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Human Fingerprint Dating In Forensic Analysis

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If you only read the books that everyone else is reading, you can only think what everyone else is thinking.

Haruki Murakami

To my family and friends. To my father, my mother and my sister.

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Abstract

Fingerprints consist of ridges and valleys that form a pattern that is unique to the individual. Metabolites from sweat and other secretions as well as chemical residues that are present on a fingertip leave behind not only an individual specific pattern, a latent fingerprint, but also a wealth of information that may be exploited for forensic purposes [1]. Latent fingerprints have provided evidentiary value in forensic investigations for over a century, mainly through pattern comparison, as a means of identification. As science and technology have progressed, so has the collection and interpretation of evidence associated with latent fingerprints [1]. The latest developments focus on the assessment of the chemical and biochemical information contained in a fingerprint with several studies being initiated into the chemical composition of a fingerprint [2].

Simply visualizing the fingerprint and running the image through a database in search of a match may lead to no match being actually found, ending any further use of the fingerprint as a means of identification [3]. However, the fingerprint analysis procedure does not have to end with a database search, and that is what we are proposing in this work, to extend information retrieval from these latent fingerprints. The work described here focuses on exploring the knowledge which can be obtained from the chemical information provided by a fingerprint, particularly the composition of a fingerprint at the time of deposition as well as the chemical changes that occur over time. This is of very high relevance as age dating of fingerprints could have a significant impact in forensic science, as it has the potential to facilitate the judicial process by assessing the relevance of a fingerprint found at a crime scene. Moreover, there is presently no current method that is capable of reliably predicting the age of a fingerprint [4]. However, mass spectrometry has become an area of increasing interest when it comes to the study of fingerprints due to the massive amount of chemical information that can be extracted regarding both the fingerprint and the donor [4].

Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) was the chosen analytical method for it allows the precise determination of a molecule's chemical formula from its measured mass as well as greatly facilitating database search due to its extreme mass accuracy. Regarding our work, by knowing the elementary composition of small molecules found in fingerprints, it is possible to identify them. Chemical compound extraction from non-porous, inert surfaces was optimized and used to identify the compounds present in fingermark residue before and after an incubation period of either a week or a month. Besides confirming variations in classes of compounds that had previously been described in the literature, it was also possible to specifically identify not only those compounds but compounds that had not been described in former research studies. These compounds' time variations should be further analysed to confirm their consistency and their potential as target compounds for fingerprint dating. Although the results presented and the conclusions reached are preliminary, it is possible to show that a high-resolution technique such as FT-ICR mass spectrometry may come to play a pivotal role in forensic analysis and dating of human fingerprints in the near future.

It is intended that these results will potentiate a further study into how small molecules found in fingerprints change with time, as well as contributing to what could be the future of fingerprint analysis in a forensic perspective.

Keywords: ***Fingerprints; Ageing; Mass spectrometry; Small molecules.***

Resumo

As impressões digitais, constituídas por cristas e vales, formam um determinado padrão que é único a cada indivíduo. Metabolitos do suor e outras secreções, bem como resíduos químicos presentes na ponta de um dedo deixam não só um padrão específico para o indivíduo, uma impressão digital latente, como também vasta informação que pode ser explorada para propósitos forenses. As impressões digitais latentes fornecem um forte valor probatório no que diz respeito a investigações forenses há mais de meio século, particularmente através da comparação de padrões como meio de identificação. Do progresso da ciência e da tecnologia, adveio a progressão no que diz respeito à aquisição e interpretação de provas e evidências associadas a impressões digitais latentes [1]. Os mais recentes desenvolvimentos estão associados à determinação da informação química e bioquímica contida numa impressão digital, sendo que vários estudos foram já iniciados no sentido de avaliar a composição química de uma impressão digital [2].

A simples visualização de uma impressão digital e o processamento da mesma numa base de dados em busca de uma correspondência pode terminar sem que qualquer correspondência tenha sido efetivamente encontrada. Desta forma, cessa qualquer uso futuro da impressão digital como meio de identificação ou de investigação [3]. No entanto, o processo de análise da impressão digital não tem de terminar obrigatoriamente com a pesquisa numa base de dados; e é isso que é proposto e investigado ao longo deste trabalho – amplificar as informações que podem ser recuperadas a partir de impressões digitais latentes. O trabalho aqui descrito concentra-se em explorar e aprofundar o conhecimento que pode ser adquirido a partir da informação química de uma impressão digital. Particularmente, a composição de uma impressão digital no momento em que esta é depositada, bem como as alterações químicas que ocorrem nos compostos que a constituem ao longo do tempo. Tal é de extrema importância, uma vez que a datação das impressões digitais pode ter um impacto significativo na área das ciências forenses, visto que tem o potencial de auxiliar o processo judicial ao avaliar a relevância e o valor de uma impressão digital encontrada na cena do crime. Assim, permitiria aos investigadores não só identificar as pessoas com acesso à cena do crime, mas também criar uma cronologia dos eventos sucedidos a fim de perceber se um dado indivíduo teve ou não acesso a esse local na altura em que o crime ocorreu [2]. É ainda de salientar que não existe atualmente um método que seja capaz de prever a idade de uma impressão digital com rigor e exatidão, não sendo por isso possível determinar quando é que ocorreu a deposição da mesma [4]. Porém, a espetrometria de massa tem-se revelado uma metodologia analítica de crescente interesse no que diz respeito ao estudo de impressões digitais. Este interesse crescente está associado à elevada quantidade de informação que pode ser extraída de uma impressão digital, tanto no que diz respeito à própria impressão digital, como no que concerne ao dador da mesma [4].

Relativamente à investigação, a análise foi realizada num número variável de voluntários – dois voluntários para a análise de uma gama de solventes, nove voluntários para a análise de amostras incubadas durante um mês e oito voluntários para amostras incubadas durante uma semana. Os voluntários fazem parte do grupo de investigação do Laboratório de FT-ICR e Espectrometria de Massa Estrutural (FT-ICR-MS-Lisboa) da Faculdade de Ciências da Universidade de Lisboa, abrangendo elementos de diferentes faixas etárias e sexos. Deste modo, os resultados obtidos e as conclusões alcançadas apenas refletem uma tendência média da amostra em estudo. Adicionalmente, não houve qualquer tipo de controlo relativamente à dieta de cada voluntário nem sobre a força exercida pelos dedos dos mesmos nas

lâminas de vidro, uma vez que se procurou reproduzir o mais próximo possível o cenário encontrado numa cena do crime.

A espetrometria de massa de ressonância ciclotrónica de ião com transformada de Fourier (FT-ICR MS) foi a metodologia analítica escolhida, uma vez que permite a determinação precisa da fórmula química de uma molécula a partir da massa medida, facilitando também a pesquisa numa base de dados, devido à sua extrema precisão de massa. Neste caso, ao conhecer a composição elementar das pequenas moléculas encontradas numa impressão digital é possível identifica-las.

A extração de compostos químicos de superfícies não porosas e inertes foi otimizada. Para tal, foi analisada uma gama de solventes de modo a determinar qual o melhor para a análise de impressões digitais. Esta determinação teve por base não só o risco químico associado a cada solvente, como a sua disponibilidade e o número de compostos cujo solvente permitia identificar por espetrometria de massa. Deste modo, tendo permitido a identificação de 19 compostos, tratando-se do mais seguro e do mais adequado para o método de ionização utilizado (ESI), o solvente constituído por acetonitrilo : metanol : água foi considerado o mais adequado para prosseguir com o estudo do envelhecimento de impressões digitais.

A aplicação da espetrometria de massa permitiu a identificação e confirmação da presença de vários compostos previamente descritos na literatura como presentes em impressões digitais. Contudo, permitiu também a identificação de novos compostos, presentes habitualmente no suor, que aparentam variar com o envelhecimento dos resíduos da impressão digital.

No que diz respeito ao aparecimento de compostos após a incubação, foi possível identificar compostos associados à decomposição de colesterol, ácidos carboxílicos e derivados, ácidos gordos saturados e insaturados e aminoácidos. Para além destas classes de compostos, que já haviam sido descritas como aparecendo em impressões digitais com o passar do tempo, foram identificados compostos como o esqualeno e lactapiperanol D.

Relativamente aos compostos descritos como desaparecendo após um determinado período de incubação, alguns dos mencionados são ácidos gordos insaturados, esqualeno e derivados do esqualeno. Neste caso, para além da identificação de ácidos gordos insaturados, foi detetada a presença de novos compostos como o ácido esteárico e lactapiperanol D, cujo desaparecimento ainda não foi descrito na literatura.

O aumento da quantidade de um dado composto foi medido pelo aumento da intensidade do mesmo. Ácidos gordos saturados de cadeia curta têm tendência a sofrer um aumento, uma vez que podem derivar de ácidos gordos de cadeia longa. Epóxidos de esqualeno e derivados do esqualeno foram também previamente descritos como aumentando ao longo do tempo. Para amostras incubadas durante um mês, verificou-se o aumento da quantidade de alguns ácidos gordos saturados de cadeia média, nomeadamente os ácidos azelaico e láurico. O ácido adípico, uma forma oxidada que deriva do esqualeno, foi também identificado.

Por outro lado, a diminuição da quantidade de um determinado composto foi medida através da diminuição da intensidade do mesmo. Ácidos gordos insaturados, triglicéridos, colesterol e esqualeno são compostos cuja quantidade tende a diminuir com o passar do tempo. Neste caso, nenhum composto foi identificado para amostras incubadas durante o período de um mês. Porém, tanto o ácido colnelénico como o metilgingerol foram identificados como tendo diminuído após uma semana de incubação, sendo que o ácido colnelénico se trata de um ácido gordo insaturado.

Assim sendo, foi possível confirmar não só variações de compostos previamente descritos na literatura, como também identificar novos compostos que aparentam variar similarmente com o envelhecimento dos resíduos deixados pela impressão digital. Embora os resultados apresentados e as conclusões alcançadas sejam preliminares, é possível mostrar que uma técnica de alta resolução como a espectrometria de massa FT-ICR pode vir a ter um papel fulcral na análise forense e na datação de impressões digitais humanas num futuro próximo.

Em suma, pretende-se que as conclusões tiradas ao longo desta dissertação potencializem um estudo futuro e mais aprofundado de como as pequenas moléculas e os compostos encontrados em impressões digitais variam com o tempo. Apesar do interesse pelas impressões digitais ter surgido há várias décadas, o seu estudo constitui ainda uma área consideravelmente inexplorada que requer mais tempo, dedicação e investigação. Deste modo, pretende-se que esta dissertação contribua para o que pode constituir o futuro da análise de impressões digitais numa perspetiva forense.

Palavras-chave: ***Impressões digitais; Envelhecimento; Espetrometria de massa; Pequenas moléculas.***

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Acronyms and Abbreviations

$[M+H]^+$	Protonated adduct
$[M+K]^+$	Potassiated adduct
$[M+Na]^+$	Sodiated adduct
ESI	Electrospray ionization
FID	Free induction decay
FT-ICR	Fourier transform ion cyclotron resonance
GC-MS	Gas chromatography mass spectrometry
ICR	Ion cyclotron resonance
LC-MS	Liquid chromatography coupled to mass spectrometry
leuENK	Leucine-enkephalin
M	Mass
m/z	Mass-to-charge ratio
MALDI	Matrix-assisted laser desorption ionization
MMA	Mass measurement accuracy
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
Nano-ESI	Nano-electrospray ionization
PCA	Principal component analysis
ppm	Parts per million
R	Resolving power
S/N	Signal-to-noise ratio
TG	Triacylglycerol

1

Framework

In this chapter, a background of the subject matter under study and a description of the main goals will be carried out.

1.1. Background

For over a century, fingerprints have been one of the major means of identification in the criminal justice system [3], being the most commonly used form of evidence worldwide [5]. As primary evidence in criminal investigations, fingerprints are the most frequently used, being presented as evidence five times more often than DNA, indicating their valuable strength and reliability [6]. However, the current procedure consists of visualizing latent prints with a forensic carbon-based powder or other methods to reveal the image, before running it through a database for a match. This procedure is limited and has not advanced over time. If the database search does not yield a match, the evidence provides no further information until the suspect is identified by other means. With modern advances in technology, such as using mass spectrometry to obtain chemical information from a fingerprint, the procedure does not need to end with a database search. Further evidence can be acquired from the composition of both endogenous compounds – amino acids, cholesterol, fatty acids, and lipids – and exogenous compounds – contaminants from the environment such as drugs or explosives – in the latent print [3].

Fingerprint evidence is at the forefront of most criminal cases, with important issues raised regarding the age of the mark [6]. Was the fingermark deposited during the crime, or at a time not related for legitimate reasons? Dating can be defined as the attribution of a relative or absolute age [7]. It can be applied to past events, objects, people or traces. It mainly consists of the determination of the time interval, separating the elements to date and the present time and the determination of the relative order of past elements [7].

There have been several past attempts to develop a method to estimate the age of fingerprints to determine relevance at a crime scene, but so far with limited success. An in-depth understanding of their change over time from the initial to the aged stage is vital to apply a robust fingerprint ageing model [6]. Once successfully developed, it would allow investigators to screen suspect pools to focus only on people with access to the crime scene, and also hypothetically create a timeline for the crime [3].

Primary investigations of fingerprint ageing focus on the fingerprint's morphological quality, and how easy the latter was to enhance using standard forensic techniques. Despite methods such as these being extremely subjective, they are the most commonly cited in court cases [2]. More recently, the investigation has changed towards the chemical composition of the fingerprint, and how it changes over time, allowing for a more quantitative ageing model [2].

In this sense, it is of extreme relevance to try and understand the fingerprint ageing process. Hence, this work intends to contribute to the preliminary characterization of an ageing profile in a small cohort of volunteers from the Faculty of Sciences of the University of Lisbon. A small cohort was decided upon for two particular reasons. Firstly, this study was designed for continuity, which means that volunteers must always be available over time. Secondly, due to the pandemic situation the world is experiencing at the time of writing this dissertation, it was neither possible nor recommended to proceed to large-scale analysis and data collection.

The work's main goal was to identify chemical compounds from a fingerprint and study its chemical changes over time. We hope to potentiate additional studies on this topic, as well as contribute to what could be the future of forensic fingerprint research.

1.2. Aims

The main goal of this dissertation was to identify the chemical compounds present on a fingerprint and study how those change with time.

The first approach focuses on determining the most suitable solvent system for fingerprint analysis, based on its composition and the number of identified compounds.

The second aim is to identify the small molecules that can be found on fingerprints through mass spectrometry. A database of compounds previously identified along with compounds known to be present in sweat was created to confirm their presence in our samples. Alongside, it was significant to study the variation of these compounds with time. Therefore, increases and decreases in the compounds' signal intensity in a mass spectrum after a defined incubation period were considered, as well as their appearance or disappearance after that same incubation period.

Another goal was to understand the chemical differences between samples incubated for different time periods, namely samples incubated for a week and samples incubated for a month. Lastly, it would be remarkable to determine if some sort of pattern can be found among the chemical changes within our samples and those previously described in the available literature.

A better understanding of the chemical alterations that take place in a fingerprint will provide researchers with insightful knowledge about the subject matter. In consequence, the incentive on this topic will pave the way for developing effective fingerprint dating methods and to make them a reality and an investigation tool in the future.

2

Theoretical Framework

In this chapter, the concepts for understanding the dissertation theme will be addressed. These concepts relate to fingerprints and their role in forensic science, fingerprints' chemical composition and how their chemical compounds are known to change with time. The preferred way to study the chemistry of fingerprints will also be addressed, which leads us to mass spectrometry and more specifically to FT-ICR MS.

2.1. Fingerprints and their role in Forensic Science

For over a century, fingerprints have been one of the major means of identification in the criminal justice system [3], being the most commonly used form of evidence worldwide [5].

Fingerprints consist of ridges and valleys that form a pattern which is unique to the individual. The sweat and chemical residues that are present on a finger can leave behind a ridge detail on a surface, or a latent fingerprint, meaning the fingerprint is not visible to the naked eye and needs some sort of detection [1]. Some of the preferred methods for the detection and acquisition of latent fingerprints include the use of reagents like ninhydrin and DFO (1,8-Diazafluoren-9-one), carbon-based powders, photos, scanners, and lights with a particular wavelength (infrared or ultraviolet) [8]. On the other hand, prints deposited in blood or any other coloured substances are readily visible to the naked eye and can usually be photographed with no further treatment to acquire an image for comparison purposes [9]. The work described in this report was based on the use of latent fingerprints. This particular type of fingerprints, despite not being visible, is present in everything an individual touches. For this reason, latent fingerprints have provided evidentiary value in forensic investigations, mainly through pattern comparison, as a means of identification.

2.2. Fingerprints' Composition

Fingerprints are composed not only of endogenous compounds that are inherent to the fingerprint, such as amino acids, cholesterol, fatty acids, and lipids but also of exogenous compounds. The exogenous compounds are chemical contaminants from the environment with which the finger has come into contact such as drugs or explosives.

2.2.1. Endogenous and Exogenous Compounds

The numerous endogenous substances found in fingerprints can have four different sources: eccrine sweat, apocrine sweat, sebum secretions and substances from the epidermis. 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, because of contamination through the touching of the face and

hairs, apocrine secretions are less frequent in fingerprints, however, they are generally significant in crimes of sexual nature. Eccrine and apocrine secretions result in a mixture of inorganic compounds and water-soluble organic compounds such as sodium chloride, urea and amino acids. On the other hand, sebaceous secretions contain fat-soluble compounds such as glycerides, fatty acids, wax esters, squalene, sterols and sterols esters. Additionally, proteins are known to be present in fingerprints but have only recently been the subject of forensic research [10]. Intrinsic components including metabolites and traces of medications and drugs and extrinsic contaminants, such as blood, dirt, grease, make-up, food contaminants, moisturisers, and hair care products can also be found in fingerprints [5].

The intrinsic and extrinsic constituents can vary significantly not only between individuals – intervariability –, but also within the same individual from one day to another – intravariability – and even at different times, on the same day [5]. Despite the presence of secretions from the epidermis and the secretory glands, the chemical composition of a fingerprint differs both qualitatively and quantitatively from the general chemical composition of sweat, as it contains a complex mixture of compounds that come from different glands and not exclusively from the eccrine ones [11]. Furthermore, water is a major component of a fingerprint, as the intrinsic components of a fingerprint are comprised of 95–99% water and organic and inorganic compounds [5].

2.2.2. Lipids as the Major Component of Fingermark Residue

The majority of the compounds that have been previously identified in fingermark residue are lipids including fatty acids, glycerides and long-chain fatty acid esters, as well as squalene, sterols – such as cholesterol – and numerous lipid esters [5]. Some of the compounds to highlight include squalene and cholesterol, both frequently identified in fingermark residues. Squalene is the precursor of many steroids including cholesterol, which is the most abundant sterol in animal tissues. While the sebaceous glands do not normally secrete cholesterol, it is contained in sebum. It seems that cholesterol enters into the sebum through blood circulation and the plasma, probably originating from the sebum or epidermis [11]. Additionally, palmitic acid, stearic acid, myristic acid and oleic acid, are also often observed in samples, and for that reason are worth highlighting as well [12].

2.2.3. Variables that Affect Fingermark Residue Composition

Fingermark residue composition depends on a various number of factors. For example, donor characteristics influence the fingermark composition; these characteristics being age, gender, ethnic origin, medication, psychological state, health, metabolism and diet. Research studies showed that the age of the donor influences fingermark residue in a way that secretions from children were observed to disappear much faster after deposition on a surface than those of adults [11]. This phenomenon was explained in the literature by the fact that residue from children mainly consists of aqueous saline compounds and fewer free fatty acids. These compounds are very volatile and thus disappear quickly. On the contrary, the residue from adults includes squalene, cholesterol, large fatty acid esters and glycerides. These compounds are far less volatile and thus remain on substrates. Regarding the inorganic compounds present in fingermark residue, it is relevant to note that the amount of chloride in fingermark residue is inversely proportional to the age of the donor, therefore the amount of chloride decreases as the age of a person increases [11].

It was observed that clinical conditions and medications significantly influence the recovered fingermark residue. For example, concerning the lipid composition, a person affected by acne has a higher amount of fatty acids and squalene than an otherwise healthy person. However, if treated – for example, with an anti-acne cream –, these compounds decrease dramatically [11]. Some other contaminants are associated with contact with products such as drugs, food or cosmetics. Cosmetics, like hair products, perfume residue, face or body creams are often identified in fingermark residue. They can be difficult to differentiate from intrinsic fingermark residue, because they may contain lipid compounds that are naturally present in fingermark secretions, fatty acids like palmitic acid, or wax esters. Nicotine contamination can also be easily identified in fingermark residue [11].

Another aspect to take into consideration is the deposition conditions, referring to the pressure, the contact duration, the time of day – morning, afternoon and night-time, associated with metabolism regulation under circadian control –, the finger itself as well as hand washing. Regarding the fingers themselves, two main observations have been made. Firstly, the fingers of the left hand contained a larger amount of chloride than the fingers of the right hand. Secondly, the thumb, index and middle fingers originate fingermarks containing a significantly smaller amount of chloride than the ring and little fingers. It is suggested that these observations can be explained by the fact that most people are right-handed and thus use this hand more than the left one. Moreover, the thumb, index and middle fingers are more used than the ring and little fingers. Consequently, the most commonly used fingers lose their secretions because of frequent contact with different surfaces, while the less used fingers can build up and keep a larger amount of secretions before coming into contact with a surface [11].

Environmental conditions such as humidity, light exposure, temperature, dust, rain, friction, air circulation and contaminants present in the atmosphere or on adjacent materials or surfaces are also factors which influence the fingermark residue composition [11].

2.3. Fingerprints' Ageing Process

In practice, a forensic scientist will never collect fingermarks right after deposition. Like all materials, fingermarks undergo modifications over time – they age. Therefore, chemical, physical and biological alterations over time will affect the fingermark residue left on surfaces and modify its initial composition [11]. Dating can be defined as the attribution of a relative or absolute age [7]. It can be applied to past events, objects, people or traces. It mainly consists of the determination of the time interval, separating the elements to date and determining the relative order of past elements [7]. At present, the precise assessment of the age of a fingermark is not possible [13]. Nevertheless, the demand for age determination of latent fingerprints is high in forensic science and this issue has bothered fingerprint experts and forensic scientists severely in the past [14].

2.3.1. The Importance of Fingerprint Dating

The question of the age of fingermarks is often raised in investigations and trials when suspects admit that they have left their fingermarks at a crime scene but allege that the contact occurred at a different time than the crime, raising alternative explanations for the presence of their fingermarks at the crime scene. After a survey of more than two dozen American court cases, it became clear that there was no consensus regarding how these courts admitted testimony related to the age of crime scene fingermarks [15]. There are numerous reported instances of attorneys requesting expert witnesses to provide precise

age determinations, nonetheless, those age estimations were never supported by robust scientific procedures. As a matter of fact, they were often generally based solely on the experience of police officers and fingermark experts, stated in the form of subjective evaluations of the quality and contrast of the developed fingermarks. On several occasions, the experts actually stated that it was impossible to estimate the exact age of fingermarks, nevertheless provided the court with a personal opinion based on their experience [15]. The existing uncertainty shows that the development and validation of a reliable fingermark dating methodology would be particularly important in forensic science casework [15].

2.3.2. The First Steps to Fingerprint Dating

As long ago as 1970, the Dactyloscopy Department of the Main Police Headquarters in Warsaw developed a method of evaluating the age of fingerprint traces which was routinely used at the time. This method concerned the traces appearing on a smooth and non-absorptive surface. To infer the age of fingerprint traces at the crime scene, close attention was paid to the reconstruction of the conditions in which the evidential traces were formed [16]. In addition, a comparative examination of evidential traces with fingerprints of the suspect was performed, the fingerprint traces were subjected to experimental examination concerning ageing in a given environment. Lastly, it would also be taken into consideration the knowledge and experience of an expert in the field of skin physiology and the mechanism of formation and ageing of fingerprints [16].

Alcaraz-Fossoul et al. have attempted to age fingerprints based on degradation by studying the colour contrast between the powder on the fingerprint ridges and lack thereof in the valleys, along with the number of minutiae visualized with the powder over time [17]. This approach is similar to others that look at the quality of the fingerprint, and how easy it is to enhance using standard forensic techniques. Despite methods such as these being extremely subjective, they are the most commonly cited in court cases [3].

Contrariwise, Champod et al. recommend that "*an age estimation should never be based solely on the quality of a developed mark*" [18]. A fingermark contains numerous compounds, mainly eccrine and sebaceous in nature that can be potentially taken advantage of [15].

Several decades ago, changes in chemical compounds found in fingerprints were already being recognized as a way to study the ageing process in fingerprints. In 1977, it was reported that the perspiration of humans is composed of phospholipids containing amino acids, therefore, an advantage could then be taken of the nature of those organic substances. Dalrymple et al. findings point out that there is a definite change in the molecular architecture of the components of palmar sweat based on fingerprints' luminescence when exposed to high-intensity argon laser light [19]. They concluded that the presence unsaturation and chemical modifications like association, dissociation or transformation is expected [19]. As for 1986, Dikshitulu et al. stated that chemical substances of perspiration found in latent fingerprint residue could hold the key for dating latent fingerprints [20]. By then, some components had already been detected and the variation in the quantity of those major components with time was observed [20].

2.3.3. Fingerprint's Ageing

Determining the composition of fingermark residue is an analytical challenge because of its complex and multifaceted nature and the existence of numerous influence factors. Consequently, the composition

of fingermark residue can be described as a system evolving between different states over time, the initial composition and the aged composition [11]. The initial composition corresponds to the transferred fingermark residue immediately after the contact between the finger and a substrate. The residue transferred depends on the different influence factors formerly mentioned. These can be classified into three categories: the donor characteristics – diet, age, gender –, the deposition conditions – deposition pressure, contact duration – and the substrate nature – porous, semi-porous and non-porous. These factors may be significantly different from fingermark to fingermark and thus lead to variability in initial composition. The elapsed time between the transfer of a fingermark on a surface and its discovery results in the emergence of the aged composition. The latter corresponds to the evolution of the initial composition over time and the products emerging in fingermark residue. During this elapsed time, three types of factors influence the ageing process, leading to the great variability among the aged composition: the substrate nature, the environmental conditions and the enhancement techniques used to visualize latent marks. Thus, the variability of the aged composition is the addition of the variability of the initial composition with the variability of the influence factors occurring over time [11]. The final aged composition is therefore a combination of all of the factors from both the deposition and ageing stages [5].

Ageing can follow many different pathways at varying rates. Alteration and disappearance of the initial compounds will occur over time in a continuous process involving a large number of phenomena such as degradation, metabolism, drying, evaporation, migration, oxidation or polymerization [11]. The general effect of ageing on eccrine fingermark residue is the loss of water, with water being the main constituent of palmar eccrine sudation. In fact, a study reported a loss of 85% of the fingermark weight over a two-week timeframe and presumed that it was primarily due to the loss of water [11]. Yet, an initial increase in detected material has been observed, possibly due to component decomposition [5].

Several studies on the ageing of the initial composition of fingermarks have been carried out to gain a better understanding of those mechanisms and their kinetics. These studies mainly concentrate on the ageing of amino acids, proteins, fatty acids, squalene, cholesterol and wax esters [11].

2.3.4. Changes in a Fingerprint's Chemical Composition with Time

Concerning the effect of ageing on amino acids, a study on the development of ninhydrin analogues for the visualization of fingermarks highlighted the general stability of amino acids over time. In fact, this study showed that old fingermarks could still be developed on paper with amino acid visualizing reagents; the given explanation is that the amino acids have an affinity for cellulose and can thus remain stable for long periods of time [21]. However, Cuthbertson observed that over a period of 236 days, the amino acid content of a fingermark left on paper decreased from 0,083 mg/cm^2 to 0,046 mg/cm^2 [22]. These results tend to suggest that the amino acids are not fully stable over time, but that their concentration remains high enough to be detected with amino acid visualizing reagents. Similar observations were also made for proteins present in fingermark residue [11].

The ageing of sebaceous compounds is mainly illustrated by their qualitative and quantitative decrease over time, principally squalene, cholesterol and fatty acids. These compounds undergo significant degradation as a function of time, resulting in the production of new constituents, mostly small oxidized molecules [11].

Squalene contains six double bonds and is ramified, giving this molecule a high capacity to react and degrade, for example, through microbial processes. This explains why oxidation products of squalene

were identified in fresh fingermark residue using mass spectrometry. These oxidation products include epoxides, ketones, alcohols and mainly squalene hydroperoxides, in particular squalene monohydroperoxide [11]. These reactions end with the formation of molecules in fully oxidized forms, such as adipic and glutaric acids. Concerning the ageing of squalene samples, it was observed that after one month of exposure to ambient conditions, 10% of each sample was composed of hydroperoxides [23]. Recent findings indicate squalene decomposes rapidly over time and is rarely detected in older fingerprints, on glass substrates it is almost undetectable after only one week. Squalene tetra- and penta-hydroperoxides were still detectable after 20 days, whereas squalene epoxide was detected in freshly deposited marks and increased in concentration over 5 days from deposition [9]. It was undetectable after 7 days however, indicating squalene epoxide undergoes further decomposition [9]. The fact that the concentration of squalene decreases rapidly over time and is rarely detected in older fingerprints, makes it a potentially useful target compound for age determination [12]. The presence or absence of squalene epoxide could also be used for age estimation, as the concentration increases up to 5 days after deposition, but is undetectable after 7 days [9]. A range of hydroperoxides is produced over time, including the main oxidation product squalene monohydroperoxide to squalene pentahydroperoxide, through the di-, tri- and tetrahydroperoxide versions [9]. Squalene tetra- and pentahydroperoxides were still detectable after 20 days [9]. This is particularly useful for age estimations, as the concentrations of squalene, squalene epoxide and squalene hydroperoxides could all be compared. A recently deposited fingerprint would have higher concentrations of squalene, squalene epoxide and the monohydroperoxide form compared to an older fingerprint, which would have lower concentrations of squalene and squalene epoxide, and higher concentrations of hydroperoxides [5].

Cholesterol also degrades upon ageing. Cholestadiene or cholestenones are possible degradation products but were not clearly identified in aged fingermark residue [11]. Cholesterol could be a suitable target compound for fingerprint age determination, as it has been observed to decrease in concentration over time, indicating the presence of decomposition mechanisms [10]. Research in other areas has determined cholesterol can form multiple products through oxidation. The identification of these products within a fingerprint with the rate at which they form could allow for an age estimation method [5].

The concentration of unsaturated fatty acids has also been observed to decrease with time, such as unsaturated C16 and C18 acids [24]. Both acids are significantly reduced in concentration over a 30-day ageing period, due to the unsaturated moiety being open to attack through aerobic and anaerobic degradation processes. Anaerobic conditions are associated with hydrogenation processes, which transform unsaturated bonds, increasing the concentration of saturated fatty acids and decreasing the proportion of unsaturated fatty acids [24]. On the other hand, aerobic degradation produces oxidized compounds, such as peroxide linkages, aldehydes and ketones, through a chain reaction process [5]. Besides chemical degradation, there is also evidence of microbial degradation of long-chain fatty acids, originating odd-numbered fatty acids, under the necessary conditions [14].

Tetracosane, a 24-carbon chain alkane, was identified in one study in samples of “intermediate age”, indicating it may be an intermediate in the decomposition of longer chain compounds [12]. No other study has identified this compound and further research is needed to explore possible formation and degradation mechanisms [5]. Saturated fatty acids remain in fingerprints for longer than unsaturated fatty acids, predominantly due to the lack of a targetable functional group [5]. Lower molecular weight compounds produced from fatty acid degradation are more volatile and disappear easily. Saturated lipids are less affected by change over time, resulting in higher levels of saturated compounds, such as wax esters [5]. Moreover, unsaturated lipids are more abundant than saturated lipids in a fresh fingerprint however, they rapidly decrease over time and are overtaken by saturated lipids at day seven [16].

Regarding the temperature, the higher it is, the faster the degradation. Furthermore, small molecules appear and are identified as volatile degradation products, while acid salts seem to be much more resistant to high temperatures [25]. When it comes to exposure to light, the compounds' degradation happens faster when compared to those that age in the dark [11].

The initial composition of a fingerprint is used to determine the starting point of a potential ageing curve [10]. This causes some difficulty when exploring the effect of time on composition, as there is significant variation between donors, as well as due to the effects of environmental variables [12]. Nonetheless, this can be overcome through an exploration of the relative amounts of constituents and key compounds inherent to fingerprints. Research focusing on the amounts of specific components relative to each other is potentially more reliable and accurate for age exploration, as it does not require knowledge of the initial concentrations. One study used this method to compare relative concentrations of squalene and cholesterol through the relative peak areas [10]. The relative standard deviation of the ratio of the two (less than 20%) was significantly less compared to the standard deviation of the individual compounds (up to 80%). This also resulted in more reproducible results over time, as the squalene-cholesterol peak area ratio produced more accurate concentration changes over the first few hours of ageing [10]. The use of compound ratios appears to yield more reliable data, which indicates further research is needed to explore this as a method that has the potential to follow changes to the composition of a fingerprint in a more reproducible way than proposed by previous research [5].

2.3.5. Target Compounds for Fingerprint Dating

A fingermark contains numerous compounds, mainly eccrine and sebaceous in nature. However, for these compounds to be relevant target compounds to study fingerprint ageing, they should ideally fulfil some criteria. Firstly, the compounds should be detectable in all donors' fingermarks and these compounds should be endogenous compounds, which means differentiable from contaminants often found on fingertips [15]. Additionally, the compounds should be detectable and measurable with analytical techniques easily available in operational forensic laboratories. Furthermore, the compounds should show variability as low as possible when exposed to influence factors that can affect fingermark composition over time and lastly, the compounds should show reproducible and measurable modifications over time, for example, a decrease or increase of their initial quantity, whether it is absolute or relative [15].

2.4. Mass Spectrometry as the Answer

Fingerprint evidence is of paramount importance in most criminal cases, with vital issues raised concerning the age of the mark [6]. There have been several attempts in the past to develop a method to estimate the age of fingerprints in order to determine relevance at a crime scene, but so far with limited success. An in-depth understanding of their change over time from the initial to the aged stage is vital for applying a robust ageing model and studying how the chemical composition of fingerprints changes over time allows for more quantitative models [6],[3].

Some criteria should be met by the techniques chosen to analyse certain compounds, regardless of what compounds are actually targeted. Firstly, the technique should be easily available in forensic laboratories at affordable prices and it should be able to differentiate endogenous compounds of fingermarks from

possible contaminants – it should be specific [15]. Also, the technique should be able to analyse compounds available in fingermarks left on different kinds of substrates. The technique should ideally be non-destructive to the fingermarks to allow for other analyses to be conducted afterwards and should be applicable after enhancement [15]. Lastly, the technique should allow quick, easy, and reproducible sample preparation and analysis steps, in other words, it should be reliable and robust [15].

Besides all the criteria that should be taken into consideration, the success of a given analytical technique depends upon the nature of the selected target compounds [15]. Biomolecular constituents of fingermark residue, such as amino acids, lipids and proteins, may provide excellent means for fingermark age determination [4]. To clearly identify and quantify these biomolecules, a robust methodology is necessary. Therefore, performing mass spectrometry on latent fingerprints is an area of increasing interest due to the enormous amount of chemical information that can be extracted regarding the donor.

Surprisingly, mass spectrometers do not measure mass, but they do measure a property related to mass, the mass-to-charge (*m/z*) ratio. Therefore, understanding how a mass spectrometer works, requires understanding how ions move in electric and magnetic fields. There are some components essential to a mass spectrometer, represented in Figure 2.1, namely the sample inlet system that may include chromatography and the ion source. Also, another fundamental component is the analyser which determines mass range, accuracy, and resolving power [26]. Additionally, there is the ion detection system as well as the data system. The latter controls the instrument and usually includes computational tools to assist the data processing.

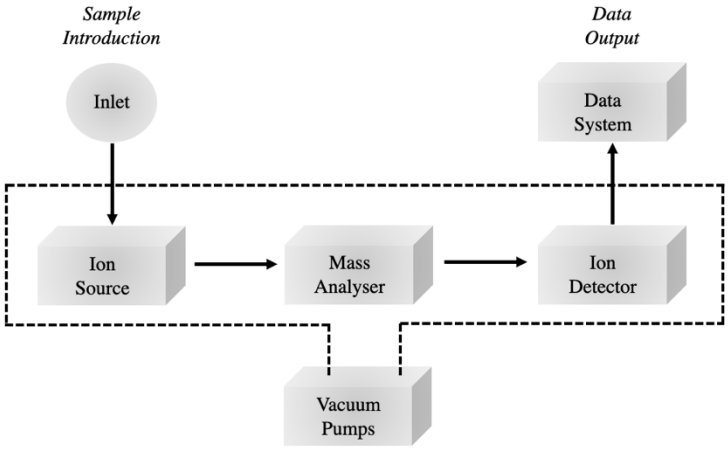

Figure 2.1: Components of a mass spectrometer. Figure courtesy of Professor Peter O'Connor.

Some of the advantages of mass spectrometry include its near-universal applicability since almost all substances can be detected in a mass spectrum and its selectivity, as the high resolving power allows selection of one component from a complex mixture. Moreover, mass spectrometry is an analytical technique with high specificity, the exact molecular weight is often unique to the compound under investigation and the observation of a chosen fragmentation in MS often identifies a given component in a mixture. Lastly, mass spectrometry is also known for its sensitivity and speed. The high resolution that can be obtained is limited by the magnetic/electric field homogeneities, whilst the high sensitivity is limited by the efficiency in ion transfer.

2.5. Why FT-ICR-MS?

Fourier transform ion cyclotron resonance mass spectrometer is a preferred instrument at the forefront of research [27]. Despite its size and cost, it has notoriously superior performance, 1-2 orders of magnitude above the next best instruments in terms of resolution and mass accuracy. Mass spectrometry using an FT-ICR offers the highest mass resolution and mass measurement accuracy of all mass analysers [28]. The FT-ICR offers ultra-high mass resolving power – R > 1.000.000 at an *m/z* of 400 – offers flexibility for hybrid instrumentation, and it also offers compatibility with a diverse range of fragmentation methods and ion sources [29]. The achieved mass accuracy value is typically 1 ppm or better, whereas sensitivity depends on the source and ion transfer optics [29]. Furthermore, FT-ICR is compatible with tandem mass spectrometry, providing unlimited fragmentation depth and different physical fragmentation mechanisms [29].

Mass accuracy is important for determining the ion's elemental composition [30]. Many types of modern mass spectrometers, such as the ion trap, quadrupole and time-of-flight, offer the mass accuracies in the level of at best tens of ppm in a limited mass range, whereas FT-ICR can achieve mass accuracy in sub-ppm, or even ppb (parts per billion) level in some instruments [27]. Mass measurement accuracy (MMA) is the key measurement parameter of a mass spectrometer. Nonetheless, mass accuracy depends upon the mass resolving power and signal-to-noise ratio, since a requirement for accuracy is that the peak of interest must be well resolved and distinguished from others. Thus, FT-ICR is known best for its ultra-high mass resolving power [27].

Consequently, the inherently ultra-high resolution and the mass accuracy that can be achieved, allow the unequivocal mass assignment and resolution of species which could not otherwise be distinguished if using some other types of mass spectrometer [31].

2.5.1. FT-ICR Mass Spectrometry Dynamics

The Fourier transform ion cyclotron resonance mass spectrometer is an ion trap, based on the classical motion of ions under the influence of both electric and magnetic fields [26]. At present, there is a wide variety of both custom and commercial FT-MS instruments throughout the world. Regardless of the type of instrument, it consists of five basic components. These components are the magnet, analyser cell, vacuum system, data system, and the ion source. Additionally, this type of mass spectrometer utilizes a magnet, most recently the magnet utilized is a superconducting magnet with the capacity to achieve higher magnetic fields [28]. The magnetic field is of high importance as many FT-ICR properties scale linearly – mass resolving power and mass accuracy – or quadratically – dynamic range and upper *m/z* limit – with magnetic field strength [28].

The analyte can be introduced as a solid, liquid, or a gas, depending on the type of ion source used [28]. In the case of FT-ICR mass spectrometry, ions are usually generated externally in a separate ion source and then injected into a container known as the *cell*. The analyser cell, represented in Figure 2.2, is the heart of the FT-ICR instrument where ions are stored, translationally excited, mass selectively isolated or ejected, and detected. The analyser cell may also be used to dissociate ions for tandem mass spectrometry (MS/MS) [28].

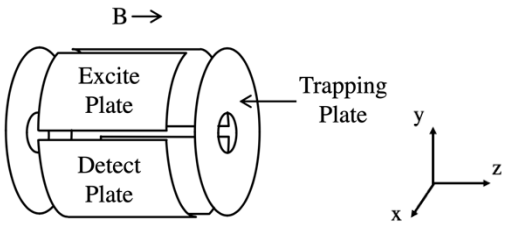

Figure 2.2: Representation of a closed cylindrical cell. The cell is oriented so that the trapping axis (z-axis) is aligned with the magnetic field, as indicated by the vector, B. In this particular representation, the excitation plates are located along the y-axis and the detection plates along the x-axis [28].

The middle cylinder is divided into four plates, two for excitation of ion motion and two for detection of the trapped ions, and the trapping plates are used to contain the ions within the analyser cell [28]. A low potential – typically of an order of 1 V – is applied to the trapping plates to restrain the ions within the FT-ICR analyser cell, so that they may be excited and detected [27].

A third component of the FT-ICR mass spectrometer is the vacuum system. During detection, ions travel several kilometres as they orbit the analyser cell. Therefore, the ultrahigh vacuum conditions in the analyser region have the purpose of avoiding that the ions are moving around randomly, and providing a long mean free path between collisions, otherwise there would be a resolution decrease [28].

Figure 2.3 shows a representation of how the inside of an ICR cell works. In the FT-ICR cell, excitation sweeps resonant ions into a large, coherent cyclotron orbit – cyclotron resonance – before they reach the detection plates. Ions are trapped and oscillate with low, incoherent, thermal amplitude. Once the ion is trapped, the magnet bends its trajectory into a circular path.

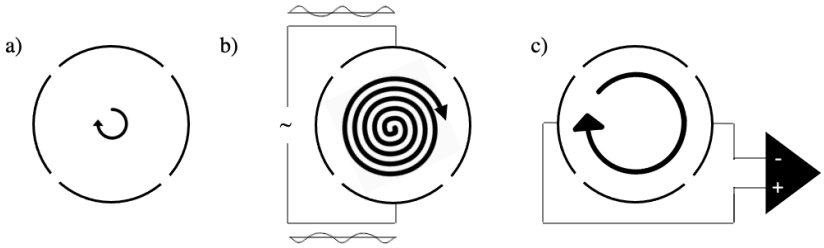

Figure 2.3: Inside the FT-ICR analyser cell. a) Ions are trapped and oscillate with low, incoherent, thermal amplitude. b) Excitation sweeps resonant ions into a large, coherent cyclotron orbit. c) Preamplifier and digitizer pick up the induced potentials on the cell. Figure courtesy of Professor Peter O'Connor.

A fourth component of the FT-ICR is the data system. The FTMS data system consists of a pulse sequence generator to control the timing of the various events in a measurement cycle, a frequency synthesizer, a wideband excitation amplifier for ion isolation and detection, and a transient digitizer. A computer controls all of these components and is used to acquire and process the data [28]. The detection of the ions occurs as they pass two detector plates. As a spiralling ion gets near a detection plate, it induces a current that is detected by the instrument. A preamplifier and digitizer pick up the induced potentials on the cell, the signal is recorded for a period of time and then displayed by the software [31].

The potential – voltage – change between the detection plates can be measured as a function of time and allows the obtainment of the raw data [31]. The raw data is also known as a "transient", "time-domain" data, or sometimes as "FID" or "free induction decay". It should be noted that the ions repeatedly pass

the detector plates for the duration of the acquisition time, as non-destructive detection is employed, thus the higher the transient duration, the higher the resolution [31].

Moreover, since ion masses are related to the frequency of cyclotron motion and frequency can be measured with as much precision as any physical quantity, precise mass measurement is relatively straightforward in FT-ICR [26]. The frequency (ω) is measured, to calculate the mass of the ion (m) since the magnetic field (B) is known as shown in Equation 2.1. A higher frequency is associated with "lighter" ions, while "heavy" ions have a lower frequency [31].

$$\omega = \frac{qB}{m} \tag{2.1}$$

The ion's cyclotron frequency is independent of its velocity, and hence, independent of its kinetic energy. This extraordinary characteristic is one of the key reasons why FT-ICR is capable of ultra-high resolving power, as the performance of most other types of mass spectrometers is affected by the ion kinetic energy spread [28].

The magnitude of the signal is proportional to the total charge and the proximity of the ions to the detection plates and is independent of magnetic field strength [31]. The raw data will represent the detection at the same time of all the ions, with their different cyclotron frequencies. It is, therefore, necessary to extract data about the different ion packets. This is done through the usage of the mathematical procedure known as a *Fourier transform* (FT). The mathematical process of Fourier transformation of the time-domain signal results in a frequency-domain spectrum, showing peaks of all ions in the sample at their cyclotron frequencies. This frequency spectrum can be easily converted into a mass spectrum if the magnetic field strength is known, as represented in Figure 2.4. In practice, a very precise magnetic field value is computed from peaks of known mass and measured frequency, produced by a calibration compound [26]. The peaks in an FT-ICR spectrum represent the frequency of the ions' cyclotron motion, while artefacts that result from transient distortion and radio-frequency interference also exist as peaks in the spectrum. The artefacts are inevitable spectral features, they contain no chemical information, and may complicate data interpretation [27].

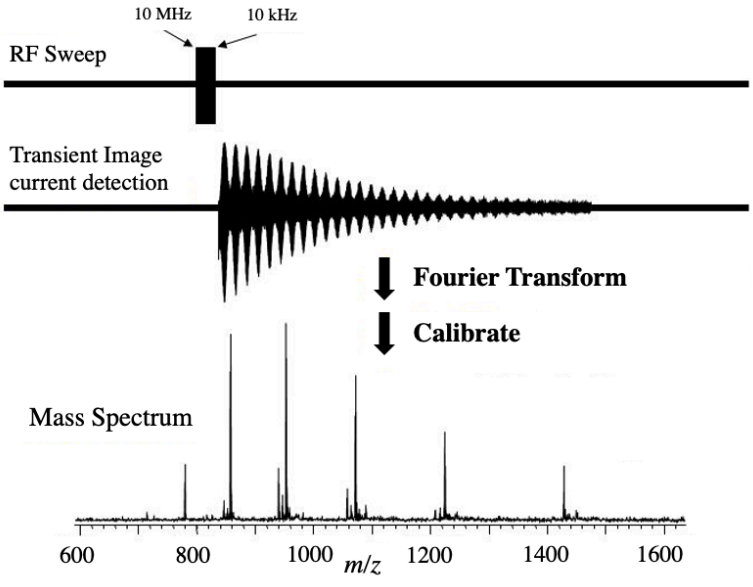

Figure 2.4: How a mass spectrum is produced. Figure courtesy of Professor Peter O'Connor.

All varieties of mass spectrometers must involve the analysis of gas-phase ions to obtain an *m/z* ratio and ion sources must be used to generate the gas-phase ions from a neutral sample [31]. Ionization requires addition or removal of one or more charged particles, usually electrons or protons, that can be done by hitting the sample with photons, electrons, fast neutral molecules, other ions, heat or a combination of methods [31]. With early FT-ICR instruments, the ion source was an integral part of the analyzer cell, or located adjacent to the analyzer cell, within the homogeneous magnetic field region of the instrument. In the 1980s, instruments were developed with ion sources located outside the magnetic field. This configuration is called an external ion source. By placing the source outside the magnetic field where it can be differentially pumped, almost any type of ion source can be interfaced to the FT-ICR. When the source is located outside the magnet, ion optics such as a quadrupole or a series of electrostatic lenses are used to transfer the ions from the source to the analyser cell.

Because the FTMS instrument separates its operations in time, it is classified as a pulsed mass spectrometer. In a pulsed mass spectrometer, the ions are formed in small packets that are, in turn, analysed as a group. This is in contrast to a scanning instrument (e.g., magnetic sector and quadrupole) in which ions are produced continuously, and the mass analyser is scanned, detecting ions one mass-to-charge value at a time.

2.5.2. Sample Calibration

For calibration, an unknown sample can be doped with a standard and the mass spectrum can be ‘‘internally’’ calibrated [31]. Internal calibration provides the best mass accuracy; better than 1ppm is easily achieved using internal calibration in modern instruments with magnetic field strengths of 7 T or higher [28]. However, internal calibration is not always practical to use, particularly for LC/MS and tandem mass spectrometry experiments. With external calibration, it is important to ensure that all experimental parameters are kept identical between the acquisition of the mass spectra of the calibrant and the analyte, particularly the trapping potential, the excitation waveform, and the number or ions [28].

2.5.3. Sample Ionization

Regarding the ionization technique, electrospray ionization (ESI) is the technique most frequently coupled with FT-ICR mass spectrometry nowadays. ESI has the advantage of being a *soft* ionization technique, resulting in the vaporization and ionization of a sample whilst minimizing fragmentation [31]. The fact ESI is a *soft* ionization technique allows ionization of non-covalently bound species, such as protein complexes [32].

In electrospray ionization, represented in Figure 2.5, a solution containing the sample is sprayed out of a needle into a large electric field. Charged droplets are formed and are transferred into the mass spectrometer by the electrostatic fields. The solvent on the charged parent droplet evaporates and ultimately leaves charges on the analytes [33].

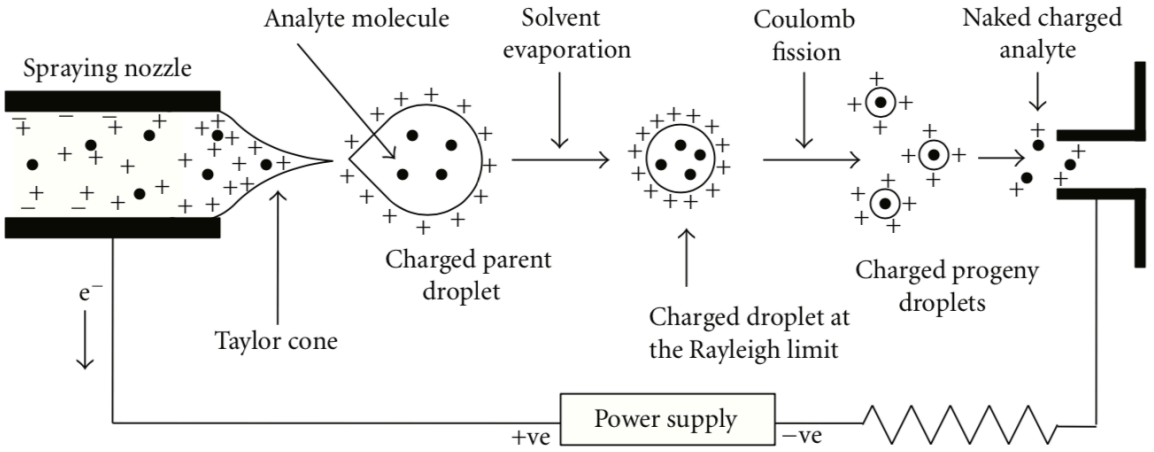

Figure 2.5: The electrospray ionization process [34].

ESI frequently leads to the formation of multiply charged ions, particularly in the case of large biomolecules. As mass spectrometry is based upon the determination of *m/z*, not mass alone, it is frequently the case that a mass spectrum will contain the same molecules but in a variety of charge states, and therefore at several different *m/z* values [31]. As ions become more highly charged, the *m/z* becomes lower and the spacing between the *isotopomers* – peaks due to the presence of other isotopes – becomes narrower [31]. As a result, it becomes more difficult to resolve the signals and the resolution of the mass analyser becomes more important. Resolution is extremely important for resolving closely spaced signals, such as in the cases of complex mixtures or multiply-charged ions. FT-ICR mass spectrometers can routinely reach resolutions of hundreds of thousands in *broadband mode* – "normal" experimental conditions – or even reach a resolution of a few million in *heterodyne mode* – where a very narrow *m/z* range is studied but under very high-resolution conditions [31].

In summary, the multiple-charging that is characteristic of ESI compresses peaks into a narrower range of *m/z* values, and the high resolution of FT-ICR MS allows simple identification of ion charge state. The combination of ESI and FT-ICR MS produces a powerful analytical tool, allowing the ionization and rapid simultaneous analysis of many labile molecules [34].

Nano-electrospray ionization (nano-ESI) is another ionization technique that can be coupled to FT-ICR instruments, as shown in Figure 2.6. Nano-ESI is generally recognized as the most efficient method of introducing a liquid sample for direct analysis by mass spectrometry [35]. As the name implies, nano-ESI is a form of electrospray, with the same fundamental ionization process of droplet formation followed by multiple divisions, and finally desorption of pre-formed ions from the droplet. The technique is distinguished from conventional ESI mainly by its low flow rate, usually of a few nL/min. The low flow rate can be controlled by the diameter of the tip, the voltage applied, and the backpressure which is sometimes applied to the tube content [35].

It was shown that, in comparison with electrospray, nano-ESI reduces interference effects from salts and other species and provides better sensitivity toward a variety of analytes, including peptides and oligosaccharides, in samples contaminated by high levels of salts [35]. This reduction in interference effects can be attributed to the reduced droplet size compared with electrospray at higher flow rates. Along with its low flow rate, nano-ESI has the ability to analyse a small volume of sample and to make the sample last for many minutes so that various experiments can be performed.

In conclusion, the defining characteristic of low flow is assumed to be responsible for the beneficial effect of high ionization and sampling efficiency, presumably because the droplets are very small and therefore can become well desolvated. The droplets in the 100 nm diameter range, cause sensitivity to go up due to greatly reduced space charge and improved capture efficiency [35].

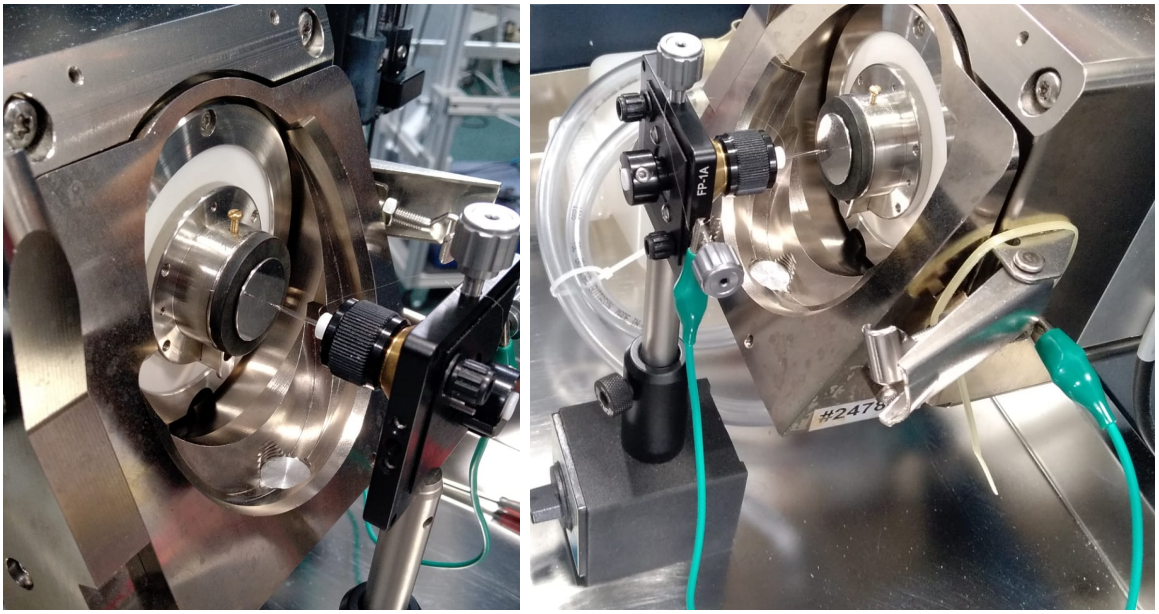

Figure 2.6: Nano-electrospray ionization source from the Fourier-Transform Ion Cyclotron Resonance Mass Spectrometry research laboratory, at the University of Warwick. Picture courtesy of Professor Peter O'Connor.

3

Experimental Procedure

The data collected and analysed for this dissertation was acquired at the Faculty of Sciences of the University of Lisbon and at the University of Warwick from volunteers who provided written informed consent before participating in the experiment.

3.1. Sample Definition

A Nanoelectrospray analysis was performed on fingerprint molecules using a full range of solvent systems to determine the most suitable one for fingerprint analysis. The examined solvent systems were chosen based on their properties and their former use regarding the study of fingerprints. The examined solvent systems are listed in Table 3.1.

Table 3.1: Range of solvent systems for the study of fingerprints' chemical composition.

Solvent 1	Acetonitrile : Methanol : Water (1:1:1)
Solvent 2	Hexane
Solvent 3	Chloroform
Solvent 4	Chloroform : Methanol (1:1)
Solvent 5	Isopropanol : Methanol : Chloroform (4:2:1)
Solvent 6	Methanol
Solvent 7	Dichloromethane
Solvent 8	Dichloromethane : Methanol (1:1)

At this point, the goal was to compare the number of identified compounds for each solvent system and for that reason, it was not necessary to use a large number of volunteers. Ideally, a single donor should have been used for all solvent systems to minimise donor variability. However, the participants of this study consist of two volunteers. Two volunteers were chosen instead of one to avoid using the same finger twice which would be an external factor of loss of chemical compounds from one's fingerprint. To minimise some of the variability factors, the chosen donors were of the same age, same nationality and ethnicity, lived under similar circumstances and had a similar diet. The fingerprint deposition force was not controlled.

Regarding the study of chemical changes in a fingerprint's composition with time, the participants were volunteers from different age groups and genders from the Faculty of Sciences of the University of Lisbon. The only inclusion criterion for volunteers to be able to participate in the study was to be over 18 years old. All participants were 18 years or older, and gave written informed consent before participating in the experiment.

The sample consists of a total of twelve volunteers, of which seven are females and six are males. The volunteers' ages roughly vary between 20 and 80 years old.

For the study to be as representative as possible, it was important that the sample reflected either the world population or the Portuguese population. For that reason, there were no restrictions in terms of the donors' age, gender, diet, lifestyle and possible pathologies. To assert if our sample was representative, a brief statistical description of both the world and the Portuguese population as of 2019 is presented [36].

Table 3.2 presents a comparison of the population data of the sample under study with the data referring to the world population as well as the Portuguese.

Table 3.2: Comparison between the sample population, the world population and the population of Portugal as of 2019.

		Sample Population	**World Population**	**Portuguese Population**
Gender	Female	58,3%	49,6%	52,7%
	Male	41,7%	50,4%	47,3%

In terms of gender balance, there is an approximation between the gender balance of the sample population and the world and Portuguese populations. Nevertheless, our sample consists of volunteers of Portuguese residence and nationality only, and all volunteers are of Caucasian ethnicity. Such facts can be considered a limitation as individuals of other nationalities, ethnicities and from different geographical areas are missing, and it is known that such factors influence a fingerprint's composition. Thus, it is not possible to infer that the sample under study is representative of neither the world nor the Portuguese population, as the sample is too small for this approach to be valid.

3.2. Questionnaire and Data Collection

The analysed data were collected after each participant filled a questionnaire (Figure A.1 from Appendix A, Supplementary Data) containing relevant information regarding each participant. Three transients were acquired from the FT-ICR for each volunteer's fingermark residue.

3.2.1. Questionnaire

The characterization of each participant was done by completing the questionnaire in Supplementary Data, Appendix A. This survey gathers information about gender, birth date and ethnicity, as well as information on diseases/health status and any taken medication. Questionnaires are kept confidential, subject identity is randomized and all information eliminated at the end of the study. Samples are not preserved beyond the required time needed for processing, ageing and analysis.

3.2.2. Data Collection

The procedures adopted to collect the volunteers' fingerprints were non-invasive and did not invade on any privacy of the donors. All participants were 18 years or older, and gave informed consent before

participating in the experiment. The collection of all data was carried out by researchers from the *Laboratório de FT-ICR-MS e Espectrometria de Massa Estrutural* from the Faculty of Sciences of the University of Lisbon. Due to the pandemic situation the world is experiencing at the time of writing this dissertation, it was not possible to collect any further data after February 2020.

Data collection took place at the *ICR Laboratory* on the University of Warwick for the solvent systems study and at the *Laboratório de FT-ICR-MS e Espectrometria de Massa Estrutural* on the Faculty of Sciences of the University of Lisbon for the study of fingerprints' ageing. Before any data was collected, the participants' eligibility was confirmed and written informed consent was given.

Finally, each volunteer was asked to rub their indexes – both indexes and thumbs for the solvent systems study – together to create homogeneity and donate their fingerprints by pressing the fingers against a glass slide each. For the solvent system study, aluminium foil was used instead of glass slides. The glass slides (VWR Microscope Slides, and NORMAX Micro Slides) were pre-cleaned with ethanol (Sigma Aldrich). The deposition force used by participants was not controlled.

Fresh fingerprints were collected and immediately prepared and analysed. For the ageing study, the homologous samples were stored in an incubator in the dark (VWR, INCU-Line IL53) at 30ºC for two different time points, either a week or a month.

For the solvent systems analysis, eight different extraction solutions were prepared. These solutions consisted of 1000 µL of each solvent system and 1 µL of formic acid (Sigma Aldrich, MS grade). Solvent system 1 was composed of acetonitrile (Merck, LC-MS grade), methanol (Merck, LC-MS grade) and water (Merck, LC-MS grade) (1:1:1), while solvent system 2 consisted of only hexane (Merck, LC-MS grade), and solvent system 3 of chloroform (Sigma Aldrich, HPLC grade). Solvent system 4 contained chloroform and methanol (2:1), whereas solvent system 5 was composed of isopropanol (Sigma Aldrich, LC-MS grade), methanol and chloroform (4:2:1). Additionally, solvent system 6 was composed of methanol only, solvent system 7 of dichloromethane (Sigma Aldrich, LC grade), and lastly, solvent system 8 had both dichloromethane and methanol (1:1). The solvent systems mentioned above are organized in Table 3.1. The various fingerprints deposited on aluminium foil were extracted with 30 µL of each of the previously prepared solutions and they were then subjected to a 10 fold dilution. A final volume of 20 µL was used to perform a Nanoelectrospray analysis on a *Bruker 12T Solarix XR* FT-ICR mass spectrometer, with arginine clusters (Sigma Aldrich) being used as external standard calibrant. For the solvent analysis, an average of 10 scans was acquired and an accumulation of 0,100 s was used.

Regarding the sample preparation for mass spectrometry analysis, the fingerprints deposited on glass slides were extracted with 20 µL of a solution containing Milli-Q water (Merck, LC-MS grade), acetonitrile (Merck, LC-MS grade), and methanol (Merck, LC-MS grade) at a proportion of 1:1:1, with 0,1% (v/v) formic acid (Sigma Aldrich, MS grade). An electrospray solution was prepared with Milli-Q water and methanol (1:1) and 1 µL of formic acid. The extraction solution was then added to the electrospray solution in a 1:100 proportion, followed by the addition of 0,2 µL of leucine–enkephalin (YGGFL Sigma Aldrich) at a concentration of 0,1 mg/mL as an internal standard. The latter solution was injected into a *Bruker 7T Solarix XR* FT-ICR mass spectrometer. For seven-day aged fingerprints, 50 scans were acquired, and a time of flight of approximately 0,800 s and an accumulation of 0,020 s were used. The parameters were changed for one-month aged fingerprints, the average scans increased to 100 and the accumulation also increased to 0,100 s due to the samples' low concentration. The syringe and ESI sprayer were washed with methanol between sample injections.

3.2.3. Data Analysis Process

The analysis of the spectra was performed using Bruker's software, *Compass DataAnalysis V. 5.0* (Bruker Daltonics) for data acquisition and processing, as well as *MetaboScape (Version 2.0)* for preliminary identification of compounds and statistical analysis. Due to the pandemic situation the world is experiencing at the time of writing this dissertation, it was not possible to use *MetaboScape* for statistical analysis after January 2020, so the latter analysis was performed on *Microsoft Excel V. 16.42* (Microsoft) exclusively.

4

Results and Data Analysis

In this chapter, a characterization of the sample under study is presented, as well as the results obtained from the analysis of the range of solvent systems tested and the changes in the chemical composition of fingerprints with time. The analysis was performed on a sample of 12 participants. Consequently, the results obtained and the conclusions reached reflect the average trend of the sample under study. Nevertheless, statistical methods were used to make sure the results are sound and statistically significant.

4.1. Sample Characterization

As previously mentioned, data from 12 volunteers were analysed. Of these 12 volunteers, seven are female and five are male. Figure 4.1 shows that out of the seven female individuals, five are between the age of 20 and 40, one is between 40 and 60 years old and the last one is between the age of 60 and 80. Out of the five male individuals, three of them are between the age of 20 and 40 while the remaining two are between 40 and 60 years old.

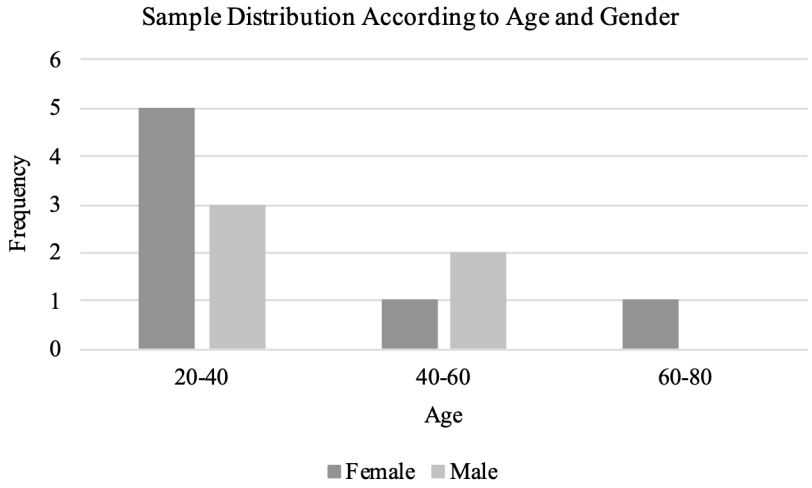

Figure 4.1: Sample distribution according to age and gender. Three age gaps were defined, from 20 to 40, 40 to 60 and 60 to 80 years old. Female individuals are represented in a darker shade and male individuals in a lighter one.

4.2. Solvent Systems' Suitability for the Study of Fingerprints' Chemical Composition

Eight different solvent systems were studied to determine which one was the most suitable for the chemical characterisation of fingermark residue. The suitability of each solvent system was determined based on the number of compounds identified for each solvent, on the solvents' availability and hazard level associated with them.

To facilitate the identification of compounds that could be potentially found in fingerprint's residue, a list of compounds was created (Table B.1 from Appendix B, Supplementary Data). This list includes compounds described in the literature ([5], [6], [10], [11], [11], [12], [15], [16], [23], [37], [38], [42]) as existing in fingermark residue as well as metabolites present in sweat, according to *The Human Metabolome Database* [43]. The list includes 141 compounds, their chemical formulas, their monoisotopic masses, the masses of the protonated molecules and those of $[M+Na]^+$ and $[M+K]^+$ adducts. Moreover, the hazard level associated with each solvent was based on their number of "hazard statements" and "precautionary statements" according to GHS US (Table B.2 from Appendix B, Supplementary Data).

Each solvent system sample was compared with the corresponding blank that consists of the solvent system without the fingerprint extraction. This comparison was performed to reveal the appearance of any of the listed compounds, based on the changes in the compounds' intensity over time. A "compound appearance" was considered when a peak was found in the solvent system sample but not in the corresponding blank. The masses associated with the identified peaks were searched for in the compounds' list created. The corresponding deviations (ppm) were calculated according to Equation 4.1, described below.

$$Deviation\ (ppm) = \frac{observed\ m/z\ -\ theoretical\ m/z}{theoretical\ m/z} \times 10^6 \tag{4.1}$$

Deviations with a module value greater than two ppm were excluded, deviations with a module value between zero and two ppm were accepted. The number of identified compounds for each solvent system is presented in Figure 4.2.

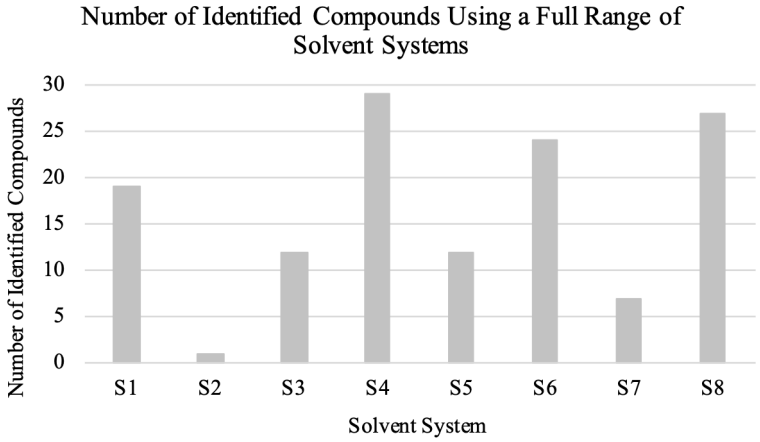

Figure 4.2: Number of compounds identified using a full range of solvent systems.

Solvent System 1 (acetonitrile : methanol : water) allowed for the identification of 19 compounds but using Solvent 2 (hexane) only one compound was identified. Solvent 3 (chloroform) permitted the identification of a total of 12 compounds, while Solvent 4 (chloroform : methanol) allowed for the identification of 29. With Solvent 5 (4 isopropanol : 2 methanol : 1 chloroform), 12 compounds were identified whereas with Solvent 6 (methanol), a total of 24 compounds detected. Regarding Solvent 7 (dichloromethane), only seven compounds were identified while Solvent 8 (dichloromethane : methanol) allowed for the identification of 27 compounds.

Even though solvent systems 4, 6 and 8 allow for the identification of a higher number of compounds, they were not considered the most adequate when compared to solvent system 1. Methanol was present in the four solvent systems last mentioned, so the main difference was the other solvents that constituted the system. When comparing acetonitrile (present in solvent system 1), chloroform (solvent system 4)

and dichloromethane (solvent system 8), acetonitrile showed a lower number of hazard statements. Furthermore, the number of detected compounds is not significantly different and could be attributed to donor characteristics such as the donor itself, the finger used for deposition – certain fingers are known to have a higher amount of material than others – and lastly, the hand from which the fingers belonged to. Hence, it was determined that solvent system 1, was the most suitable. Not only is solvent system 1 the most balanced and safe, but it is also the most compatible with electrospray ionization. Generally polar solvents such as water, methanol, and acetonitrile, easily undergo electrochemical reactions in the spraying nozzle, being the most suitable for ESI-MS experiments [34].

4.3. Changes in the Chemical Composition of Fingerprints with Time

To identify chemical changes that occur to a fingerprint's composition over time, samples were analysed by mass spectrometry at different time points, either right after deposition or after an incubation period of seven days or a month. The number of donors varied according to their availability on the experiment day. For the seven-day aged samples, eight volunteers donned their fingerprints, whereas, for the samples that were aged for a month, there were a total of nine donors. In all cases, three transients were acquired for each volunteer. A new spectrum is recorded by the data acquisition system each time a high voltage is applied to the plates of the ion pulser, this spectrum is called a single transient. This means a total of 24 spectra were acquired for seven-day aged samples and a total of 27 spectra for one-month aged samples. Two of the acquired spectra – one for each incubation period – are represented below in Figure 4.3 and Figure 4.4. By simply looking at the spectra, it is noticeable that despite considerable overlapping, differences in peak intensities are clearly visible.

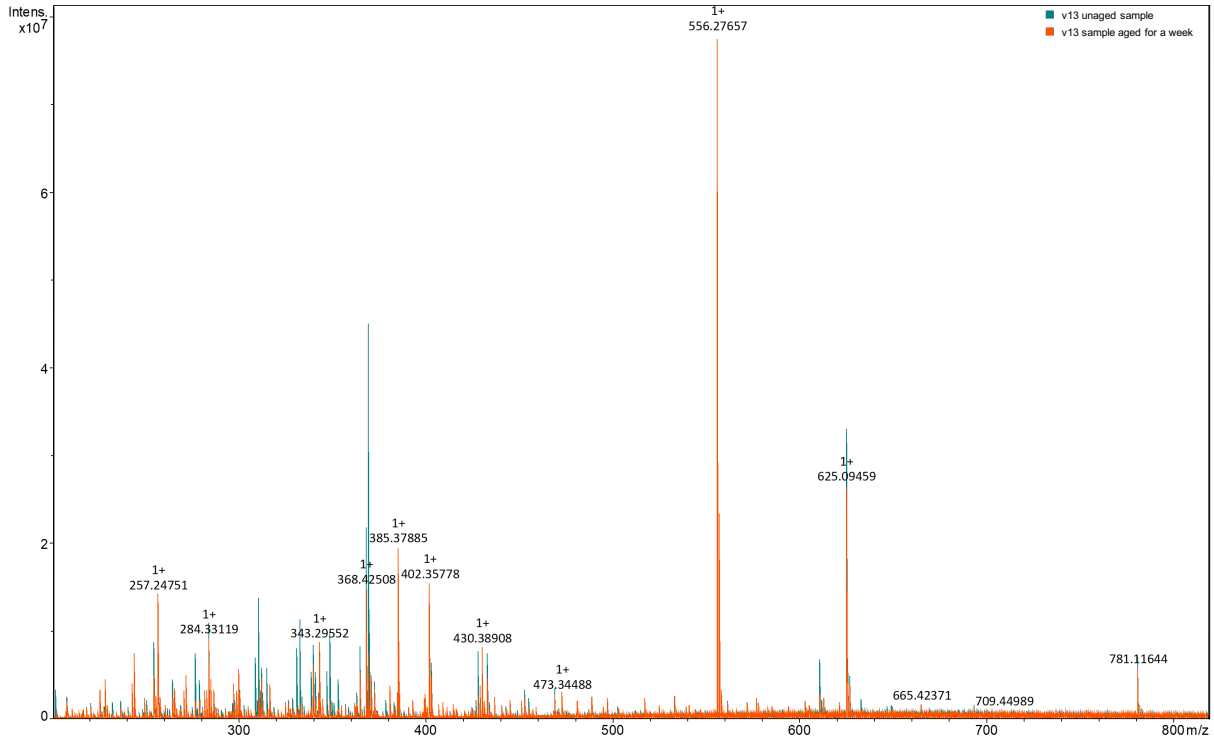

Figure 4.3: Mass spectrum of samples from the same individual before (blue) and after (orange) a seven-day incubation period.

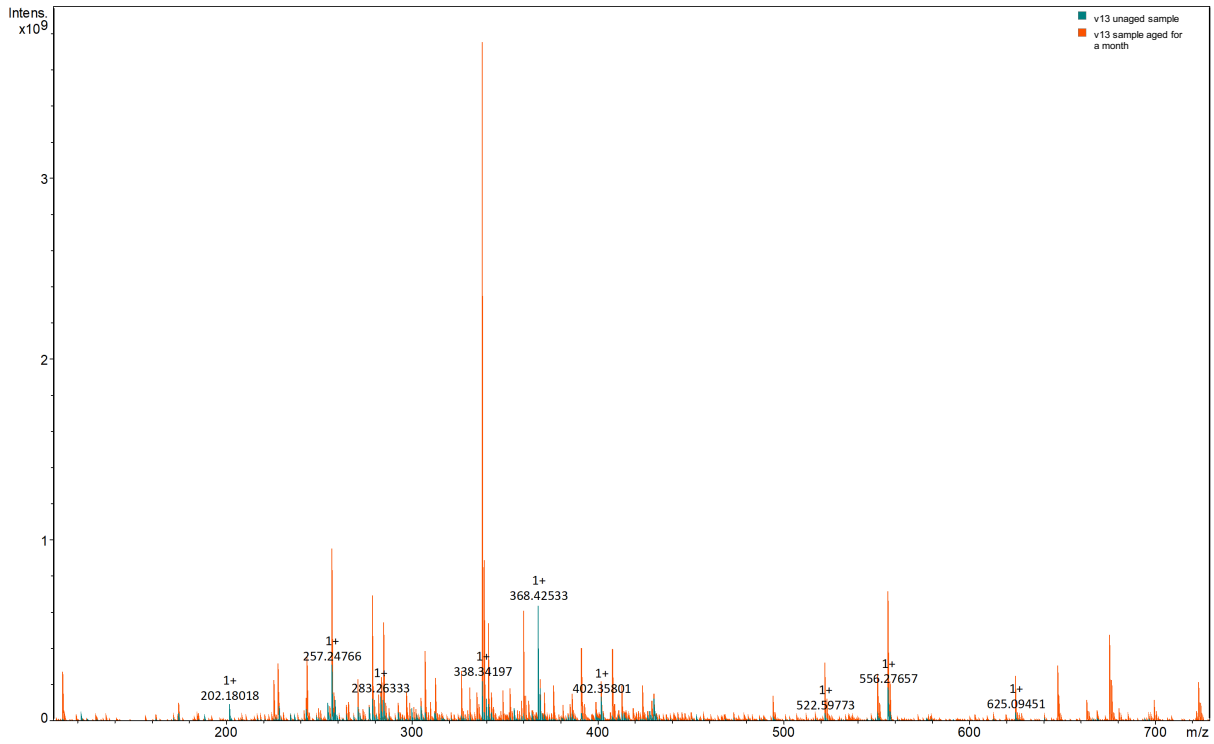

Figure 4.4: Mass spectrum of samples from the same individual before (blue) and after (orange) a one-month incubation period.

Before proceeding to the study of how the compounds present in fingermark residue varied with time, and before proceeding to their identification, some tests were performed in *MetaboScape* to assess if the aged and the unaged populations were statistically different. A Principal Component Analysis (PCA) was performed and data was plotted using a Volcano plot. The Volcano plot results from the statistical analysis effected. The chemical compositional space was displayed with a Van Krevelen diagram.

Firstly, a PCA analysis was performed. PCA is a statistical procedure that converts a set of observations of possibly correlated variables into a set of values of linearly uncorrelated variables – principal components. In other words, PCA reduces the existing dimensions (from a large set of variables) to smaller dimensions that contain most of the information in the large set. PCA is a form of unsupervised learning, meaning it finds regularities without requiring any form of supervision. The model works on its own to discover existing patterns in data, finding features which can be useful for categorization.

On the other hand, a Volcano plot is a type of scatter-plot that is used to quickly identify changes in large data sets composed of replicate data. Plotting data in this way results in two regions of interest in the plot. The data points with low p-values, which appear towards the top of the plot are the ones that vary the most. These represent values that display a large magnitude of fold changes and high statistical significance. In contrast, the data points that appear towards the bottom of the plot are the ones that vary the least.

Lastly, a Van Krevelen diagram is presented. The Van Krevelen diagram shows two ratios, the Oxygen to Carbon ratio (O:C), and the Hydrogen to Carbon ratio (H:C). It displays the areas with the highest point density for certain metabolites, including lipids, amino acids and peptides [44]. This diagram is often used to describe the change of biomass composition during decomposition. Processes such as oxidation, methylation and dehydration will cause a shift in the molecular ratios previously mentioned

which can be compared to the initial composition. Certain ratios are associated with certain classes of metabolites, which facilitates their identification in the Van Krevelen diagram [45].

The PCA in Figure 4.5 was obtained from *MetaboScape* and it illustrates the difference between the two groups under study, aged and unaged fingerprints. The unaged fingerprints donned by each volunteer are represented in blue, while the aged fingerprints from the different volunteers are represented in orange. The two groups – aged and unaged fingerprints – are evidently separated which confirms that the two groups are essentially different.

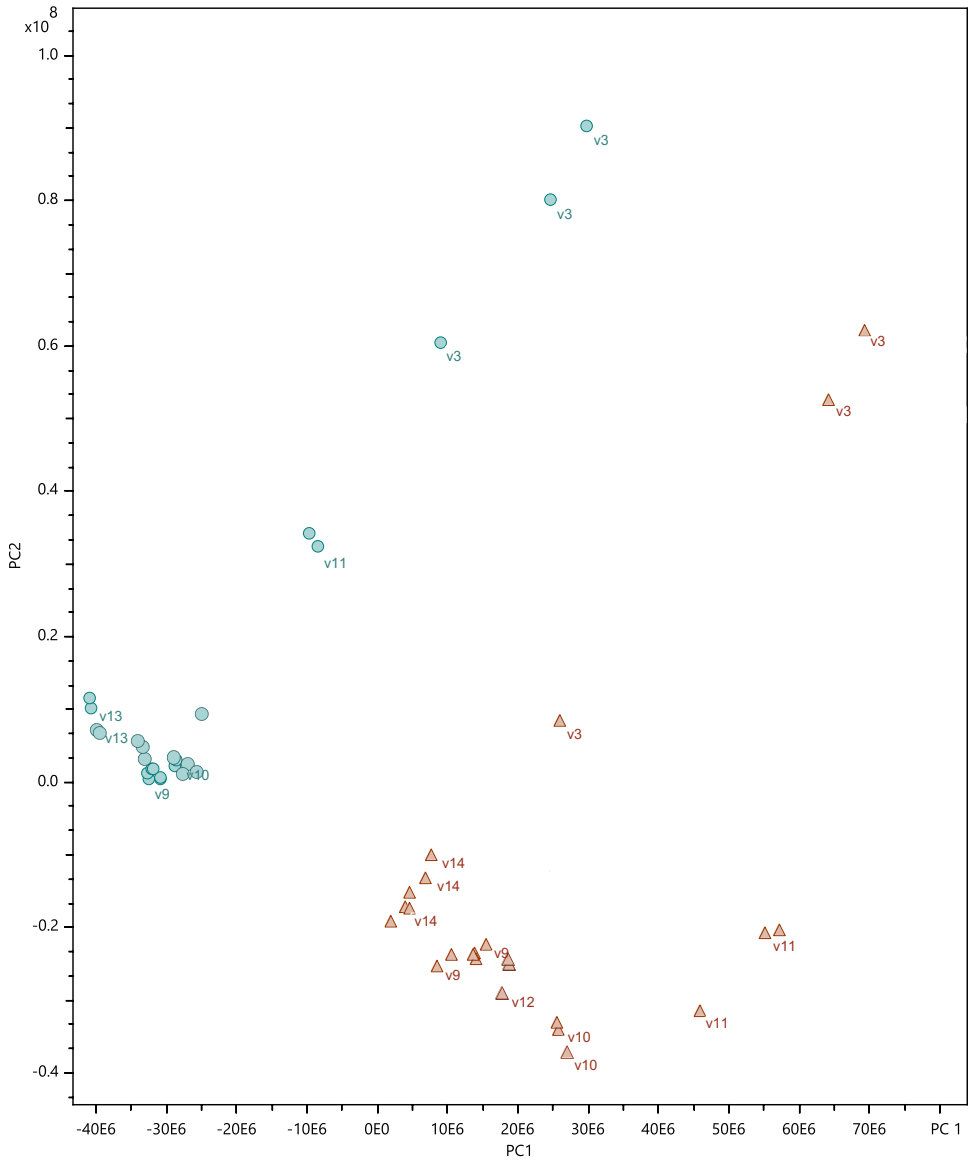

Figure 4.5: PCA analysis obtained from MetaboScape illustrating the difference between aged and unaged fingerprints. The unaged fingerprints are represented in blue, while the aged ones are represented in orange.

The Volcano plot in Figure 4.6 is a preliminary representation of the compounds that differ between the aged and the unaged fingerprints of a group of eight individuals. The compounds that vary with ageing are represented in yellow.

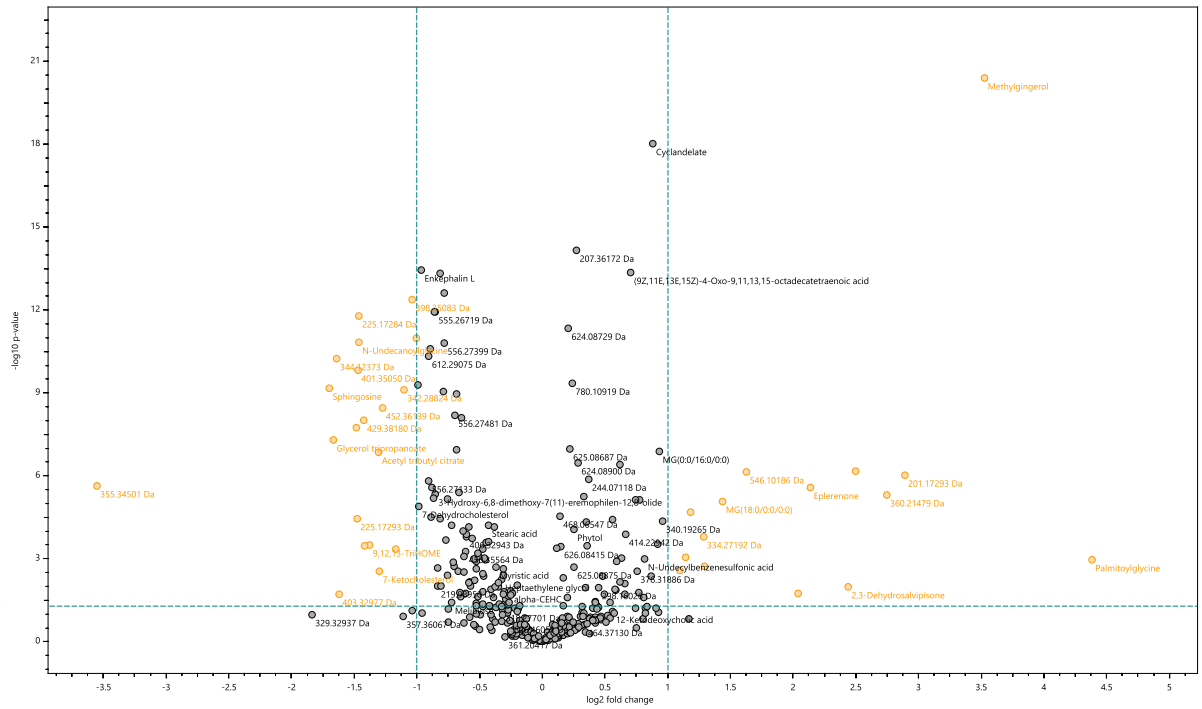

Figure 4.6: Volcano plot of a set of data non-incubated and data incubated for a week. The compounds that significantly differ between the two data sets are represented in yellow.

The Van Krevelen diagrams in Figure 4.7 display the areas with the highest point density. In this case, we can see a high-density area between H:C values of 1,5 and 2,2. This value defines the area where lipids are found [45]. Lipids are known for being present and easily detected in fingerprints so the Van Krevelen diagrams are a good representation of such fact. For the aged samples, there is a slight shift from low to higher O:C ratios which is known to occur in the oxidation process. Considering that oxidation is commonly found in ageing processes, the shift that is visible in the data points from the diagrams could be attributed to that.

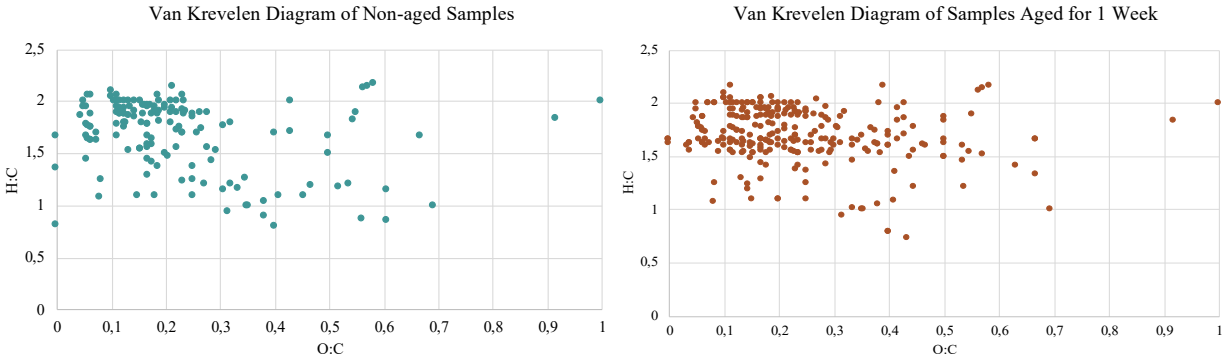

Figure 4.7: Van Krevelen diagrams for the samples that were not aged (blue) and for the samples aged for a period of 7 days (orange).

The 141 compound list previously mentioned was used once again to identify compounds and their presence. Alongside, it was relevant to study the variation of these compounds with time. Therefore, increases and decreases in the compounds' intensity after a defined incubation period were considered, as well as their appearance or disappearance after that same incubation period.

Any increase, decrease, appearance or disappearance of a compound over time can be of significant relevance when it comes to studying the process of fingerprint ageing. The mass spectra of the unaged

samples from each donor were compared with the corresponding spectra of the seven-day aged or one-month aged samples, to try and find changes in the compounds' intensity over time. The masses associated with the identified peaks were searched for in the compounds' list created (Table B.1 from Appendix B, Supplementary Data). The corresponding deviations (ppm) were calculated according to Equation 4.1.

The identified compounds – with a deviation module value lower than 2 ppm – from each donor, within the same incubation periods, were compared and the percentage of the presence of each compound was calculated. Those calculations were performed individually for all four categories – "compound appearance", "compound disappearance", "compound increase" and "compound decrease". Despite considering the existence of adducts, the presence of a compound was confirmed if any of the species – $[M+H]^+$, $[M+Na]^+$ or $[M+K]^+$ – was present. Additionally, the identification of one of the forms or the identification of the multiple forms regarding a compound led to the same conclusion, that the compound was in fact present.

There is a high intravariability between transients, meaning the same donor will originate three substantially different transients. This can be mainly attributed to the electrospray ionization method, which gives rise to high variability in signals' intensity. Besides, considering how complex the sample is, some compounds end up not ionizing. The percentage of compounds that were common to all three transients from the same donor was calculated, along with the percentage of common compounds to two transients and the percentage of compounds that were unique and only present once within the three transients. These values were calculated for each volunteer according to the compounds' variation – appearance, disappearance, increase and decrease – and for the different incubation periods. Figure 4.8 exemplifies what happens in most cases, there is often a higher percentage of non-common compounds.

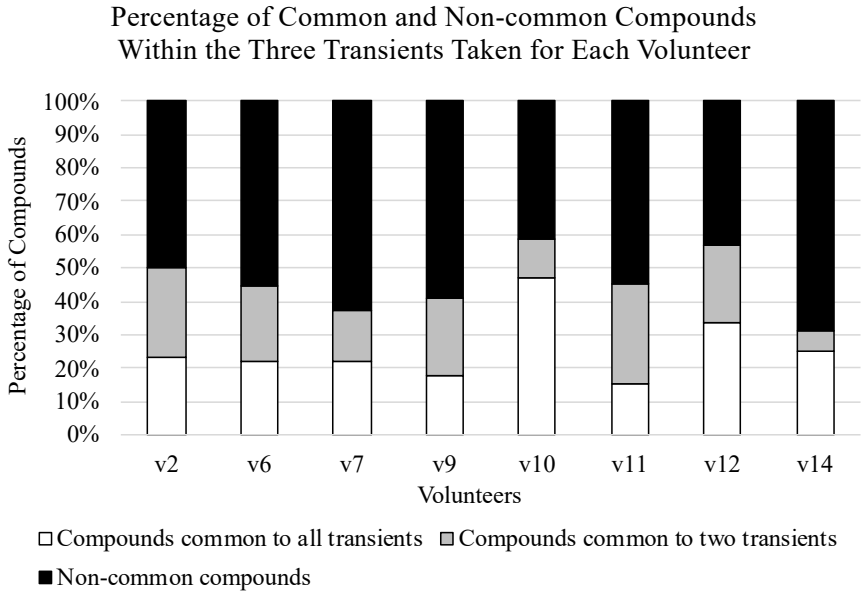

Figure 4.8: Percentage of common and non-common compounds within the three transients taken for each volunteer. The compounds correspond to those identified as being a "compound appearance" for samples incubated for a week (from 29-10-2019 to 05-11-2019).

A single factor ANOVA ($\alpha = 0,05$) was performed to assess if the means of the percentages previously mentioned were in fact different. Afterwards, a paired t-test ($\alpha = 0,05$) was performed to evaluate if the mean of the percentages of the compounds that were common to all three transients was different from

the mean of the percentages of the compounds that were only found once in the same three transients. This data is included in Appendix C. Statistical analysis was performed; two tests were performed for each of the four forms of compounds' variations (appearance, disappearance, increase and decrease), for all three sets of samples analysed (two sets of samples incubated for a week and a set of samples incubated for a month).

Five of them revealed a difference not only in the means of the three types of percentages calculated (common compounds to all three transients, common compounds to two transients, and compounds that were only found once) but also a difference in the mean of the percentages of the compounds that were common to all three transients and the mean of the percentages of the compounds that were only found once. Therefore, we considered that these samples were in fact statistically different. Moreover, two of the statistical analyses showed a difference in the means of the three types of percentages calculated but the mean of the percentages of the compounds that were common to all three transients and the mean of the percentages of the compounds that were only found once were not considered statistically different.

Lastly, the rest showed no difference between the calculated means for both the ANOVA and the t-test. In this case, the samples were not considered statistically different. However, these cases refer to situations of either "compounds' decrease" or "compounds' increase" where a low number of compounds was identified. In this cases, two scenarios were noticeable; either the low number of compounds identified was common to a vast majority of the samples, or the opposite happened and the identified compounds had extremely low incidence values. In some of these cases, identified compounds were present in a high number of samples (79,2% or 100% of the samples), making all transients from all donors similar between themselves. This may explain why it was not possible to assess that some transients were statistically different. In other cases, the number of identified compounds was particularly low and at the same time, their incidences were so low (with no compounds having an incidence greater than 50%) that did not allow for any conclusion to be taken. For this reason, the fact the means for both the ANOVA and the t-test are not statistically different for the "compounds' decrease" and "increase" categories can be mainly attributed to either the high number of common compounds or to how inconclusive some results were, and not necessarily because the transients are not statistically different between themselves.

Due to the high intravariability demonstrated within transients from the same donor, they were considered independent samples and analysed as so. Hence, a total of 24 samples were considered as seven-day aged samples, while 27 were considered one-month aged samples.

4.3.1. Compound Appearance

A "compound appearance" was pondered when a peak was found in the aged sample spectrum but not in the unaged sample spectrum.

Seven-day Incubated Samples

Two sets of seven-day incubated samples were analysed, to distinguish them they will be differentiated according to their incubation date. Set 1 corresponds to the samples incubated from 29-10-2019 to 05-11-2019, while Set 2 corresponds to the samples incubated from 4-11-2019 to 11-11-2019.

Set 1 allowed for the identification of 41 compounds that "appeared" after the seven-day incubation period. Of these 41 identified compounds, only 10 appeared in at least 50% of the 24 samples. These compounds are listed in Table 4.1.

Table 4.1: Compounds that appeared after an incubation period of seven days from Set 1. The average deviation in ppm, the relative incidence and its percentage are shown for the identified compounds, with a minimum incidence percentage of 50%.

Compound	Average Deviation (ppm)	Incidence	Incidence (%)
Stearic acid	-0,05159	19/24	79,2%
Azelaic acid	-0,04103	17/24	70,8%
Cholestenone	-0,01588	17/24	70,8%
7-Ketocholesterol	-0,02891	16/24	66,7%
Oleic acid	-0,00178	15/24	62,5%
Lauroyl diethanolamide	-0,04247	15/24	62,5%
Heptadecanoic acid	0,16466	14/24	58,3%
Ricinoleic acid	-0,03377	12/24	50,0%
Squalene	-0,01742	12/24	50,0%
Tetracosanoic acid	-0,03700	12/24	50,0%

Stearic acid was the most common compound, being identified in 19 of the 24 samples analysed, meaning it appeared in 79,2% of the samples after seven days of incubation. Azelaic acid and cholestenone appeared in a total of 17 samples, which corresponds to appearing in 70,8% of the cases. With an incidence between 60% and 70%, 7-ketocholesterol, oleic acid and lauroyl diethanolamide were identified. Lastly, ricinoleic acid, squalene and tetracosanoic acid appeared in 50% of the cases after the mentioned incubation period.

Stearic acid's isotopic distribution is shown in Figure 4.9, along with its location in the spectrum. The isotopic pattern was previously simulated and confirmed with *Compass DataAnalysis*.

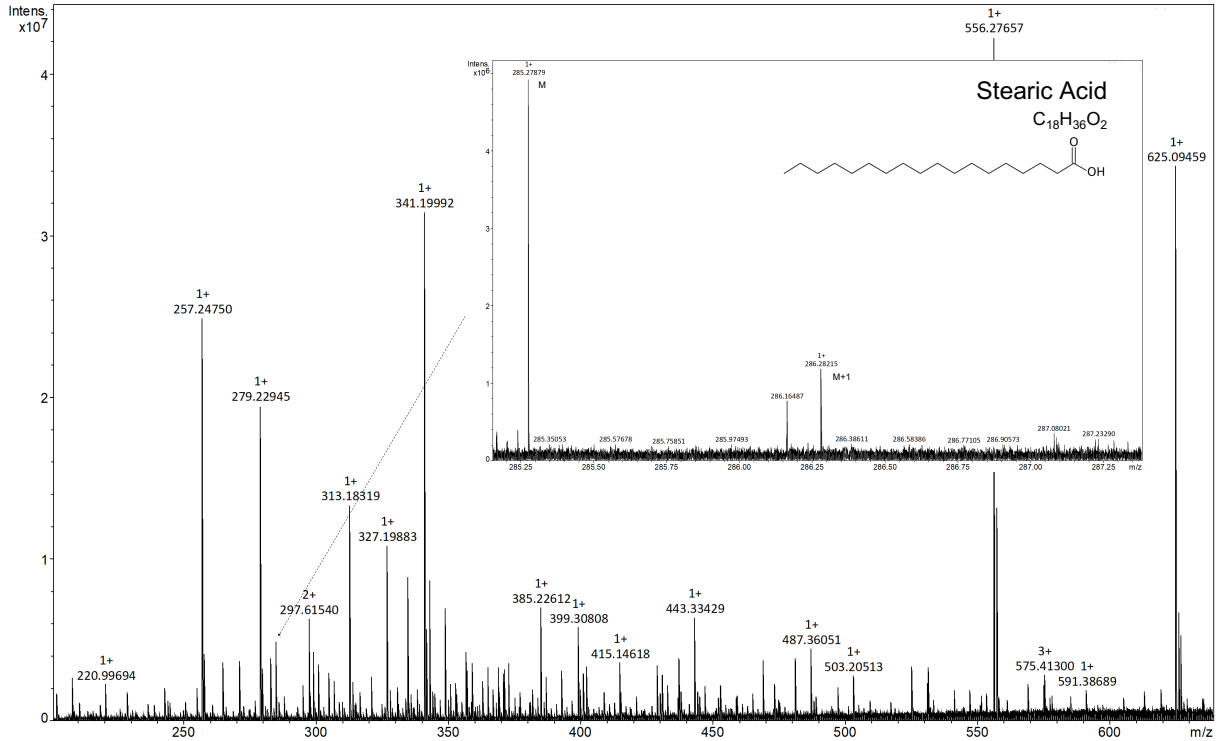

Figure 4.9: Stearic acid's isotopic distribution of the $[M+H]^+$ species, acquired in a Bruker 7T Solarix XR FT-ICR mass spectrometer after a week of incubation (Set 1, 5th of November 2019).

Set 2 allowed for the identification of a smaller number of compounds, in this case, 37 compounds were identified as appearing after a seven-day incubation period. Nevertheless, only four compounds appeared in at least 50% of the 24 samples. The compounds are listed in Table 4.2.

Table 4.2: Compounds that appeared after an incubation period of seven days from Set 2. The average deviation in ppm, the relative incidence and its percentage are shown for the identified compounds, with a minimum incidence percentage of 50%.

Compound	Average Deviation (ppm)	Incidence	Incidence (%)
Azelaic acid	0,15053	21/24	87,5%
Glucose	0,18788	14/24	58,3%
Octyl 4-methoxycinnamic acid	0,03112	14/24	58,3%
Prolylhydroxyproline	0,13967	14/24	58,3%

Azelaic acid was the most identified common compound, appearing in a total of 21 of the 24 samples analysed, being present in 87,5% of the samples incubated for seven days. With an incidence of 58,3%, glucose, octyl 4-methoxycinnamic acid and prolylhydroxyproline were identified.

Octyl 4-methoxycinnamic acid's isotopic pattern is presented in Figure 4.10, along with its location in the spectrum. The isotopic pattern was previously simulated and confirmed with *Compass DataAnalysis*.

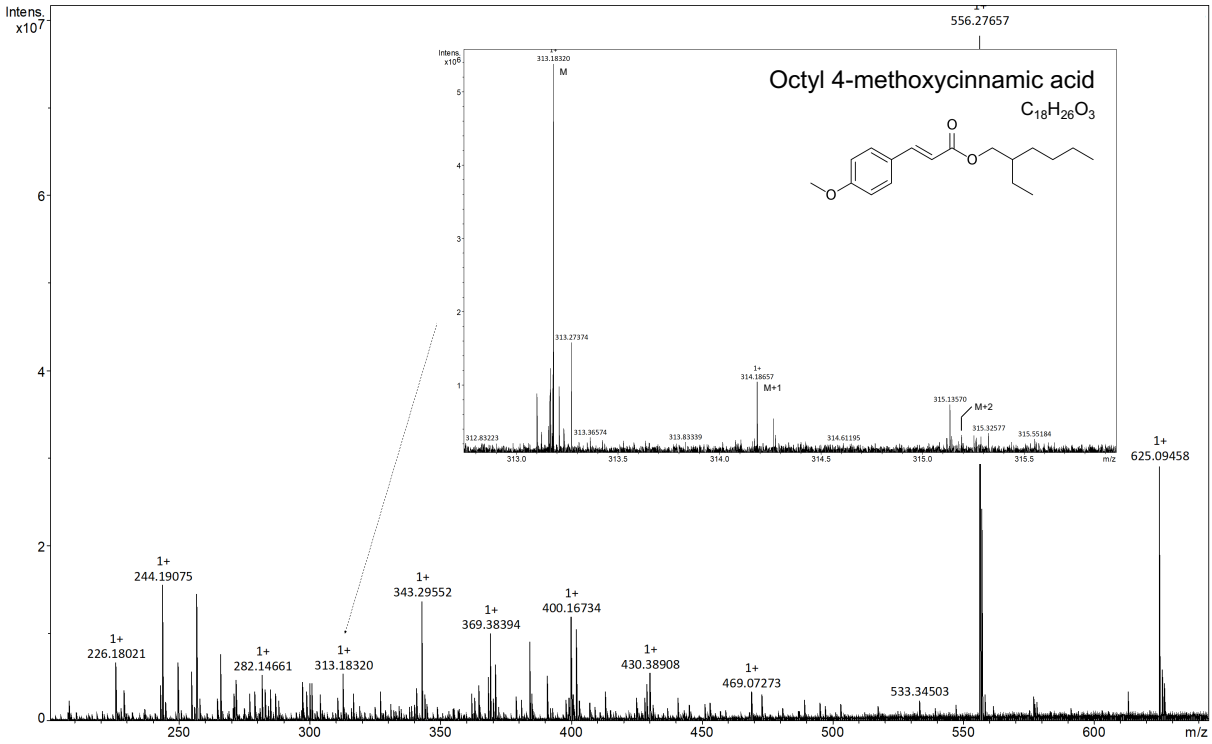

Figure 4.10: Octyl 4-methoxycinnamic acid's isotopic distribution of the $[M+Na]^+$ species, acquired in a Bruker 7T Solarix XR FT-ICR mass spectrometer after a week of incubation (Set 2, 11th of November 2019).

One-month Incubated Samples

The samples that were left ageing for a whole month often allowed for the detection of a lot more compounds, namely 97 compounds were identified as appearing in the aged samples. Of these 97 compounds, 54 had an incidence percentage of at least 50%. These compounds are listed in Table 4.3.

Table 4.3: Compounds that appeared after an incubation period of a month. The average deviation in ppm, the relative incidence and its percentage are shown for the identified compounds, with a minimum incidence percentage of 50%.

Compound	Average Deviation (ppm)	Incidence	Incidence (%)
Diethyl tartrate	0,05437	27/27	100,0%
Lactapiperanol D	0,07888	27/27	100,0%
Methylgingerol	0,15207	27/27	100,0%
Tartaric acid	0,39858	27/27	100,0%
Nonanoic acid	0,36716	25/27	92,6%
Cholesta-4,6-dien-3-one	-0,23456	24/27	88,9%
Citric acid	0,27745	24/27	88,9%
Glutaric acid	0,71192	24/27	88,9%
Adipic acid	0,35540	23/27	85,2%
Aspartic acid	0,26384	23/27	85,2%
Glutamic acid	0,37946	23/27	85,2%
Octanoic acid	-1,24724	23/27	85,2%
Pantothenol	-0,09884	23/27	85,2%
Pentadecanoic acid	-0,33306	23/27	85,2%
Propylparaben	0,36629	23/27	85,2%
Myristic acid	-0,20692	23/27	85,2%
4β-Hydroxycholesterol	0,06401	22/27	81,5%
7-Ketocholesterol	0,28486	22/27	81,5%
(2'E,4'Z,7'Z,8E)-Colnelenic acid	0,14288	21/27	77,8%
4-Hydroxybenzoic acid	1,03055	21/27	77,8%
Decanoic acid	0,30219	21/27	77,8%
Stearic acid	0,17211	21/27	77,8%
Prolylhydroxyproline	0,61881	21/27	77,8%
Tetracosanoic acid	0,33101	21/27	77,8%
Arachidic acid	0,25794	20/27	74,1%
Lauric acid	-0,00242	19/27	70,4%
Glyceryl monostearate	0,52287	19/27	70,4%
Pyroglutamic acid	0,46942	19/27	70,4%
Succinic acid	-0,42935	18/27	66,7%
Docosanoic acid	0,25384	18/27	66,7%
Eicosapentaenoic acid	0,40314	18/27	66,7%
Leucine	0,45419	18/27	66,7%
Serine	0,94646	18/27	66,7%
Oleic acid	0,09272	17/27	63,0%
Cholestenone	0,50821	17/27	63,0%
Methylparaben	0,80139	17/27	63,0%
Octyl 4-methoxycinnamic acid	0,24438	17/27	63,0%
Ricinoleic acid	-0,79245	16/27	59,3%
Glycyl-Histidine	0,44522	16/27	59,3%
Nonadecanoic acid	0,30827	16/27	59,3%
2-Palmitoylglycerol	-1,44963	16/27	59,3%
7β-Hydroxycholesterol	0,09861	15/27	55,6%
Heptadecanoic acid	0,12999	15/27	55,6%
Hexaethylene glycol	0,51518	15/27	55,6%
Lysine	0,40332	15/27	55,6%
Valine	0,38573	15/27	55,6%
Ornithine	0,52593	15/27	55,6%

Table 4.3 (Continued)

Polyoxyethylene (600) monoricinoleate	0,23565	15/27	55,6%
Squalene	-0,02295	15/27	55,6%
1,5-Anhydrosorbitol	0,35218	14/27	51,9%
12-Ketodeoxycholic acid	-0,42229	14/27	51,9%
Linoleic acid	0,27957	14/27	51,9%
Threonic acid	-1,19347	14/27	51,9%
Urocanic acid	0,16810	14/27	51,9%

Diethyl tartrate, lactapiperanol D, methylgingerol and tartaric acid always appeared after an incubation period of a month, being identified in 100% of the samples. Between incidence values of 70% and 99%, there is a total of 25 compounds, from which the most common are nonanoic acid, cholesta-4,6-dien-3-one, citric acid and glutaric acid. Additionally, with an incidence percentage between 50% and 69%, 27 compounds were identified, namely cholestenone and 7β-hydroxycholesterol.

Adipic acid's isotopic pattern is shown in Figure 4.11, along with its location in the spectrum. The isotopic pattern was previously simulated and confirmed with *Compass DataAnalysis*.

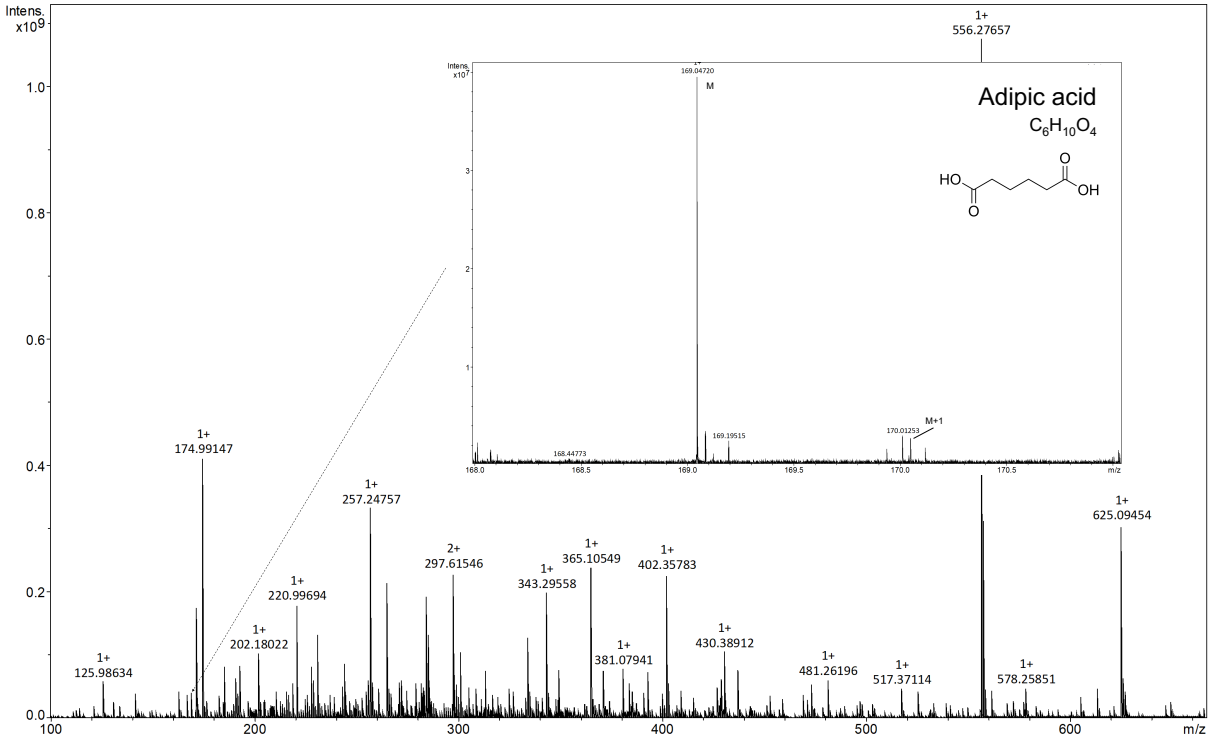

Figure 4.11: Adipic acid's isotopic distribution of the $[M+Na]^+$ species, acquired in a Bruker 7T Solarix XR FT-ICR mass spectrometer after a month of incubation (17th of January 2020).

4.3.2. Compound Disappearance

A "compound disappearance" was pondered when a peak was found in the unaged sample but not in the corresponding aged sample.

Seven-day Incubated Samples

Regarding the identification of compounds that disappeared with ageing, Set 1 allowed for the identification of 31 compounds. Of these 31 identified compounds, only four disappeared in at least 50% of the 24 samples. These compounds are listed in Table 4.4.

Table 4.4: Compounds that disappeared after an incubation period of seven days from Set 1. The average deviation in ppm, the relative incidence and its percentage are shown for the identified compounds, with a minimum incidence percentage of 50%.

Compound	Average Deviation (ppm)	Incidence	Incidence (%)
(2'E,4'Z,7'Z,8E)-Colnelenic acid	-0,01380	24/24	100,0%
Lactapiperanol D	0,02370	21/24	87,5%
Myristoleic acid	0,02062	19/24	79,2%
Octyl 4-methoxycinnamic acid	-0,11847	15/24	62,5%

(2'E,4'Z,7'Z,8E)-Colnelenic acid disappeared after a week of incubation in all 24 samples analysed. Lactapiperanol D and myristoleic acid disappeared in 87,5% and 79,2% of the samples respectively. Lastly, octyl 4-methoxycinnamic acid disappeared in 15 of the 24 samples after a seven-day incubation period.

(2'E,4'Z,7'Z,8E)-Colnelenic acid's isotopic pattern is presented in Figure 4.12, along with its location in the spectrum. The isotopic pattern was previously simulated and confirmed with *Compass DataAnalysis*.

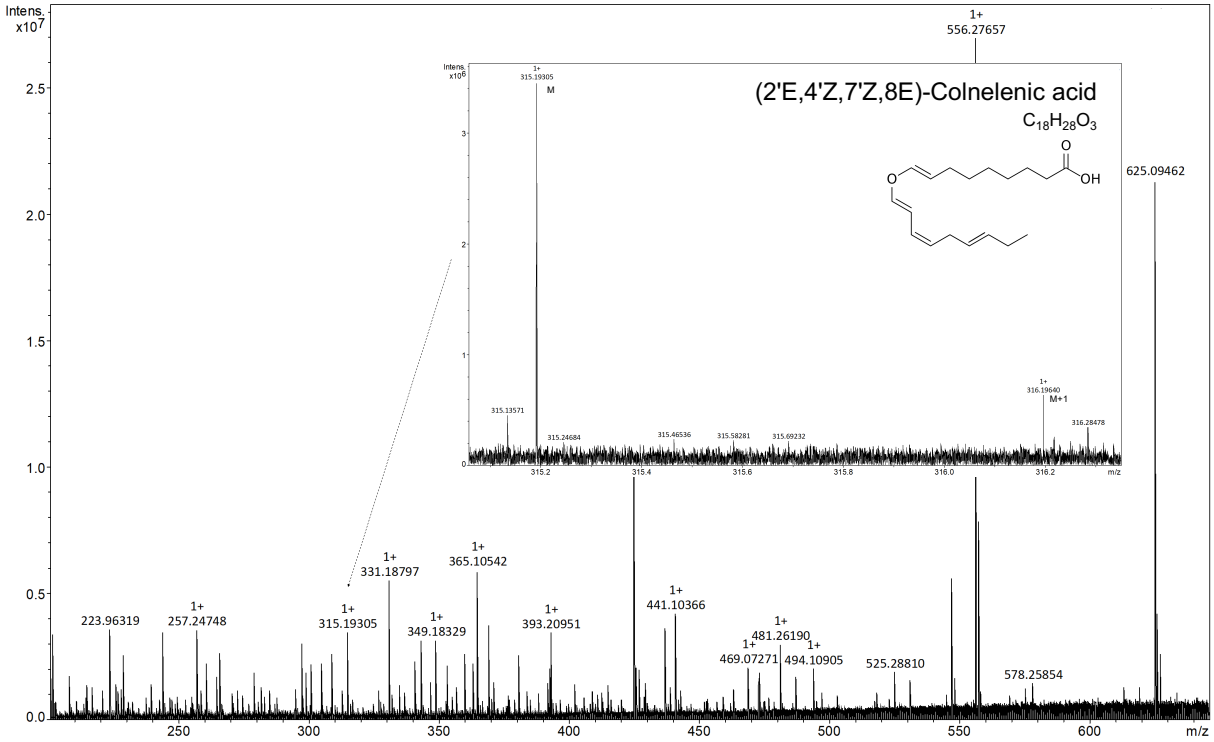

Figure 4.12: (2'E,4'Z,7'Z,8E)-Colnelenic acid's isotopic distribution of the $[M+Na]^+$ species, acquired in a Bruker 7T Solarix XR FT-ICR mass spectrometer before a week of incubation (Set 1, 29th of October 2019).

On the other hand, Set 2 allowed for the identification of 37 compounds, which disappeared after a seven-day incubation period. As for Set 1, only four compounds disappeared in at least 50% of the samples. The compounds are listed in Table 4.5.

Table 4.5: Compounds that disappeared after an incubation period of seven days from Set 2. The average deviation in ppm, the relative incidence and its percentage are shown for the identified compounds, with a minimum incidence percentage of 50%.

Compound	Average Deviation (ppm)	Incidence	Incidence (%)
Lactapiperanol D	-0,00815	22/24	91,7%
(2'E,4'Z,7'Z,8E)-Colnelenic acid	-0,01482	18/24	75,0%
Glyceryl monostearate	-0,01515	12/24	50,0%
Stearic acid	-0,03760	12/24	50,0%

Lactapiperanol D disappeared in 22 of the 24 samples incubated for a week, which corresponds to a percentage of 91,7%. The disappearance of *(2'E,4'Z,7'Z,8E)*-colnelenic acid was identified in 75,0% of the cases while both glyceryl monostearate and stearic acid disappeared after incubation in 50,0% of the analysed samples.

Glyceryl monostearate's isotopic pattern is shown in Figure 4.13, along with its location in the spectrum. The isotopic pattern was previously simulated and confirmed with *Compass DataAnalysis*.

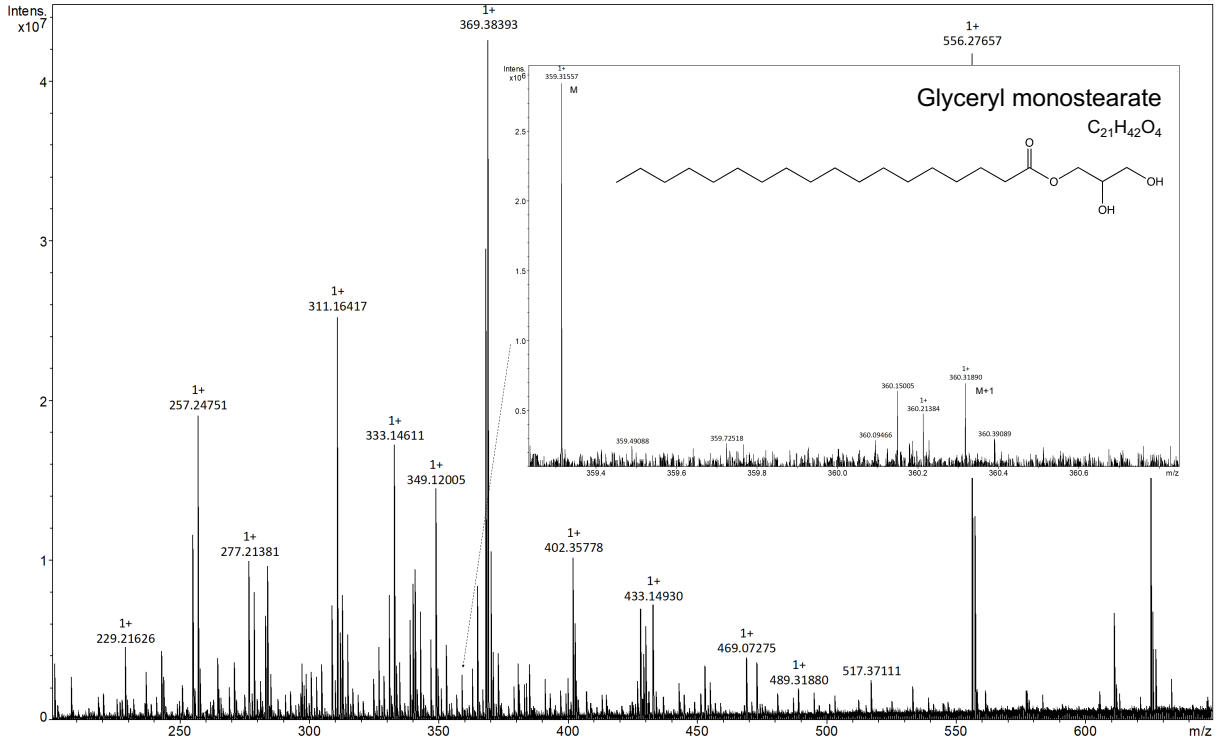

Figure 4.13: Glyceryl monostearate's isotopic distribution of the $[M+H]^+$ species, acquired in a Bruker 7T Solarix XR FT-ICR mass spectrometer before a week of incubation (Set 2, 4th of November 2019).

One-month Incubated Samples

The samples that aged for a month allowed once more for the detection of a higher number of compounds; 72 compounds were identified as disappearing after incubation. However, of the 72 compounds, only two disappeared in at least 50% of the samples. These compounds are listed in Table 4.6.

Table 4.6: Compounds that disappeared after an incubation period of a month. The average deviation in ppm, the relative incidence and its percentage are shown for the identified compounds, with a minimum incidence percentage of 50%.

Compound	Average Deviation (ppm)	Incidence	Incidence (%)
Glycerol	0,22175	15/27	55,6%
Octyl 4-methoxycinnamic acid	-0,24199	15/27	55,6%

Both glycerol and octyl 4-methoxycinnamic acid disappeared after a month of incubation in 15 of the 27 samples analysed, which corresponds to a percentage of 55,6%. There were no well-defined isotopic patterns for the compounds identified as "disappearing" over time.

4.3.3. Compound Increase

Any peak with an intensity increase of over an order of magnitude was considered a "compound increase".

Seven-day Incubated Samples

Regarding the identification of compounds whose amount increased with ageing, Set 1 allowed for the identification of 14 compounds. Of these 14 identified compounds, none appeared in at least 50% of the 24 samples. The same happened for Set 2, where four compounds were identified as increasing with time, however, none of those compounds was present in at least half of the cases.

One-month Incubated Samples

The samples incubated for a month allowed for the detection of a greater number of compounds; 49 compounds were identified as increasing during incubation. Of the 49 compounds, eight increased in at least 50% of the samples. These compounds are listed in Table 4.7.

Table 4.7: Compounds that increased after an incubation period of a month. The average deviation in ppm, the relative incidence and its percentage are shown for the identified compounds, with a minimum incidence percentage of 50%.

Compound	Average Deviation (ppm)	Incidence	Incidence (%)
Adipic acid	0,24335	21/27	77,8%
Ricinoleic acid	0,42681	18/27	66,7%
12-Ketodeoxycholic acid	0,12377	17/27	63,0%
Stearic acid	0,18949	17/27	63,0%
Azelaic acid	0,21271	17/27	63,0%
Hexaethylene glycol	0,19346	14/27	51,9%
Lauric acid	1,32452	14/27	51,9%
Methylgingerol	0,13903	14/27	51,9%

Adipic acid showed an increase after a month of incubation in 21 of the 27 samples analysed, which corresponds to a percentage of 77,8%. The following compounds, which increased in around 60% of the cases, include ricinoleic acid, 12-ketodeoxycholic acid, stearic acid and azelaic acid. Lastly, some worth mentioning compounds include hexaethylene glycol, lauric acid and methylgingerol, which increased after incubation in 51,9% of the samples.

12-Ketodeoxycholic acid's isotopic pattern is presented in Figure 4.14, along with its location in the spectrum. The isotopic pattern was previously simulated and confirmed with *Compass DataAnalysis*.

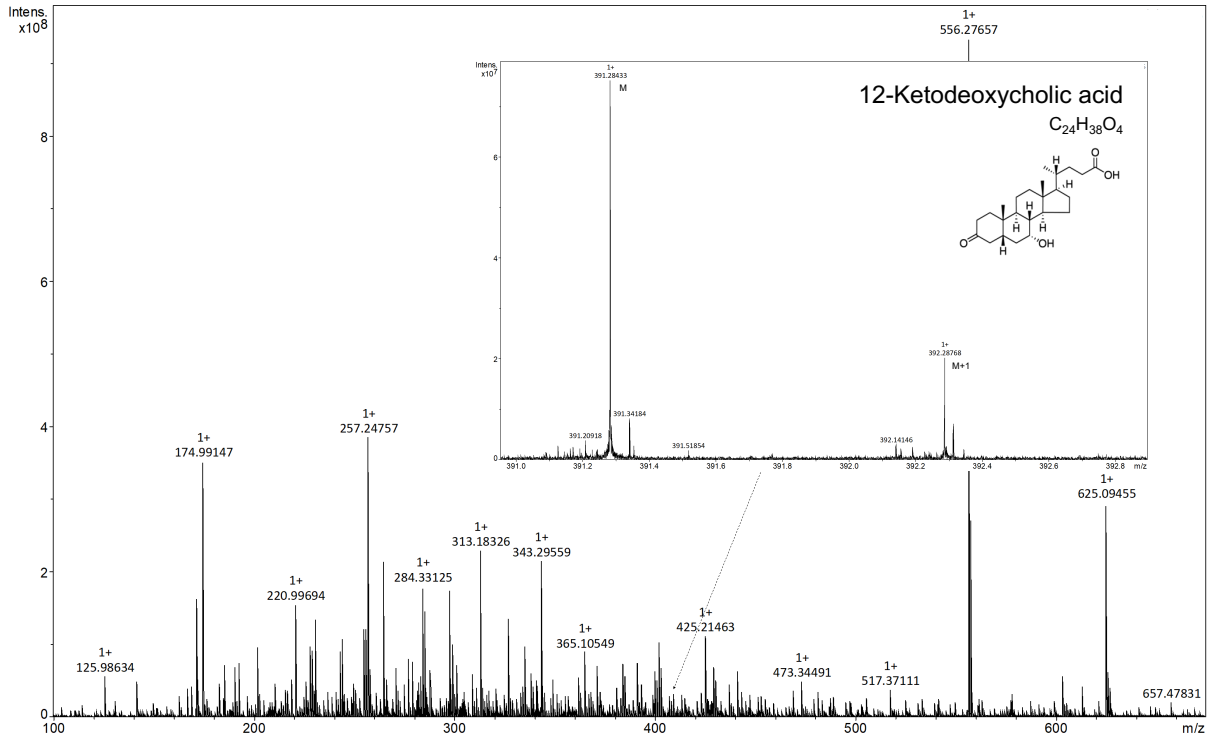

Figure 4.14: 12-Ketodeoxycholic acid's isotopic distribution of the $[M+H]^+$ species, acquired in a Bruker 7T Solarix XR FT-ICR mass spectrometer after a month of incubation (17th of January 2020).

4.3.4. Compound Decrease

Any peak with an intensity decrease of over an order of magnitude was considered a "compound decrease".

Seven-day Incubated Samples

Set 1 allowed for the identification of six compounds whose amount decreased after the seven-day incubation period. Of these five identified compounds none appeared in at least 50% of the 24 samples.

Set 2 allowed for the identification of seven compounds, which decreased after the samples were incubated for a week. Of the 7 compounds, 2 were identified in at least 50% of the 24 samples. These compounds are listed in Table 4.8.

Table 4.8: Compounds that decreased after an incubation period of seven days from Set 2. The average deviation in ppm, the relative incidence and its percentage are shown for the identified compounds, with a minimum incidence percentage of 50%.

Compound	Average Deviation (ppm)	Incidence	Incidence (%)
Methylgingerol	-0,00947	24/24	100,0%
(2'E,4'Z,7'Z,8E)-Colnelenic acid	-0,00665	19/24	79,2%

A decrease in methylgingerol was observed in all 24 samples, while a decrease in the amount of *(2'E,4'Z,7'Z,8E)*-colnelenic acid was observed in 19 of the 24 cases, which corresponds to 79,2%.

Methylgingerol's isotopic pattern is presented in Figure 4.15, along with its location in the spectrum. The isotopic pattern was previously simulated and confirmed with *Compass DataAnalysis*.

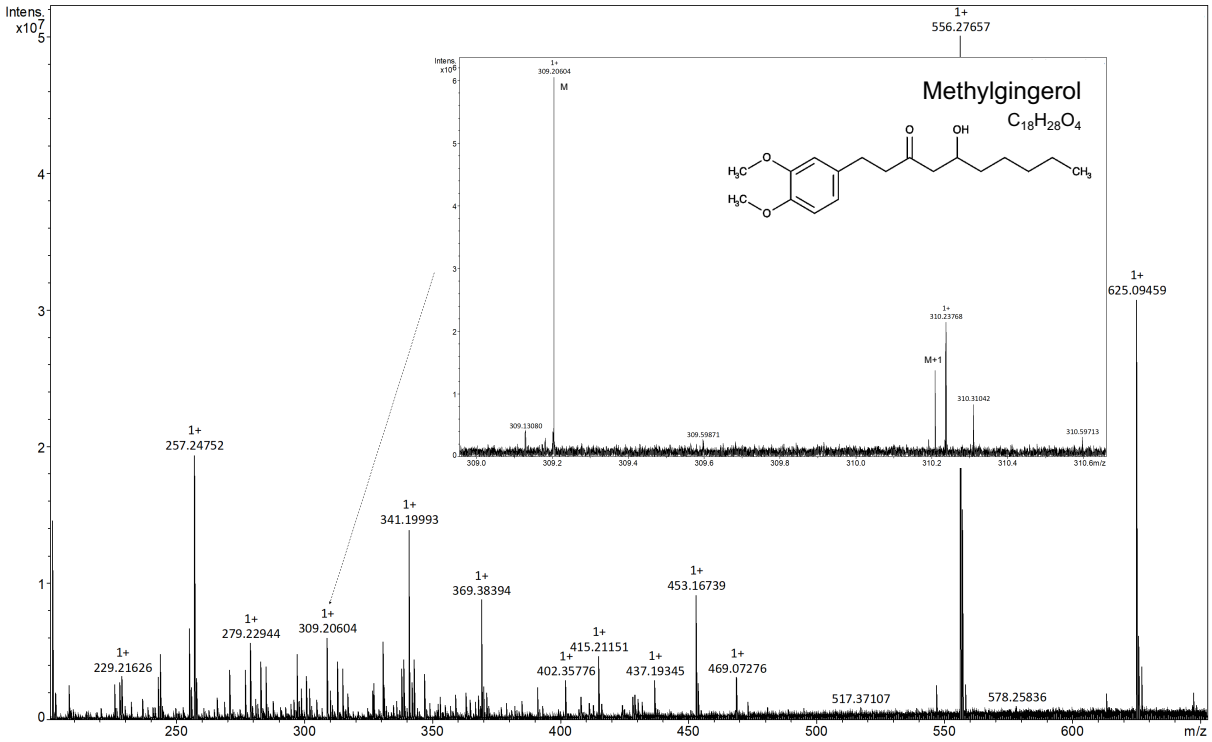

Figure 4.15: Methylgingerol's isotopic distribution of the $[M+H]^+$ species, acquired in a Bruker 7T Solarix XR FT-ICR mass spectrometer before a week of incubation (Set 2, 4th of November 2019).

One-month Incubated Samples

The samples incubated for a month allowed for the identification of nine compounds whose amount decreased with time. However, of these nine compounds, none appeared in at least 50% of the 27 samples.

4.4. Comparison of the Changes in the Chemical Composition of Fingerprints with Time

A comparison was performed between the two sets of samples incubated for a week, as well as between those sets and the set of samples incubated for a month to assess if any of the compound's variations were common to them.

4.4.1. Compound Appearance

The compounds that were labelled as "appearing" after a certain period of incubation were compared between themselves.

Seven-day Incubated Samples Comparison

The two sets of seven-day incubated samples were compared to determine if there were any compounds in both sets with a mean incidence greater than 50%. In this case, the incidence of a compound within its set did not have to be greater than 50%. A total of 3 compounds, common to the two sets were identified as appearing after a seven-day incubation period under the previously mentioned circumstances. The compounds are listed in Table 4.9.

Table 4.9: Compounds that appeared after an incubation period of seven days from Set 1 and 2. The average deviation in ppm, relative incidences and their percentages are shown for the identified compounds, with a minimum mean incidence percentage of 50%.

Compound	Average Deviation (ppm)	Set 1 Incidence (%)	Set 2 Incidence (%)	Mean Incidence (%)
Azelaic acid	0,05475	70,8%	87,5%	79,2%
Cholestenone	-0,02351	70,8%	37,5%	54,2%
Lauroyl diethanolamide	-0,03172	62,5%	37,5%	50,0%

Azelaic acid had the highest mean incidence of 79,2% being present in more than 50% of the samples for each set. Despite cholestenone and lauroyl diethanolamide having mean incidences around 50%, both showed up in less than 50% of the samples in Set 2.

Seven-day and One-month Incubated Samples Comparison

The two sets of seven-day incubated samples were compared with the samples incubated for a month. The main goal was to determine if there were any compounds from the three sample sets with a mean incidence greater than 50%. The conditions previously mentioned regarding each sets' incidence still apply. In this case, five appearing compounds were identified as being common to the two sets of seven-day incubated samples and the one-month incubated set. The common compounds are listed in Table 4.10.

Table 4.10: Compounds that appeared after incubation periods of seven days (from Set 1 and 2) or a month. The average deviation in ppm, relative incidences and their percentages are shown for the identified compounds, with a minimum mean incidence percentage of 50%.

Compound	Average Deviation (ppm)	1 Week (Set 1) Incidence (%)	1 Week (Set 2) Incidence (%)	1 Month Incidence (%)	Mean Incidence (%)
7-Ketocholesterol	0,09604	66,7%	29,2%	81,5%	59,1%
Stearic acid	0,02385	79,2%	16,7%	77,8%	57,9%
Cholestenone	0,15373	70,8%	37,5%	63,0%	57,1%
Azelaic acid	0,30739	70,8%	87,5%	11,1%	56,5%
Tetracosanoic acid	0,10716	50,0%	29,2%	77,8%	52,3%

7-Ketocholesterol had the highest mean incidence of 59,1% being present in less than 50% on the seven-day aged samples from Set 2. For stearic acid, cholestenone, azelaic acid and tetracosanoic acid the mean incidences were around 50% and for all of them, there was at least one set of samples where the compound had an incidence lower than 50%.

4.4.2. Compound Disappearance

The compounds labelled as disappearing after a certain period of incubation were compared between themselves. Despite the fact there were no common compounds to all three tables (Tables 4.4, 4.5 and 4.6) associated with "compound disappearance", there were some compounds common to two of the tables.

Seven-day Incubated Samples Comparison

The two sets of seven-day incubated samples were compared to determine if there were any compounds in both sets with a mean incidence greater than 50%. In this case, the incidence of a compound within its set did not have to be greater than 50%. A total of four compounds, common to the two sets were identified as disappearing after a seven-day incubation period under the previously mentioned circumstances. The compounds are listed in Table 4.11.

Table 4.11: Compounds that disappeared after an incubation period of seven days from Set 1 and 2. The average deviation in ppm, relative incidences and their percentages are shown for the identified compounds, with a minimum mean incidence percentage of 50%.

Compound	Average Deviation (ppm)	Set 1 Incidence (%)	Set 2 Incidence (%)	Mean Incidence (%)
Lactapiperanol D	0,00778	87,5%	91,7%	89,6%
(2'E,4'Z,7'Z,8E)-Colnelenic acid	-0,01431	100,0%	75,0%	87,5%
Myristoleic acid	0,07219	79,2%	33,3%	56,5%
Octyl 4-methoxycinnamic acid	-0,05793	62,5%	37,5%	50,0%

Lactapiperanol D had the highest mean incidence of 89,6%, followed by *(2'E,4'Z,7'Z,8E)*-colnelenic acid, and both compounds were present in more than 50% of the samples for each set. Despite myristoleic acid and octyl 4-methoxycinnamic acid having mean incidences around 50%, both showed up in less than 50% of the samples from Set 2.

Seven-day and One-month Incubated Samples Comparison

The two sets of seven-day incubated samples were compared with the samples incubated for a month. The main goal was to once again determine if there were any compounds from the three sample sets with a mean incidence greater than 50%. In this case, a total of three disappearing compounds were identified as being common to the two sets of seven-day incubated samples as well as the one-month incubated set. The common compounds are listed in Table 4.12.

Table 4.12: Compounds that disappeared after incubation periods of seven days (from Set 1 and 2) or a month. The average deviation in ppm, relative incidences and their percentages are shown for the identified compounds, with a minimum mean incidence percentage of 50%.

Compound	Average Deviation (ppm)	1 Week (Set 1) Incidence (%)	1 Week (Set 2) Incidence (%)	1 Month Incidence (%)	Mean Incidence (%)
Lactapiperanol D	0,09720	87,5%	91,7%	7,4%	62,2%
(2'E,4'Z,7'Z,8E)-Colnelenic acid	0,14280	100,0%	75,0%	7,4%	60,8%
Octyl 4-methoxycinnamic acid	-0,11928	62,5%	37,5%	55,6%	51,9%

Lactapiperanol D had the highest mean incidence of 62,2% being present in less than 50% on the one-month aged samples. While *(2'E,4'Z,7'Z,8E)*-colnelenic acid also showed an incidence lower than 50% for the samples incubated for a month, octyl 4-methoxycinnamic acid showed an incidence lower than 50% on the Set 2 samples.

4.4.3. Compound Increase

The compounds that were labelled as "increasing" after a week or month of incubation were compared between themselves. No compounds were identified with a mean incidence greater than 50% for both the seven-day incubated samples comparison and the seven-day and one-month incubated samples comparison.

4.4.4. Compound Decrease

The compounds labelled as "decreasing" over a certain incubation period were compared between themselves. Despite the fact there were no common compounds to the three tables associated with a "compound decrease", there were some compounds common to two of the tables.

Seven-day Incubated Samples Comparison

Both sets of seven-day incubated samples were compared to determine if there were any compounds in the two sets with a mean incidence greater than 50%. In this case, two compounds common to the two sets were identified as disappearing after a seven-day incubation period under the previously mentioned circumstances. The compounds are listed in Table 4.13.

Table 4.13: Compounds that decreased after an incubation period of seven days from Set 1 and 2. The average deviation in ppm, relative incidences and their percentages are shown for the identified compounds, with a minimum mean incidence percentage of 50%.

Compound	Average Deviation (ppm)	Set 1 Incidence (%)	Set 2 Incidence (%)	Mean Incidence (%)
Methylgingerol	-0,00179	25,0%	100,0%	62,5%
(2'E,4'Z,7'Z,8E)-Colnelenic acid	-0,01468	37,5%	79,2%	58,3%

Methylgingerol had the highest mean incidence of 62,5%, with incidences superior to 50% in only one of the sets of seven-days incubated samples. *(2'E,4'Z,7'Z,8E)*-Colnelenic acid had a mean incidence of 58,3%, its incidence was also less than 50% in Set 1.

Seven-day and One-month Incubated Samples Comparison

The two sets of seven-day incubated samples were compared with the samples incubated for a month. The main goal was to once again determine if there were any compounds from the three sample sets with a mean incidence greater than 50%. In this case, no decreasing compound was identified as being common to the two sets of seven-day incubated samples as well as the one-month incubated set.

5

Discussion, Conclusions and Future Work

In this chapter, a discussion of the results is presented, as well as the general conclusions of the study and the prospects for future work.

5.1. Discussion

The main goal of this dissertation project was to identify the chemical compounds present on a fingerprint and study how those change with time. Another aim was to understand the chemical differences between samples incubated for different time periods, namely samples incubated for a week and samples incubated for a month. Lastly, it would be remarkable to determine if some sort of pattern could be found among the chemical changes within our samples and those previously described in the available literature. It is intended that the analysis performed can be used in the development of a fingerprint ageing profile, which could be carried out in the future.

The results obtained and the conclusions reached only reflect an average trend of the sample under study.

Regarding the analysis of the range of solvent systems, their suitability for fingerprint analysis was determined based on the number of compounds identified for each solvent, the solvents' availability and associated hazard level. Despite the fact solvent systems 4, 6 and 8 allowed for the identification of a higher number of compounds, they were not considered the most adequate when compared to solvent system 1. The fact this solvent system is the most balanced and safe, along with it being the most compatible with electrospray ionization had great influence in its election as the most adequate solvent system.

Concerning the identification of compounds for the samples under analysis, several compounds were identified with deviation module values lower than 2 ppm. The analysis was performed on samples aged for two different time points, either one week or one month. The compounds were identified for all four categories – "compound appearance", "compound disappearance", "compound increase" and "compound decrease".

Regarding the identified cases of "compound appearance", both incubation periods allowed for the identification of a great number of compounds. Previous research studies report the appearance of certain classes of compounds, including cholesterol-derived compounds and carboxylic acids and aromatic compounds deriving from the degradation of triglycerides [11].

For the first set of one week incubated samples, it was possible to identify two products of cholesterol degradation, cholestenone and 7-ketocholesterol, and six fatty acids. Stearic, azelaic, heptadecanoic and tetracosanoic acids correspond to the identified saturated fatty acids, while oleic acid and ricinoleic acid correspond to the unsaturated. Lauroyl diethanolamide was also identified, however fatty amides have

not been described as “appearing” on fingermark residue with time. Nevertheless, fatty amides are carboxylic acid amide derivatives of fatty acids, that are formed from a fatty acid and an amine, which might justify its appearance. Additionally, the presence of squalene was verified in half of the analysed samples despite its ease of degradation. Squalene is known for being present in fresh fingermark residue only, rapidly degrading and not being detected after seven days on non-porous surfaces, such as glass, despite being detected after 30 days on porous surfaces [11]. For that reason, its appearance in the analysed samples should be further investigated, for example through MS/MS to confirm its identity.

The second set of one week incubated samples also allowed for the identification of azelaic acid, a saturated fatty acid, and two carboxylic acids deriving compounds, octyl 4-methoxycinnamic acid and prolylhydroxyproline. Despite having no previous mention in past research studies, the “appearance” of glucose was identified in 58,3% of the analysed samples. Glucose is synthesized from non-carbohydrate intermediates, such as pyruvate and glycerol. A “disappearance” of glycerol was identified after a month of incubation, there is the possibility that this variation could be related and that should be explored, namely for the presence of other related compounds, such as pyruvate.

Concerning the samples incubated for a month, a total of 54 compounds were identified as “appearing” with time, of which 18 are fatty acids. Nonanoic, octanoic, pentadecanoic and myristic acids are the saturated fatty acids with highest incidence values, while *(2'E,4'Z,7'Z,8E)*-colnelenic acid and eicosapentaenoic acid are the most common unsaturated fatty acids. Moreover, 12 carboxylic acids deriving compounds were detected, including diethyl tartrate, and propylparaben with incidences over 80%. Regarding other carboxylic acids, tartaric, citric, glutaric and adipic acids were some of the detected compounds which showed an “appearance” after incubation. Glutaric and adipic acids are both fully oxidized forms that derive from squalene, meaning their appearance may relate to squalene degradation. Five products of cholesterol degradation were identified, namely cholesta-4,6-dien-3-one, 4β-hydroxycholesterol, 7-ketocholesterol, cholestenone, and 7β-hydroxycholesterol. Several amino acids, including arginine, aspartic acid and glutamic acid were also detected. Lastly, the presence of an aromatic compound was also reported, namely, methylgingerol. Regarding the classes of compounds which have not been described as “appearing” on fingermark residue after a certain incubation period, lactapiperanol D was identified. This compound belongs to the class of organic compounds known as diterpenoids that are terpene compounds formed by four isoprenes [43]. The mechanism of lactapiperanol D formation could be further studied to assess the relevance of this compound’s presence. The same applies for squalene, pantothenol, which is a part of the alcohols and polyols class, choline, an organonitrogen compound, hexaethylene glycol, an ether, 1,5-Anhydrosorbitol, a part of the carbohydrates and conjugates class and lastly uric acid which belongs to the imidazopyrimidines class. Further research is needed to confirm that these compounds indeed appear after incubation as they may hold relevant and new information on the fingerprint ageing process.

Squalene has been considered a good target compound because of its recurrent absence in older samples. Nevertheless, an “appearance” of squalene was detected after the samples were incubated for both a week and a month. To assess if this increase was associated with a specific adduct, squalene’s conjugation with the different adducts was further studied. The samples aged for a week only revealed the presence of protonated squalene, $[M+H]^+$. On the other hand, for one-month aged samples, all adducts were detected, $[M+H]^+$ had the highest incidence of 48,1% for the 27 analysed samples, while the $[M+Na]^+$ and $[M+K]^+$ species had incidences of 44,4% and 29,6% respectively. The appearance of squalene does not seem to be related to its adducts and for that reason, it should be further explored.

A comparison was performed between the different sets of incubated samples to assess if there were any equal compound variations, with an overall incidence greater than 50%. When comparing the two sets of samples incubated for a week, a common appearance of azelaic acid, cholestenone and lauroyl diethanolamide was found. However, when comparing these two sets with the set of samples incubated for a month, besides azelaic acid and cholestenone, 7-ketocholesterol, stearic acid and tetracosanoic acid all had an overall incidence greater than 50%. These compounds showed a certain behaviour consistency amongst the different sets and should be given special attention in further research studies.

Regarding the identified cases of "compound disappearance", a smaller number of compounds were identified. Previous research studies report the disappearance of certain classes of compounds, including squalene and unsaturated fatty acids [5].

For Set 1 of one week incubated samples, it was possible to identify two unsaturated fatty acids who "disappeared" after incubation, namely *(2'E,4'Z,7'Z,8E)*-colnelenic acid and myristoleic acid. Unsaturated acids tend to disappear with time due to the unsaturated moiety being open to attack through aerobic and anaerobic degradation processes [5]. The "disappearance" of lactapiperanol D was unexpected as the same compound "appeared" after a month of incubation. All adducts were present for the two cases, which does not justify this compound's variation. Consequently, this compound's presence should be confirmed by MS/MS. The same applies to octyl 4-methoxycinnamic acid, a carboxylic acid deriving compound which also "appeared" after the samples' incubation.

Set 2 of one week incubated samples also allowed for the identification of the unsaturated fatty acid *(2'E,4'Z,7'Z,8E)*-colnelenic. A "disappearance" of lactapiperanol D was once again identified. Furthermore, the disappearance of both glyceryl monostearate and stearic acid had not been reported in previous research studies, and both compounds were actually identified as "appearing" after incubation. Nevertheless, stearic acid belongs to the class of organic compounds known as long-chain fatty acids and glyceryl monostearate also has a long chain which may have broken down, causing the "disappearance" of these compounds after incubation [43].

Regarding the compounds that "disappeared" for one-month incubated samples, only two compounds were identified. Octyl 4-methoxycinnamic acid, which also "disappeared" for the first set of one week incubated samples, and glycerol. As formerly mentioned, glycerol's "disappearance" could be related to the "appearance" of glucose in one-week incubated samples.

A comparison was performed to assess if there were any equal compound "disappearances" for the tested incubation periods with an overall incidence greater than 50%. Lactapiperanol D, *(2'E,4'Z,7'Z,8E)*-colnelenic acid and octyl 4-methoxycinnamic acids were identified as "disappearing" with time for both the comparison of the two sets of samples incubated for a week and the comparison of the two sets of samples incubated for a week and the set incubated for a month. For that reason, those compounds should be further explored in the future.

Regarding the identified cases of "compound increase", no compounds were identified for the samples aged for a week. Preceding research studies report the increase of short-chain saturated fatty acids, squalene epoxides and squalene-derived compounds [5].

Compounds were only identified as "increasing" after a month of incubation. Adipic acid, one of the most fully oxidized forms of squalene was identified in 77,8% of the analysed samples. Azelaic acid and lauric acid both belong to the class of medium-chain fatty acids, and may derive from the long-chain

fatty acids' decomposition. Stearic acid, a saturated fatty acid, whose amount is known to increase before decreasing was also identified. The increase of ricinoleic acid and 12- ketodeoxycholic acid should be verified by MS/MS, as well as hexaethylene glycol and methylgingerol. Some of methylgingerol's "alternative parents" and "substituents" include ethers [43]. Due to ethers stability, their formation may occur over time.

No common compounds were identified as "increasing" with time, between the three sets of analysed data.

Concerning the identified cases of "compound decrease", compounds with an incidence greater than 50% were only identified for the second set of one-week incubated samples. Previous research studies report the decrease of certain classes of compounds, namely unsaturated fatty acids, triglycerides and cholesterol and squalene [5].

Compounds were only identified as "decreasing" for one set of the seven-day incubated samples. A "decrease" in *(2'E,4'Z,7'Z,8E)*-colnelenic acid, an unsaturated fatty acid was reported, along with a "decrease" in methylgingerol. Methylgingerol's variation should be further explored, since this compound also "increased" after a month of incubation, indicating possible fluctuations in its behaviours.

The comparison performed between the two sets of one-week incubated samples was the only who showed results of equal compound variations with an overall incidence greater than 50%. As formerly described, both *(2'E,4'Z,7'Z,8E)*-colnelenic acid and methylgingerol "decreased" with time for these two sets of data.

For the most part, it was possible to confirm variations of compounds that had already been described in previous works, but it was also possible to identify new compounds that also seem to variate with fingermark residue ageing. Moreover, former research studies focused on variations of classes of compounds, such as carboxylic acids and triglycerides-derived compounds. With this work, more specific identification of the compounds was achieved within those classes. Even though the results presented are still preliminary, it is possible to show how a high-resolution technique such as FT-ICR mass spectrometry may come to play a pivotal role in forensic analysis and dating of human fingerprints in the near future.

5.2. Conclusions

Determining the composition of fingermark residue is an analytical challenge because of its complex and multifaceted nature and the existence of numerous factors that exert a strong influence, exogenous, endogenous and environmental. The compounds should show reproducible and measurable modifications over time to be of value for fingermark ageing studies. However, due to the significant intervariability and intravariability, and the difficulties in obtaining homogeneous samples of fingerprints, it is difficult to make absolute statements concerning changes in the composition of fingerprints over time.

Notwithstanding the difficulties, a large number of compounds were identified, some with high incidence values for the analysed data sets. Regarding how compounds variated with time, "appearances" and "disappearances" allowed for the identification of a higher number of compounds when compared to "increases" and "decreases". Also, "appearances" and "disappearances" seem to be more reliable when it comes to studying compounds' variations. These correspond to crude and drastic variations,

while “increases” and “decreases”, in addition to being harder to define, consist of subtle and slight changes.

It is intended that the results achieved and presented in this dissertation help in building the fundamental basis for further forensic method development and potential application in forensic investigation. Despite the interest in fingerprint dating from several decades ago, it is still a considerably unexplored field that requires more time, dedication and research.

5.3. Future Work

As the interest in fingerprint dating increases, a larger flux of material and studies is expected, which will help consolidate the results obtained in this dissertation.

It should be noted that there was a considerable amount of identified compounds which had not been previously described in the literature as fingermark components. Therefore, they should be further explored to confirm their identity, namely through MS/MS. Additionally, once a set of compounds is identified, quantitative targeted methods can be used to assess their variations with time. For that reason, target triple quadrupole mass spectrometry analysis for quantitative purposes may be the following step.

Moreover, to strengthen and confirm the presented results, different incubation periods should be tested. Also, the increase in the study population will allow obtaining more reliable conclusions that could lead to the future trace of a fingerprint ageing profile, which may be used in future forensic investigation.

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Supplementary Data

Appendix A

Appendix A contains the questionnaire that the volunteers were asked to complete before donating their fingerprints.

S. R.

MINISTERIO DA JUSTIÇA

POLÍCIA JUDICIÁRIA

DIRECTORIA NACIONAL

Laboratório de Polícia Científica
Faculdade de Ciências da Universidade de Lisboa

Projecto FINGERPRINTS - Ficha de Identificação

V1

Nome: _____

Data de nascimento:_____/_____/_____

Género:_____

Raça:_____

Profissão:_____

Local de trabalho:_____

Medicação:_____

Doenças/estado de saúde:_____

Contacto:_____

_____

V1- 1

Data da recolha : Hora:

Procedimento de recolha:

Observações:

_____

V1- 2

Data da recolha : Hora:

Procedimento de recolha:

Observações:

Figure A.1: Questionnaire to be filled by each of the volunteers before proceeding to their fingerprint's donation.

S. R.

MINISTÉRIO DA JUSTIÇA

POLÍCIA JUDICIÁRIA

DIRECTORIA NACIONAL

V1

V1- 3
Data da recolha : Hora:
Procedimento de recolha:

Observações:

V1- 4
Data da recolha : Hora:

Procedimento de recolha:

Observações:

V1- 5
Data da recolha : Hora:

Procedimento de recolha:

Observações:

V1- 6
Data da recolha : Hora:

Procedimento de recolha:

Observações:

Figure A.1 (continued)

S. R.

MINISTÉRIO DA JUSTIÇA

POLÍCIA JUDICIÁRIA

DIRECTORIA NACIONAL

V1

V1- 7
Data da recolha : Hora:
Procedimento de recolha:

Observações:

V1- 8
Data da recolha : Hora:
Procedimento de recolha:

Observações:

V1- 9
Data da recolha : Hora:
Procedimento de recolha:

Observações:

V1- 10
Data da recolha : Hora:
Procedimento de recolha:

Observações:

Figure A.1 (continued)

S. R.

MINISTÉRIO DA JUSTIÇA

POLÍCIA JUDICIÁRIA

DIRECTORIA NACIONAL

V1

V1- 11
Data da recolha : Hora:
Procedimento de recolha:

Observações:

V1- 12
Data da recolha : Hora:
Procedimento de recolha:

Observações:

V1- 13
Data da recolha : Hora:
Procedimento de recolha:

Observações:

V1- 14
Data da recolha : Hora:
Procedimento de recolha:

Observações:

Figure A.1 (continued)

Appendix B

Appendix B's section contains supplementary data. The treatment and presentation of the most relevant aspects of these data can be found in Chapter 4.

Table B.1 displays the list of compounds described in the literature as existing in fingermark residue as well as metabolites present in sweat, according to *The Human Metabolome Database*.

Table B.1: Compounds previously found in either fingermark residue or sweat, their chemical formula, monoisotopic mass, the mass of the protonated molecule and the Na^+ and K^+ adducts.

	Chemical Formula	**Mass, M (g/mol)**	**M + H (g/mol)**	**M + Na (g/mol)**	**M + K (g/mol)**
Water	H_2O	18,01530	19,02314	41,00507	57,11360
Ammonium	H_4N	18,03850	19,04634	41,02827	57,13680
Formaldehyde	CH_2O	30,01002	31,01784	52,99979	68,97372
Methylamine	CH_5N	31,04165	32,04948	54,03142	70,00536
Chlorine anion	Cl^-	35,45300	36,46084	58,44277	74,55130
Acetaldehyde	C_2H_4O	44,05260	45,06044	67,04237	83,15090
Dimethylamine	C_2H_7N	45,08370	46,09154	68,07347	84,18200
Formic acid	CH_2O_2	46,02540	47,03324	69,01517	85,12370
Ethanol	C_2H_6O	46,06840	47,07624	69,05817	85,16670
Urea	CH_4N_2O	60,05530	61,06314	83,04507	99,15360
Malondialdehyde	$C_3H_4O_2$	72,02058	73,02841	95,01035	110,98429
Propionic acid	$C_3H_6O_2$	74,03623	75,04406	97,02600	112,99994
Glycine	$C_2H_5NO_2$	75,03148	76,03931	98,02125	113,99519
Glycolic acid	$C_2H_4O_3$	76,01550	77,02332	99,00527	114,97920
Propylene glycol	$C_3H_8O_2$	76,05188	77,05971	99,04165	115,01559
Pyruvic acid (2-Oxopropanoic acid)	$C_3H_4O_3$	88,01550	89,02332	111,00527	126,97920
Alanine	$C_3H_7NO_2$	89,04713	90,05496	112,03690	128,01084
Oxalic acid	C2H2O4	89,99476	91,00259	112,98453	128,95847
Lactic acid	$C_3H_6O_3$	90,03115	91,03897	113,02092	128,99485
Glycerol	$C_3H_8O_3$	92,04680	93,05462	115,03657	131,01050
Phenol	C_6H_6O	94,04132	95,04914	117,03109	133,00502
Sulfate	SO_4^{2-}	95,95118	96,95901	118,94095	134,91489
Maleimide	$C_4H_3NO_2$	97,01583	98,02366	120,00560	135,97954
Sulfuric acid	H_2SO_4	97,96683	98,97466	120,95660	136,93054
Phosphoric acid	H_3PO_4	97,97635	98,98417	120,96612	136,94005
Malonic acid	$C_3H_4O_4$	104,01041	105,01824	127,00018	142,97412
2-Hydroxybutyric acid	$C_4H_8O_3$	104,04680	105,05462	127,03657	143,01050
Choline	$C_5H_{14}NO$	104,10699	105,11482	127,09676	143,07070
Serine	$C_3H_7NO_3$	105,04205	106,04987	128,03181	144,00575
Glyceric acid	$C_3H_6O_4$	106,02606	107,03389	129,01583	144,98977
Creatinine	$C_4H_7N_3O$	113,05836	114,06619	136,04813	152,02207
3-Aminopiperidin-2-one	$C_5H_{10}N_2O$	114,07876	115,08659	137,06853	153,04247
Proline	$C_5H_9NO_2$	115,06278	116,07061	138,05255	154,02649
Maleic acid	$C_4H_4O_4$	116,01041	117,01824	139,00018	154,97412
Hexanoic acid	$C_6H_{12}O_2$	116,08318	117,09101	139,07295	155,04689
Valine	$C_5H_{11}NO_2$	117,07843	118,08626	140,06820	156,04214
Succinic acid	$C_4H_6O_4$	118,02606	119,03389	141,01583	156,98977
Aminomalonic acid	$C_3H_5NO_4$	119,02131	120,02913	142,01108	157,98502
Threonine	$C_4H_9NO_3$	119,05770	120,06552	142,04746	158,02140
Cysteine	$C_3H_7NO_2S$	121,01920	122,02703	144,00897	159,98291
Benzoic Acid	$C_7H_6O_2$	122,03623	123,04406	145,02600	160,99994
6-Methyl-3-hepten-2-one	$C_8H_{14}O$	126,10392	127,11174	149,09369	165,06762
Pyroglutamic acid	$C_5H_7NO_3$	129,04205	130,04987	152,03181	168,00575

Table B.1 (Continued)

Itaconic acid	$C_5H_6O_4$	130,02606	131,03389	153,01583	168,98977
Creatine	$C_4H_9N_3O_2$	131,06893	132,07675	154,05870	170,03264
Leucine	$C_6H_{13}NO_2$	131,09408	132,10191	154,08385	170,05779
Glutaric acid	$C_5H_8O_4$	132,04171	133,04954	155,03148	171,00542
Asparagine	$C_4H_8N_2O_3$	132,05294	133,06077	155,04271	171,01665
Ornithine	$C_5H_{12}N_2O_2$	132,08933	133,09715	155,07910	171,05304
Aspartic acid	$C_4H_7NO_4$	133,03696	134,04478	156,02673	172,00067
Malic Acid	$C_4H_6O_5$	134,02098	135,02880	157,01074	172,98468
Threonic acid	$C_4H_8O_5$	136,03663	137,04445	159,02639	175,00033
4-Hydroxybenzoic acid	$C_7H_6O_3$	138,03115	139,03897	161,02092	176,99485
Urocanic acid	$C_6H_6N_2O_2$	138,04238	139,05020	161,03215	177,00609
Maleic anhydride	$C_7H_8O_3$	140,04680	141,05462	163,03657	179,01050
Alanine anhydride	$C_6H_{10}N_2O_2$	142,07368	143,08150	165,06345	181,03739
Octanoic acid	$C_8H_{16}O_2$	144,11448	145,12231	167,10425	183,07819
Adipic acid	$C_6H_{10}O_4$	146,05736	147,06519	169,04713	185,02107
Glutamine	$C_5H_{10}N_2O_3$	146,06859	147,07642	169,05836	185,03230
Lysine	$C_6H_{14}N_2O_2$	146,10498	147,11280	169,09475	185,06869
Glutamic acid	$C_5H_9NO_4$	147,05261	148,06043	170,04238	186,01632
Methionine	$C_5H_{11}NO_2S$	149,05050	150,05833	172,04027	188,01421
Triethanolamine	$C_6H_{15}NO_3$	149,10465	150,11247	172,09441	188,06835
Tartaric acid	$C_4H_6O_6$	150,01589	151,02371	173,00566	188,97960
Xanthine	$C_5H_4N_4O_2$	152,03288	153,04070	175,02265	190,99658
Methylparaben	$C_8H_8O_3$	152,04680	153,05462	175,03657	191,01050
Histidine	$C_6H_9N_3O_2$	155,06893	156,07675	178,05870	194,03264
Decanal	$C_{10}H_{20}O$	156,15087	157,15869	179,14064	195,11457
Nonanoic acid	$C_9H_{18}O_2$	158,13013	159,13796	181,11990	197,09384
Glycyl-Serine	$C_5H_{10}N_2O_4$	162,06351	163,07133	185,05328	201,02722
Nicotine	$C_{10}H_{14}N_2$	162,11515	163,12298	185,10492	201,07886
1,5-Anhydrosorbitol	$C_6H_{12}O_5$	164,06793	165,07575	187,05769	203,03163
Phenylalanine	$C_9H_{11}NO_2$	165,07843	166,08626	188,06820	204,04214
Uric acid	$C_5H_4N_4O_3$	168,02779	169,03562	191,01756	206,99150
Decanoic acid	$C_{10}H_{20}O_2$	172,14578	173,15361	195,13555	211,10949
Aconitic acid	$C_6H_6O_6$	174,01589	175,02371	197,00566	212,97960
Arginine	$C_6H_{14}N_4O_2$	174,11113	175,11895	197,10090	213,07483
Citrulline	$C_6H_{13}N_3O_3$	175,09514	176,10297	198,08491	214,05885
Cotinine	$C_{10}H_{12}N_2O$	176,09441	177,10224	199,08418	215,05812
Glucose	$C_6H_{12}O_6$	180,06284	181,07067	203,05261	219,02655
Propylparaben	$C_{10}H_{12}O_3$	180,07810	181,08592	203,06787	219,04180
Tyrosine	$C_9H_{11}NO_3$	181,07335	182,08117	204,06311	220,03705
Azelaic acid	$C_9H_{16}O_4$	188,10431	189,11214	211,09408	227,06802
Citric acid	$C_6H_8O_7$	192,02645	193,03428	215,01622	230,99016
Lauric acid	$C_{12}H_{24}O_2$	200,17708	201,18406	223,16685	239,14079
Tryptophan	$C_{11}H_{12}N_2O_2$	204,08933	205,09715	227,07910	243,05304
Pantothenol	$C_9H_{19}NO_4$	205,13086	206,13869	228,12063	244,09457
Diethyl tartrate	$C_8H_{14}O_6$	206,07849	207,08632	229,06826	245,04220
Pilocarpine	$C_{11}H_{16}N_2O_2$	208,12063	209,12845	231,11040	247,08434
Glycyl-Histidine	$C_8H_{12}N_4O_3$	212,09039	213,09822	235,08016	251,05410
Tridecanoic acid	$C_{13}H_{26}O_2$	214,19273	215,20056	237,18250	253,15644
Myristoleic acid	$C_{14}H_{26}O_2$	226,19273	227,20056	249,18250	265,15644
Prolylhydroxyproline	$C_{10}H_{16}N_2O_4$	228,11046	229,11828	251,10023	267,07417
Myristic acid	$C_{14}H_{28}O_2$	228,20838	229,21621	251,19815	267,17209
3-(5,5,6-Trimethylbicyclo[2.2.1]hept-2-yl) cyclohexanol	$C_{16}H_{28}O$	236,21347	237,22129	259,20324	275,17717
3-Methylcyclopentadecanone	$C_{16}H_{30}O$	238,22912	239,23694	261,21889	277,19282
Pentadecanoic acid	$C_{15}H_{30}O_2$	242,22403	243,23186	265,21380	281,18774
Palmitoleic acid	$C_{16}H_{30}O_2$	254,22403	255,23186	277,21380	293,18774

Table B.1 (Continued)

Palmitic acid	$C_{16}H_{32}O_2$	256,23968	257,24751	279,22945	295,20339
Heptadecanoic acid	$C_{17}H_{34}O_2$	270,25533	271,26316	293,24510	309,21904
Dibutyl phthalate	$C_{16}H_{22}O_4$	278,15126	279,15909	301,14103	317,11497
Linoleic acid	$C_{18}H_{32}O_2$	280,23968	281,24751	303,22945	319,20339
Hexaethylene glycol	$C_{12}H_{26}O_7$	282,16731	283,17513	305,15707	321,13101
Oleic acid	$C_{18}H_{34}O_2$	282,25533	283,26316	305,24510	321,21904
Stearic acid	$C_{18}H_{36}O_2$	284,27098	285,27881	307,26075	323,23469
Lauroyl diethanolamide	$C_{16}H_{33}NO_3$	287,24550	288,25332	310,23527	326,20920
Octyl 4-methoxycinnamic acid	$C_{18}H_{26}O_3$	290,18765	291,19547	313,17742	329,15135
(2'E,4'Z,7'Z,8E)-Colnelenic acid	$C_{18}H_{28}O_3$	292,20330	293,21112	315,19307	331,16700
Ricinoleic acid	$C_{18}H_{34}O_3$	298,25025	299,25807	321,24002	337,21395
Nonadecanoic acid	$C_{19}H_{38}O_2$	298,28663	299,29446	321,27640	337,25034
Eicosapentaenoic acid	$C_{20}H_{30}O_2$	302,22403	303,23186	325,21380	341,18774
Methylgingerol	$C_{18}H_{28}O_4$	308,19821	309,20604	331,18798	347,16192
Arachidic acid	$C_{20}H_{40}O_2$	312,30228	313,31011	335,29205	351,26599
Lactapiperanol D	$C_{18}H_{28}O_5$	324,19313	325,20095	347,18290	363,15683
Heneicosanoic acid	$C_{21}H_{42}O_2$	326,31793	327,32576	349,30770	365,28164
2-Palmitoylglycerol	$C_{19}H_{38}O_4$	330,27646	331,28429	353,26623	369,24017
Tetracosane	$C_{24}H_{50}$	338,39070	339,39853	361,38047	377,35441
Polyoxyethylene (600) monoricinoleate	$C_{21}H_{40}O_3$	340,29720	341,30502	363,28697	379,26090
Docosanoic acid	$C_{22}H_{44}O_2$	340,33358	341,34141	363,32335	379,29729
Sucrose	$C_{12}H_{22}O_{11}$	342,11566	343,12349	365,10543	381,07937
Tricosanoic acid	$C_{23}H_{46}O_2$	354,34923	355,35706	377,33900	392,31294
Glyceryl monostearate	$C_{21}H_{42}O_4$	358,30776	359,31559	381,29753	397,27147
Tetracosanoic acid	$C_{24}H_{48}O_2$	368,36488	369,37271	391,35465	407,32859
Riboflavin	$C_{17}H_{20}N_4O_6$	376,13774	377,14556	399,12751	415,10144
Cholesta-4,6-dien-3-one	$C_{27}H_{42}O$	382,32302	383,33084	405,31279	421,28672
Cholestenone	$C_{27}H_{44}O$	384,33867	385,34649	407,32844	423,30237
Cholesterol	$C_{27}H_{46}O$	386,35432	387,36214	409,34409	425,31802
12-Ketodeoxycholic acid	$C_{24}H_{38}O_4$	390,27646	391,28429	413,26623	429,24017
Octacosane	$C_{28}H_{58}$	394,45330	395,46113	417,44307	433,41701
Hexacosanoic acid	$C_{26}H_{52}O_2$	396,39618	397,40401	419,38595	435,35989
7-Ketocholesterol	$C_{27}H_{44}O_2$	400,33358	401,34141	423,32335	439,29279
7β-Hydroxycholesterol	$C_{27}H_{46}O_2$	402,34923	403,35706	425,33900	441,31239
Squalene	$C_{30}H_{50}$	410,39070	411,39853	433,38047	449,35441
4β-Hydroxycholesterol	$C_{27}H_{46}O_3$	418,34415	419,35197	441,33392	457,30785
Squalene epoxide	$C_{30}H_{50}O$	426,38562	427,39344	449,37539	465,34932
Squalene monohydroperoxide	$C_{30}H_{51}O_2$	443,38836	444,39618	466,37813	482,35206
Squalene dihydroperoxide	$C_{30}H_{52}O_4$	476,38601	477,39384	499,37578	515,34972
Squalene trihydroperoxide	$C_{30}H_{53}O_6$	509,38367	510,39149	532,37344	548,34737
Squalene tetrahydroperoxide	$C_{30}H_{54}O_8$	542,38132	543,38915	565,37109	581,34503
Squalene pentahydroperoxide	$C_{30}H_{55}O_{10}$	575,37897	576,38680	598,36874	614,34268
Glycogen	$C_{24}H_{42}O_{21}$	666,22131	667,22914	689,21108	705,18502

Table B.2 displays the number of "Hazard Statements" and "Precautionary Statements" according to GHS US (Globally Harmonized System of Classification and Labeling of Chemicals of the United States) for the solvents used on the solvent systems analysis.

Table B.2: Number of "Hazard Statements" and "Precautionary Statements" according to GHS US for each solvent used.

	Acetonitrile	***Chloroform***	***Dichloromethane***	***Hexane***	***Isopropanol***	***Methanol***	***Water***
Number of "Hazard Statements"	2	7	7	9	3	5	0
Number of "Precautionary Statements"	21	35	25	36	25	26	0

Appendix C

This section contains statistical information regarding the analysed data. The discussion of the most relevant aspects of these data can be found in Chapter 4. Table C.1 to C.24 display the statistical analyses performed on the transients data within the same donor for the two sets of seven-days aged samples and one-month aged-samples, to assess if they were statistically different.

Seven-day Incubated Samples

Set 1's statistical analysis is portrayed below.

Table C.1: Single Factor ANOVA (α = 0,05) performed on compounds labelled as "appearing after incubation", for Set 1 of one-week incubated samples. The analysis was performed on three groups defined as "compound common to three transients", "compound common to two transients" and "compounds different to the three transients".

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0,54546	2	0,27273	31,52302	4,74101E-07	3,46680
Within Groups	0,18169	21	0,00865			
Total	0,72715	23				

Table C.2: Paired t-Test (α = 0,05) performed on compounds labelled as "appearing after incubation", for Set 1 of one-week incubated samples. The analysis was performed on two variables defined as "compound common to three transients" and "compounds different to the three transients".

	Variable 1	*Variable 2*
Mean	0,25695	0,54391
Variance	0,01036	0,00897
Observations	8	8
Pearson Correlation	-0,65919	
Hypothesized Mean Difference	0	
df	7	
t Stat	-4,53402	
P(T<=t) one-tail	0,00134	
t Critical one-tail	1,89458	
P(T<=t) two-tail	0,00269	
t Critical two-tail	2,36462	

Table C.3: Single Factor ANOVA (α = 0,05) performed on compounds labelled as "disappearing after incubation", for Set 1 of one-week incubated samples. The analysis was performed on three groups defined as "compound common to three transients", "compound common to two transients" and "compounds different to the three transients".

Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	0,39063	2	0,19532	15,78404	6,54215E-05	3,46680
Within Groups	0,25986	21	0,01237			
Total	0,65049	23				

Table C.4: Paired t-Test (α = 0,05) performed on compounds labelled as "disappearing after incubation", for Set 1 of one-week incubated samples. The analysis was performed on two variables defined as "compound common to three transients" and "compounds different to the three transients".

	Variable 1	***Variable 2***
Mean	0,22400	0,51230
Variance	0,00525	0,01378
Observations	8	8
Pearson Correlation	-0,05443	
Hypothesized Mean Difference	0	
df	7	
t Stat	-5,77307	
P(T<=t) one-tail	0,00034	
t Critical one-tail	1,89458	
P(T<=t) two-tail	0,00068	
t Critical two-tail	2,36462	

Table C.5: Single Factor ANOVA (α = 0,05) performed on compounds labelled as "increasing after incubation", for Set 1 of one-week incubated samples. The analysis was performed on three groups defined as "compound common to three transients", "compound common to two transients" and "compounds different to the three transients".

Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	0,77833	2	0,38917	3,94955	0,03500	3,46680
Within Groups	2,06922	21	0,09853			
Total	2,84756	23				

Table C.6: Paired t-Test (α = 0,05) performed on compounds labelled as "increasing after incubation", for Set 1 of one-week incubated samples. The analysis was performed on two variables defined as "compound common to three transients" and "compounds different to the three transients".

	Variable 1	***Variable 2***
Mean	0,20833	0,54175
Variance	0,11705	0,14883
Observations	8	8
Pearson Correlation	-0,55630	
Hypothesized Mean Difference	0	
df	7	
t Stat	-1,46791	
P(T<=t) one-tail	0,09279	
t Critical one-tail	1,89458	
P(T<=t) two-tail	0,18557	
t Critical two-tail	2,36462	

Table C.7: Single Factor ANOVA (α = 0,05) performed on compounds labelled as "decreasing after incubation", for Set 1 of one-week incubated samples. The analysis was performed on three groups defined as "compound common to three transients", "compound common to two transients" and "compounds different to the three transients".

Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	0,35583	2	0,17792	0,89868	0,42220	3,46680
Within Groups	4,15750	21	0,19798			
Total	4,51333	23				

Table C.8: Paired t-Test (α = 0,05) performed on compounds labelled as "decreasing after incubation", for Set 1 of one-week incubated samples. The analysis was performed on two variables defined as "compound common to three transients" and "compounds different to the three transients".

	Variable 1	***Variable 2***
Mean	0,40000	0,16250
Variance	0,25143	0,09696
Observations	8	8
Pearson Correlation	-0,32938	
Hypothesized Mean Difference	0	
df	7	
t Stat	1,00000	
P(T<=t) one-tail	0,17531	
t Critical one-tail	1,89458	
P(T<=t) two-tail	0,35062	
t Critical two-tail	2,36462	

Set 2's statistical analysis is depicted below.

Table C.9: Single Factor ANOVA (α = 0,05) performed on compounds labelled as "appearing after incubation", for Set 2 of one-week incubated samples. The analysis was performed on three groups defined as "compound common to three transients", "compound common to two transients" and "compounds different to the three transients".

Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	0,52761	2	0,26380	25,59512	2,34034E-06	3,46680
Within Groups	0,21644	21	0,01031			
Total	0,74405	23				

Table C.10: Paired t-Test (α = 0,05) performed on compounds labelled as "appearing after incubation", for Set 2 of one-week incubated samples. The analysis was performed on two variables defined as "compound common to three transients" and "compounds different to the three transients".

	Variable 1	***Variable 2***
Mean	0,17513	0,53162
Variance	0,00703	0,01029
Observations	8	8
Pearson Correlation	-0,21843	
Hypothesized Mean Difference	0	
df	7	
t Stat	-6,95204	
P(T<=t) one-tail	0,00011	
t Critical one-tail	1,89458	
P(T<=t) two-tail	0,00022	
t Critical two-tail	2,36462	

Table C.11: Single Factor ANOVA (α = 0,05) performed on compounds labelled as "disappearing after incubation", for Set 2 of one-week incubated samples. The analysis was performed on three groups defined as "compound common to three transients", "compound common to two transients" and "compounds different to the three transients".

Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	0,77773	2	0,38887	43,35049	3,50749E-08	3,46680
Within Groups	0,18838	21	0,00897			
Total	0,96611	23				

Table C.12: Paired t-Test (α = 0,05) performed on compounds labelled as "disappearing after incubation", for Set 1 of one-week incubated samples. The analysis was performed on two variables defined as "compound common to three transients" and "compounds different to the three transients".

	Variable 1	***Variable 2***
Mean	0,21575	0,58767
Variance	0,00354	0,01227
Observations	8	8
Pearson Correlation	-0,35724	
Hypothesized Mean Difference	0	
df	7	
t Stat	-7,34397	
P(T<=t) one-tail	0,00008	
t Critical one-tail	1,89458	
P(T<=t) two-tail	0,00016	
t Critical two-tail	2,36462	

Table C.13: Single Factor ANOVA (α = 0,05) performed on compounds labelled as "increasing after incubation", for Set 2 of one-week incubated samples. The analysis was performed on three groups defined as "compound common to three transients", "compound common to two transients" and "compounds different to the three transients".

Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	0,43750	2	0,21875	1,28947	0,29634	3,46680
Within Groups	3,56250	21	0,16964			
Total	4	23				

Table C.14: Paired t-Test (α = 0,05) performed on compounds labelled as "increasing after incubation", for Set 2 of one-week incubated samples. The analysis was performed on two variables defined as "compound common to three transients" and "compounds different to the three transients".

	Variable 1	***Variable 2***
Mean	0,31250	0,37500
Variance	0,20982	0,26786
Observations	8	8
Pearson Correlation	-0,56493	
Hypothesized Mean Difference	0	
df	7	
t Stat	-0,20473	
P(T<=t) one-tail	0,42180	
t Critical one-tail	1,89458	
P(T<=t) two-tail	0,84361	
t Critical two-tail	2,36462	

Table C.15: Single Factor ANOVA (α = 0,05) performed on compounds labelled as "decreasing after incubation", for Set 2 of one-week incubated samples. The analysis was performed on three groups defined as "compound common to three transients", "compound common to two transients" and "compounds different to the three transients".

Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	0,54215	2	0,27108	3,42570	0,05157	3,46680
Within Groups	1,66174	21	0,07913			
Total	2,20389	23				

Table C.16: Paired t-Test (α = 0,05) performed on compounds labelled as "decreasing after incubation", for Set 2 of one-week incubated samples. The analysis was performed on two variables defined as "compound common to three transients" and "compounds different to the three transients".

	Variable 1	***Variable 2***
Mean	0,54583	0,22292
Variance	0,10371	0,04515
Observations	8	8
Pearson Correlation	-0,44086	
Hypothesized Mean Difference	0	
df	7	
t Stat	1,99689	
P(T<=t) one-tail	0,04301	
t Critical one-tail	1,89458	
P(T<=t) two-tail	0,08601	
t Critical two-tail	2,36462	

One-month Incubated Samples

Table C.17: Single Factor ANOVA (α = 0,05) performed on compounds labelled as "appearing after incubation", of one-month incubated samples. The analysis was performed on three groups defined as "compound common to three transients", "compound common to two transients" and "compounds different to the three transients".

Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	0,06416	2	0,03208	5,42867	0,01135	3,40283
Within Groups	0,14182	24	0,00591			
Total	0,20597	26				

Table C.18: Paired t-Test (α = 0,05) performed on compounds labelled as "appearing after incubation", of one-month incubated samples. The analysis was performed on two variables defined as "compound common to three transients" and "compounds different to the three transients".

	Variable 1	***Variable 2***
Mean	0,40215	0,30248
Variance	0,00811	0,00488
Observations	9	9
Pearson Correlation	-0,65606	
Hypothesized Mean Difference	0	
df	8	
t Stat	2,05121	
P(T<=t) one-tail	0,03718	
t Critical one-tail	1,85955	
P(T<=t) two-tail	0,07437	
t Critical two-tail	2,30600	

Table C.19: Single Factor ANOVA (α = 0,05) performed on compounds labelled as "disappearing after incubation", of one-month incubated samples. The analysis was performed on three groups defined as "compound common to three transients", "compound common to two transients" and "compounds different to the three transients".

Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	1,25666	2	0,62833	39,28039	2,69624E-08	3,40283
Within Groups	0,38390	24	0,01600			
Total	1,64056	26				

Table C.20: Paired t-Test (α = 0,05) performed on compounds labelled as "disappearing after incubation", of one-month incubated samples. The analysis was performed on two variables defined as "compound common to three transients" and "compounds different to the three transients".

	Variable 1	***Variable 2***
Mean	0,11439	0,62682
Variance	0,01079	0,02593
Observations	9	9
Pearson Correlation	-0,76096	
Hypothesized Mean Difference	0	
df	8	
t Stat	-6,16455	
P(T<=t) one-tail	0,00013	
t Critical one-tail	1,85955	
P(T<=t) two-tail	0,00027	
t Critical two-tail	2,30600	

Table C.21: Single Factor ANOVA (α = 0,05) performed on compounds labelled as "increasing after incubation", of one-month incubated samples. The analysis was performed on three groups defined as "compound common to three transients", "compound common to two transients" and "compounds different to the three transients".

Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	0,24960	2	0,12480	2,65935	0,09053	3,40283
Within Groups	1,12628	24	0,04693			
Total	1,37587	26				

Table C.22: Paired t-Test (α = 0,05) performed on compounds labelled as "increasing after incubation", of one-month incubated samples. The analysis was performed on two variables defined as "compound common to three transients" and "compounds different to the three transients".

	Variable 1	***Variable 2***
Mean	0,29237	0,46610
Variance	0,02877	0,07763
Observations	9	9
Pearson Correlation	-0,76184	
Hypothesized Mean Difference	0	
df	8	
t Stat	-1,23400	
P(T<=t) one-tail	0,12611	
t Critical one-tail	1,85955	
P(T<=t) two-tail	0,25222	
t Critical two-tail	2,30600	

Table C.23: Single Factor ANOVA (α = 0,05) performed on compounds labelled as "decreasing after incubation", of one-month incubated samples. The analysis was performed on three groups defined as "compound common to three transients", "compound common to two transients" and "compounds different to the three transients".

Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	1	2	0,50000	2,50000	0,11560	3,68232
Within Groups	3	15	0,20000			
Total	4	17				

Table C.24: Paired t-Test ($\alpha = 0,05$) performed on compounds labelled as "decreasing after incubation", of one-month incubated samples. The analysis was performed on two variables defined as "compound common to three transients" and "compounds different to the three transients".

	Variable 1	***Variable 2***
Mean	0,16667	0,66667
Variance	0,16667	0,26667
Observations	6	6
Pearson Correlation	-0,63246	
Hypothesized Mean Difference	0	
df	5	
t Stat	-1,46385	
P(T<=t) one-tail	0,10156	
t Critical one-tail	2,01505	
P(T<=t) two-tail	0,20311	
t Critical two-tail	2,57058	