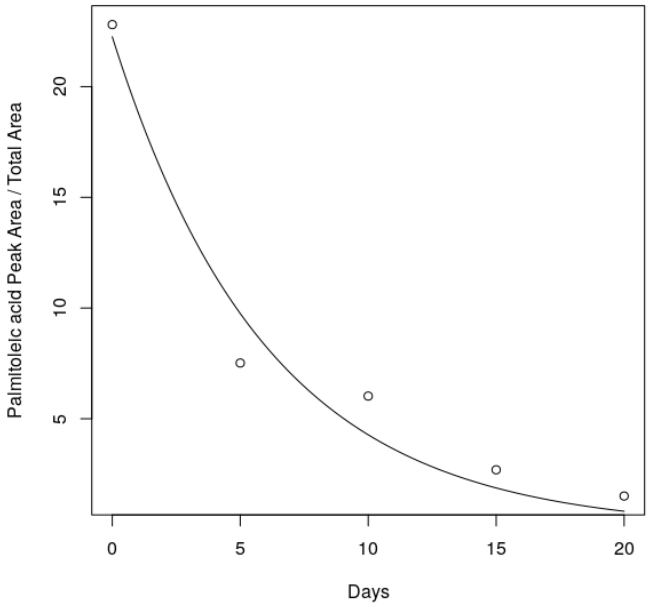

Figure 9. Exponential model fitted to the palmitoleic acid (C16:1*n*-7c) concentration relative to the quantifiable total, as a function of time.

Table 5. Parameters of the exponential model of palmitoleic acid concentration relative to the total concentration (RPA), as a function of time, including residual standard error (RSE), half-lives ($t_{1/2}$), and parameter 95% confidence intervals (CI).

$RPA = b_0 e^{-b_1 t}$	Palmitoleic acid
b_0	22.25
b_1 (days^{-1})	0.17
RSE	0.56
b_0 CI	[16.64; 27.93]
b_1 CI	[0.09; 0.32]
$t_{1/2}$ (days)	4.20

Due to the findings above mentioned, palmitic acid (**8**) and its monounsaturated derivative, palmitoleic acid, appear to be valuable temporal fingermark constituents due to the pronounced content variation **(Fig. 9)**, as statistically validated. Furthermore, it is worth also considering the SFAs **10**, **16** and **19** as potential markers for the age determination of fingermarks **(Fig.10)**.

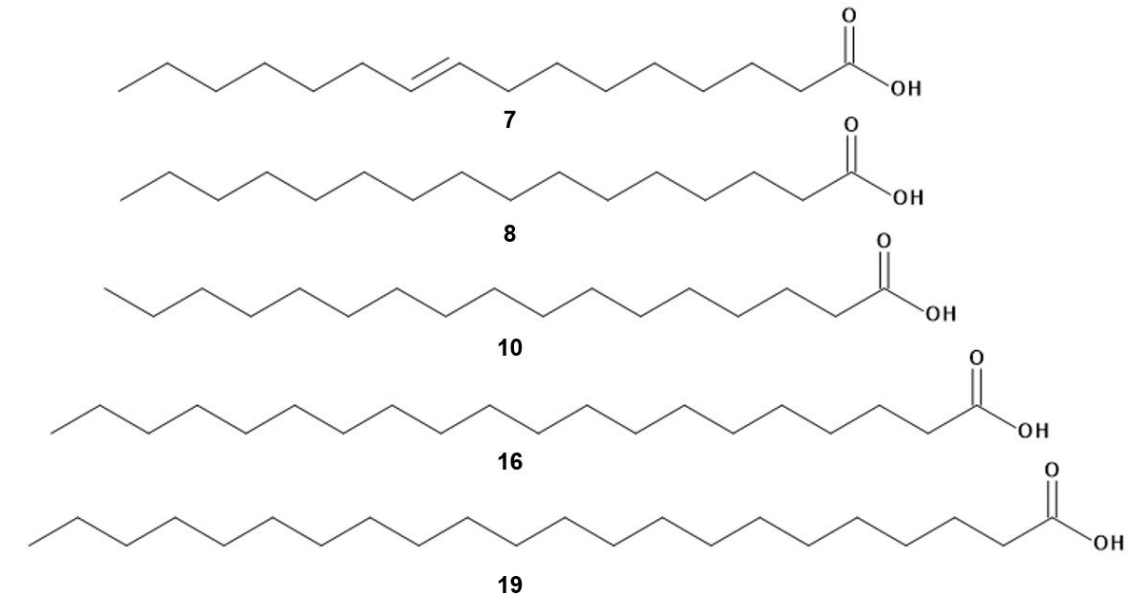

Figure 10. Potential temporal fatty acid markers. **7.** *Cis*-9-hexadecenoic acid; **8**. Palmitic acid; **10.** Heptadecanoic acid; **16.** Eicosanoic acid; **19.** Docosanoic acid.

4.3. FATTY ACIDS ANALYSIS BY GC-MS

4.3.1. Initial composition

In order to provide further insights on the chemical composition of fresh and aged fingermark residues, as well to attempt the identification of additional constituents for dating purposes, the samples were further analysed through GC-MS. Compounds were identified through comparison with computerized databases (NIST and WILEY libraries), published data and through the examination of the mass spectra for diagnostic fragment ions **(Hartzell-Baguley et al., 2007; Frick et al., 2015).**

A total of 39 endogenous metabolites were identified in the residue obtained from an extract of fresh fingermarks **(Fig. 11** and **Table 6)**, predominantly fatty acids, including

short- (**1**), medium- (**6**) and long-chain (**15, 16, 18-20, 24, 26, 27**) free fatty acids, as well as esterified fatty acids (**7, 9, 12, 21-23, 31, 35, 37-39**).

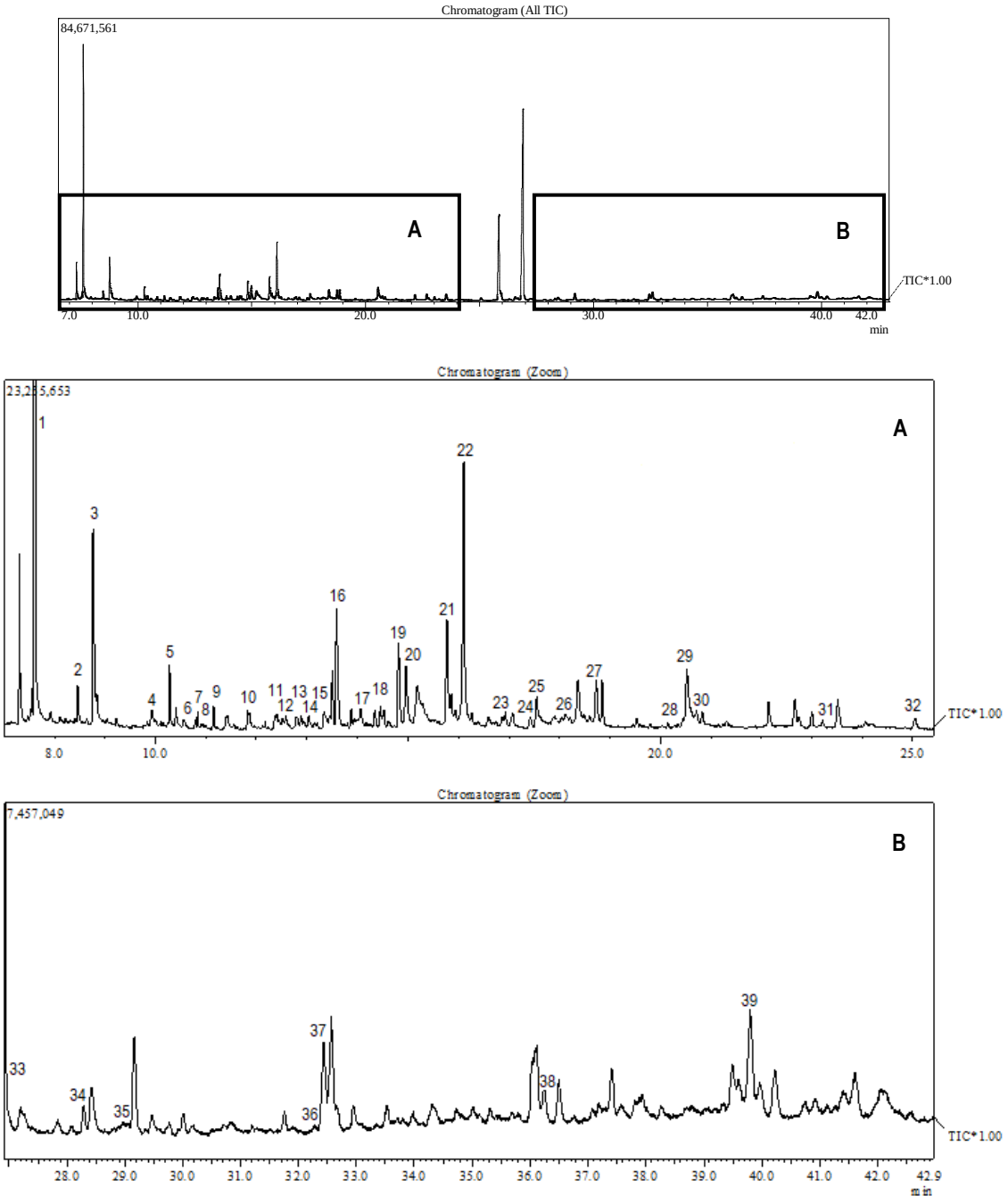

Figure 11. GC-MS characterization of compounds **A) 1-32; B) 33-39** of fresh residues obtained from fingermarks.
Identity of compounds as in **Table 6**.

Table 6. GC-MS based characterization of an extract obtained from fresh fingermark residues[1].

Peak	Compound	Relative %
1	Butanoic acid	$0.09 \pm 5.8 \times 10^{-6}$
2	Dodecanol	$0.22 \pm 1.5 \times 10^{-5}$
3	Pentose monosaccharide derivative	$0.14 \pm 9.4 \times 10^{-6}$
4	Pentose monosaccharide derivative	$0.26 \pm 1.8 \times 10^{-5}$
5	Pentose monosaccharide derivative	$0.52 \pm 3.5 \times 10^{-5}$
6	Dodecanoic acid	$0.28 \pm 1.9 \times 10^{-5}$
7	Methyl-6-palmitoyl-α-D-glucopyranoside	$0.42 \pm 2.8 \times 10^{-5}$
8	Hexose monossaccharide derivative	$0.16 \pm 1.1 \times 10^{-5}$
9	Glycerol palmitate	$0.23 \pm 9.4 \times 10^{-6}$
10	Hexose monossaccharide derivative	$0.32 \pm 2.2 \times 10^{-5}$
11	Tetradecanol	$0.15 \pm 1.0 \times 10^{-5}$
12	Hexanedioic acid dibutyl ester	$0.20 \pm 1.4 \times 10^{-5}$
13	Hexose monossaccharide derivative	$0.15 \pm 1.0 \times 10^{-5}$
14	Hexadecanol	$0.21 \pm 1.4 \times 10^{-5}$
15	Myristoleic acid	$0.52 \pm 3.5 \times 10^{-5}$
16	Myristic acid	$4.23 \pm 2.8 \times 10^{-4}$
17	Hexose monossaccharide derivative	$0.50 \pm 3.4 \times 10^{-5}$
18	13-Methyltetradecanoic acid	$0.63 \pm 9.4 \times 10^{-6}$
19	Palmitoleic acid	$2.86 \pm 1.9 \times 10^{-4}$
20	Palmitic acid	$3.24 \pm 2.2 \times 10^{-4}$
21	Isopropyl palmitate	$0.65 \pm 4.4 \times 10^{-5}$
22	Palmitic acid derivative	$9.00 \pm 6.1 \times 10^{-4}$
23	Oleic acid methyl ester	$0.84 \pm 5.6 \times 10^{-5}$
24	Heptadecanoic acid	$0.45 \pm 3.0 \times 10^{-5}$
25	Octadecanol	$1.13 \pm 7.6 \times 10^{-5}$
26	Oleic acid	$1.53 \pm 1.0 \times 10^{-4}$
27	Stearic acid	$1.36 \pm 9.2 \times 10^{-5}$
28	Tetramethylhexadecanol	$0.06 \pm 4.3 \times 10^{-6}$
29	9-Octadecenamide	$2.98 \pm 2.0 \times 10^{-4}$
30	Docosane	$0.39 \pm 2.6 \times 10^{-5}$
31	Monopalmitin derivative	$0.12 \pm 8.4 \times 10^{-6}$
32	Nonacosane	$0.39 \pm 2.6 \times 10^{-5}$
33	Squalene	$58.4 \pm 3.9 \times 10^{-3}$
34	Cholesta-3,5-diene	$0.74 \pm 5.0 \times 10^{-5}$
35	Docosanyl docosanoate	$1.63 \pm 1.0 \times 10^{-5}$
36	Cholesterol	$1.55 \pm 1.0 \times 10^{-5}$
37	Palmitoleic acid derivative	$1.18 \pm 7.9 \times 10^{-5}$
38	*cis*-9-Tetradecenoic acid heptyl ester	$0.46 \pm 3.1 \times 10^{-5}$
39	Arachidyl palmitoleate	$1.81 \pm 6.7 \times 10^{-4}$
	Free Fatty Acids	$15.12 \pm 1.0 \times 10^{-3}$
	Esterified Fatty Acids	$16.53 \pm 1.1 \times 10^{-3}$
	Fatty alcohols	$1.76 \pm 1.2 \times 10^{-4}$
	Carbohydrates	$2.05 \pm 1.4 \times 10^{-4}$

[1] Results are expressed as the mean (standard deviation) of a triplicate analysis. "-" not detected

Furthermore, in addition to the major component squalene (**33**) and 9-octadecenamide (**29**), a series of fatty alcohols (**2, 11, 14, 25, 28**), carbohydrates (**3-5, 8, 10, 13, 17**), acyclic alkanes (**30, 32**) and sterols (**34, 36**), were also identified **(Table 6)**.

While not classified as endogenous components, it is worth to mention the identification of several exogenous components (contaminants), such as surfactants and agents used in the production of unsaturated polyester resins and plasticizers. Identified contaminants include diethylene glycol, 2-aminobenzoxazole, surfadol 541, citroflex and phthalates (Supplementary materials). Additionally, several endogenous compounds can also be found in cosmetic formulations, such as dodecanol (**2**), dodecanoic acid (**6**), glycerol palmitate (**9**), tetradecanol (**11**), dibutyl adipate (**12**), hexadecanol (**14**), myristic acid (**16**), palmitoleic acid (**19**), palmitic acid (**20**), isopropryl palmitate (**21**), oleic acid methyl ester (**23**), octadecanol (**25**), oleic acid (**26**), stearic acid (**27**), 9-octadecenamide (**29**), docosane (**30**), squalene (**33**) and cholesterol (**36**). However, it is worth to emphasize that the fingermark residues under study were obtained from donors devoid of cosmetics, thus the identified components being identified as endogenous metabolites.

Qualitative analysis of the residues obtained from fresh fingermarks allowed to identify several unreported constituents such as butanoic acid (**1**), dodecanol (**2**), methyl-6-palmitoyl-α-D-glucopyranoside (**7**), glycerol palmitate (**9**), tetradecanol (**11**), hexanedioic acid dibutyl ester (**12**), hexadecanol (**14**), 13-methyltetradecanoic acid (**18**), oleic acid methyl ester (**23**), heptadecanoic acid (**24**), octadecanol (**25**), tetramethylhexadecanol (**28**), 9-octadecenamide (**29**), docosane (**30**), nonacosane (**32**), cholesta-3,5-diene (**34**), docosanyl docosanoate (**35**), *cis*-9-tetradecenoic acid heptyl ester (**38**) and arachidyl palmitoleate (**39**). In agreement with previous studies **(Girod & Weyermann, 2014)**, quantitative analysis revealed that the sterol precursor squalene (**33**) was the main component of the fresh fingermark residues (ca. 58%), followed by an uncharacterized palmitic acid derivative (**22**), myristic acid (**16**), palmitic acid (**20**) and palmitoleic acid (**19**) **(Table 6)**. Concerning each structural class, the extract obtained from fresh fingermarks was predominantly characterized by free fatty acids and esterified fatty acids, constituting *ca.* 15.1 % and 16.5 % of the quantifiable total **(Table 6)**.

As previously discussed, squalene undergoes oxidation, degradation products such as hydroperoxides and hydroxyl radicals being formed, which may influence the decomposition of the triglycerides and the initial increase of SFAs. It has been shown

that, also with squalene, cholesterol also influences the intermolecular lipid fingermarks interactions **(Johnston & Rogers, 2018)**.

A study by **Hartzell-Baguley (2007)**, also on a glass surface, showed that squalene would have the most abundant peak in fingermark samples, like in the most chromatograms in these studies **(Frick et al., 2015)**. However, more compounds were identified, such as certain long chain fatty acids, namely myristic (**16**) and oleic acids (**26**), and cholesterol (**36**), corroborating the results obtained in our work.

Additional studies on the chemical profiling of latent fingermarks have previously allowed the identification of myristic (**16**) and oleic acids (**26**), squalene (**33**), cholesterol (**36**) and isopropyl palmitate (**21**), as in the current work **(Girod & Weyermann, 2014)**.

4.3.2. Aged composition

Study on the endogenous components of aged fingermarks was performed with residues obtained over 15 days **(Table 7)**. Similarly to what has been found on the fatty acid profiling study, comparison between fresh and aged fingermark residues demonstrated a notable variation on the content of several constituents **(Table7)**. Due to the variation in the extraction yields, quantitative data analysis is represented in relative percentage, insofar as the total quantifiable contents are variable (Supplementary materials). As evidenced in **Table 7**, an increase on the content of free fatty acids is noted in comparison with esterified fatty acids, which may result from the ester hydrolysis and due to oxidation. In fact, it is worth to highlight that, while detected on residues obtained from fresh fingermarks, the palmitic acid esterified derivative (**22)** was not detected in aged residues. In contrast, an obvious increase was observed concerning the relative content of the free fatty acids palmitic (**20**) and oleic (**26**) acids, which explain the variation on the ratio between free and esterified fatty acids over time.

Table 7. GC-MS based characterization of an extract obtained from fresh and aged fingermark residues[1].

Peak	Compound	Fresh	5	10	15
1	Butanoic acid	$0.09\pm5.8\times10^{-6}$	-	-	-
2	Dodecanol	$0.22\pm1.5\times10^{-5}$	-	-	-
3	Pentose monosaccharide derivative	$0.14\pm9.4\times10^{-6}$	-	$1.39\pm3.7\times10^{-6}$	$0.30\pm6.1\times10^{-6}$
4	Pentose monosaccharide derivative	$0.26\pm1.8\times10^{-5}$	$0.09\pm1.8\times10^{-5}$	$0.55\pm1.5\times10^{-6}$	$0.86\pm1.7\times10^{-5}$
5	Pentose monosaccharide derivative	$0.52\pm3.5\times10^{-5}$	$0.01\pm1.3\times10^{-6}$	$0.83\pm2.2\times10^{-6}$	$1.85\pm1.7\times10^{-5}$
6	Dodecanoic acid	$0.28\pm1.9\times10^{-5}$	-	-	-
7	Methyl-6-palmitoyl-α-D-glucopyranoside	$0.42\pm2.8\times10^{-5}$	-	-	$2.08\pm4.2\times10^{-5}$
8	Hexose monossaccharide derivative	$0.16\pm1.1\times10^{-5}$	-	-	-
9	Glycerol palmitate	$0.23\pm9.4\times10^{-6}$	-	-	-
10	Hexose monossaccharide derivative	$0.32\pm2.2\times10^{-5}$	-	$0.36\pm9.7\times10^{-7}$	$0.58\pm1.2\times10^{-5}$
11	Tetradecanol	$0.15\pm1.0\times10^{-5}$	$0.01\pm2.7\times10^{-6}$	$0.46\pm1.2\times10^{-6}$	$0.20\pm3.9\times10^{-6}$
12	Hexanedioic acid dibutyl ester	$0.20\pm1.4\times10^{-5}$	$0.07\pm1.4\times10^{-5}$	$1.12\pm3.0\times10^{-6}$	$1.98\pm4.0\times10^{-5}$
13	Hexose monossaccharide derivative	$0.15\pm1.0\times10^{-5}$	$5.18\pm1.1\times10^{-3}$	-	-
14	Hexadecanol	$0.21\pm1.4\times10^{-5}$	$0.42\pm8.6\times10^{-5}$	$0.02\pm4.8\times10^{-8}$	$0.95\pm1.9\times10^{-5}$
15	Myristoleic acid	$0.52\pm3.5\times10^{-5}$	$0.51\pm1.9\times10^{-5}$	$0.64\pm1.7\times10^{-6}$	-
16	Myristic acid	$4.23\pm2.8\times10^{-4}$	$1.17\pm2.4\times10^{-4}$	$1.17\pm3.1\times10^{-6}$	$3.13\pm6.3\times10^{-5}$
17	Hexose monossaccharide derivative	$0.50\pm3.4\times10^{-5}$	-	$0.66\pm1.7\times10^{-6}$	$1.66\pm3.3\times10^{-5}$
18	13-Methyltetradecanoic acid	$0.63\pm9.4\times10^{-6}$	$0.01\pm2.5\times10^{-6}$	-	-
19	Palmitoleic acid	$2.86\pm1.9\times10^{-4}$	$9.08\pm1.9\times10^{-3}$	$4.62\pm1.2\times10^{-5}$	$2.90\pm5.8\times10^{-5}$
20	Palmitic acid	$3.24\pm2.2\times10^{-4}$	$11.26\pm2.3\times10^{-3}$	$6.95\pm1.9\times10^{-5}$	$9.37\pm1.9\times10^{-4}$
21	Isopropyl palmitate	$0.65\pm4.4\times10^{-5}$	$2.29\pm4.7\times10^{-4}$	$1.61\pm4.3\times10^{-6}$	$0.60\pm1.2\times10^{-5}$
22	Palmitic acid derivative	$9.00\pm6.1\times10^{-4}$	-	-	-
23	Oleic acid methyl ester	$0.84\pm5.6\times10^{-5}$	$0.10\pm1.9\times10^{-5}$	-	-

24	Heptadecanoic acid	$0.45\pm3.0\times10^{-5}$	-	-	-
25	Octadecanol	$1.13\pm7.6\times10^{-5}$	$0.67\pm1.4\times10^{-4}$	-	$1.04\pm2.1\times10^{-5}$
26	Oleic acid	$1.53\pm1.0\times10^{-4}$	$1.67\pm3.4\times10^{-4}$	$12.06\pm3.2\times10^{-5}$	$22.58\pm4.5\times10^{-4}$
27	Stearic acid	$1.36\pm9.2\times10^{-5}$	$2.21\pm4.5\times10^{-4}$	$0.62\pm1.7\times10^{-6}$	-
28	Tetramethylhexadecanol	$0.06\pm4.3\times10^{-6}$	$0.06\pm1.8\times10^{-5}$	-	$0.77\pm1.6\times10^{-5}$
29	9-Octadecenamide	$2.98\pm2.0\times10^{-4}$	$4.32\pm8.8\times10^{-4}$	$6.08\pm1.6\times10^{-5}$	$1.99\pm4.0\times10^{-5}$
30	Docosane	$0.39\pm2.6\times10^{-5}$	$1.19\pm2.4\times10^{-4}$	$5.36\pm1.4\times10^{-5}$	$9.01\pm1.8\times10^{-4}$
31	Monopalmitin derivative	$0.12\pm8.4\times10^{-6}$	$2.21\pm4.5\times10^{-4}$	$1.56\pm4.2\times10^{-6}$	$3.11\pm6.2\times10^{-5}$
32	Nonacosane	$0.39\pm2.6\times10^{-5}$	$3.30\pm6.7\times10^{-4}$	$0.94\pm2.5\times10^{-6}$	-
33	Squalene	$58.41\pm3.9\times10^{-3}$	$31.32\pm6.4\times10^{-3}$	$20.84\pm5.5\times10^{-5}$	$10.45\pm2.1\times10^{-4}$
34	Cholesta-3,5-diene	$0.74\pm5.0\times10^{-5}$	$5.56\pm1.1\times10^{-3}$	$1.95\pm5.2\times10^{-6}$	$3.02\pm6.1\times10^{-5}$
35	Docosanyl docosanoate	$1.63\pm1.0\times10^{-5}$	$6.21\pm1.3\times10^{-3}$	$6.80\pm1.8\times10^{-5}$	$6.44\pm1.3\times10^{-4}$
36	Cholesterol	$1.55\pm1.0\times10^{-5}$	-	-	-
*	Epicholesterol	-	$1.21\pm2.5\times10^{-4}$	$1.86\pm4.9\times10^{-6}$	$9.84\pm2.0\times10^{-4}$
37	Palmitoleic acid derivative	$1.18\pm7.9\times10^{-5}$	$2.73\pm5.5\times10^{-4}$	-	-
38	*cis*-9-Tetradecenoic acid heptyl ester	$0,46\pm3.1\times10^{-5}$	$1.61\pm3.3\times10^{-4}$	-	-
39	Arachidyl palmitoleate	$1,81\pm6.7\times10^{-4}$	$6.14\pm1.3\times10^{-3}$	$3.64\pm9.7\times10^{-6}$	$4.45\pm8.9\times10^{-5}$
	Free Fatty Acids	$15.12\pm1.0\times10^{-3}$	$25.95\pm5.3\times10^{-3}$	$26.06\pm6.9\times10^{-5}$	$37.98\pm7.6\times10^{-4}$
	Esterified Fatty Acids	$16.53\pm1.1\times10^{-3}$	$21.35\pm4.3\times10^{-3}$	$14.73\pm3.9\times10^{-5}$	$28.49\pm5.7\times10^{-4}$
	Fatty alcohols	$1.76\pm1.2\times10^{-4}$	$1.16\pm2.4\times10^{-4}$	$0.48\pm1.3\times10^{-6}$	$2.00\pm4.0\times10^{-5}$
	Carbohydrates	$2.05\pm1.4\times10^{-4}$	$5.27\pm1.1\times10^{-3}$	$3.79\pm1.0\times10^{-5}$	$3.67\pm7.4\times10^{-5}$

[1] Results are expressed as the mean (standard deviation) of a triplicate analysis. "-" not detected

Regarding the contents of fatty alcohols and carbohydrates, displaying values ranging from 0.48 to 2.00% and 2.05 to 5.27%, respectively, no evident temporal variation was observed, their relative contents remaining relatively stable over time (**Table 7**).

Since factors such as donor characteristics, substrate nature, deposition and storage conditions, and enhancement techniques, affect the reproducibility of fingermark composition, and in order to obtain a reproducible dating model, target compounds were selected based on their presence in all fingermarks' residues, as well as their reasonable abundance and resolution. Consequently, and in agreement with such criteria, compounds **19**, **20**, **26**, **30**, **33, 34** and **35** were targeted (**Fig. 12**).

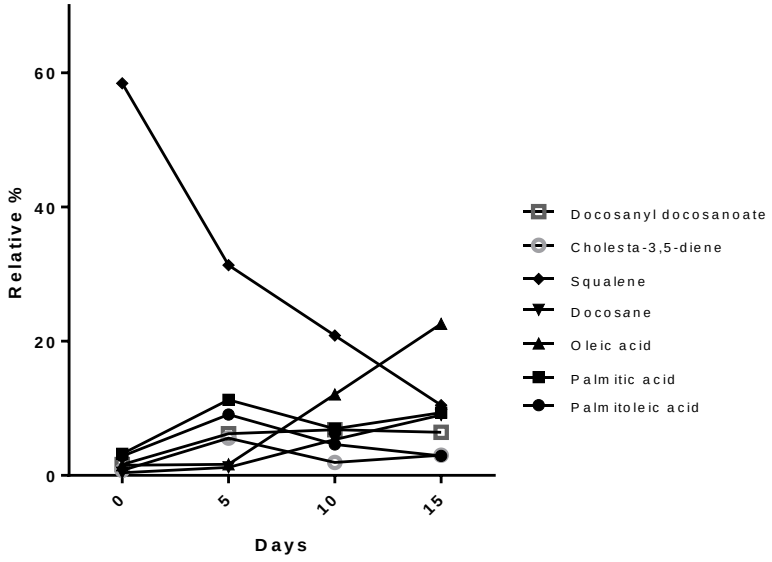

Figure 12. Content variation (relative %) of compounds **19**, **20**, **26**, **30**, **33**, **34** and **35** in residues obtained from fresh and aged fingermarks.

As previously mentioned, while the esterified palmitic acid derivative (**22**) was solely detected on the residue obtained from the fresh fingermark (**Table 7**), its degradation and absence in aged fingermarks is expected, probably due to the hydrolysis of the ester linkage. It is also relevant to note the detection of cholesterol (**36**) in the fresh fingermark residue, remaining undetected in aged residues, while its steroisomer epicholesterol (**36a**) was quantitated in increasable amounts in aged fingermarks (**Table 7**). It is well known that cholesterol (**36**) is particularly vulnerable to degradation upon aging, not only through isomerization but particularly by oxidation in the presence of

fatty acids, leading to the production of cholesta-3,5-diene (**34**) and the hydrolysis of esterified derivatives (**Fig. 13**) **(Mong et al., 1999)**. However, and to the best of our knowledge, identification and quantitation of such degradation products is so far restricted to the identification of the cholesterol ester cholesteryl propionate by **Girod and Weyermann (2014)**, no reports being available on the identification and quantitation of cholesta-3,5-diene (**34**) and epicholesterol (**36a**).

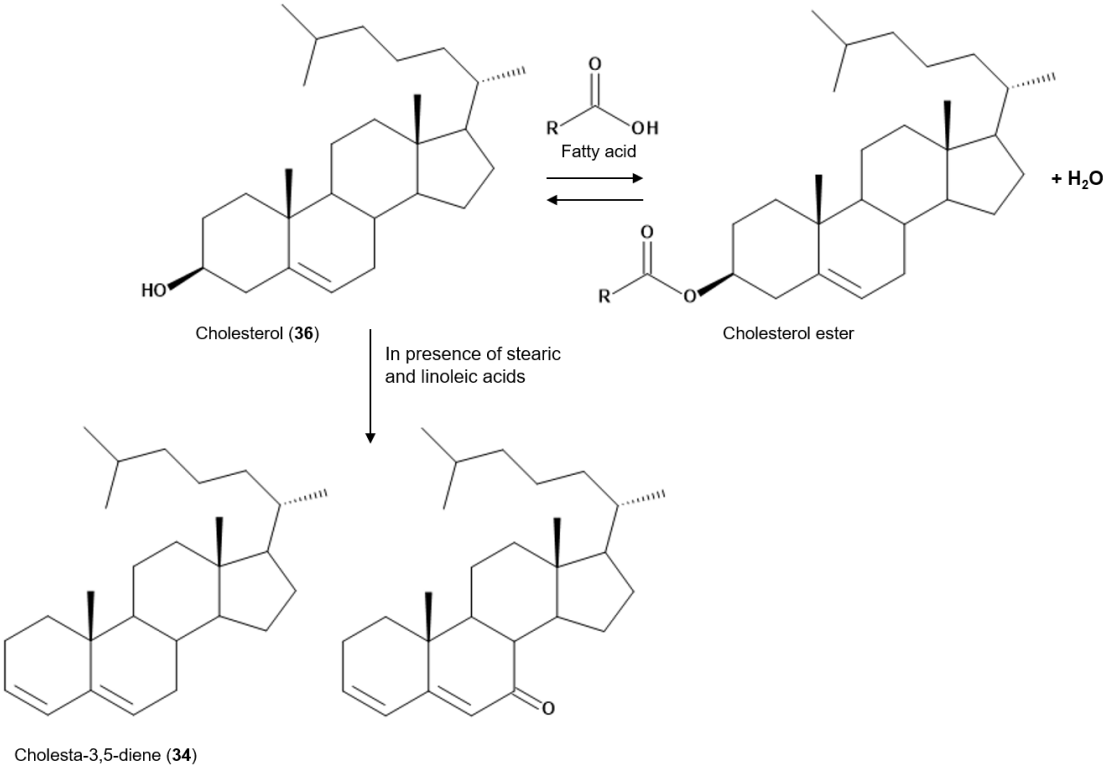

Figure 13. Cholesterol degradation in the presence of fatty acids (Cadd et al., 2015).

Analysis of the relative content of targeted fatty acid constituents (**Fig. 14**), clearly demonstrates an increase on the content of the MUFA oleic acid (**26**) and the esterified fatty acid docosanyl docosanoate (**55**) in aged residues, in comparison with the residues obtained from the fresh fingermark. Relevantly, while GC-FID analysis revealed only a slight increase on the relative amounts of oleic acid (**26**), a linear increase was observed in samples obtained from 10- and 15-day aged fingermarks (**Fig. 14**). Furthermore, and similarly to what has been found on the GC-FID profiling, a notable increase on the content of palmitic acid (**20**) was once again observed in contrast with its MUFA derivative (**19)**, whose content remained low in the 15-day aged fingermark (**Fig. 14**).

The increased content of palmitoleic acid (**19**) in the 5-day aged fingermark and the subsequent decreased levels in 10- and 15-day aged residues can be explained by the bacterial breakdown of triglycerides and wax esters into fatty acids, leading to an initial increase on the content of certain fatty acids, followed by a decrease after total breakdown of triglycerides and wax esters in free fatty acids, and the subsequent oxidation of UFAs to SFAs **(Archer et al., 2005; De Paoli et al., 2010)**.

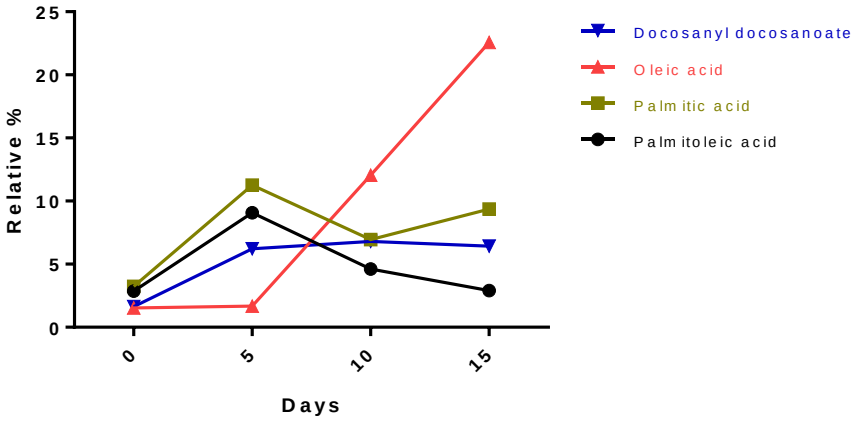

Figure 14. Relative content in fatty acid constituents in residues obtained from fresh and aged fingermarks.

Concerning the remaining targeted constituents, it is relevant to note the accumulation of cholesta-3,5-diene (**34**) in aged fingermarks (**Fig. 15**), particularly in the residue obtained after 5 days, which may result from the direct oxidation of cholesterol (**36**) as well as a result of the hydrolysis of esterified cholesta-3,5-diene analogues, as previously mentioned. Also the previously unreported linear alkane docosane (**30**) displayed a tendentially linear content increase over the course of 15 days, suggesting its suitability as a time-dependent endogenous marker.

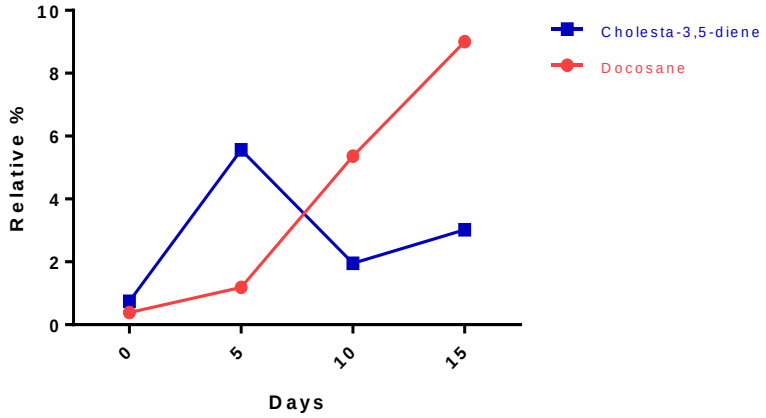

Figure 15. Relative content in cholesta-3,5-diene and docosane in residues obtained from fresh and aged fingermarks.

In agreement with the results obtained through HPLC-DAD analysis, while the content in squalene (**33**) correspond to ca. 58.4% of the quantifiable total in the residue obtained from the fresh fingermark, a linear decrease was observed in aged samples, being 5-fold lower in the residue obtained from 15-day aged fingermarks (**Table 7 and Fig. 12**). Despite the previous reports on the degradation of squalene in aged fingermarks **(Weyermann et al., 2011)**, it is instructive to observe that the peak area of squalene (**33**), relative to the total areas, as a function of time, is well fitted by simple exponential decay models (**Fig. 16**).

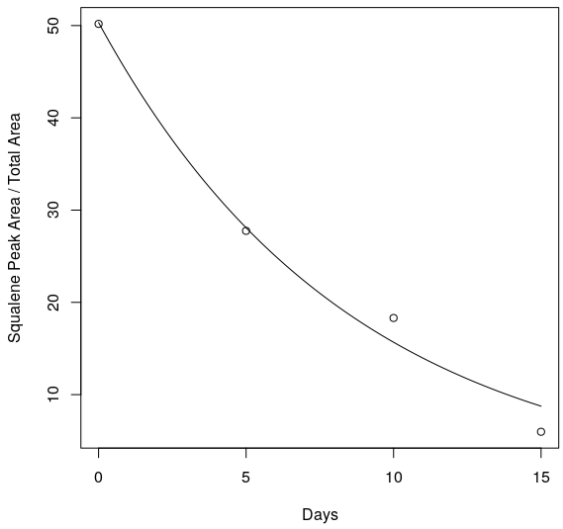

Figure 16. Exponential model fitted to the squalene peak area relative to the total area, as a function of time.

As an exploratory analysis, exponential models of the peak area of squalene (**33**) divided by the peak area of each of the other constituents, is also fitted to the data. Peak area of squalene (**33**) divided by docosane's (**30**) area revealed model improvements which, if not spurious due to the low number of data samples, should be investigated **(Table 8)**.

Table 8. Parameters of the exponential model of squalene content relative to the total content (RPA), as a function of time, including residual standard error (RSE), half-lives ($t_{1/2}$), and parameter 95% confidence intervals (CI).

$RPA = b_0 e^{-b_1 t}$	Squalene
b_0	50.33
b_1 (days^{-1})	0.12
RSE	2.72
b_0 CI	[39.19; 61.64]
b_1 CI	[0.07; 0.18]
$t_{1/2}$ (days)	5.94

The results on the GC-MS quantitative analysis of fresh and aged fingermarks obtained herein, allowed to pinpoint new potential endogenous components that may provide evidence on the age of fingermarks (**Fig. 17**).

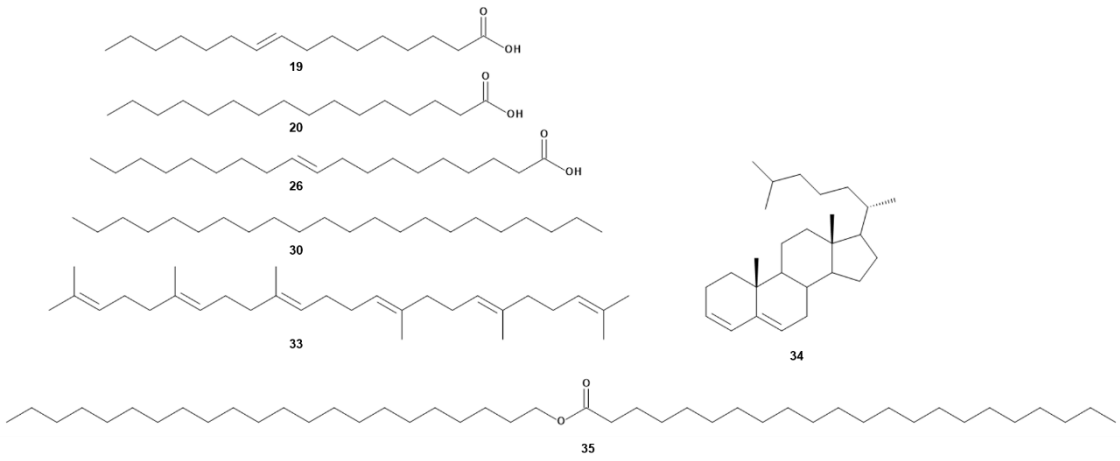

Figure 17. Potential temporal endogenous markers. **19.** Palmitoleic acid; **20**. Palmitic acid; **26.** Oleic acid; **30.** Docosane**; 33.** Squalene; **34.** Cholesta-3,5-diene; **35.** Docosanyl docosanoate (Girod et al., 2012).

Other studies based on GC-MS analysis showed reproducible results for the creation of fingerprint aging models, due to lipid degradation and the calculation of lipid ratios **(Weyermann et al., 2011; Girod et al., 2016)**.

Part V: Conclusion and future perspectives

The deposition of fingermarks on a surface and the chemical characterization of their residues is a challenge, mainly due to their variation over time and the difficulty in extracting fingermark samples, frequently occurring loss of material, being difficult to compare absolute quantifications of different compounds between different studies. In the present work, there are some general observations that can be made, although no absolute statements, about the variation of fingermark compounds over time, can be affirmed, since further studies with a higher sampling are needed, in order to corroborate the results obtained herein. However, there are results that are considered reliable, due to the repeatability of the same results in many other works.

It has been found that squalene is the major compound probably due to its function of biosynthesis of sterols, as well as the origin of degradation products formed by its oxidation. Following the squalene, and also according to literature, the compounds that had prevalence were palmitic acid, palmitoleic acid, stearic acid, myristic acid, tetradecanoic acid and pentadecanoic acid. The knowledge regarding the chemical constitution of endogenous components of fingermarks was extended since, to the best of our knowledge, new compounds were identified in this work. Polyunsaturated fatty acid *cis*-11,14-eicosadienoic was detected through GC-FID analyses and several novel compounds were identified by GC-MS namely, butanoic acid, dodecanol, methyl-6-palmitoyl-α-D-glucopyranoside, glycerol palmitate, tetradecanol, hexanedioic acid dibutyl ester, hexadecanol, 13-methyltetradecanoic acid, oleic acid methyl ester, heptadecanoic acid, octadecanol, tetramethylhexadecanol, 9-octadecenamide, docosane, nonacosane, cholesta-3,5-diene, docosanyl docosanoate, *cis*-9-tetradecenoic acid heptyl ester, arachidyl palmitoleate and epicholesterol. Furthermore, cholesterol is considered as a potential temporal marker, being detected only in fresh latent fingermarks and docosane is likely to be also a good temporal marker since its quantity tends to increase linearly over time. Additionally, palmitic and palmitoleic acid appear to be also valuable temporal fingermark constituents due to their concentration variations, i.e. the amount of palmitic acid that tends to increase as the amount of palmitoleic acid decreases.

In the present study, the SFA/UFA ratio tends to increase 4-fold over time, from day 0 to day 20 of aging. It is noticeable the UFAs decrease, as their saturated portions oxidize,

leading to a visible consequently increase in SFAs over time. We could also observe an increase in the content of free fatty acids in relation to the esterified fatty acids, due to hydrolysis and oxidation processes. Free fatty acids undergo several processes over time, which consequently increase their content in the endogenous components. The variation of the ratio between free and esterified fatty acids can be explained by the increase in the relative content of the free fatty acids palmitic and oleic acids, together with the disappearance of esterified palmitic acid after 20 days of aging.

The limitation in our sampling is already a starting point to perspective future work, in order to try to give consistency to the results obtained herein. More knowledge is needed, with this type of approach, to achieve a correct correspondence between the chemical profile of fingermarks and specific characteristics of the donor, such as gender, age, race, pathological state and diet and also for dating latent fingermarks assertively. When an individual is suspected of having committed a crime, and he assumes that he was at the crime scene but denies having been in a specific period of time, being able of dating fingermarks is extremely important, mainly after the application of revealing agents by police forces. In this way, it would also be interesting for future work, to try to understand the interference of the revealing agents with the chemical compounds of fingermarks. For instance, the use of ninhydrin in this study would not be a limitation as it reacts only with amino acids. However, the use of iodine crystals would be a problem since interferes with lipidic compounds. Oxidation occurs between iodine crystals and fatty acids, interacting with the double bonds of unsaturated fatty acids, blocking their unsaturations.

A deeper and more robust knowledge of the chemical composition of fingermarks, and its variation over time, will undoubtedly be an excellent tool to aid the Judicial System.

Part VI: References

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Part VII: Attachments

7.1. CHROMATOGRAMS OF LIPIDIC COMPONENTS OF AGED FINERMARKS ANALYZED BY GC-FID

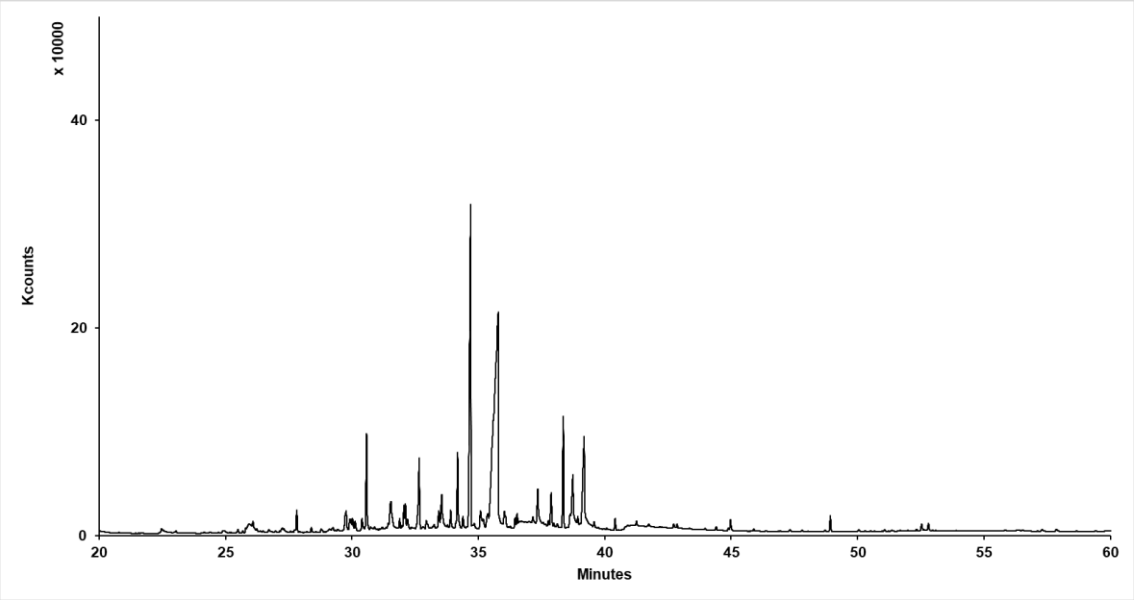

Figure 18. Fatty acid methyl esters content in an extract obtained from 5-days aged fingermark residues.

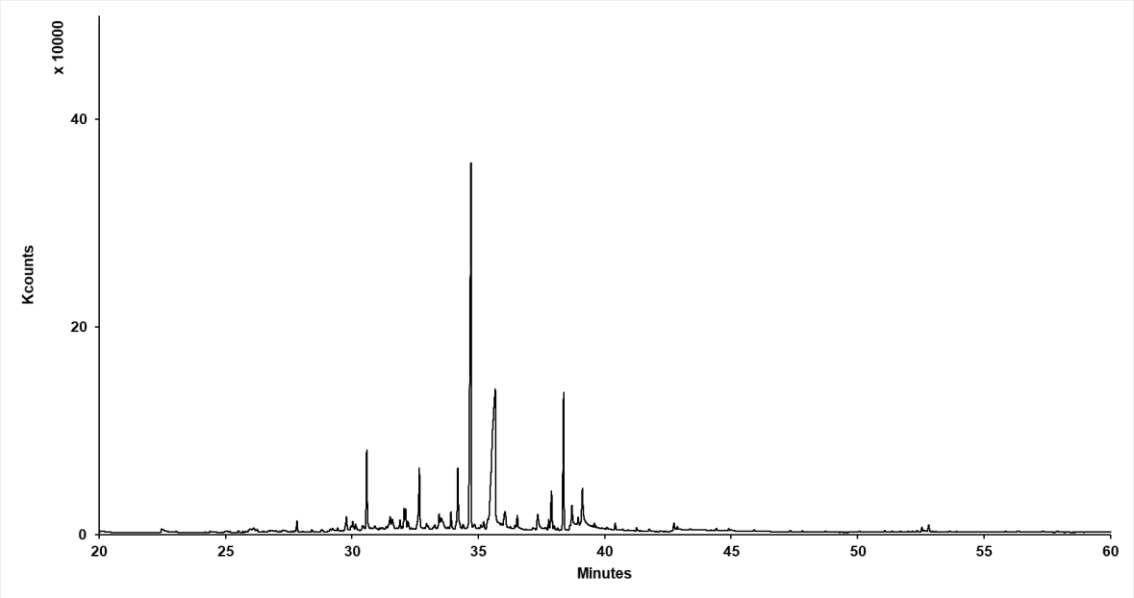

Figure 19. Fatty acid methyl esters content in an extract obtained from 10-days aged fingermark residues.

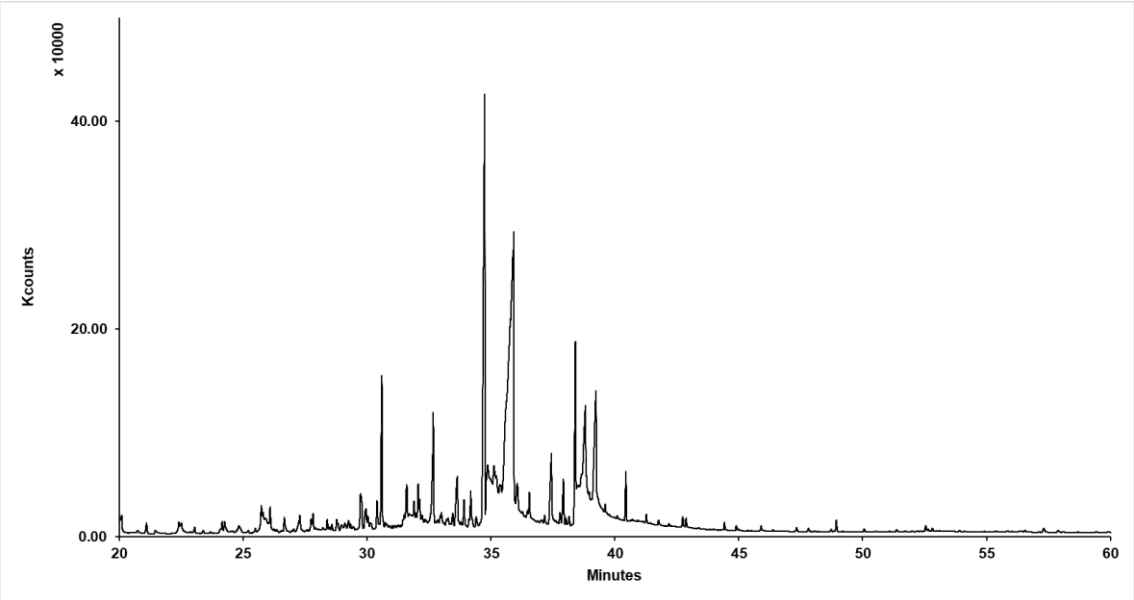

Figure 20. Fatty acid methyl esters content in an extract obtained from 15-days aged fingermark residues.

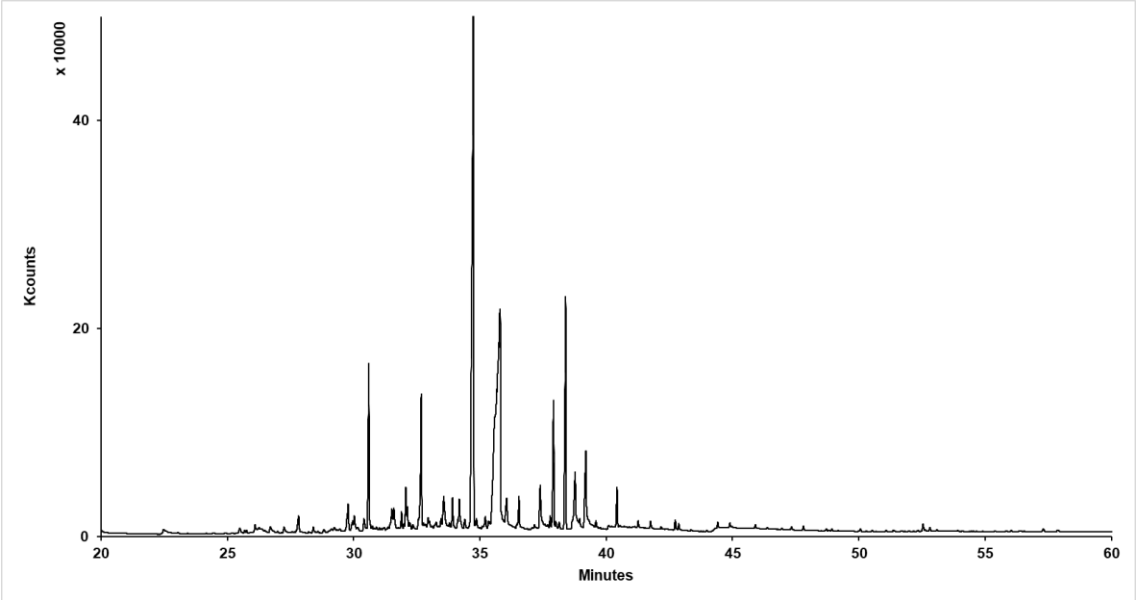

Figure 21. Fatty acid methyl esters content in an extract obtained from 20-days aged fingermark residues.

7.2. CHROMATOGRAMS OF LIPIDIC COMPONENTS OF AGED FINERMARKS ANALYZED BY GC-MS

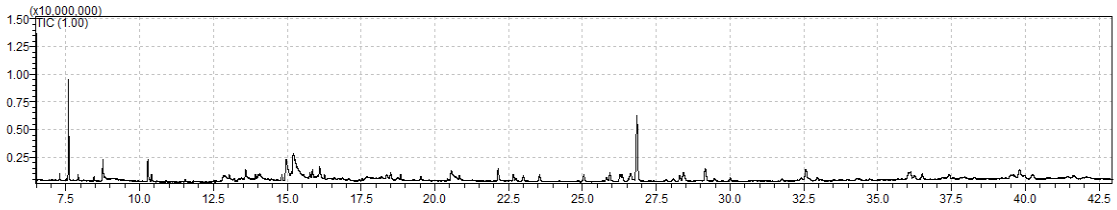

Figure 22. GC-MS characterization of lipid compounds of 5-days aged fingermark residues.

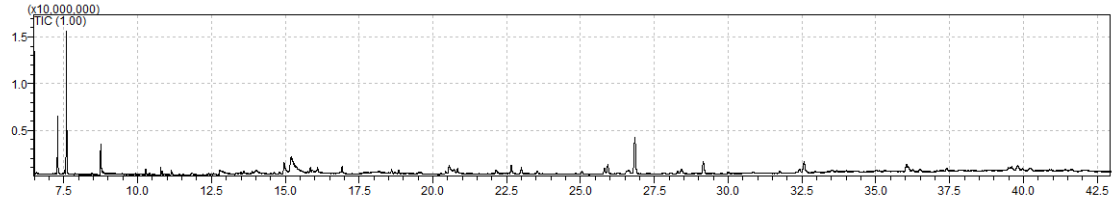

Figure 23. GC-MS characterization of lipid compounds of 10-days aged fingermark residues.

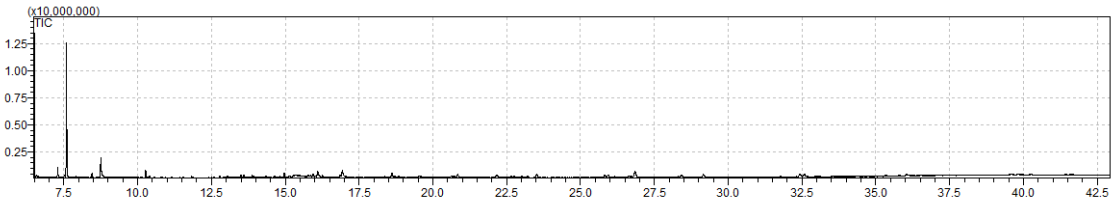

Figure 24. GC-MS characterization of lipid compounds of 15-days aged fingermark residues.

7.3. MASS SPECTRA OF LIPIDIC COMPONENTS ANALYZED BY GC-MS

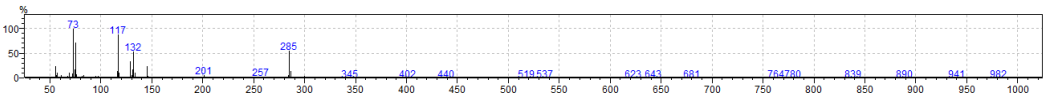

Figure 25. Mass spectrum of myristic acid (RT=13.59).

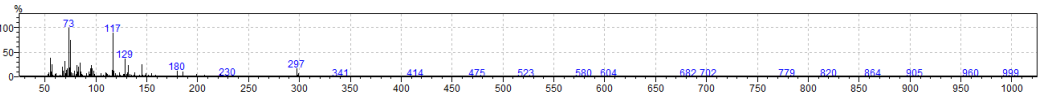

Figure 26. Mass spectrum of 13-Methyltetradec-9-enoic acid (RT=14.51).

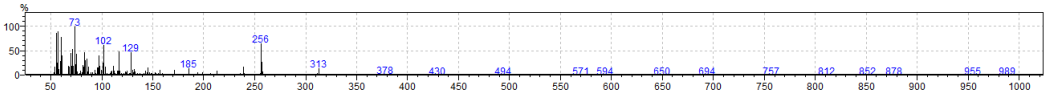

Figure 27. Mass spectrum of Isopropyl palmitate (RT=15.86).

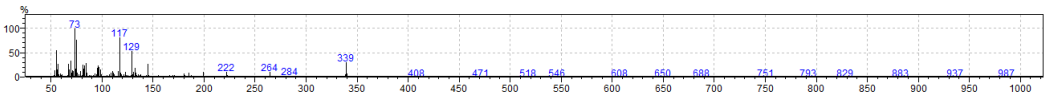

Figure 28. Mass spectrum of Oleic acid (RT=18.37).

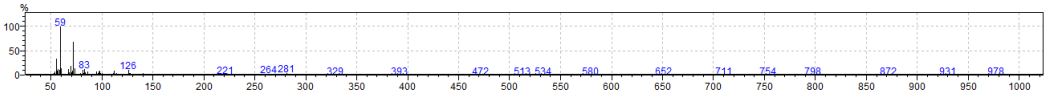

Figure 29. Mass spectrum of 9-Octadecenamide (RT=20.53).

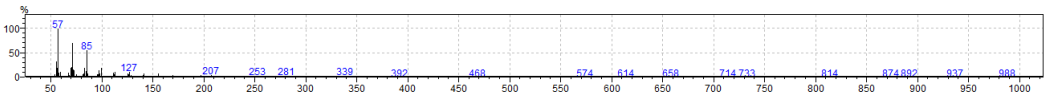

Figure 30. Mass spectrum of Docosane (RT=20.83).

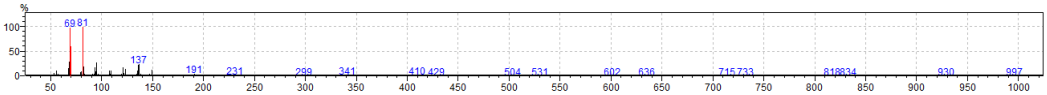

Figure 31. Mass spectrum of Squalene (RT=26.84).

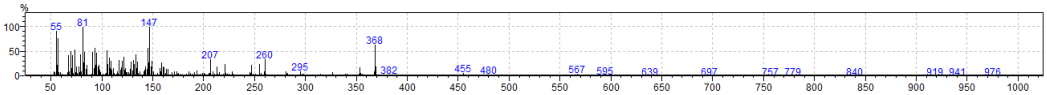

Figure 32. Mass spectrum of Cholesta-3,5-diene (RT=28.42).

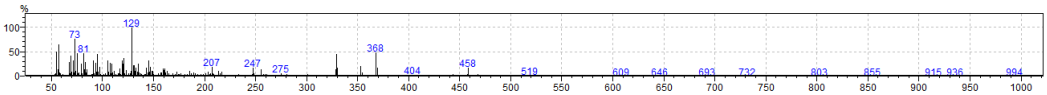

Figure 33. Mass spectrum of Cholesterol (RT=32.43).

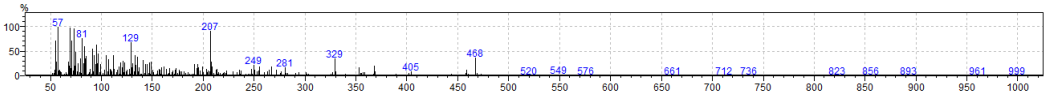

Figure 34. Mass spectrum of Epicholesterol (RT=32.43).

7.3.1. MASS SPECTRA OF CONTAMINANTS

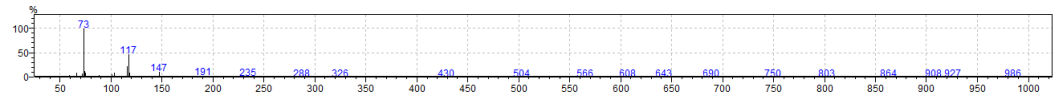

Figure 35. Mass spectrum of Diethylene glycol (RT=7.30, 9.92).

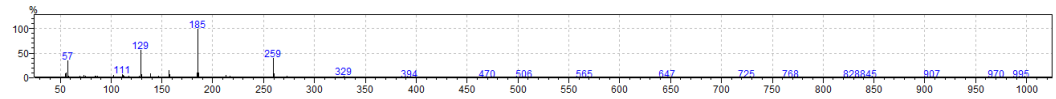

Figure 36. Mass spectrum of Citroflex (RT=18.84).

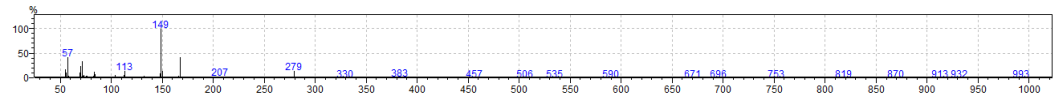

Figure 37. Mass spectrum of Phtalate (RT=22.66, 25.80).

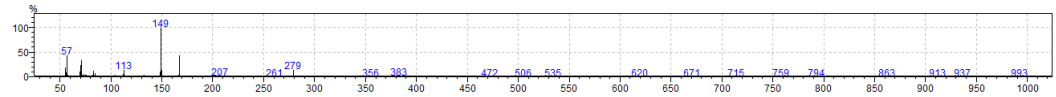

Figure 38. Mass spectrum of Sucrose (RT=23.51).