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Proefschrift

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Anybody who has been seriously engaged in scientific work of any kind realizes that over the entrance to the gates of the temple of science are written the words: 'Ye must have faith.'

Max Planck

Contents

Summary

Fingerprints are a commonly exploited type of evidence and can be crucial in a criminal investigation. The process of individualization or exclusion of a donor relies on the comparison of ridge detail characteristics between a fingermark, found at a crime scene, and reference fingerprints, collected under controlled conditions (either or not stored in a database). Although this process has been successfully used for over a century, fingermarks found at a crime scene are of limited value for a criminal trial if the corresponding reference fingerprint is not available, or the found fingermark is of poor quality. Fingerprints consist of donor secretion, mainly eccrine and sebaceous, of which the exact composition is likely influenced by many (both endogenous and exogenous) factors, including donor traits, habits and activities. Analysis of the chemical composition could thus potentially lead to the retrieval of donor information from those fingerprints that yielded no information in the traditional comparison process. The main aim of this dissertation was to determine what donor information can reliably and validly be derived from the chemical analysis of the fingerprint composition, in order to be used in forensic investigations.

For this purpose, mass spectrometry based methods were developed, facilitating the compositional analysis of fingerprints. To analyze the relation between certain donor traits and the compounds they leave behind in a fingerprint, the chemical variability (both between and within donors) was studied. Additionally, the derivation of information from the presence of exogenous compounds in fingerprints was investigated. Another key forensic question is related to fingerprint dating, for which the applicability of amino acid enantiomers was analyzed. Lastly, a method was developed which potentially renders the chemical analysis of fingerprints compatible with the traditional fingerprint comparison process. The research described in this dissertation has shown that both donor information as well as information about the trace or crime can potentially be deduced from the chemical analyses of fingerprints. Additionally, it was shown that chemical analyses can be performed without destructing the fingerprint ridge detail in such a way that traditional fingerprint comparison is no longer possible. This is described in Chapters 2 to 6 of this dissertation.

Chapter 2 describes the development of a novel ultra-high-performance liquid chromatography mass spectrometry (UPLC-MS) method for the analysis of polar compounds in fingerprint residue. Sweat, an important component of fingerprint residue, consists of water and many water soluble, polar, compounds. Exogenous compounds can be encountered in fingerprints as well, as a result of contact. To investigate the intra- and inter-variability of these compounds, in particular amino acids, in fingerprints, a large amount of samples needs to be analyzed. Existing methods are often based on a relatively long analysis time, as well as a labor-intensive sample preparation. Therefore, a method was developed that facilitates a relatively easy and fast sample preparation and analysis of polar compounds, such as amino acids, from fin-

gerprints. This method is based on UPLC-MS and circumvents the need to derivatize amino acids by using a polar, amide-based, stationary phase, thereby simplifying and shortening the sample preparation. To investigate if the developed method is sufficiently sensitive for quantitative analyses, data of the analysis from fingerprints from 19 donors were compared, using either a time-of-flight mass spectrometer (TOF-MS) or triple quadrupole tandem MS (QqQ-MS/MS) as detector. Although the triple quadrupole had a higher sensitivity for most amino acids, both mass spectrometers were able to retrieve the amino acid profiles of fingerprints from the 19 donors. Between these profiles, only minor differences were observed between the separate analyses on the different mass analyzers.

Chapter 3 investigates the variability in fingerprint composition both between and within donors. To investigate the inter-variability, fingerprints from 463 donors were analyzed, and it was examined if the composition of these fingerprints differed systematically depending on donor traits. For this purpose, the developed method UPLC method in combination with a TOF-MS, as well a previously developed gas chromatography mass spectrometry (GC-MS) method to quantify common lipid compounds from fingerprints, were used. A data set of 1852 fingerprints donated by 463 donors, collected at the Dutch music festival Lowlands was analyzed. Four fingerprints per donor were collected; 2 natural, 1 eccrine (sweat-rich) and 1 sebaceous (lipid-rich). The natural fingerprints were collected without any preparation, except the rubbing together of both hands to homogenize the fingerprint residue. The eccrine fingerprint, containing mainly sweat, was collected after wearing nitrile gloves for some time after hand-washing. This results in a fingerprint mainly containing eccrine components. Finally, before the collection of the sebaceous fingerprint, after hand-washing and glove wearing as well, the donors rubbed their hands over their forehead, a sebum-rich area. For each donor, 1 of the natural fingerprints and the eccrine fingerprint were analyzed using UPLC-MS. The other natural fingerprint and sebaceous fingerprint were subjected to GC-MS analysis. The data was analyzed in two separate approaches. In a targeted approach, the quantified compounds (amino acids, fatty acid, cholesterol and squalene) were analyzed. In an untargeted approach all full-scan mass spectra were analyzed, to find potentially new relevant compounds in fingerprints that could provide donor information. In the targeted approach, a large inter-variability in the quantified fingerprint components was found. Total amino acid ranged from below 100 ng to over 10 µg and was generally higher in natural fingerprints compared to eccrine fingerprints, which is likely to be a result of the experimental design. Total quantified lipid compounds ranged between 100 ng and 100 µg and was found to be higher in sebaceous fingerprints compared to natural fingerprints. Statistical analysis of the targeted metabolites revealed 5 potential markers (quantifiable indicators of certain donor traits). L-phenylalanine, L-(iso)leucine and palmitoleic acid were found to be higher in fingerprints from male donors compared to fingerprints from female donors. Moreover, L-alanine was found to be increased in fingerprints from donors that indicated to have used cannabis in the last 24-hours than people who did not. Similarly, L-proline was found to be higher in fingerprints from donors that consumed large amounts of alcoholic consumption in the past 24 hours, compared to people who consumed less, or no alcohol. Subsequently, data were

analyzed in an untargeted approach, to discover potential markers for donor factors in the full-scan data. As a proof of principle, a classification model was developed to distinguish between fingerprint type (i.e. natural and eccrine for LC-MS data, natural and sebaceous for GC-MS) to investigate the performance of the untargeted approach. In case of the LC-MS data, the model predicted the fingerprint type with 95.3% accuracy, based on the test set. In this model, compounds putatively annotated as urea and multiple amino acid degradation products were important predictors. When using the GC-MS data to classify fingerprint type, an accuracy of 86.8% was obtained. Here, mainly fragments putatively annotated to originate from squalene were used. Then, classification models were developed for the two forensic relevant donor traits: gender and smoking habit. Predicting if the fingerprint donor was either male or female was most successful using the LC-MS data of natural fingerprints, with an accuracy of 77.9%. In this model, compounds putatively annotated as products from the guanosine monophosphate (GMP) degradation pathway were important predictors. Donor smoking habit could be predicted with high accuracy using LC-MS data from natural as well as eccrine fingerprints (90.4% and 90.2%, respectively), based on the putatively annotated predictors nicotine and cotinine.

Additionally, this chapter investigates the intra-variability in fingerprint composition. Determining the variability within a donor, within a certain time frame, is an important factor in the possible application of the method. In total, 56 natural fingerprints from 4 donors were collected during 5 days. The total amino acid content was determined using UPLC-MS. The variability within donors ranged from 39.1% to 66.9% (RSD) during the 5 days, based on total amino acid content (15.0-100% relative range), without controlling deposition pressure, time and surface area. Based on a similar untargeted approach as was used studying the inter-variability, using the full-scan MS-data, a classification model was developed to distinguish between the 4 fingerprint donors, using the fingerprints collected during the 5 days. Based on the small data set, the generated model was able to distinguish the fingerprints from different donors. This indicated that although intra-variability is large, there seem to be certain distinguishing components present that facilitate the discrimination between donors based on the fingerprint composition. These are possibly exogenous compounds, a result of the daily routine of the fingerprint donor. With increasing data set however, the classification accuracy is likely to decrease.

Chapter 4 addresses the age determination of fingerprints. The chemical compounds present in fingerprint residue, which can potentially be applied to retrieve donor information, are likely to change over time (after deposition), and thus could potentially be used to estimate the time of deposition. With the potential to place a fingerprint found at a crime scene in the time frame around a crime, methods to estimate fingerprint age have been getting increasing attention. Amino acids are a common component in fingerprints, and due to a chiral carbon atom, every amino acid (except glycine) occurs in two enantiomers. In nature, the L-enantiomer of amino acids is usually synthesized. It was investigated if amino acid racemization, the process towards a 50-50 equilibrium of L- and D-enantiomer, could be a viable option for fingerprint age estimation. Because this method is based on the relative ratio of enantiomers, it could offer a decreased sensitivity to the unknown starting amount and pos-

sible degradation that has taken place, when compared to existing methods. A method was developed to separate and relatively quantify amino acid enantiomers from fingerprints using 1-(9-fluorenyl) ethyl chloroformate (FLEC) and ultra-high-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS). After analysis of fingerprints up to 6 months old, a relative increase in D-serine was found. This proves as a promising marker for the further development of fingerprint age estimation methods. In general, D-serine showed a promising trend for fingerprints up to 30 days old. Some key parameters, such as temperature, humidity, light exposure and surface were controlled in this study. Despite the potential advantages that D-serine offers as age marker, the influence of these parameters, which likely influence the racemization rate, remains unknown. In addition to confirming the potentially useful trend observed for D-serine, further research is needed to investigate the influence of these parameters and study the potential racemization of the other amino acids present in fingerprints.

Chapter 5 investigated the extent to which specific exogenous factors - such as condom lubricants - can be derived from fingerprints. This is forensically relevant as these can be found on crime scenes of sexual assault. A desorption electrospray ionization mass spectrometry imaging (DESI-MSI) method was developed, compatible with cyanoacrylate fuming, to analyze common lubricant components from fingerprints, while allowing the possibility to generate chemical images simultaneously. A database of 32 different condoms from 21 different brands was built. Using a PCA-LDA (principal component analysis-linear discriminant analysis) model, condom lubricant spectra were classified with 99.0% accuracy based on the analysis of lubricant spots, and 90.9% accuracy when analyzing fingerprints containing condom lubricant. Additionally, full chemical images of the fingerprint can be obtained, showed the distribution of common lubricant components, such as PEG and PDMS, throughout the fingerprint.

Chapter 6 describes the development of a method to sample fingerprints in a non-marking manner (i.e. without physically marking the fingerprint ridge detail). Chemical analyses of fingerprint composition are usually not compatible with the current fingerprint process. The potential of hydrogels to retrieve analytes of interest from fingerprints in a non-marking manner was investigated. Fingerprints could still be successfully visualized using cyanoacrylate fuming and subsequent basic yellow dyeing after being treated with the hydrogel solution, with only light smearing of ridge detail observable. Using an amino acid stock solution as well as fingerprints from 5 donors, the extraction efficiency of the hydrogel was compared to completely dissolving the trace or fingerprint. Both methods resulted in high extraction efficiencies, although the hydrogel underperformed in case of L-lysine, L-histidine, L-tryptophan and L-cystine. Additionally, the extraction efficiency of DNA from fingerprints using the hydrogel method was compared to that of using conventional cotton swabs. This resulted in a 20–60% extraction efficiency compared to conventional swabbing.

This dissertation shows there is potential for the extraction of donor information from fingerprints by chemical analysis. The research shows there is the possibility to retrieve both donor information, such as the classification of smokers, as well as information about the trace (time of deposition) or crime (through exogenous com-

pounds) from fingerprints. Additionally it was shown, using hydrogels, that both amino acids and DNA can be retrieved and analyzed from fingerprints without marking the ridge detail in such a way that traditional comparison is no longer possible. Common metabolites such as amino acids and fatty acids show large variability both between and within donors, thereby complicating the extraction of donor information from these compounds. The relative quantification of enantiomers of serine however, poses as a potential marker for fingerprint age estimation studies. Further research should reveal if this marker is suitable for forensic applications. Additionally, the detection of stimulants and their metabolites in fingerprints proves promising in retrieving donor information, such as the detection of nicotine and its metabolite cotinine in the fingerprints of smokers. Moreover, the detection of exogenous compounds in fingerprints as a result of contact with certain products or items has the potential to contribute valuable information to the investigation, as was shown by the detection and discrimination of lubricants from fingerprints that had been in contact with a condom. More research is needed to bring chemical profiling of fingerprints another step closer to practice. The markers put forward in this research were not subjected to ageing, prior to analysis. Potential ageing that has taken place before analysis, could possibly interfere with accurate classification, by degradation of found markers. Additionally, for the analysis of every (class of) analyte(s) of interest, methods are needed that render the chemical analysis compatible with the current fingerprint detection processes. The chemical profiling approach based on the full-scan MS data described in this dissertation could find an application in other fields, both within and outside forensic science. Chemical profiling in forensic science is potentially interesting in multiple areas of expertise, which use similar mass spectrometry-based methods, such as, among others, explosives, toxicology and illicit drugs investigations. In general, the approach may be applied in any field using full-scan MS-data to discover new markers, potentially answering more fundamental (bio)chemical research questions.

Overall, the research presented in this dissertation shows that the compositional analysis of fingerprints is a promising tool and could be a valuable contribution to the continuously developing field of forensic science.

Samenvatting

Vingerafdrukken worden veel gebruikt binnen de forensische wetenschap en kunnen doorslaggevend zijn in een misdaadonderzoek. De mogelijkheid om een donor te individualiseren of uit te sluiten is gebaseerd op de vergelijking van details in de papillairlijnen tussen een vingerspoor, gevonden op een plaats delict, en een referentie vingerafdruk, afgenomen onder gecontroleerde omstandigheden (al dan niet opgeslagen in een database). Hoewel dit proces al meer dan een eeuw succesvol wordt gebruikt, zijn vingersporen van een plaats delict niet bruikbaar in een strafproces wanneer de bijbehorende referentie niet beschikbaar is, of wanneer het spoor van slechte kwailiteit is. Vingerafdrukken bestaan uit afscheiding van de donor, voornamelijk zweet en vettige uitscheiding, waarvan de samenstelling wordt beïnvloed door vele endogene en exogene factoren, waaronder mogelijk ook donoreigenschappen, gewoontes en activiteiten. Analyse van de chemische samenstelling van een vingerafdruk zou dus potentieel informatie kunnen opleveren over kenmerken van vingerafdrukken die niet geschikt zijn voor het standaard vergelijkingsproces. Het doel van het onderzoek dat in dit proefschrift wordt beschreven is te bepalen welke donorinformatie betrouwbaar en valide afgeleid kan worden uit de chemische samenstelling van vingerafdrukken, zodat deze informatie in het forensisch onderzoek kan worden gebruikt.

Om dit te kunnen onderzoeken werden op massaspectrometrie gebaseerde methoden opgezet waarmee de chemische samenstelling kan worden geanalyseerd. Daarna werd, om de samenhang te onderzoeken tussen bepaalde donorkenmerken en de stoffen die zij achterlaten in een vingerspoor, de chemische variabiliteit zowel binnen als tussen personen bestudeerd. Ook het afleiden van informatie uit de aanwezigheid van exogene stoffen in vingerafdrukken werd onderzocht. Een ander forensisch vraagstuk is het dateren van vingerafdrukken, waar in dit onderzoek de bruikbaarheid van aminozuur enantiomeren voor werd geanalyseerd. Als laatst werd een methode ontwikkeld waarmee de chemische analyse van vingerafdrukken mogelijk verenigbaar wordt met traditioneel dactyloscopisch onderzoek. Het onderzoek heeft laten zien dat er in potentie zowel donorinformatie als informatie over het spoor of misdrijf kan worden afgeleid uit de chemische samenstelling van vingersporen. Tevens is laten zien dat het mogelijk is chemische analyses te verrichten zonder daarmee het papillairlijnenpartroon van de vingerafdruk zodanig aan te tasten dat vergelijkend onderzoek niet meer mogelijk is. In de verschillende hoofdstukken van dit proefschrift wordt dit achtereenvolgens beschreven.

Hoofdstuk 2 beschrijft de ontwikkeling van een nieuwe ultra-high-performance vloeistof chromatografie massa spectrometrie (UPLC-MS) methode voor de analyse van polaire verbindingen uit vingerspoorresidue. Zweet, een belangrijke component in vingerspoorresidu, bevat naast water veel wateroplosbare, polaire verbindingen. Ook exogene polaire stoffen kunnen, door bijvoorbeeld aanraking, in het vingerspoor-

residu terechtkomen. Om de intra- en inter-variabiliteit van deze polaire verbindingen, in het bijzonder aminozuren, in vingerafdrukken te bepalen, is de analyse van grote aantallen monsters nodig. Bestaande methoden hebben vaak een relatief lange analysetijd en arbeidsintensieve sample voorbewerking. Hiertoe werd een methode ontwikkeld die een snelle en gemakkelijke opwerking en analyse van polaire verbindingen uit vingerafdrukken, zoals aminozuren, faciliteert. Deze method is gebaseerd op UPLC-MS en omzeilt de noodzaak om te derivatiseren door gebruik te maken van een polaire (amide) stationaire fase, waardoor de monstervoorbewerking vereenvoudigd en verkort wordt. Om te onderzoeken of de ontwikkelde methode geschikt en voldoende sensitief is voor kwantitative analyse, werden de resultaten van vingerafdrukken van 19 donoren vergeleken tussen het gebruik van een time-of-flight massa spectrometer (TOF-MS) en een triple quadrupole tandem MS (QqQ-MS/MS) als detector. Hoewel de triple quadrupole een hogere gevoeligheid bleek te hebben voor de meeste aminozuren, waren beide massa spectrometers in staat de aminozuur profielen van de 19 donoren te bepalen. Binnen deze profielen waren slechts kleine verschillen waarneembaar tussen de verschillende analyses met de verschillende massa spectrometers.

Hoofdstuk 3 behandelt de variabiliteit in vingerspoor samenstelling binnen en tussen personen. Om interpersoonlijke variabiliteit te kunnen onderzoeken werden de vingerafdrukken van 463 donoren geanalyseerd en werd onderzocht of de chemische samenstelling van deze afdrukken systematisch verschilden afhankelijk van de kenmerken van de donor. Hiertoe werd de ontwikkelde UPLC methode in combinatie met een TOF-MS gebruikt. Daarnaast werd een eerder ontwikkelde gas chromatografie massa spectrometrie (GC-MS) methode om veelvoorkomende vettige stoffen uit vingerafdrukken te kwantificeren gebruikt. Een dataset van 1852 vingerafdrukken, gedoneerd door 463 personen op het muziekfestival Lowlands, werd geanalyseerd. Per donor werden 4 vingerafdrukken verzameld; 2 natuurlijk, 1 eccrien (zweet) en 1 vettig. De natuurlijke afdrukken werden afgenomen zonder enige voorbewerking, behalve het tegen elkaar wrijven van de handen om te homogeniseren. De eccrine afdruk, een afdruk met veel zweet-afscheiding, werd afgenomen na het dragen van nitril handschoenen na het handenwassen. Op deze manier bevat de afdruk voornamelijk componenten uit zweet-afscheiding; het zweten werd gestimuleerd door het dragen van handschoenen. Voor het doneren van de vettige afdruk werden ook de handen gewassen en handschoenen gedragen, maar werd voor het zetten met de handen over het voorhoofd, een plek waar veel talgklieren zitten, gewreven. Per donor werd zowel één van de natuurlijke vingerafdrukken, als de eccriene afdruk geanalyseerd met UPLC-MS. De overgebleven natuurlijke vingerafdruk en de vettige afdruk werden analyseerd met GC-MS. De verkregen data werd op twee manieren geanalyseerd. In een gerichte aanpak, werd gekeken naar bekende componenten in vingerafdrukken: aminozuren en vetzuren, cholesterol en squaleen. Daarna werden in een ongerichte aanpak alle vastgelegde massa-spectra geanalyseerd, om zo mogelijk nieuwe relevante verbindingen in vingerafdrukken te vinden. Bij de gerichte aanpak werd een grote inter-variabiliteit gevonden in de gekwantificeerde vingerafdruk componenten. De totale hoeveelheid aminozuur in een vingerafdruk varieerde van minder dan 100 ng tot meer dan 10 µg en was over het algemeen hoger in natuurlijke vingerafdrukken

dan in eccriene vingerafdrukken. Dit was vermoedelijk een gevolg van de experimentele opzet. De totale hoeveelheid gekwantificeerde vetten varieerde tussen 100 ng en 100 µg en was hoger in vettige vingerafdrukken dan in natuurlijke afdrukken. Statistische analyse van de gekwantificeerde verbindingen leverde 5 potentiële markers op (meetbare indicatoren van bepaalde donoreigenschappen). L-fenylalanine, L-(iso)leucine en palmitoleïnezuur werden in hogere concentraties teruggevonden bij mannelijke donoren dan bij vrouwelijke donoren. Daarnaast werd L-alanine in hogere concentratie gemeten in vingerafdrukken van donoren die aangaven cannabis te hebben gebruikt in de voorafgaande 24 uur vergeleken met donoren die dat niet hadden gedaan. Ook werd L-proline in hogere concentraties gevonden in vingerafdrukken van donoren die grote hoeveelheden alcohol hadden geconsumeerd in de voorafgaande 24 uur vergeleken met mensen die minder, of geen alcohol hadden geconsumeerd. Vervolgens werd de data ook in de ongerichte aanpak geanalyseerd, om mogelijke markers te vinden in de full-scan data. Eerst werd een classificeringsmodel gemaakt om onderscheid te maken tussen de verschillende typen vingerafdrukken (natuurlijk versus eccrien voor de LC-MS data, natuurlijk versus vettig voor de GC-MS), om de werking van de ongerichte aanpak te onderzoeken. Voor de LC-MS data kon het model dit onderscheid maken met 95.3% nauwkeurigheid, gebaseerd op een testset. In dit model speelden stoffen vermoedelijke geïdentifieerd als ureum en diverse afbraakproducten van aminozuren een belangrijke rol als predictor. In het geval van de GC-MS data werd een nauwkeurigheid van 86.8% behaald voor de verschillende vingerafdruk typen. Hiervoor waren vooral fragmenten vermoedelijk afkomstig van squaleen als predictors verantwoordelijk. Vervolgens werden classificeringsmodellen ontwikkeld voor twee forensisch relevante donoreigenschappen: geslacht en rookgewoonte. Het voorspellen van het geslacht van de donor was het meest succesvol op basis van de LC-MS data van natuurlijke vingerafdrukken, met een nauwkeurigheid van 77.9%. In dit model waren verbindingen vermoedelijke gerelateerd aan GMP afbraak belangrijke predictors. Rookgewoonte kon worden voorspeld met hoge nauwkeurigheid op basis van LC-MS data van zowel natuurlijke als eccriene vingerafdrukken (90.4% en 90.2%, respectievelijk), op basis van de vermoedelijke predictors nicotine en cotinine.

Daarnaast wordt in dit hoofdstuk de intrapersoonlijke variabiliteit in de samenstelling van vingerafdrukken onderzocht. Het is van belang om te bepalen hoe variabel de samenstelling binnen een bepaalde tijdsspanne in een donor is voor de mogelijke toepassing van de methode. Van 4 donoren werden 56 natuurlijke vingerafdrukken afgenomen, verdeeld over 5 dagen. De totale hoeveelheid aminozuur in deze afdrukken werd bepaald met UPLC-MS. De variatie binnen donoren liep van 39.1% tot 66.9% (RSD) gedurende de 5 dagen, gebaseerd op de totale hoeveelheid aminozuur (relatief bereik van 15.0-100%), zonder het controleren van het oppervlak, de tijd en druk van de zetting. Gebaseerd op dezelfde ongerichte aanpak als gebruikt bij het bestuderen van de inter-variabiliteit, gebruikmakend van de full-scan MS-data, werd wederom een classificeringsmodel gemaakt, ditmaal om de vingerafdrukken van de 4 donoren, gezet gedurende de 5 dagen, te onderscheiden. Gebaseerd op deze kleine dataset, kon het gegenereerde model dit onderscheid nauwkeurig maken. Dit wijst erop dat, hoewel de intra-variabiliteit groot is, unieke verbindingen aanwezig lijken te

zijn in vingerafdrukken van verschillende donoren, waardoor deze te onderscheiden zijn. Mogelijk zijn dit exogene componenten die een gevolg zijn van de dagelijkse routine van de donor. In geval van grotere datasets zal het onderscheidend vermogen waarschijnlijk afnemen.

Hoofdstuk 4 behandelt het bepalen van de leeftijd van een vingerafdruk. De chemische componenten in vingerspoorresidu, die mogelijk gebruikt kunnen worden om donorinformatie af te leiden, veranderen over tijd (na zetting van de afdruk), en kunnen dus mogelijk toegepast worden om een schatting te geven van het moment van zetten. Dit biedt de mogelijkheid om vingerafdrukken die zijn aangetroffen op een plaats delict te koppelen aan de tijdsspanne rond de misdaad. De ontwikkeling van zulke methoden heeft om die reden in recente jaren meer aandacht gekregen. Aminozuren zijn een veelvoorkomende verbinding in vingerafdrukken en dankzij een chiraal koolstof atoom komt elk aminozuur (behalve glycine) in 2 enantiomeren voor. In de natuur wordt meestal de L-enantiomeer gesynthetiseerd. Er werd onderzocht of aminozuur racemizatie, het proces richting een 50-50 evenwicht van L- en D-enantiomeer, een mogelijkheid is om vingerafdrukken te dateren. Een voordeel van deze methode ten opzichte van bestaande methodes is dat deze mogelijke minder gevoelig is voor een onbekende uitgangshoeveelheid en mogelijk afbraak die al heeft plaatsgevonden. Er werd een methode ontwikkeld om aminozuur enantiomeren uit vingerafdrukken te scheiden en relatief te kwantificeren, gebruikmakend van 1-(9-fluorenyl) ethyl chloroformate (FLEC) en ultra-high-performance vloeistof chromatografie tandem massa spectrometrie (UPLC-MS/MS). Na analyse van vingerafdrukken tot 6 maanden oud, werd een relatieve toename waargenomen voor D-serine. Deze stof dient zich aan als mogelijke marker voor de ontwikkeling van een methode om vingerafdrukken te dateren. Over het algemeen vertoont D-serine een veelbelovende trend voor vingerafdrukken tot 30 dagen oud. Echter werden in dit onderzoek enkele relevante parameters zoals temperatuur, luchtvochtigheid, licht en ondergrond gecontroleerd. Ondanks de mogelijke voordelen van D-serine als leeftijdsmarker, blijft de invloed van deze parameters, die mogelijk ook de racemisatiesnelheid beïvloeden, dus nog onduidelijk. Naast het bevestigen van de mogelijk bruikbare trend van D-serine, is verder onderzoek nodig om meer over de invloed van deze parameters evenals de racemisatie van overige aminozuren te weten te komen.

Hoofdstuk 5 onderzocht in hoeverre specifieke exogene factoren - zoals glijmiddelen uit een condoom - uit vingersporen kunnen worden afgeleid. Forensisch gezien is dit relevant omdat deze kunnen worden aangetroffen op een plaats delict in het geval van zedenzaken. Er werd een desorption electrospray ionization mass spectrometry imaging (DESI-MSI) methode ontwikkeld, die verenigbaar is met cyanoacrylaat opdamping, om glijmiddel componenten uit vingerafdrukken te kunnen analyseren, terwijl tegelijkertijd een chemische afbeelding wordt verkregen. Een database van 32 verschillende condooms van 21 verschillende merken werd verzameld en geanalyseerd met deze methode. Met het gebruik van een PCA-LDA (principal component analysis-linear discriminant analysis) model konden glijmiddel spectra met een nauwkeurigheid van 99.0% worden geclassificeerd op basis van glijmiddel druppels, en 90.9% nauwkeurigheid op basis van vingerafdrukken die glijmiddel bevatten. Daarnaast konden volledige chemische afbeeldingen van vingerafdrukken worden ge-

genereerd die de verdeling van glijmiddel componenten zoals PEG en PDMS in de vingerafdruk laten zien.

Hoofdstuk 6 beschrijft de ontwikkeling van een methode om vingerafdrukken te bemonsteren op een niet-markerende manier. De chemische analyse van vingerafdrukken is vaak niet verenigbaar met het huidige vingerafdrukken vergelijkingsproces. Er werd onderzocht of met hydrogels mogelijk analieten uit vingerafdrukken kunnen worden geëxtraheerd zonder het spoor optisch te beïnvloeden (niet-markerend). Vingerafdrukken konden worden gevisualiseerd na behandeling met de hydrogel oplossing door middel van cyanoacrylaat opdamping en basic yellow, met slechts een kleine afname van papillairlijnen details. Op basis van experimenten met zowel een aminozuuroplossing als vingerafdrukken van 5 donoren werd de extractie-efficiëntie van de hydrogel vergeleken met het compleet oplossen van een vingerafdruk/spoor. Met beide methoden werd een hoge efficiëntie behaald, hoewel de hydrogel het slechter deed in het geval van L-lysine, L-histidine, L-tryptofaan en L-cystine. Daarnaast werd de extractie-efficiëntie van DNA uit vingerafdrukken met de hydrogel vergeleken met die van veelgebruikte katoenen swabs. Dit resulteerde in een 20-60% extractie-efficiëntie ten opzichte van de swabs.

Dit proefschrift laat zien dat er in potentie forensisch relevante informatie kan worden afgeleid uit de chemische samenstelling van vingersporen. Het onderzoek toont aan dat er mogelijkheden liggen om zowel donorinformatie, zoals het classificeren van donoren als rokers, als informatie over het spoor (moment van zetting) of misdrijf (door exogene stoffen) uit vingerafdrukken te halen. Tevens is, gebruik makend van hydrogels, laten zien dat het mogelijk is analyse van aminozuren en DNA uit vingerafdrukken te verrichten zonder daarmee het papillairlijnenpartroon zodanig wordt aangetast dat traditioneel dactyloscopisch onderzoek niet meer mogelijk is. Veelvoorkomende metabolieten zoals aminozuren en vetzuren laten een grote variabiliteit zien, zowel binnen als tussen donoren, waardoor ze minder geschikt zijn voor het extraheren van informatie. De bepaling van de relatieve verhouding van enantiomeren van serine daarentegen lijkt een potentiële marker voor het bepalen van de leeftijd van een vingerafdruk. Nader onderzoek moet uitwijzen of deze marker daadwerkelijk bruikbaar is voor forensische toepassingen. Daarnaast laat de analyse van stimulerende middelen of afbraakproducten daarvan veelbelovende resultaten zien, zoals de detectie van nicotine en het afbraakproduct cotinine in vingeradrukken van rokers. Ook informatie die voortkomt uit de analyse van exogene stoffen, die het gevolg zijn van aanraking met bepaalde producten of items in vingerafdrukken, kan van groot belang zijn om een specifieke donor te relateren aan een misdrijf. In dit proefschrift werd de analyse en onderscheiding van glijmiddelen uit vingerafdrukken beschreven. Meer onderzoek is nodig om het chemisch profileren van vingerafdrukken een stap dichter bij de praktijk te brengen. De gevonden markers in de beschreven onderzoeken waren niet onderhevig aan veroudering. Mogelijk veroudering die al heeft plaatsgevonden kan de classificering beïnvloeden, doordat afbraakprocessen hebben plaatsgevonden. Ook is voor elke analyse van potentiële nieuwe markers een methode nodig om de analyse verenigbaar te maken met het huidige detectie proces. Het chemisch profileren op basis massaspectrometrische data beschreven in dit proefschrift is mogelijk ook toepasbaar in andere gebieden, zowel binnen als buiten de forensische wereld. Che-

misch profileren binnen de forensische wetenschap is mogelijk interessant voor meerdere gebieden waar gebruikt wordt gemaakt van vergelijkbare analytisch chemische methoden zoals, onder andere, explosieven-, toxicologie- en verdovende middelen onderzoek. Over het algemeen is de methode toepasbaar in elk gebied waar full-scan MS-data wordt gebruikt om nieuwe markers te vinden, waarmee mogelijk ook meer fundamentele (bio)chemische onderzoeksvragen kunnen worden beantwoord.

Al met al heeft het onderzoek dat in dit proefschrift is beschreven laten zien dat de chemische analyse van vingerafdrukken een waardevolle bijdrage kan leveren aan het voortdurend ontwikkelende onderzoeksgebied van de forensische wetenschap.

1

Introduction

The whole of science is nothing more than a refinement of everyday thinking.

Albert Einstein

1

1.1. Fingerprints in forensic science

For over 100 years, fingermarks have been used to individualize perpetrators. In the early 1900s, Edmond Locard was the first to establish rules for personal identification using fingerprint details and proved to be one of the key contributors of fingerprint science [1]. The potential value of fingerprints as a means of personal identification was recognized long before. In 1788, Johann Mayer was likely the first to state that fingerprints were unique to each individual [2]. Even today, fingerprint evidence is still one of the most used types of evidence in forensic science. A fingerprint originates from contact between a bare fingertip and a surface and mainly consists of eccrine and sebaceous secretion [3]. Because the exocrine glands are only located on the friction ridges and not in the furrows, the secretion is often left behind on the surface in the distinctive fingerprint pattern. These patterns, made up by the flow of the friction ridges, can be divided into three different categories, namely loops, whorls and arches (Figure 1.1). Back in 1686, Marcello Malpighi was the first to describe the different patterns found in palmar surfaces [4]. These patterns are nowadays subcategorized further into at least 35 different patterns [1, 5].

Figure 1.1: Principal fingerprint types, from left to right: the whorl, loop and arch. Figure adapted from Bleay et al. [6].

The friction ridges, and with that the general pattern, start to develop in the fetal stage, as early as when the foetus is 11 weeks old [1]. The development of the general pattern appears to be influenced by several factors such as the shape and size of the volar pads (pads of tissue under the epidermis), their time of regression, and the bone morphology [1]. The discriminatory power of fingerprints however, is found on a lower level, and lies in the details of the friction ridges, the so-called minutiae (Figure 1.2). Among the most common minutiae are ridge endings, bifurcations and dots. Many more are classified, which consist of a combination of these three, of which some are more frequently encountered than others. The development process of the minutiae is a random process and remains largely unknown. Because the process is stochastic and an individual fingerprint may contain over 100 minutiae, there is an extreme variability in fingerprints. Moreover, as a result of the stochasticity, even identical twins possess different fingerprints. Despite the technological advances in the past century, such as the use of AFIS (Automated Fingerprint Identification System), the process is based on the same principle as a century ago; the comparison between

a fingermark found at a crime scene and a reference fingerprint, which can lead to identifying the perpetrator.

Figure 1.2: Fingerprint showing common minutiae. Figure adapted from Bleay et al. [6].

Fingermarks at a crime scene, however, often appear latent, and thus need to be visualized first. Many methods have been developed and optimized to detect and visualize latent fingermarks on different surfaces. Methods to detect fingermarks on non-porous surfaces at the crime scene are often based of powders, such as aluminium, fluorescent or magnetic powders, which physically "stick" to the fingerprint residue [1]. Additionally, items collected at a crime scene are often subjected to fingerprint detection techniques in forensic laboratories. Common detection techniques for latent fingermarks on porous surfaces such as paper include ninhydrin, DFO (diazafluorenone) and 1,2-indanedione. These techniques are based on a chemical reaction with the amino acids present in fingerprints to induce a color change. The most commonly used technique for latent fingeprints on non-porous surfaces is cyanoacrylate fuming, also known as superglue fuming. In this reaction, the cyanoacrylate vapor selectively polymerizes on the fingerprint residue to form a white polymer [1]. There are many more techniques such as physical developer (PD) and vacuum metal deposition (VMD). New techniques are continuously being developed as well, often to maximize performance on specific substrates, such as S_2N_2, which visualizes latent fingermarks on metal surfaces [7, 8].

The overview of fingerprint analysis and detection presented here is concise and simplistic, while in practice these may be considered a science on their own. A wealth of information on these topics is comprehensively described by Champod et al. and Bleay et al. [1, 6]. Clearly, there are numerous detection techniques, and whereas the mechanism behind many of these techniques greatly differs, ranging from physical (i.e. powder adhering to a fingerprint) to chemical (i.e. various chemical reactions

with fingerprint components, inducing a color change), all of them are designed to reach the same end result: creating contrast between a fingermark and the substrate.

However, not every fingermark found at a crime scene can be directly used for individualizing purposes. This happens when there is no reference fingerprint available (i.e. the suspect is unknown and his or her fingerprints are not available in the database), or if the fingermark is of poor quality in terms of ridge detail, for example due to distortion of the fingerprint pattern. As a result, crimes may remain unsolved. In the Netherlands, annually about 20.000 fingermarks collected from crime scenes are searched in the national database HAVANK (Het Automatisch Vinger Afdrukkensysteem Nederlandse Kollektie). In 2015, out of the 17,371 fingermarks searched in the system, 19.5% resulted in a match [9]. Therefore, there is great interest among forensic investigators in techniques that can still retrieve basic donor information from the circa 80% of fingermarks that did not lead to a match. Is there a way in which we can retrieve information from these fingermarks, which could directly be used in the investigation process?

1.2. Chemical composition of fingerprints

The answer to this question may lie in the chemical composition of the fingermark itself. The eccrine and sebaceous secretions that make up a large part of a fingerprint, which are also the basis of many fingerprint detection techniques, consist of compounds excreted by the donor, and thus may contain information about them. The eccrine gland is the only type of gland that is found on the palms and fingers (Figure 1.3), yet sebaceous compounds are often found in fingerprints as well. This is a result of contact between the fingers and other parts of the body, for instance the sebaceous-rich forehead. Eccrine secretion (sweat) mainly consists of water, salts and smaller metabolites such as amino acids, sebaceous secretion (sebum) largely consists of lipid compounds such as triacylglycerols, fatty acids, cholesterol and squalene (Table 1.1) [3, 6, 10]. Other potential sources of fingermark constituents are the apocrine sweat glands, the skin surface itself and external contaminants through contact. The concentration of these metabolites in a donor's secretion is believed to be influenced by the donor's traits, such as gender, age and diet, and thus studying the fingerprint composition could retrieve information about the donor's traits [6].

Additionally, an advantage that the analysis of the composition of fingerprints offers, is that it may provide habit and activity level information due to the presence of exogenous compounds (Table 1.1) [6, 11, 12]. The detection of illicit compounds or their metabolites in fingerprints, could be valuable information for forensic investigators [13]. The presence of a drug and/or their metabolites can indicate that the person has taken this drug, whereas the presence of an explosive can indicate that the donor has been in touch with that compound. Moreover, recent examples of lifestyle profiling based on exogenous compounds present in donor secretion were given by Hinners et al. and Bouslimani et al. [11, 12]. These studies showed that the presence of exogenous compounds may reveal information about the donors lifestyle, such as the use of bug sprays, sunscreens, beauty products as well as food consumption and medication use.

Several studies have been performed on the chemical composition of fingerprints.

Figure 1.3: Close-up of a fingertip, showing the secretion of sweat from the eccrine glands on the fingerprint ridges. Figure adapted from Bleay et al. [6].

In particular, common metabolites such as amino acids and fatty acids have been investigated. Gas chromatography coupled to mass spectrometry (GC-MS) was used in various studies both to study amino acid and fatty acid profiles from fingerprints [14–16]. Besides GC-MS, multiple other analytical techniques have been used in studies into the amino acid profiles of fingerprints. Capillary Electrophoresis (CE) and (Ultra) High Performance Liquid Chromatography ((U)HPLC), using either a UV or MS as detector, are examples of techniques that have been successfully applied to separate and quantify amino acids retrieved from fingerprints [17].

In addition to the chromatography-based approaches, studies have been performed based on mass spectrometry imaging (MSI), combining the retrieval of chemical information with spatial information. These approaches enable the reconstruction of the fingerprint ridge detail by mapping the chemical information on the ridges, thus also providing associative evidence by linking intelligence around the donor. Studies have been performed using Matrix Assisted Laser Desorption Ionization (MALDI), Desorption Electrospray Ionization (DESI) and Secondary Ion Mass Spectrometry (SIMS). Whereas MALDI and DESI are soft ionization techniques, allowing the analysis of organic molecules, SIMS is more appropriate for the analysis of inorganic constituents, such as inorganic salt ions and trace metal ions. MALDI has been used to analyze the molecular composition of fingerprints, and components such as lipids, peptides

Table 1.1: Fingermark residue constituents and sources. Table adapted from Bleay et al. [6].

Source	Location	Constituents
Eccrine sweat	Eccrine sweat glands found all over the body and particularly abundant on the palms of hand and fingertips.	Water, urea, uric acid, creatinine, amino acids, ammonia, choline, glucose, and other reducing sugars, lactic acid and lactate, sodium, chloride, potassium, calcium, trace metal ions, phosphate, sulphate, enzymes, peptides, proteins, vitamins.
Sebum	Sebaceous glands on the face, head and other locations associated with hair follicles.	Free fatty acids, cholesterol esters, mono-, di- and triacylglycerols, wax esters, cholesterol, squalene and other hydrocarbons.
Apocrine sweat	Apocrine sweat glands found in axillary regions of the body, namely, the armpits and the genital area.	Ammonia, androgenic steroids, cholesterol, glycogen, iron, proteins and water.
Epidermal lipids (skin surface)	From touching other areas of the body (the epidermis) and migration of material from the non-palm side of hand.	Free fatty acids, glycerides, proteins, sterols, sterol esters.
External contaminants (exogenous substances)	Picked up as a consequence of touching other objects and surface.	Illicit drugs, nicotine, cosmetics, explosives, foodstuffs, dust, grease.

and proteins in more detail [18, 19]. Moreover, MALDI was the first technique to show potential for discrimination of donor gender based on the chemical analysis of fingerprints [20]. DESI has been successfully used to analyze fingerprints as well, showing distinguishing capabilities between donor gender, ethnicity and age [21]. SIMS has been applied to study exogenous compounds in fingerprint residue, for instance in traces originating from contact between the finger and amphetamine drugs [22]. Lastly, alternative approaches based on spectroscopic techniques such as FT-IR (Fourier-transform infrared spectroscopy), immunolabeling and fluorescence spectroscopy have been described. Using fluorescence spectroscopy, Van Dam et al. developed a method to estimate the time since deposition of fingerprints, based on autofluorescent properties [23]. Moreover, Van Dam et al. showed that immunolabeling can be used both for fingerprint detection and donor profiling based on fingerprint composition [24]. Lastly, FT-IR spectral imaging has been used to detect illicit substances in fingerprint residue [25].

There have been several studies into the chemical composition of fingerprints that have shown promising leads for the retrieval of donor information. These need to be confirmed and built upon using various analytical techniques.

1.3. Thesis aims

The aim of the research work described in this thesis is to determine which information about the donor, and which additional information, such as the time of deposition or the presence of exogenous compounds, potentially linking a found fingerprint to the crime, can be retrieved by analyzing the chemical composition of fingerprints by employing GC-MS, UPLC-MS(/MS) and DESI-MS. To be successful in achieving this aim, the following key questions need to be addressed:

- The concentration of common metabolites, such as amino acids, in fingerprints is generally low. Reproducible analysis of these metabolites therefore proves challenging. How amino acids from fingerprints be analyzed in a fast and reliable way?
- In order to find out which donor traits influence the chemical composition, it needs to be analyzed how the chemical composition varies both between donors and within donors (e.g. during a day and week). What is the inter- and intra-variability of fingerprint chemical composition?
- A challenge in studying the chemical composition of fingerprints is the influence of the time of deposition. With the analysis of the degradation processes of compounds in fingerprints rises opportunity to estimate the time of deposition, and thus link a fingermark to a crime scene by their time of deposition. Can fingerprint age information be derived by analyzing compounds present in an aged fingerprint using UPLC-MS/MS?
- In addition to endogenous compounds that make up for a large part of the fingerprint composition, exogenous compounds can be found in fingerprints as well. This could be through contact with exogenous compounds. Can habit or activity level information be derived from the detection of exogenous compounds in fingerprints using DESI-MS?
- To be compatible with the current fingerprint process, methods need to be developed that can retrieve analytes of interest from fingerprints without altering the fingerprint ridge detail. Can compounds of interest from fingerprints be collected in a non-marking way (i.e. without physically marking the ridge detail)?

1.4. Outline of this thesis

This thesis is divided in 7 chapters. Chapters 2 to 6 present the different studies that were conducted as part of this dissertation:

Chapter 2 describes the development of a new method to extract and quantify amino acids from fingerprints. The method is based on ultra high performance liquid chromatography coupled to a mass spectrometer. A polar stationary phase was used, circumventing the need for amino acid derivatization prior to analysis.

Chapter 3 presents how the chemical composition of fingerprints differs within (intra) and between (inter) donors, and what donor traits possibly influence the composition. To study the inter-variability of fingerprints, 1852 fingerprints from 463 donors at the Dutch musical festival Lowlands were collected. These were analyzed using the described method in Chapter 2, as well as a gas chromatography-based analytical method. To investigate the intra-variability, 56 fingerprints from 4 donors were analyzed.

Chapter 4 describes how the racemization of amino acids, a process that starts as soon as the endogenous L-amino acids are excreted by the eccrine glands, poses as

potential marker for the age of a fingerprint. A method was developed to separate and relatively quantify D/L-enantiomers from 7 amino acids from fingerprints, after which fingerprints up to 6 months old were analyzed.

Chapter 5 presents an example of how exogenous compounds found in fingerprints can serve as an extra source of information in the case of fingermarks found at scenes of sexual assault. Different brands of condoms were classified based on traces of lubricants found in fingerprints.

Chapter 6 is dedicated to the proof of concept of using hydrogels to retrieve compounds of interest from fingerprints in a non-marking manner. Hydrogel lifting was used to retrieve amino acids and DNA from fingerprints without destruction of ridge detail.

Chapter 7 provides the overall conclusions and recommendations.

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2

Analysis of common metabolites from fingerprints

I often say that when you can measure what you are speaking about, and express it in numbers, you know something about it; but when you cannot measure it, when you cannot express it in numbers, your knowledge is of a meagre and unsatisfactory kind.

Lord Kelvin

Previous research has focused on quantifying lipid compounds and amino acids from fingerprints using both GC-MS and LC-MS. However, there is no method to facilitate the large-scale quantification of common polar metabolites such as amino acids from fingerprints. Therefore, the aim of this chapter is to develop such a method using a straightforward sample preparation. The first part of the chapter presents an overview of current methods to analyze amino acid from fingerprints. Then, the development of the analytical method, which is based on UPLC-TOF-MS, using an amide stationary phase is described. Finally, the quantification results of a TOF-MS and a QqQ MS/MS as detectors are compared by using both methods to examine the amino acid content in fingerprints from 19 donors.

Parts of this chapter have been published in *Analytical Methods* **9**, 38 (2017) [1].

2.1. Introduction

Fingermarks make up an important part of criminal investigations. The deposition is regarded as a proof of contact, with the odd exception, and the individualization has proven to contain a strong evidential value [2]. As such, a crime related fingermark found at the crime scene is regarded as an incriminating piece of evidence. The chemical composition of fingerprints has been investigated extensively, as comprehensively described by Girod et al. and Cadd et al. [3, 4]. The analysis of the chemical compounds present in fingerprints, in particular lipids and amino acids, has been investigated several times in the recent past. Croxton et al. published the use of gas chromatography coupled with mass spectrometry (GC-MS) for amino and fatty acids [5, 6]. Previously, Girod and Weyermann have described the use of fatty acid analysis for donor classification [7], and more recently, Delgado-Povedano et al. described a method for enhancing metabolomics from sweat [8]. By extracting the fatty components from fingerprints using $CHCl_3$, Girod et al. succeeded in obtaining lipid profiles of a substantial amount of fingerprints. Even though it appeared later that the extraction methods used may not have been optimal,[8, 9] Girod et al. have shown the great potential of chemical profiling of fingerprints on the basis of exogenous and endogenous materials. Although the fatty acids, squalene, cholesterol and triglycerides found in fingerprints may not be directly related to the finger or palm deposition, more information on what has been touched previous to the crime related deposition can be invaluable. One example was described by Weyermann et al., where the disappearance, or chemical breakdown, of certain compounds in a fingerprint could potentially be used as an indication for the age of a fingerprint [10]. Liquid chromatography coupled with mass spectrometry (LC-MS) has been applied previously for the analysis of amino acids [11] and non-polar materials in fingerprints [12]. Although the chromatographic separation was lengthy, parameters such as the resolution, repeatability and linearity are within an acceptable range.

To investigate the intra- and inter-variability of polar compounds, such as amino acids, in fingerprints, large data sets need to be analyzed. Existing methods are often based on a relatively long analysis time, as well as a labor-intensive sample preparation including derivatization. Only one attempt on the non-derivatized separation of amino acids from fingerprints, using electrophoresis, has been described [13]. Although the separation of the analytes was satisfactory, the sensitivity of the separation method in combination with mass spectrometric detection was not satisfactory.

In this chapter, the development of a method for the extraction and analysis of non-derivatized amino acids from fingerprints using ultra-high performance liquid chromatography (UHPLC or UPLC) with an amide stationary phase is described. This form of hydrophilic interaction chromatography (HILIC) enables the separation of polar compounds through liquid-liquid partitioning between a water-enriched layer of stagnant eluent, on the stationary phase, and a more hydrophobic eluent [14]. Secondly, the differences in performance between the use of a tandem quadrupole (QqQ) MS/MS and a time-of-flight (TOF) MS are described; both detectors are frequently used in metabolomic profiling studies [15]. The TOF-MS offers high resolution and mass accuracy, whereas the triple quadrupole MS/MS provides structural information through fragmentation. The methods have been used for the analysis of the amino acid profiles

from the fingerprints of 19 donors.

2.2. Materials and methods

2.2.1. Solvents and solutions

Amino acids L-alanine (98%), L-lysine (97%), L-leucine (98%), L- glutamic acid (99%), L-glutamine (99%), L-tyrosine HCl monohydrate (98%), L-histidine HCl monohydrate (98%), L-ornithine HCl monohydrate (99%), hydroxy-L-proline (99%), L-asparagine (98%), L-threonine (98%), L-isoleucine (98%), L-serine (99%), L-methionine (98%), L-tryptophan (98%), L-valine (98%), L-phenylalanine (Phe, 98%), L- arginine HCl (98%), L-cystine (98%), L-aspartic acid (98%) and L-proline (99%) were purchased from Sigma-Aldrich (Zwijndrecht, the Netherlands). Isotope labelled amino acids L-glutamic acid D_5 (98.5 atom% D), L-glutamine D_5 (98.8 atom% D), L-histidine HCl monohydrate D_3 (98.4 atom% D), L-threonine D_2 (98.8 atom% D), L-serine D_7 (98.6 atom% D), DL- cystine D_6 (99.5 atom% D), hydroxy-L-proline D_3 (99.2 atom% D), DL-valine D_8 (99.1 atom% D) and L-ornithine D_6 HCl (99.1 atom% D) were purchased from CDN isotopes (Pointe-Claire, Canada). L-Aspartic acid D_3 (98 atom% D), L- lysine D_4 (98 atom% D), L-leucine D_{10} (98 atom% D), L-phenylalanine D_5 (98 atom% D) and L-asparagine D_8 (97 atom% D) were purchased from Sigma-Aldrich (Zwijndrecht, the Netherlands). L-Tyrosine D_2 (98 atom% D), L-tryptophan D_5 (97 atom% D) and L-methionine D_3 (98 atom% D) were purchased from Isotec (Zwijndrecht, the Netherlands). L-Isoleucine $^{13}C_6$ (99%) ^{15}N (99%), L-alanine $^{13}C_3$ (97–99%) D_4 (97–99%) ^{15}N (97–99%), L-arginine HCl D_7 (98%) $^{15}N_4$ (98%), and L-proline $^{13}C_5$ (99%) ^{15}N (99%) were purchased from Cambridge Isotope Laboratories (Tewksbury, MA, USA). UPLC-grade acetonitrile, methanol and formic acid were purchased from Biosolve (Valkenswaard, the Netherlands).

A mixture of 21 amino acids was used to identify and quantify the amino acids with UPLC-MS. Every amino acid was individually dissolved in MeOH (containing 5 v/v% formic acid), making up a stock solution with a concentration of 100, 250 or 500 mg/L. An amino acid working solution of 2.0 mg/L of the 21 amino acids was prepared by adding the required volumes of stock solutions of each individual amino acid, followed by dilution with MeOH (containing 5% v/v formic acid) to a final volume of 25 mL. Similarly, an internal standard working solution of 2.0 mg/L of the isotope labeled internal standards was prepared. A calibration range from 0.10 to 1.60 mg/L was produced, which corresponds to 5–80 ng of a single amino acid in a sample volume (50 µL) per fingerprint. These solutions were prepared by adding 2.5, 5, 10, 15, 30, and 40 µL of 2.0 mg/L working solution into a glass screw neck injection vial. Internal standard solution (20 µL) was added to each vial, and MeOH containing 5 v/v% formic acid was added to the total volume of 100 µL.

2.2.2. Fingerprint collection and processing

The fingerprints were donated voluntarily by colleagues, who all gave informed consent. No ethical approval was obtained as the material is gathered in a non- invasive manner and did not infringe on any privacy of the donors; no images were taken of the fingerprints. All experiments were carried out following institutional guidelines

and are in compliance with relevant laws. Fingerprints were collected from the right and left hand index fingers from 19 donors, 10 females and 9 males, ranging from 20 to 66 years old (Table A.1). Donors were asked to wear nitrile gloves for 10 minutes and rub their hands together prior to the print deposition, to create homogeneity in the composition on both hands of the donor. Fingerprints were collected on 2.5 x 5 cm pieces of aluminum foil. Aluminum foil was chosen as a substrate as it is non-porous and flexible and hence it is convenient in the sample preparation process. The aluminum foil sheets were transferred into a 15 mL polypropylene conical tube (Fisherbrand) and internal standard solution (20 µL) was added. MeOH (2 mL, containing 5% v/v formic acid) was added and the solution was mixed using a vortex. After mixing, the aluminum foil was removed from the tube and the sample solution was evaporated under nitrogen flux. The material was dissolved in MeOH (100 µL, containing 5% v/v formic acid) and then transferred into an injection vial. A blank sample was prepared by the addition of 25 µL MeOH solvent to a clean aluminum foil sheet and a positive control was prepared by adding 25 µL of 2.0 mg/L amino acid working solution to a clean aluminum foil sheet. After drying in air, these samples were processed as described above.

2.2.3. UPLC-TOF MS

The separation was carried out using a 150 mm UPLC ethylene bridged hybrid (BEH) amide column (Waters, Milford, MA, USA). An Acquity I-class UPLC autosampler and binary solvent pump (Waters, Milford, MA, USA) were used to inject and elute the sample solution. The used flow rate was 0.500 mL/min. The solvents used for LC were (A) 0.4% formic acid in acetonitrile (ACN) and (B) 0.4% formic acid in ultra pure water (purified deionized water, to attain a resistivity of 18 MΩ-cm at 25 °C). The gradient applied was 95% A for 3 minutes followed by a linear gradient from 5 to 50% B in 19 minutes and then 1 minute of 50% B. Finally, the column was reconditioned for 2 minutes with 95% A (the total run time for each sample was 25 minutes). For each analysis 2.5 µL of sample was injected. The eluent of the column was transferred into a dual electrospray ionisation (ESI) ion source coupled with a accurate mass time-of-flight (TOF) mass spectrometer (Agilent 6220, Santa Clara, CA, USA). The system was operated in the positive ion mode and MS spectra from m/z 40–1200 were acquired at a resolution of 7500 at m/z 400. The spectra were acquired at a rate of 1 spectrum per second. The capillary voltage was set at 3.5 kV, the source gas temperature was set at 325 ºC and a drying gas flow of 5 L/min was used. The nebulizer pressure used was 30 psig. The fragmentor, skimmer and octapole 1 RF voltages were set at 160, 65 and 250 V, respectively. MS full scan data were acquired using Agilent Mass Hunter Data Acquisition software (version B.04.00) and data were processed using Agilent Mass Hunter Qualitative Analysis software (version B.05.00) and Quantitative Analysis software (version B.05.00).

2.2.4. UPLC-QqQ MS/MS

The UPLC conditions were identical to those of the UPLC-TOF MS method described above. All samples were analyzed on a tandem quadrupole mass spectrometer (MicroMass Quattro Premier XE, Waters, Milford, MA, USA) which was operated in pos-

itive electrospray ionization (ESI) mode. The ESI-parameters were: capillary voltage 0.5 kV; source temperature 130 ºC; desolvation gas temperature 400 ºC at a flow rate of 1000 L/h (N2); and cone gas flow rate 100 L/h (N_2). Argon was used as the collision gas at a flow rate of 0.27 mL/min. Multiple reaction monitoring (MRM) transitions, cone voltages and collision energies for all analytes and internal standards are summarized in Table A.2. MassLynx (version 4.1) and QuanLynx were used for data analysis.

2.3. Results and discussion

Based on the developed UPLC method, the non-derivatized amino acid mixture was separated in 17 minutes (extracted ion chromatograms shown in Figure A.1). For both the triple quadrupole MS/MS and the TOF-MS the limit of detection (LOD), limit of quantification (LOQ), linearity (R^2) and intra-day reproducibility were determined for all amino acids (Table 2.1). In the case of the triple quadrupole, the linearity for all amino acids exceeded 0.99 except for L-methionine and L-phenylalanine (>0.98). For the TOF-MS, L-iso-/leucine, L-lysine and L-methionine were the only amino acids with a linearity not exceeding 0.99 (0.988, 0.987 and 0.964, respectively). The respective LODs and LOQs achieved with the triple quadrupole were found to be lower for all amino acids, except L-alanine, L-phenylalanine, L-tryptophan, L-tyrosine, L-iso-/leucine and hydroxy-L-proline, than achieved using TOF-MS. The intra-day reproducibility determined for the 21 amino acids was found to be comparable in both cases. Overall, the mean intra-day RSD of the peak areas was 9.54% ± 3.07% for the triple quadrupole and 8.22% ± 1.94% for the TOF-MS. The extraction efficiency of the amino acids was calculated and in general was between 96 and 117 percent (Table A.3). The fact that efficiencies higher than 100% are found might be due to methanol evaporation, slightly concentrating the samples. The extraction efficiency of L-methionine is somewhat lower (89.1%), which may be caused by the oxidation of L-methionine to L-methionine sulfoxide, although this was not investigated any further.

Next, the amino acid profiles (relative amino acid abundances to serine, the most abundant amino acid in fingerprints) from fingerprints from 19 donors were analyzed on both mass spectrometers (Figure 2.1). Four amino acids (L-cystine, L-methionine, L-tyrosine, and hydroxy-L-proline) were excluded from further analysis, as they were unsuccessfully quantified in either method. In general, the profiles obtained from the 19 donors appear to be very similar. However, some striking differences were found in the abundances of L-proline, L-lysine and L-asparagine, which appear to be slightly higher when analyzed with the triple quadrupole. The abundance of L-phenylalanine however, appears to be slightly higher in some donors when analyzed with the TOF-MS. Surprisingly, the mean RSD of the amino acid profiles based on duplicate fingerprints is generally higher in the case of the triple quadrupole analyses, when compared to the TOF-MS analyses (18.6% ± 6.6% vs. 13.2% ± 3.8%).

The total amount of amino acid per fingerprint was calculated for each donated fingerprint (Figure 2.2). Although the amino acid profiles of the 19 donors did not show large variability at first sight, the differences in the total amino acid deposited per fingerprint are significant, ranging from about 60 to 1200 ng per fingerprint de-

Table 2.1: Linearity (R^2), limit of detection (LOD), limit of quantification (LOQ) and intra-day reproducibility of the 21 amino acids on both the triple quadrupole and the time of flight MS. Limits of detection and quantification were estimated based on S/N ratios (3:1 and 10:1). Intra-day reproducibility (RSD) is based on the relative standard deviation of peak areas from isotopically labeled amino acids from 5 different standards.

	Triple quadrupole MS/MS				TOF-MS			
Compound	R^2	LOD (ng/fingerprint)	LOQ (ng/fingerprint)	RSD (Intra-day)	R^2	LOD (ng/fingerprint)	LOQ (ng/fingerprint)	RSD (Intra-day)
L-alanine	0.992	1.04	3.46	6.63	0.997	0.89	2.97	9.53
L-arginine	0.996	0.17	0.58	13.4	0.993	0.33	1.11	7.30
L-asparagine	0.998	0.06	0.19	13.9	0.994	0.35	1.15	7.26
L-aspartic acid	0.995	0.38	1.28	13.2	0.999	0.75	2.49	6.13
L-cystine	0.993	0.71	2.35	12.8	0.998	1.96	6.54	13.6
L-glutamic acid	0.993	0.16	0.55	7.98	0.994	0.21	0.70	7.86
L-glutamine	0.998	0.08	0.27	6.78	0.999	0.57	1.90	8.79
L-histidine	0.999	0.58	1.95	9.04	0.994	0.67	2.24	9.33
hydroxy-L-proline	0.999	0.11	0.37	9.20	0.991	0.10	0.34	8.19
L-iso-/leucine	0.997	0.05	0.17	4.64	0.988	0.04	0.15	10.7
L-lysine	0.995	0.58	1.92	13.2	0.987	1.45	4.84	9.47
L-methionine	0.982	0.23	0.77	11.2	0.964	0.63	2.09	5.77
L-ornithine	0.997	0.19	0.64	10.9	0.997	0.74	2.48	6.97
L-phenylalanine	0.981	0.32	1.08	7.77	0.992	0.05	0.18	6.98
L-proline	0.992	0.03	0.11	13.6	0.992	0.04	0.14	6.57
L-serine	0.992	0.11	0.38	6.12	0.992	0.34	1.14	7.40
L-threonine	0.992	0.07	0.24	9.15	0.998	0.17	0.58	5.79
L-tryptophan	0.995	0.11	0.38	5.94	0.995	0.04	0.14	8.48
L-tyrosine	0.999	0.78	2.58	5.53	0.991	0.09	0.29	7.54
L-valine	0.991	0.03	0.12	9.93	0.992	0.11	0.35	10.8

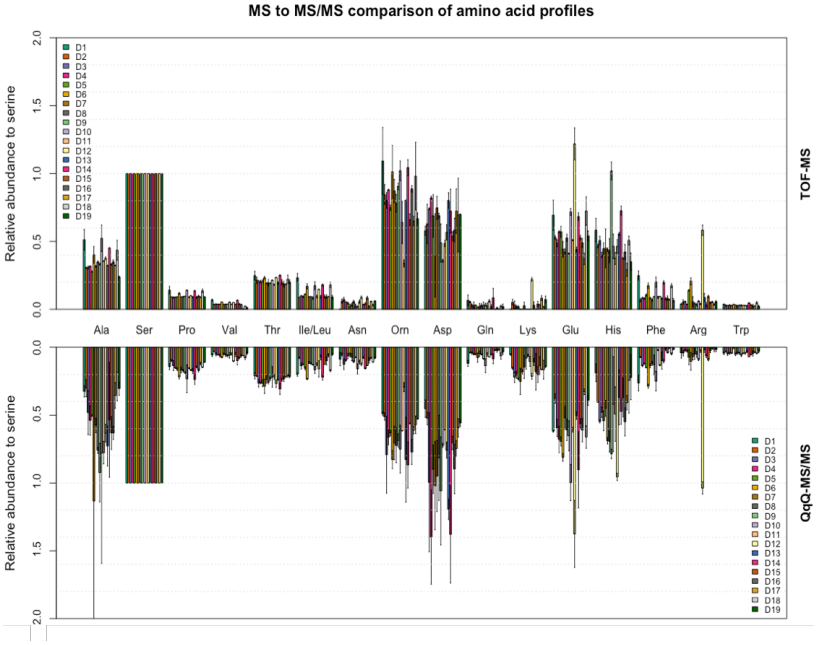

Figure 2.1: Amino acid profiles of the 16 most abundant amino acids found in fingerprints of 19 donors (labeled D1 through D19) analyzed on both the triple quadrupole and time of flight MS. All amino acid abundances are relative to serine. Amino acid abundances for each donor were calculated as mean of the two donated prints with standard deviation as shown.

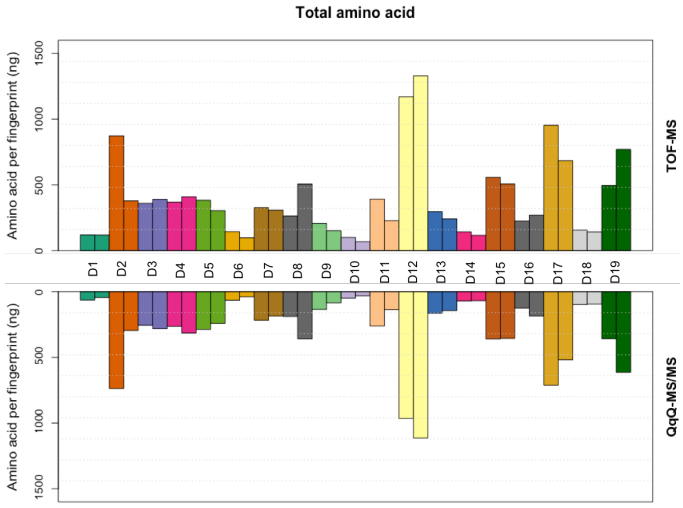

Figure 2.2: Total amino acid (ng) per fingerprint, calculated by summing all amino acids abundances found in each fingerprint sample of the 19 donors (labeled D1 through D19), for both the triple quadrupole and time of flight MS.

position. This may be due to the fact that the fingerprint deposition was not precisely controlled and factors such as deposition pressure, time and surface area may have varied between depositions. Despite this lack of controlled fingerprint deposition, prints from the same donors often yielded comparable amounts of total amino acid. While the absolute abundances in each fingerprint are largely similar, the calculated total amounts of amino acid were overall higher for the TOF-MS. This may be explained by the fact that the samples were analyzed on the TOF-MS after analysis on the triple quadrupole MS/MS and some methanol might have evaporated, yielding slightly higher concentrations in the fingerprint samples.

2.4. Conclusion

In this chapter, a method for the separation and quantification of amino acids from fingerprints using UPLC and both a triple quadrupole MS/MS and a TOF-MS is described. The chromatographic separation is achieved in 17 minutes. The use of the amide stationary phase circumvents the need to derivatize amino acids prior to LC-MS analysis, thereby shortening and simplifying the sample preparation process as well. This facilitates the analyses of the large numbers of samples needed to study the intra- and inter-variability of amino acids profiles from fingerprints. Although the triple quadrupole had a higher sensitivity for most amino acids, the TOF-MS showed sufficient sensitivity to yield the same amino acid profiles obtained from 19 donors. Interestingly, the results obtained with the TOF-MS had lower mean RSDs in amino

acid profiles from duplicate fingerprints.

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3

Intra- and inter-variability of fingerprint chemical composition

Numbers are the highest degree of knowledge.
It is knowledge itself.

Plato

The previous chapter presented the developed analytical method to retrieve and quantify common polar metabolites, such as amino acids, from fingerprints. This method facilitates the quantitative analysis of large numbers of fingerprints. However, to investigate what the chemical composition can reveal about the fingerprint donor, larger data sets are needed. The aim of this chapter is to determine how certain donor traits, such as gender, influence the chemical composition of fingerprints. Using the method described in the previous chapter, a large data set of fingerprints was analyzed to establish how their composition differs both between and within donors. First, background about the compositional analysis of fingerprints is given. Then, the collection and analysis of the fingerprint samples is presented. Finally, the statistical analysis and models that were developed to classify donor traits based on the collected data set are described.

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3.1. Introduction

Techniques that can retrieve donor information from fingermarks without being dependent on the availability of a reference or the quality of the ridge detail would be of great added value. Several studies have been carried out into the chemical composition of fingerprints with exactly this aim. Gas chromatography coupled to mass spectrometry (GC-MS) was used in numerous studies both to study amino acid and fatty acid profiles from fingerprints [2–4]. Capillary Electrophoresis (CE) and (Ultra) High Performance Liquid Chromatography ((U)HPLC), using a mass spectrometer as detector, have been successfully applied to separate and quantify amino acids retrieved from fingerprints [5–7]. In addition, several mass spectrometry imaging approaches, often using Matrix Assisted Laser Desorption Ionization (MALDI), have been used to investigate the composition of fingerprints [8–11].

A major drive behind the research into the chemical composition of fingerprints is that eccrine or sebaceous secretion is likely to be influenced by several donor factors, such as the change in the composition of sebaceous secretion before and after puberty, and thus donor information may be derived from fingerprint residue [12, 13]. The chemical composition of a fingerprint has been reported to be influenced by a wide variety of donor traits [14, 15]. Extensive research has shown the influence of key donor factors, such as age and gender [9, 16–19]. Recently, Heaton et al. showed to be able to discriminate between donor gender with a predictive power of 86%, based on the analysis of proteins and peptides in fingermarks using MALDI [20]. The presence of exogeneous compounds has been investigated as well, and is reported to provide information about an individual's lifestyle [21, 22]. Additionally, illicit drugs and their metabolites have been detected in fingerprints using various surface mass spectrometry approaches [23, 24]. There have been several studies into the chemical composition of fingerprints that have shown promising leads for the retrieval of different types of donor information. These need to be confirmed and built upon using various analytical techniques. The aim of this study is to do this by analyzing the relation between fingerprint composition (natural, eccrine and sebaceous) and the characteristics of a large set of donors, using GC-MS and UPLC-MS. To be able to categorize donors based on their fingerprint composition, the influence of common donor factors, such as gender, age, diet, smoking habit and (medicinal) drug use needs to be analyzed.

In this study, the chemical composition of 1852 fingerprints were analyzed, donated by 463 donors (179 male, 280 female and 4 unknown, ranging from 18-63 years old, median age of 26) on the Dutch music festival Lowlands in 2016. In a targeted metabolomics approach, the amino acid profiles of 926 fingerprints (463 natural and 463 eccrine) were analyzed using LC-MS and the fatty acid, squalene and cholesterol content in the other 926 fingerprints (463 natural and 463 sebaceous) were quantified using GC-MS. Permutation tests were used to find significant differences in amino acid and lipid abundances. In an untargeted approach, the full-scan MS data from the analyzed fingerprints were used to generate conditional inference trees [25] as classification models to predict two forensic relevant donor traits: gender and smoking habits of fingerprint donors. Additionally, an insight in donor intra-variability is given, based on the analysis of 56 fingerprints from 4 donors.

3.2. Materials and methods

3.2.1. Solvents and solutions

The 21 amino acids L-alanine (98%), L-lysine (97%), L-leucine (98%), L-glutamic acid (99%), L-glutamine (99%), L-tyrosine hydrochloride monohydrate (98%), L-histidine hydrochloride monohydrate (98%), L-ornithine hydrochloride monohydrate (99%), hydroxy-L-proline (99%), L-asparagine (98%), L-threonine (98%), L-isoleucine (98%), L-serine (99%), L-methionine (98%), L-tryptophan (98%), L-valine (98%), L-phenylalanine (98%), L-arginine hydrochloride (98%), L-cystine (98%), L-aspartic acid (98%) and L-proline (99%) were purchased at Sigma-Aldrich (Zwijndrecht, the Netherlands). Isotope labelled internal standard (IS) amino acids L-glutamic acid D_5 (98.5 atom % D), L-glutamine D_5 (98.8 atom % D), L-histidine hydrochloride monohydrate D_3 (98.4 atom % D), L-threonine D_2 (98.8 atom % D), L-serine D_7 (98.6 atom % D), DL-cystine D_6 (99.5 atom % D), hydroxy-L-proline D_3 (99.2 atom % D), DL-valine D_8 (99.1 atom % D) and L-ornithine D_6 hydrochloride (99.1 atom % D) were purchased at CDN isotopes (Pointe-Claire, Canada). L-aspartic acid D_3 (98 atom % D), L-lysine D_4 (98 atom % D), L-leucine D_{10} (98 atom % D), L-phenylalanine D_5 (98 atom % D) and L-asparagine D_8 (97 atom % D) were purchased at Sigma-Aldrich (Zwijndrecht, the Netherlands). L-tyrosine D_2 (98 atom % D), L-tryptophan D_5 (97 atom % D) and L-methionine D_3 (98 atom % D) were purchased at Isotec (Zwijndrecht, the Netherlands). L-isoleucine $^{13}C_6$ (99%) ^{15}N (99%), L-alanine $^{13}C_3$ (97-99%) D_4 (97-99%)^{15}N (97-99%), L-arginine hydrochloride D_7 (98%)$^{15}N_4$ (98%), L-proline $^{13}C_5$ (99%)^{15}N (99%) were purchased at Cambridge Isotope Laboratories (Tewksbury, MA, USA). UPLC-grade acetonitrile (ACN), methanol (MeOH) and formic acid were purchased at Biosolve (Valkenswaard, the Netherlands). Docosane (analytical standard), squalene (99%), cholesterol (99%), caprylic acid (99%), capric acid (98%), lauric acid (99.5%), myristoleic acid (99%), myristic acid (99%), pentadecanoic acid (99%), palmitoleic acid (98.5%), palmitic acid (99%), heptadecanoic acid (98%), linoleic acid (99%), oleic acid (99%), stearic acid (98.5%), nonadecylic acid (99%), arachidic acid (99%) chloroform (99.9%) and chlorotrimethylsilane (99%) were purchased from Sigma Aldrich (Zwijndrecht, the Netherlands). Individual amino acid and individual isotopically labelled amino acid (IS) stock solutions of 100-500 mg/L, depending on their solubility, were prepared in MeOH containing 5% (v/v) formic acid using a volumetric flask and were stored at -20 °C. An amino acid stock solution of 2 mg/L of each of the 21 amino acids was prepared from the individual stocks using a volumetric flask and stored at -20 °C. Similarly, an IS mixture of 2 mg/L of each of the 21 IS amino acids was prepared using a volumetric flask from the individual IS stocks and stored at -20 °C. Individual fatty acid, squalene and cholesterol stock solutions of 500-2000 mg/L, depending on their solubility, were prepared in MeOH using a volumetric flask and were stored at -20 °C. A lipid stock solution of 500 mg/L of each of the fatty acids, squalene and cholesterol was prepared from the individual stocks using a volumetric flask, evaporated under nitrogen flow, reconstituted in MeOH, and then stored at -20 °C. Extraction solvent for amino acid analysis was prepared as MeOH containing 5% (v/v) formic acid and 0.01 mg/L IS amino acids. Extraction solvent for lipid analysis was prepared as MeOH solvent containing 1.5 mg/L docosane (IS).

3.2.2. Fingerprint collection and processing

Inter-variability

Fingerprints were donated by volunteers at Lowlands Science 2016. The material was gathered in a non- invasive manner and did not infringe on any privacy of the donors; fingerprints were purposely donated in a light smearing motion to minimize the number of individualizing characteristics. All experiments were carried out following institutional guidelines and relevant laws. Ethical approval was obtained before executing the experiment. All participants were 18 years or older, gave informed consent and received a debriefing form after participating in the experiment. All participants (463) donated two natural fingerprints, one eccrine fingerprint and one sebaceous fingerprint. Participants were asked to rub their hands together to create homogeneity and donate two fingerprints with their index finger on 2.5 by 5 cm aluminum foil sheets. The participants then transferred the sheets to two 15 mL conical tubes. To one of the tubes 2 mL MeOH containing 5% (v/v) formic acid and 0.01 mg/L IS amino acids was added by means of a dispenser. To the other, 2 mL MeOH containing 1.5 mg/L docosane was added by means of a dispenser. Both samples were mixed using a vortex mixer for 30 seconds. The participants were then asked to wash their hands thoroughly with soap, dry them with paper and then put on nitrile gloves. The participants then filled out a digital questionnaire consisting of 31 questions regarding general donor factors and habits such as age, gender, diet, smoking habit and (illicit) drug use (questionnaire can be found in supplementary material B). After completing the questionnaire, the participants were asked to remove their gloves, rub their hands together to create homogeneity and donate one fingerprint with their index finger on a 2.5 by 5 cm aluminum foil sheet. Then, participants were asked to donate a sebaceous fingerprint by grooming their foreheads before donating the last fingerprint. The aluminum sheets were transferred by the donors to 15 mL conical tubes. To the eccrine fingerprint, by means of a dispenser, 2 mL MeOH containing 5% (v/v) formic acid and 0.01 mg/L IS amino acids was added. To the sebaceous fingerprint, using a dispenser, 2 mL MeOH containing 1.5 mg/L docosane was added. Both samples were mixed using a vortex mixer for 30 seconds. After collection, all samples were stored at 4 ºC for the first 72 hours, then at -20 ºC until analysis.

Intra-variability

To analyze fingerprint intra-variability, natural fingerprints from 4 donors (aged 20-24) were collected 3 times a day for 5 days (Monday to Friday, only 2 fingerprints were collected on Friday), totaling to 56 fingerprints (14 fingerprints per donor). Fingerprints were collected and processed as described above (2 mL MeOH containing 5% (v/v) formic acid and 0.01 mg/L IS amino acids was added).

3.2.3. UPLC-MS

To study inter-variability, for each donor (463), one natural and one eccrine fingerprint were analyzed using UPLC-MS. To determine intra-variability, all 56 samples (from 4 donors) were only analyzed using UPLC-MS. The fingerprint samples were first brought to room temperature and subsequently mixed using a vortex mixer for 30 seconds. Then, the aluminum foil sheets were removed using clean tweezers. Sam-

ples were then evaporated under nitrogen flow and subsequently reconstituted in 50 µL MeOH containing 5% (v/v) formic acid and transferred to an injection vial. UPLC-MS was performed as described previously [6]. Liquid chromatography was carried out using a 150 mm UPLC ethylene bridged hybrid (BEH) amide column (Waters, Milford, MA, USA) and an Acquity I-class UPLC autosampler and binary solvent pump (Waters, Milford, MA, USA). The flow rate was set at 0.500 mL/min. Column eluent was sprayed into the ion source of the time-of-flight MS by electrospray ionization (ESI). The solvents used for UPLC were (A) 0.4% formic acid in acetonitrile (ACN) and (B) 0.4% formic acid in ultrapure water (purified deionized water, to attain a resistivity of 18 MΩ-cm at 25 °C). The gradient used was 95% A for 3 minutes, followed by a linear gradient from 5 to 50% B in 19 minutes and then 1 minute of 50% B. Finally, the column was reconditioned for 2 minutes with 95% A (run time totaling to 25 minutes). For each analysis 2.5 µL of sample solution was injected. Samples were analyzed on an accurate mass TOF with a dual ESI (Agilent 6220, Santa Clara, CA, USA). The system was operated in the positive ion mode. MS spectra from m/z 40–1200 were acquired at a resolution of 7500 at m/z 400 at a rate of 1 spectrum per second. The capillary voltage was set at 3.5 kV, the source gas temperature at 325 C and a drying gas flow of 5 L/min was used. The nebulizer pressure used was 30 psig, while fragmentor, skimmer and octapole 1 RF voltages were set at 160, 65 and 250 V, respectively. MS full scan data were acquired with Agilent Mass Hunter Data Acquisition software (version B.04.00) and data were processed using Agilent Mass Hunter Qualitative Analysis software (version B.05.00) and Quantitative Analysis software (version B.05.00). To quantify amino acid content, a calibration series was prepared ranging from 0.10 to 1.60 mg/L per amino acid. If amino acid quantification results were outside the calibration range, samples were diluted and reanalyzed. Amino acid quantification results were used for the targeted data analysis, while the full scan LC-MS data was used in for an untargeted approach.

3.2.4. GC-MS

Lipid components were extracted and quantified in a two-step method as proposed by Cadd et al. [4]. To determine inter-variability, for each donor, the fatty acid, cholesterol and squalene profile from one natural and one sebaceous fingerprint were determined. The fingerprint samples were first heated to room temperature and subsequently mixed using a vortex mixer for 30 seconds. The sample solution was then transferred to a 10 mL glass tube and 100 µL chlorotrimethylsilane was added to start derivatization. The aluminum foil sheet was transferred to a new 10 mL glass tube and 2 mL of chloroform was added and subsequently mixed using a vortex mixer for 60 seconds. Then, the chloroform sample solution was transferred to the tube already containing the methanol and chlorotrimethylsilane sample solution and subsequently samples were evaporated under nitrogen flow. After reconstitution in 50 µL of chloroform, samples were transferred to injection vials (with 50 µL inserts). Analyses were carried out on a GC–MS HP6890/5973 (Agilent Santa Clara, CA, USA). An HP-5MS column was used for separation (30 m length, 0.25 mm internal diameter, film thickness 0.25 µm). Aliquots of 5 µl were injected onto the column in split mode (1/20) using an auto sampler and a 4.5 min solvent delay. The temperature of the liner was

held at 250 °C and helium was used as a carrier gas. The column temperature was held at 80 °C for 1.0 min and heated to 230 °C at a rate of 10 °C/min. Then, the column was heated to 310 °C at a rate of 4 °C/min and this temperature was held constant for 8 min. A calibration series was prepared using the lipid stock solution in concentrations of 0, 0.01, 0.02, 0.05, 0.1 and 0.2 g/L and was processed in the same way as the fingerprints deposited on aluminum foil. If lipid quantification results were outside the calibration range, samples were diluted and reanalyzed. Lipid quantification results were used for the targeted data analysis, while the GC-MS data were used in for an untargeted approach.

3.2.5. Data analysis

Data was analyzed with R (version 3.4.2) using R studio (Version 1.1.456). R was chosen because of the wide availability of packages for preprocessing, visualization and machine learning approaches. Additionally, as it is open source software, it is available to others in the field. In the data analysis, both a targeted metabolomics approach and an untargeted profiling approach were implemented. Both the quantitative data of the targeted metabolites as well as the raw LC-MS and GC-MS data will be made publicly available within 6 months after publication (reserved doi: 10.4121/uuid:0611ccbb-1e5a-4bf4-b6da-abc115ca0c98).

Targeted metabolomics

The quantified amino acid and lipids were analyzed in a targeted metabolomics approach. The targeted metabolites were regarded as a sub composition of the fingerprint residue, as not all fingerprint components were quantified, and the analytes of interest are thus a sub composition of a larger, unknown, composition. Therefore, a compositional data analysis approach was used, as described by Aitchison [26], which is based on the additive log-ratio transformation (alr):

$$\mathrm{alr}(x) = \left[\ln\left(\frac{x_1}{x_D}\right), \ln\left(\frac{x_2}{x_D}\right), \ldots, \ln\left(\frac{x_{D-1}}{x_D}\right)\right] \tag{3.1}$$

where each part x_j of the composition is transformed to a log-ratio with common divisor x_D. L-threonine and palmitic acid yielded the lowest total variation (data not shown) and were chosen as reference part (x_D) for the eccrine and sebaceous compositions, respectively. To deal with missing values in the targeted metabolites, the following transformation was used, denoted genlog:

$$\mathrm{genlog}(x) = \ln\left(x + d\right) - c \tag{3.2}$$

where

$$c = \mathrm{int}\Big(\ln\left(m\right)\Big) \qquad \text{order of magnitude constant}$$
$$d = e^c \qquad \text{decimal constant}$$

in which m is the smallest non-zero value in a vector and $\mathrm{int}(x)$ a function that drops all digits after the decimal point. The subtraction of the constant c from each element after log transformation ensures that the lowest value in a vector remains zero. The alr

transformation from equation 3.1 adapted to equation 3.2 leads to the function that has been used to transform the targeted metabolite values:

$$\text{alr}(x) = \left[\ln\left(x_1 - x_D\right), \ln\left(x_2 - x_D\right), \ldots, \ln\left(x_{D-1} - x_D\right)\right] \tag{3.3}$$

where

$$x_j = \begin{cases} \ln\left(x_j + d\right) - c & \text{if } \min(x_j) = 0 \\ \ln\left(x_j\right) & \text{if } \min(x_j) > 0 \end{cases}$$

To reduce the number of missing values, the number of variables was reduced by applying a modified 80% rule [27]. This meant a variable was included if at least 80% of the values is non-zero in the samples of any class. Permutation tests were executed as statistical tests to find significant differences in targeted alr-transformed amino acids or lipids. The Agresti–Coull interval is used as an approximate binomial confidence interval [28].

Untargeted analyte profiling

The data were analyzed in an untargeted profiling approach using the XCMS package [29–31]. LC-MS data files were converted to mzXML using MSconvert [32]. GC-MS data files were converted to CDF using openChrom [33]. Peak picking and retention time correction were optimized using the IPO package, using 10 randomly selected datafiles [34]. Analyte difference reports were subsequently generated for the following classes; type of fingerprint, gender and smoking (every day and past 24 hours). Peak areas were normalized using the total sum of peak areas per sample. Classification models were generated using the caret package [35]. 30 features were preselected with minimum redundancy and maximum relevance using the mRMRe package, using Spearman's rho as a measure of the correlation between the features in the dataset [36]. The data was subsequently preprocessed using the Yeo-Johnson transformation. Conditional inference random forests (cforest) were used as classification models, using the party package [37–39]. The data were randomly divided in train and test sets (75% and 25%, respectively). Classification accuracy was evaluated by generating confusion matrices using the caret package [35]. Variable importance was evaluated using the varImp function (caret package), to find the most important predictors of the generated classification models. The p-values associated with fold changes were calculated using *t*-tests. Features were putatively annotated using the online METLIN mass spectral metabolite database [40].

3.3. Results

3.3.1. Inter-variability: Targeted metabolomics

In total, 463 participants donated 4 fingerprints (2 natural, 1 eccrine and 1 sebaceous fingerprint), resulting in the analysis of 1852 fingerprints. Using LC-MS, the amino acid profiles of one of the natural and the eccrine fingerprint of each donor was determined. Using GC-MS, the lipid profile of the remaining natural and sebaceous fingerprints was determined. Figure 3.1 shows the distribution of the total amino acid and lipid content for the different types of collected fingerprints. Clearly, the variability in total amino acid is large, ranging from below 100 ng to above 10 µg per

fingerprint. The amino acid content was generally higher in the natural fingerprints when compared to the eccrine fingerprints (5.4 fold on average, p-value 3.48E-04). This might be explained by the fact that the participants washed their hands and only wore gloves for a limited time before donating the eccrine fingerprint. The variability in total lipid content shows a similar pattern, ranging from 100 ng up to 100 µg per natural fingerprint. The lipid content is higher in the sebaceous fingerprints (5.9 fold on average, p-value 2.32E-11), ranging from about 1 µg to over 100 µg per fingerprint.

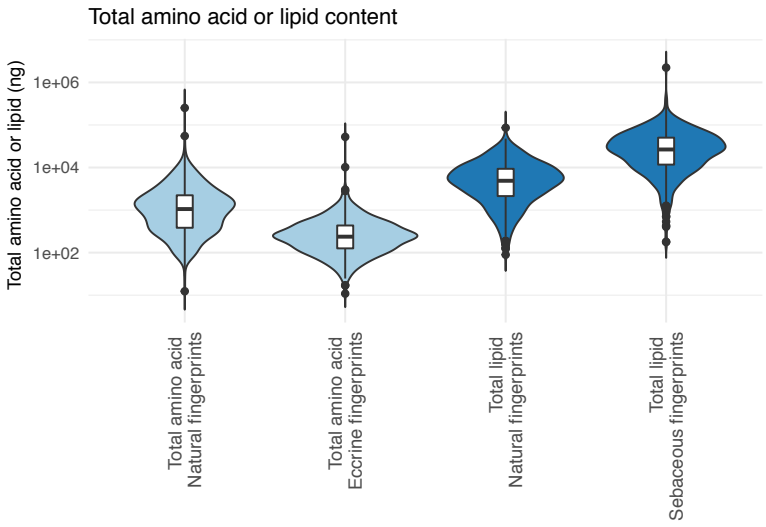

Figure 3.1: The distribution of total amino acid and lipid content (ng) in the natural, eccrine and the sebaceous fingerprints, calculated as sum of all quantified amino acids (LC-MS) or lipid compounds (GC-MS).

Subsequently, multiple permutation tests (104 permutations per test and $\alpha = 0.05$) were performed to identify potential metabolic markers using the additive log-ratio transformed metabolite concentrations (Figure B.1). Table 3.1 displays the compounds that were found to vary significantly between donor classes. Compounds were only reported if found significant in both types of fingerprint samples (natural and eccrine/sebaceous). The rationale behind this is that changes found in both types of fingerprints are more likely to be a result of metabolic changes. Additionally, as the variation in fingerprint composition is large, the data from the eccrine and sebaceous fingerprints can be used to confirm findings from the natural prints. However, significant differences, which might be of forensic relevance, found only in natural fingerprints are also reported. It must be noted that the fact that many donor traits did not lead to any significant findings in the targeted amino acid or lipid compounds, is possibly related to unbalanced sample sizes. This was the case for many of the questions regarding to medicinal drug use. Similarly, in case of donor age, over 90% of the participants was in the age range of 18 to 40 years.

Table 3.1: Fold change in additive log-ratio transformed targeted amino acids (LC-MS) and lipids (GC-MS), if found significant in both natural and eccrine or sebaceous fingerprint. Significant differences were found in gender, cannabis usage and alcohol consumption classes. Under classes is specified: M = male, F=female in case of gender; Yes = used cannabis in past 24 h, No = not used cannabis in past 24 h in case of cannabis usage, and in case of response variable alcohol: the number of alcoholic consumptions consumed in the past 24 h. The p-values were calculated using the Agresti–Coull interval as an approximate binomial confidence interval.

Response variable	Class 1	Class 2	Explanatory variable	Higher in class	Fold change natural	p-value	Fold change eccrine/sebaceous	p-value
Gender	M	F	L-phenylalanine	1	1.352	0.0003	1.134	0.0010
	M	F	L-(iso-)leucine	1	1.133	0.0357	1.079	0.0045
	M	F	Palmitoleic acid	1	1.216	0.0096	1.135	0.0121
Cannabis	Yes	No	L-alanine	1	1.186	0.0128	1.050	0.0163
Alcohol	>15	0	L-proline	1	1.767	0.0002	1.119	0.0455
	>15	1-5	L-proline	1	1.408	0.0005	1.094	0.0332
	>15	6-10	L-proline	1	1.223	0.0049	1.109	0.0056

Gender differences were found in L-(iso-)leucine and L-phenylalanine concentrations, which were found to be higher in men in both the natural and eccrine fingerprints (35.2% and 13.4% in case of L-phenylalanine, 13.3% and 7.9% for L-(iso)leucine). These findings are in line with previous findings in studies on amino acid serum levels, where six out of six studies that included both men and women also found significantly higher isoleucine levels in men, and five out of six for phenylalanine [41]. In comparison, Huynh et al. reported higher levels of L-phenylalanine in females compared to male fingerprint donors [42]. In case of the lipid compounds, palmitoleic acid was the only compound found to significantly differ between male and female donors in both the natural and sebaceous fingerprints. Palmitoleic acid was found to be 21.6% higher on average in natural fingerprints donated by male donors. In case of the sebaceous fingerprints, the palmitoleic acid content was 13.5% higher in case of male donors. Alanine concentrations were found to be 18.6% (natural) and 5.0% (eccrine) higher on average for donors who reported to have used cannabis in the last 24 hours. Donors that reported to have consumed more than 15 units of alcohol in the 24 hours prior to donating their fingerprint, showed higher proline concentrations than those that consumed no alcohol, 1-5 or 6-10 units. Acute alcohol administration is known to cause decreased utilization of proline, which could explain an increased secretion in heavy drinkers (> 15 units) compared to other classes [43].

Previous studies have shown the ability to successfully detect illicit drugs and/or their metabolites in fingerprints of drug users using either DESI, LESA or MALDI-MS [23, 24, 44]. Moreover, the detection of certain drugs of abuse in fingerprints after contact has also been shown to be possible using techniques such as SIMS and Raman spectroscopy [45, 46]. In the current study, possible indirect effects of drugs use on the chemical composition (i.e. changes in amino acid or lipid profile) were investigated. As mentioned before, only in the case of usage of cannabis and alcohol, a small but significant change in certain metabolite levels in both the eccrine as well as natural fingerprints was detected. Only in the natural fingerprints of donors who indicated to have used MDMA in the past 24 hours, tryptophan was found to be 38.5% higher compared to donors who did not use MDMA. Previous studies have

shown that MDMA inhibits tryptophan hydroxylase activity [47–49]. This could possibly explain the higher abundance of tryptophan in donors that used MDMA. In the eccrine fingerprints no significant difference was found. Similarly, significant differences were found in L-asparagine levels in only the natural fingerprints from donors who indicated to have consumed diet soda in the past 24 hours and people who consumed regular soda or no soda at all. L-asparagine levels were 27.2% and 26.0% higher in natural fingerprints from donors that drank diet soda, compared to those who did not drink soda or drank regular soda, respectively. After ingestion, the artificial sweetener aspartame is hydrolyzed into L-tryptophan and L-aspartic acid, since it is the methyl ester of the dipeptide of these amino acids [50]. To our knowledge, no relation between increased levels of L-asparagine and aspartame has been reported previously, although it is well known that L-asparagine can be readily synthesized from L-aspartate by asparagine synthetase [51].

Since data were acquired in full scan mode, potential metabolic markers were subsequently investigated based on the full scan data in an untargeted approach.

3.3.2. Inter-variability: Untargeted profiling

The aim of the untargeted approach was to develop classification models for forensic relevant donor factors such as gender, age, diet, smoking habit and (medicinal) drug use. Based on the data and the corresponding sample sizes, donor gender and smoking habit were selected to develop classification models. Conditional random forests were used as models, as they allow for easy interpretation of variable importance. As a proof of principle, models were generated based on the LC-MS and GC-MS data for the different types of collected fingerprints (i.e. natural vs eccrine for LC-MS, natural vs sebaceous for GC-MS). For these classification models, 30 features (normalized peak intensities) were preselected (using the mRMRe package, Table B.1). In case of the LC-MS data, the model was able to predict the fingerprint type (natural or eccrine) with 95.3% accuracy (CI: 91.7% - 97.6%, Table 3.2). The most important predictor, turned out to be *m/z* 147.0760, putatively annotated as the amino acid L-glutamine. Table 3.3 presents the putative annotations of other predictors used in this model (see Figures B.2 and B.3 for volcano plot and abundance data). Most of these compounds showed higher normalized intensity in the natural samples, in line with the data from the targeted approach. Among these putatively annotated compounds was urea, a well-known component of eccrine secretion [19, 52, 53]. Next to the amino acids L-glutamine and L-arginine, which were found to be higher in the natural samples, several putatively annotated amino acid degradation products such as urocanic acid, pyroglutamic acid and 4-methylene-L-glutamine were found to be higher in the natural fingerprints. The possible increased abundance of amino acid degradation products in natural fingerprints can be explained by the fact that the eccrine fingerprints only contained fresh secretion and thus no amino acid degradation products yet. Moreover, natural fingerprints may be contaminated with a range of compounds (through contact), some of them old enough to be degraded. The increased normalized intensity of L-glutamine and L-arginine in natural fingerprints might be caused by slower secretion of these amino acids. Interestingly, three *m/z* values putatively annotated to small peptides were included in the model, of which two were higher

in the eccrine fingerprints. It could be hypothesized that these small peptides are excreted by eccrine glands, but are readily hydrolyzed. The higher abundance of one of the peptides in the natural fingerprints however, does not support this hypothesis. These peptides are likely to be hydrolysis products from larger peptides or proteins. Further analysis of the data reveals the possible annotation of additional short peptides (<5 amino acids, Table B.2) which were found in the full scan data. These were not included in the classification model.

Table 3.2: Confusion matrix of cforest classification model for fingerprint type based on LC-MS data with an overall 95.3% accuracy (CI: 91.7% - 97.6%).

		Reference	
		Natural	Eccrine
Prediction	Natural	112	6
	Eccrine	5	110

Table 3.3: Features used in the classification model for fingerprint type based on the LC-MS data, their putative annotation, relative mass error (in ppm) and fold change. P-values were calculated using *t*-tests.

Experimental *m/z*	Putative annotation	Δppm	Higher in	Fold change	p-value
61.0398	Urea $[M+H]^+$	3.26	Natural	2.82	4.94E-47
139.0498	Urocanic acid $[M+H]^+$	2.88	Natural	6.30	3.73E-24
147.0760	L-glutamine $[M+H]^+$	2.72	Natural	6.85	6.26E-44
152.0316	Pyroglutamic acid $[M+H]^+$	1.31	Natural	4.75	4.32E-31
159.0758	4-methylene-L-glutamine $[M+H]^+$	3.77	Natural	11.08	1.25E-18
175.1176	L-arginine $[M+H]^+$	3.26	Natural	10.21	2.11E-21
362.1559	Val-Glu-Asp or Asp-Leu/Ile-Asp $[M+H]^+$	0.28	Natural	6.99	3.14E-47
465.2483	Lys-Lys-Ser-Cys $[M+H]^+$	3.26	Eccrine	3.56	1.36E-52
663.3338	Arg-Arg-Phe-Tyr $[M+Na]^+$	0.15	Eccrine	3.00	3.51E-30

When constructing the classification model for fingerprint type with the GC-MS data, the resulting accuracy was slightly lower: 86.8% (CI: 81.8% - 90.9%, confusion matrix in Table B.3, 30 preselected features in Table B.1). The model is mainly driven by the three most important predictors: *m/z* 96.10, 137.20 and 203.20 (volcano plot and the normalized peak intensity of these predictors as well as the other predictors used in this model are displayed in Figures B.4 and B.5). Table 3.4 summarizes the putative fragments and their sources. All *m/z* values used in the classification were found to have a higher normalized intensity in the sebaceous fingerprints compared to the natural fingerprints. Mong et al. previously found unsaturated fatty acids to decrease faster compared to saturated fatty acids [54]. Similarly, squalene is known to degrade relatively fast in fingerprints, hence it has been a compound of interest in fingerprint age estimation studies [2, 55]. These findings are confirmed by the importance of fragments from squalene and monounsaturated fatty acids in this classification model. Additionally, all lipid compounds in the sebaceous fingerprints are more abundant compared to the internal standard (docosane) than is the case in the natural fingerprints, resulting in higher normalized peak intensities.

Table 3.4: Features used in the classification model for fingerprint type based on GC-MS data, their putative source and fold change. P-values were calculated using *t*-tests.

Experimental *m/z*	Putative fragment	Fragment from	Higher in	Fold change	p-value
79.10	C_6H_5 +2H	Squalene, cholesterol	Sebaceous	6.11	5.06E-45
87.10	$C_4H_9OCH_2$, C_3H_7O–C=O, C_4H_9CHOH, $C_3H_7OCHCH_3$	FAMES*	Sebaceous	7.86	3.09E-41
93.10	C_7H_9	Squalene, cholesterol	Sebaceous	8.98	2.79E-50
96.10	C_7H_{12}	FAMES* (monounsaturated)	Sebaceous	7.16	1.13E-46
137.20	$C_{10}H_{17}$	Squalene	Sebaceous	11.75	1.45E-49
203.20	$C_{15}H_{23}$	Squalene	Sebaceous	11.29	3.86E-52

*Fatty acid methyl esters

Subsequently, a classification model was build to predict donor gender, using only 30 preselected features (Table B.1), based on the full scan LC-MS data from the collected natural fingerprints. The accuracy of this model, based on the test set, was found to be 77.9% (CI: 69.1% - 85.1%, Table 3.5). Interestingly, the sensitivity for males is significantly lower than for females (65.9% compared to 85.5%, respectively). Among the most important predictors were *m/z* 284.0988 and *m/z* 169.0361, putatively annotated as guanosine and uric acid, respectively (Table 3.6, volcano plot Figure B.6). Moreover, other *m/z* values incorporated in the classification model possibly correspond to guanine, uric acid and guanosine, all degradation products of guanosine monophosphate (GMP) (pathway depicted in Figure B.7). The normalized peak intensities of the used predictors are depicted in Figure 3.2. Further analysis of the data reveals that a compound putatively annotated as xanthine also significantly differs between males and females, but that this compound played no role in the classification model. Possibly, quantitative information from xanthine is redundant since guanine and uric acid are already incorporated. Although the compounds putatively annotated as products from GMP catabolism are included in the classification model for gender, there is, to our knowledge, no previous record of concentrations of GMP or related products in fingerprints. Studies into the concentration of cGMP and guanosine in nasal mucus and human brain tissue, respectively, found significant higher concentrations in females compared to males [56, 57]. The *m/z* 166.0865 (detected in 175 out of 179 males and in 271 out of 280 females), even though not used in the classification model, is worth mentioning, as it corresponds to the $[M+H]^+$ of L-phenylalanine ($[M+H]^+$ of 166.0863) and was found to be 32% higher in males than females, which is in line with the fold change found in the targeted approach (35% change, Table 3.1). No significant changes in L-(iso)leucine levels were found using this approach. Similarly, a classification model to predict gender was built using 30 predictors from the eccrine fingerprints. In this case the accuracy decreased to 71.7% (CI: 62.4% - 79.8%, confusion matrix in Table B.4, 30 preselected features in Table B.1). Putatively annotated products from GMP catabolism were not included in this model, although some changes in uric acid and guanine were seen (respective 1.77- and 2.06-fold increase in males, data not shown). Subsequently, the corresponding classification models for gender based on the GC-MS data were developed. The model based on the natural fingerprints had an overall accuracy of 68.1% (CI: 58.7% - 76.6%, confusion matrix in Table B.5, 30 preselected features in Table B.1). When the

model was built based on sebaceous fingerprints, the accuracy decreased even further to 64.6% (CI: 55.0% - 73.4%, confusion matrix in Table B.6, 30 preselected features in Table B.1). The low accuracy might be due to the large variation in sebaceous fingerprint content, which was also seen in the targeted approach.

Table 3.5: Confusion matrix of cforest classification model for donor gender based on LC-MS data of the natural fingerprints with an overall 77.9% accuracy (CI: 69.1% - 85.1%).

		Reference	
		Male	Female
Prediction	Male	29	10
	Female	15	59

Table 3.6: Features used in the classification model for gender based on natural fingerprint LC-MS data, their putative annotation, relative mass error (in ppm) and fold change. P-values were calculated using *t*-tests.

Experimental *m/z*	Putative annotation	Δppm	Higher in	Fold change	p-value
152.0565	Guanine $[M+H]^+$	1.32	Male	1.48	4.53E-04
169.0361	Uric acid $[M+H]^+$	2.96	Male	1.61	8.52E-05
284.0988	Guanosine $[M+H]^+$	0.35	Male	2.57	2.30E-03
153.0410	Xanthine* $[M+H]^+$	1.96	Male	1.55	5.89E-03
166.0865	L-phenylalanine* $[M+H]^+$	1.20	Male	1.32	9.87E-03

*Not used in classification model

Next, models were generated in the attempt to classify smokers versus non-smokers, using only 30 preselected features. Based on the LC-MS data from the natural fingerprints, the generated model was able to achieve a 90.4% accuracy (CI: 83.4% - 95.1%, Table 3.7). The most important predictor was *m/z* 163.1225, putatively annotated as nicotine ($[M+H]^+$ of 163.1230, Table 3.8). The second and third most important predictors were *m/z* 177.1024 and *m/z* 96.0444, respectively. The *m/z* 177.1024 possibly corresponds to cotinine ($[M+H]^+$ of 177.1022), the main degradation product of nicotine, which has been detected in fingerprints in previous studies [58, 59]. The *m/z* 96.0444 matches with hydroxypyridine ($[M+H]^+$ of 96.0444). Although hydroxypyridine itself is not a direct degradation product of nicotine in humans, the pyridine pathway is a well-known microbial degradation route of nicotine, forming 2,5-dihydroxypyridine [60–62]. Putative hydroxypyridine, nicotine and cotinine levels were, respectively, 2.76-, 11.74- and 5.70-fold higher on average in fingerprints from smokers compared to those of non-smokers (Figure 3.3, volcano plot Figure B.8). Prediction of smokers versus non-smokers was also attempted with the eccrine fingerprints and resulted in similar accuracy (90.2%, CI: 83.1% - 95.0%, confusion matrix in Table B.7, 30 preselected features in Table B.1, volcano plot Figure B.9). In this model, only the *m/z* annotated as nicotine is incorporated, which was 6.80-fold high in smokers compared to non-smokers (Figure 3.3). The absence of cotinine might be a result of the lower concentrations in the eccrine fingerprints. Hydroxypyridine, might be absent because it possibly is a microbial degradation product, and thus is

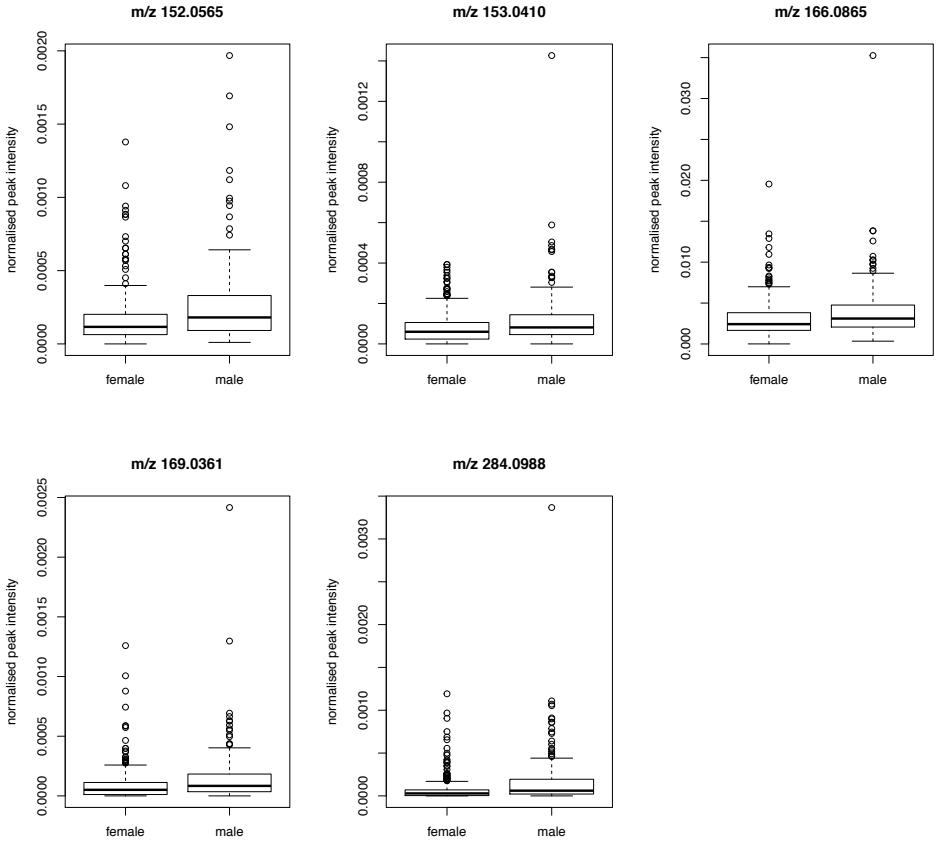

Figure 3.2: Boxplots of the features used in the model to predict gender based on natural fingerprint LC-MS data. From left to right putatively annotated compounds: guanine (*m/z* 152.0565), xanthine (*m/z* 153.0410), L-phenylalanine (*m/z* 166.0865), uric acid (*m/z* 169.0361) and guanosine (*m/z* 284.0988).

not excreted directly by the eccrine glands.

Then, the classification model to differentiate between donors who smoked in the past 24 hours and donors who did not smoke in the past 24 hours was generated. The percentage of participants that indicated to have smoked in the past 24 hours was 36%, compared to 28% that indicated to smoke on an everyday basis. In case of the natural fingerprints, this resulted in an accuracy of 87.7% (CI: 80.3% - 93.1%, confusion matrix in Table B.8, 30 preselected features in Table B.1, abundance data and feature fold changes in Figure B.10 and Table B.10). Again, the *m/z* values likely corresponding to nicotine, cotinine and hydroxypyridine are incorporated in this model, as was to be expected (respective 11.07-, 4.07- and 2.50-fold increase). This model has slightly lower accuracy as the model constructed for everyday smokers, mainly because of an increased false negative rate (i.e. predicting a smoker as non-smoker).

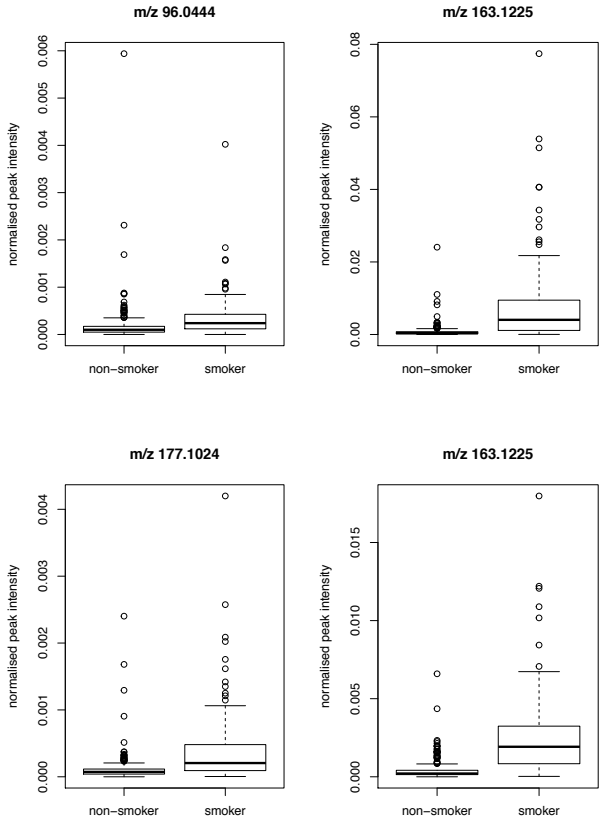

Figure 3.3: Boxplots of the features used in the model to predict everyday smoking habit based on LC-MS data (both natural and eccrine). From left to right putative annotation: hydroxypyridine (*m/z* 96.0444) in natural fingerprints, nicotine (*m/z* 163.1225) in natural fingerprints, cotinine (*m/z* 177.1024) in natural fingerprints and nicotine (*m/z* 163.1225) in eccrine fingerprints.

Table 3.7: Confusion matrix of cforest classification model for everyday smoking habit of donors, based on LC-MS data of the natural fingerprints with an overall 90.4% accuracy (CI: 83.4% - 95.1%).

		Reference	
		Non-smoker	Smoker
Prediction	Non-smoker	76	6
	Smoker	5	27

Table 3.8: Features used in the classification model for everyday smoking based on LC-MS data, their putative annotation, relative mass error (in ppm) and fold change. P-values were calculated using *t*-tests.

Experimental *m/z*	Putative annotation	Δppm	Higher in	Fold change natural	p-value	Fold change eccrine	p-value
96.0444	Hydroxypyridine $[M+H]^+$	0	Smoker	2.76	8.53E-03	n.f.	-
163.1225	Nicotine $[M+H]^+$	3.07	Smoker	11.74	1.66E-10	6.80	1.29E-12
177.1024	Cotinine $[M+H]^+$	1.13	Smoker	5.70	2.16E-05	n.f.	-

This might indicate that it takes some time before the found markers reach the fingerprint residue, or putative levels of nicotine, cotinine and hydroxypyridine are not sufficiently high in fingerprints by occasionally smoking. This trend was also seen in case of eccrine fingerprints (accuracy of 87.6%, CI: 80.1% - 93.1%, confusion matrix in Table B.9, 30 preselected features in Table B.1, abundance data and feature fold changes in Figure B.10 and Table B.10), where only the *m/z* value likely corresponding to nicotine was included in the model (6.33-fold increase). Both these models show large similarity with the everyday-smoking models, since participants who smoke every day are likely to have smoked in the past 24 hours.

In the natural fingerprints of participants that indicated to have used cosmetic or personal care products such as make-up, sunscreen or hair gel, several exogenous compounds were putatively annotated, although classification models to predict the contact with such compounds could not be constructed successfully (Figure B.11 and Table B.11). Among the putatively annotated compounds were ensulizole (sunscreen agent, change not significant), panthenol (moisturizer in cosmetic and personal care products), glycerol (humectant and lubricant in pharmaceutical and personal care products) and dimethylethanolamine (DMEA, used in skin care products, change not significant). Next to DMEA, the related compound choline was putatively annotated as well.

3.3.3. Intra-variability: Amino acid content

From 4 donors, 14 fingerprints were collected during 5 days (three per day, two on the last day). Figure 3.4 displays the total amino acid content of the quantified amino acids per donor. Clearly, variation both within and between donors is significant. Total amino acid ranged from 270 ng on average (Donor 1) to 630 ng (Donor 4) per fingerprint (Table 3.9). Additionally, intra-donor variability, quantified by the relative standard deviation, was reasonably large as well, ranging from 39.1% to 66.9% during the week. The variability within a day, quantified by the average relative standard deviations of the 5 separate days, was comparable, ranging from 36.1% to 52.5%. It must be noted that the deposition pressure, time and area were not controlled, as this would represent fingermarks found in practice best.

The relative amino acid profiles (normalized to serine) of the analyzed fingerprints showed less variability, both between and within donors (Figure 3.5). Relative variability was largest for the amino acids lysine, glutamic acid and tyrosine. These results indicate that although the variability in the amount of transferred residue (based on total amino acid) can be large, the relative concentration of the constituents seems to be more stable, as was to be expected.

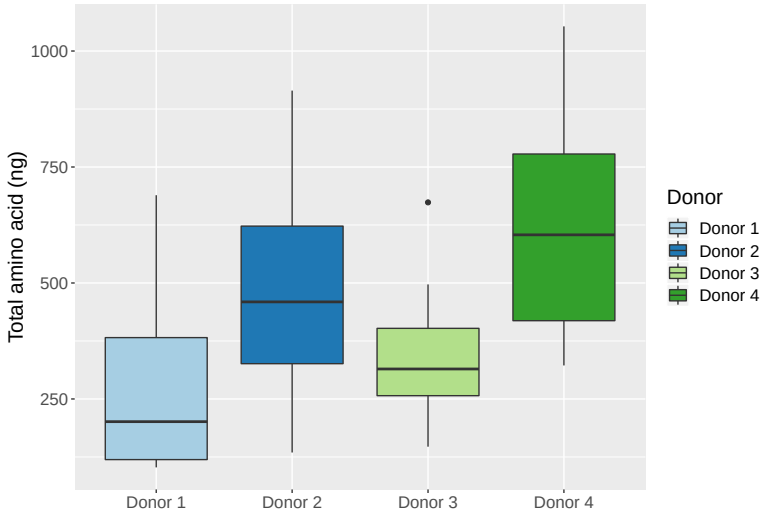

Figure 3.4: Total amino acid content of quantified amino acids in the collected fingerprints per donor during 5 days.

Table 3.9: Total amino acid in nanograms, standard deviation in nanograms, and fingerprint intra-day and intra-week variablity per donor. Intra-week variability is quantified as relative standard deviation and relative range (relative to maximum). Intra-day variability is quantified as the average relative standard deviations of the 5 days and the relative range (relative to maximum).

	Average amino acid (ng)	SD (ng)	Intra-day RSD (%)	Relative range (%)	Intra-week RSD (%)	Relative range (%)
Donor 1	270	± 180	52.5	15.0 - 100	66.9	15.0 - 100
Donor 2	504	± 231	43.1	17.1 - 100	45.9	15.2 - 100
Donor 3	338	± 134	36.1	29.6 - 100	39.7	22.1 - 100
Donor 4	630	± 246	43.1	32.2 - 100	39.1	31.5 - 100

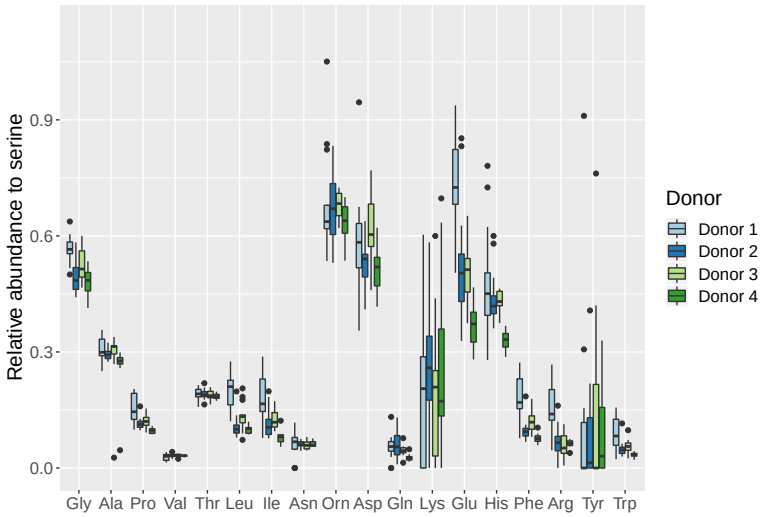

Figure 3.5: Relative abundance of the quantified amino acids (to serine) of the collected fingerprints per donor during 5 days.

3.3.4. Intra-variability: Untargeted profiling

Similar to the approach described for the inter-variability study using the data collected at the music festival Lowlands, an untargeted approach was used to analyze the intra-variability based on the full-scan MS data. The developed model was able to distinguish the fingerprints from the 4 donors based on this relatively small dataset (Table 3.10). When looking at the most important predictor per donor, it can be seen that the model incorporates *m/z* values that are differentially abundant in fingerprints from different donors (Figure 3.6). The most important predictor for donor 1, *m/z* 408.1255, is higher abundant in fingerprints from donor 1 than the other 3 donors throughout the week. Similarly, *m/z* 403.2793, the most important predictor for donor 2, is almost exclusively found in fingerprints from donor 2. Alternatively, *m/z* 868.3256, the feature essential for donor 3, is found in lower abundance in fingerprints from donor 3, compared to the other donors. Lastly, *m/z* 762.7943, the predictor of highest importance for donor 4, is found to be highly abundant in fingerprints from donor 4, although the feature is also present in fingerprints from donors 1 and 3. Overall, these results show that fairly unique markers seem to be present in fingerprints, which can potentially be used to distinguish between donors. Moreover, these markers seem to be present in the fingerprint residue relatively constant throughout the week.

3.4. Discussion

From a forensic point of view, insight into potentially distinguishing properties of fingermarks is needed to increase the chances of individualizing or categorizing perpetrators. Previous research indicated differences in the chemical composition of

Table 3.10: Confusion matrix of cforest classification model for donor fingerprint, based on LC-MS data of natural fingerprints with an overall 100% accuracy (CI: 73.5% - 100%).

		Reference			
		Donor 1	Donor 2	Donor 3	Donor 4
Prediction	Donor 1	3	0	0	0
	Donor 2	0	3	0	0
	Donor 3	0	0	3	0
	Donor 4	0	0	0	3

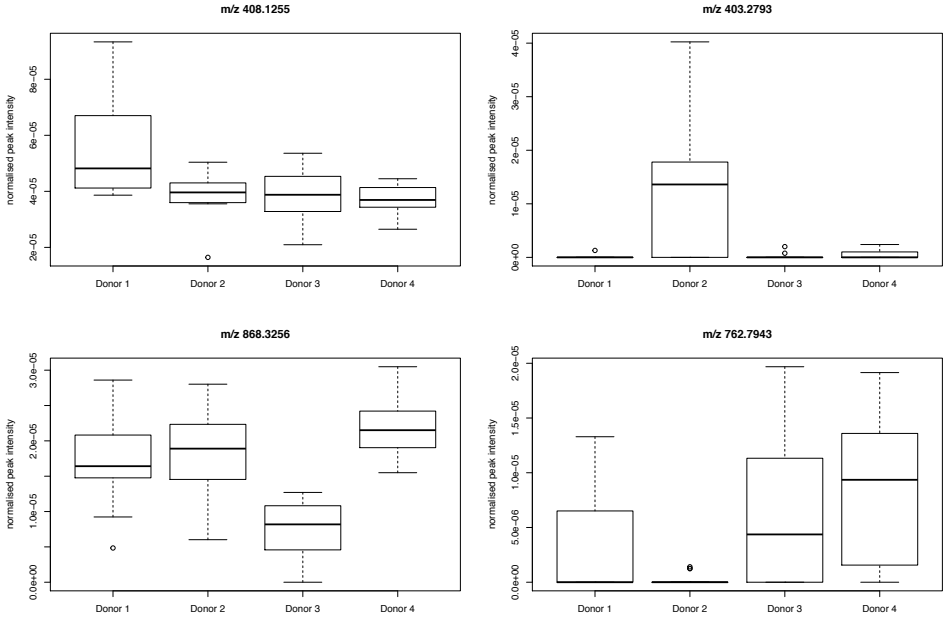

Figure 3.6: Boxplots of the most important predictors per donor. From left to right, top to bottom, the most important predict for donor 1, 2, 3 and 4, respectively.

fingermark residue, but advanced knowledge was still missing. The primary objective of this study was to identify differences in the composition of fingermark residue in relation to certain donor conditions. Various distinguishing compounds have been found in eccrine, sebaceous and natural fingerprints. The inter-variability in both natural and eccrine or sebaceous fingerprint residue was found to be large. It must be noted that deposition pressure, time and surface area were not controlled in this study. The intra-variability was found to be large as well, ranging from 39.1% to 66.9% (RSD) during 5 days. Recent work by Dorakumbura et al. into intra-donor variability, using squalene as indicator, showed that the percentage difference of squalene from deposits from two hands varies between 4-100%, when deposition pressure is not controlled [63]. In comparison, in our study, without controlling deposition pressure, time and surface area, the percentage difference of total amino acid varied between 15.0 - 100% during 5 days. Intra-day variability was found to be marginally lower than the intra-week variability. The large intra-variability partly explains the large inter-variability found in our study. The intra-variability of many compounds remains largely unknown, but clearly could be significant as well if no consistent deposition force is used. Since only a single fingerprint per donor per analysis method (LC-MS or GC-MS) and fingerprint type (natural/ eccrine/ sebaceous) was analyzed in our large data set, it remains uncertain what implications this has on found markers in practice. Moreover, since natural fingermarks can be subjected to numerous external factors, it cannot be verified that the markers found are a result of metabolic changes. Nevertheless, natural fingermarks best represent the residue that may be found in (fresh) fingermarks in practice. However, since fingerprint constituents in this study were collected under controlled conditions using only one type of substrate and directly dissolved, the influence of time, external factors (such as temperature changes, humidity and light exposure) and substrate remains unknown as well. Moreover, to better characterize the pure chemical ranges of variability (both between and within donor) in fingerprint composition, an experiment where deposition pressure, time and surface area are controlled should be performed.

Additionally, it must be noted that it remains uncertain if the detected potential markers would survive prior visualization of latent fingermarks, as fingerprints constituents were directly dissolved in this study. Further research should focus on the compatibility of analytical techniques with common fingermark detection techniques, such as cyanoacrylate fuming. Alternatively, the development of extraction techniques that leave the fingermark ridge pattern unmarked, such as the use of hydrogels [64], could potentially overcome this problem as well.

In the targeted approach, compounds were found that could serve as potential metabolic marker for gender, cannabis usage and alcohol consumption. The proposed generalized logarithm that was used in this approach to circumvent the zero-value problem in log-ratios might affect inference. Nevertheless, it was assumed this did not have any major implications, as it tends to preserve the original order of magnitudes – and it seems a more appropriate method than the commonly used 'half minimum imputation'. Alternative approaches for censored data are described by Helsel [65]. Additionally, some conditions have highly unbalanced sample sizes. These included fairly unique traits, such as use of drugs, for which research ethics committees

generally have strict rules. Therefore, in spite of the unbalanced sample sizes, these conditions were taken into consideration. It remains uncertain if this had implications on the permutation tests.

In the untargeted approach, classification models were developed based on the full scan MS-data to retrieve information about donor traits based on fingerprint composition. Accurate prediction of donor gender and smoking habit, would be valuable information to forensic investigators, as it would enable significant reduction of the suspect population. In case of the model to predict smoking habit, *m/z* values corresponding to nicotine and cotinine were the most important predictors. Classification accuracy might improve further by setting environmental cutoff levels, as was proposed by Ismail et al. for cocaine and heroin [66]. These could potentially correct for detection of nicotine or cotinine which results from environmental contamination, such as passive smoking. It must be noted that the suggested putative annotations of the *m/z* values incorporated in the classification models can only be considered to be level 2 or 3 metabolite identifications, as defined by the 2007 metabolomics standards initiative [67]. They should serve as targets for further research, in which higher levels of annotations of these predictors should be achieved. Moreover, the quantitative analyses in this approach are based on the peak intensity normalized to the total peak sum, which is arguable. The fact that the relative change in L-phenylalanine was similar to the internal standard corrected targeted approach is an indication that this approach is valid.

Several putative exogenous compounds originating from cosmetic and personal care products were found, in line with previous findings from Bouslimani et al. and Hinners et al. [19,20]. However, constructing accurate classification models based on these compounds was unsuccessful. This is likely a result of grouping different personal care and cosmetics products together in the questionnaire, while these compounds may be specific to one class of products. Analysis of these exogenous compounds on a more specific fingerprint-to-fingerprint basis, as was executed in the aforementioned studies, might be more suitable. Using an untargeted approach based on the full-scan data of the 56 fingerprints from 4 donors, a distinction could be made between the 4 different donors. These results are in line with recent findings from Gorka et al., who described the differentiation of fingerprints from 4 donors using MALDI-MSI [8]. Fairly unique features seem to be present in fingerprints from different donors, which can potentially be used in their discrimination. These could be exogenous compounds, a result of contact with certain products or items, which are part of the daily routine of the donor.

In this study, many donor traits, such as all the (medicinal) drug use and donor age, did not yield significant changes in targeted metabolite levels and were neither successfully predicted in the untargeted approach. In many of these cases, sample sizes were highly unbalanced. Future studies with larger, more balanced sample sizes, should be executed to investigate the feasibility of deriving information about these donor traits from fingerprint composition. The fact that smoking habit could be predicted with relative high accuracy based on this data set is a promising lead for the development of classification models for similar stimulants based on fingerprint chemical composition.

3.5. Conclusion

A database of chemical profiles from 1852 fingerprints, collected from 463 donors was successfully constructed. A large inter-variability in all analyzed types in fingerprints was found. Total amino acid levels were found to range from below 100 ng to 10 µg. The variability in total lipid content ranged from 100 ng up to 100 µg in natural fingerprints, while the lipid ranging from about 1 µg to over 100 µg in sebaceous fingerprints. In a targeted metabolomics approach, L-phenylalanine, L-(iso)-leucine and palmitoleic acid were found to differ significantly between male and female donors. Moreover, L-alanine levels were found to differ for donors who indicated to have used cannabis while L-proline levels differed for donors that consumed a large amount (>15 units) of alcohol. The targeted amino acid and lipid compounds alone were, however, insufficient to successfully derive donor information from fingerprint composition. Based on total amino acid content of 56 fingerprints from 4 donors, the intra-variability was analyzed as well, and was found to range between 15.0 - 100%, without controlling the deposition time, pressure and surface.

In an untargeted approach, classification models for fingerprint type, gender and smoking habit were constructed using the large data set. Based on the full-scan data, models could accurately discriminate between the fingerprint type (95.3% and 86.8% accuracy for LC- and GC-MS, respectively). Gender could only be predicted with moderate accuracy based on natural fingerprints analyzed by LC-MS (77.9%). Surprisingly, putatively annotated metabolites from the GMP degradation pathway serve as predictors in this model, which pose as interesting targets for further research. Everyday smoking habit was accurately predicted in both natural and eccrine fingerprints (90.4% and 90.2% accuracy, respectively). Smoking habits in the past 24-hours could be predicted with slightly lower accuracy. In these models, *m/z* values corresponding to nicotine and cotinine were the most important predictors. Using a similar approach based on the full-scan data of the 56 fingerprints from 4 donors, a distinction could be made between the 4 different donors. Fairly unique features seem to be present in fingerprints from different donors, enabling their discrimination. The results presented in this chapter are promising leads for further investigations into retrieving donor information from the chemical composition of fingerprints. Further analysis is needed to validate the potential metabolic markers found.

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4

Analysis of amino acid enantiomers from aged fingerprints

The task is not to see what has never been seen before, but to think what has never been thought before about what you see every day.

Erwin Schrödinger

Chapter 3 demonstrated how the chemical composition of fingerprints differs both between and within donors, and what information can be retrieved by analyzing that composition. Using these methods, information could potentially be retrieved from fingermarks of poor-quality or fingermarks that lack of a reference. However, all this data is not helpful if the fingerprint cannot be linked to the crime itself. In this chapter, another application of the analysis of the chemical composition of fingerprints is presented: estimating a fingerprint's age. In contrast to the previous chapter, this is particularly valuable for fingerprints that did lead to an individualization. The ability to estimate a fingerprint's time of deposition would enable forensic professionals to link the identified fingerprint to the time of the crime. Thus, the aim of this chapter is to pioneer a method to estimate fingerprint age based on the ratio of L- and D-amino acids. In the first part, an overview of previous research into estimation of fingerprint age will be given. Then, the development of a UPLC-QqQ-based analytical method to retrieve, separate and subsequently relatively quantify L- and D-amino acids from fingerprints is described. Finally, the results of the analysis of fingerprints that were aged up to 6 months are presented.

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4.1. Introduction

Fingerprints can be crucial evidence in criminal investigations. The unique ridge detail can be used to individualize or exclude potential perpetrators, if the fingermark is of sufficient quality and the reference fingerprint is present in the database. It is generally accepted that a fingermark found on an object is established by contact of the donor's finger and the object. However, the time at which this contact has taken place, which can be crucial to link the perpetrator to the crime, can at present not be derived from the fingermark. Therefore, fingerprint age estimation has been a topic of interest in the past decades [2].

The main focus has been on using chemical changes in the composition of fingermark residue to estimate the time of deposition. After deposition, the molecules that make up a fingerprint are subject to degradation, such as hydrolysis and oxidation reactions [2]. Several investigations aimed at these types of chemical changes to predict the age of a fingerprint. Studying fingerprint ageing using gas chromatography mass spectrometry (GC-MS), Archer et al. described the degradation of fatty acids and squalene in fingerprints after deposition on a surface [3]. Weyermann et al., based on GC-MS analyses as well, suggested a ratio between squalene and cholesterol as potential predictor for fingerprint age [4]. In subsequent research, Koenig et al. proposed to add wax ester compounds to the equation to reduce variability in initial composition [5]. Pleik et al. focused on the identification of degradation products of common fatty acids in fingerprints as potential tool for age determination [6]. Van Dam et al. used fluorescence spectroscopy to determine the relative amount of fluorescent oxidation products to estimate the age of fingerprints from male donors up to three weeks old, within several days' accuracy [7]. Alternatively, Oonk et al., using a proteomics approach, suggested several potential protein markers to estimate fingerprint age [8]. More recently, Hinners et al., suggested the ozonolysis of triacylglycerols as a means of determining the age of a fingerprint, and showed its potential as age marker in fingerprints up to one week old [9]. However, environmental factors such as temperature, humidity and light exposure often complicate accurate fingerprint age estimation.

Another potential drawback in many age estimation methods is that the starting concentrations at deposition are generally unknown and may vary largely, which could greatly affect the accuracy of the estimation. Targeting relative concentrations between fingerprint components could potentially overcome these issues, as was suggested by Van Dam et al. and Weyermann et al. [4, 7]. A method widely used in the fields of geochemistry and archaeology as dating tool for samples such as fossil bones and sediments, is amino acid racemization [10–12]. These methods are based on the fact that the biologically predominant and optically active L- enantiomer usually racemizes over time when it is isolated from the biological processes that maintain the optical activity, eventually leading to a racemic and optically inactive mixture [13]. Commonly used age determination methods are based on the ratio of D/L-enantiomers of aspartic acid [14].

In the aforementioned fields, separation of the amino acid enantiomers has been achieved using various analytical techniques. GC, capillary electrophoreses (CE) and (ultra) high performance liquid chromatography ((U)HPLC) are the most used meth-

ods to separate amino acid enantiomers [12, 15–18]. When not using chiral stationary phase columns in liquid chromatography, derivatization of the amino acids prior to analysis is often essential, which is based on the formation of diastereomers by reaction with a chiral derivatizing agent [16]. Commonly used agents are 1-fluoro-2-4-dinitrophenyl-5-L-alanine amide (FDAA or Marfey's reagent), 1-(9-fluorenyl) ethyl chloroformate (FLEC), N-(4-nitrophenoxycarbonyl)-L-phenylalanine 2-methoxyethyl ester (S-NIFE), 2,3,4,6-tetra-O-acetyl-β-d-glucopyranosyl isothiocyanate (GITC) and o-phthaldialdehyde (OPA) with chiral thiols [16, 19].

As amino acids are a commonly found component in fingerprint residues [20], presumably in the naturally predominant L-enantiomer, it was investigated if amino acid racemization could be a viable option for fingerprint age estimation. A method to separate and relatively quantify amino acid enantiomers from fingerprints using FLEC and ultra-high-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) was developed. FLEC was chosen as derivatization reagent as the reaction is relatively fast, and formed products are stable [21]. FLEC reacts with primary and secondary amines to form diastereomers, adding a nominal mass of 236 Da to the amino acid molecular mass. As a proof of principle, fingerprints from 6 different donors up to 6 months old are analyzed.

4.2. Materials and methods

4.2.1. Solvents and chemicals

L-alanine (≥98%), D-alanine (≥98%), L-serine (≥99%), D-serine (≥98%), L-threonine (≥98%), D-threonine (≥98%), L-valine (≥98%), D-valine (≥98%), L-histidine monohydrochloride monohydrate (≥98%), D-histidine monohydrochloride monohydrate (≥98%), L-methionine (≥98%), D-methionine (≥98%), L-proline (≥98%), D-proline (≥99%), L-phenylalanine (≥98%), D-phenylalanine (≥98%), cis-4-Hydroxy-D-proline (≥98%), (-)-(9-fluorenyl)ethyl chloroformate solution and sodium tetraborate decahydrate were purchased from Sigma-Aldrich (Zwijndrecht, the Netherlands). UPLC-grade acetonitrile (ACN), methanol (MeOH) and formic acid were purchased at Biosolve (Valkenswaard, the Netherlands). Ultrapure water was obtained by purifying deionized water to attain a resistivity of 18 MΩ-cm at 25 °C.

4.2.2. Fingerprints and standards

For a baseline measurement of fresh fingerprints (composition at t=0), fingerprints (left and right thumb) from 40 donors (20 male, 20 female, age ranging from 20-69) were collected on 76 x 26 mm glass microscope slides (Thermo Scientific, Breda, the Netherlands). Similarly, for aging experiments, fingerprints (left and right thumb) from 6 donors (3 male, 3 female, age ranging from 20-45) were collected on glass microscope slides and subsequently stored in the dark in a temperature-controlled room (at 21°C). Fingerprints were aged for 0, 7, 14, 21, 30, 60, 90, 120, 150 and 180 days. Fingerprint residue was collected from the surface using polyester swabs (CleanTips Polyester Alpha, Texwipe, NC, USA). Prior to swabbing, the swab was wetted with 50 µL methanol. After swabbing the swab was placed in a 0.5 mL Eppendorf tube and 200 µL methanol was added. The tube was vortexed for 1 minute and subsequently

placed in an ultrasonic bath for 10 minutes. After sonication, the sample solution was transferred to a 2 mL Eppendorf tube while the swab was transferred to a spin basket and subsequently placed on the Eppendorf tube. The tube was centrifuged for 10 minutes at 13000 rpm. After centrifugation, the sample was transferred to a 2 mL LC injection vial and 20 µL of internal standard (50 mg/L hydroxy-D-proline in methanol) was added. Subsequently, the sample was evaporated under nitrogen flow at room temperature. After evaporation, 50 µL of 0.16 M borate buffer and 50 µL of 18 mM FLEC solution in acetone were added. After 20 minutes of incubation at room temperature, 100 µL 70:30 acetonitrile: ultrapure water (containing 1.5% (v/v) formic acid) was added. Prior to LC-MS/MS analysis, the samples were filtrated using a 0.45 µm syringe filter. The calibration standards were prepared out of a 10 mg/L stock solution containing a 50:50 mixture of each of the D- and L-amino acid enantiomers in methanol. Calibration series were prepared in duplicate ranging from 0.2 to 1.0 mg/L and were prepared by transferring the required volume of stock solution directly to an injection vial. The addition of internal standard and the derivatization using FLEC were executed as described above. After derivatization, 550 µL acetonitrile, 350 µL ultrapure water and 15 µL formic acid were added and the solution was vortexed for 1 minute. All samples were filtrated using a 0.45 µm syringe filter. All samples were analyzed in triplicate. The percentage of D-amino acid is calculated by dividing the peak area of the D-enantiomer by the sum of the D- and L-enantiomer as shown in equation 4.1:

$$\%D = \frac{\text{Area}_{\text{D-enantiomer}}}{\text{Area}_{\text{D-enantiomer}} + \text{Area}_{\text{L-enantiomer}}} * 100\% \tag{4.1}$$

To test the accuracy of the determination of the ratio of D- and L- amino acid enantiomers, a calibration set with varying ratios of D- and L-amino acids was prepared from stock solutions consisting of 7 samples with L/D ratios of 100:0, 95:5, 90:10, 80:20, 70:30, 60:40, 50:50 approximately, adjusted for enantiomeric purity of amino acids.

4.2.3. UPLC-MS/MS

Separation was performed using an Acquity UPLC with an ethylene bridged hybrid (BEH) C_{18} 1.7 µm, 2.1 x 150 mm, column (Waters, Milford, MA, USA). Solvents used were acetonitrile containing 0.4% (v/v) formic acid (A) and H2O:MeOH (95:5) containing 0.4% (v/v) formic acid (B). A flow rate of 400 µL/min was used and a gradient starting at 75% A was programmed. A linear increase of solvent B to 29% after 10 minutes, followed by a increase to 33% after 20 minutes was programmed. Solvent B was then increased to 39% after 25 minutes and held constant for 20 minutes. Finally, the column is flushed for 4 minutes by increasing solvent B to 80%, followed by re-equilibrating the column for 5 minutes to 75% A (total run time of 55 minutes). An injection volume of 1 µL was used. The column eluent was directly analyzed using MS/MS using a triple quadrupole (QqQ) mass spectrometer (Thermo Scientific Quantiva, Breda, the Netherlands), operated in positive mode. The ESI conditions were set as follows: a spray voltage of 3.5 kV was used, the sheath gas, aux gas, and sweep gas

were set to 45, 13 and 1 (Arb), respectively. The ion transfer tube was set to 342 °C and the vaporizer temperature was set to 358 °C. The cycle time was set to 1 second, the Q1 and Q3 resolution were set to 0.7 FWHM. The collision gas pressure was 1.5 mTorr and the source fragmentation was set to 0 V. The optimized MRM parameters can be found in Table C.1.

4.3. Results

Using FLEC derivatization and the developed UPLC-QqQ method, baseline separation of the D/L-enantiomers of histidine, serine, threonine, alanine, proline and valine was achieved (Figure 4.1). Complete separation of the enantiomers of methionine and phenylalanine was not achieved. The baseline-separated amino acids were included in this study as well as methionine, even though complete baseline separation was not achieved. The limit of detection (LOD) and limit of quantification (LOQ) of the 7 amino acids used in this study are presented in Table C.2.

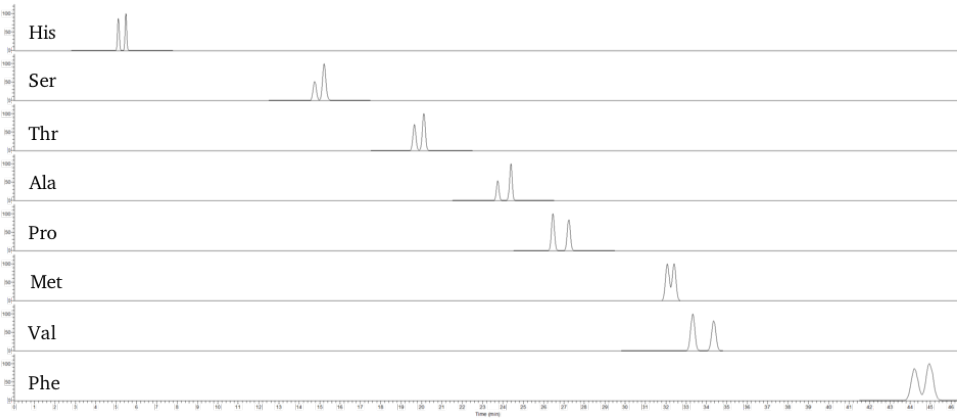

Figure 4.1: UPLC-MS/MS chromatograms of the 8 amino acid enantiomers, showing their separation in the 46-minute gradient.

To verify the ability of the method to accurately quantify different ratios of L- and D-amino acids, standards with varying ratios of these enantiomers were analysed, ranging from 100% L-amino acid to a 50:50 mixture (racemic equilibrium, Figure 4.2). For all 7 amino acids included in the method, reasonable linearity was achieved (R^2>0.98). Especially important for the application of this method is the performance in the low range, which seems to be slightly poorer for alanine. Moreover, the percentage of D-enantiomer seems to be slightly overestimated in case of threonine, proline and valine. Subsequently, the abundance of the D-enantiomer of these 7 amino acids in freshly deposited fingerprints from 40 donors (Figure 4.3) was determined, as the D/L-ratio of the amino acids in freshly deposited fingerprints is an important factor in this study. To be suitable for age estimation, the percentage of D-amino acids in different fingerprints at the time of deposition would ideally be close to zero with low variability between donors.

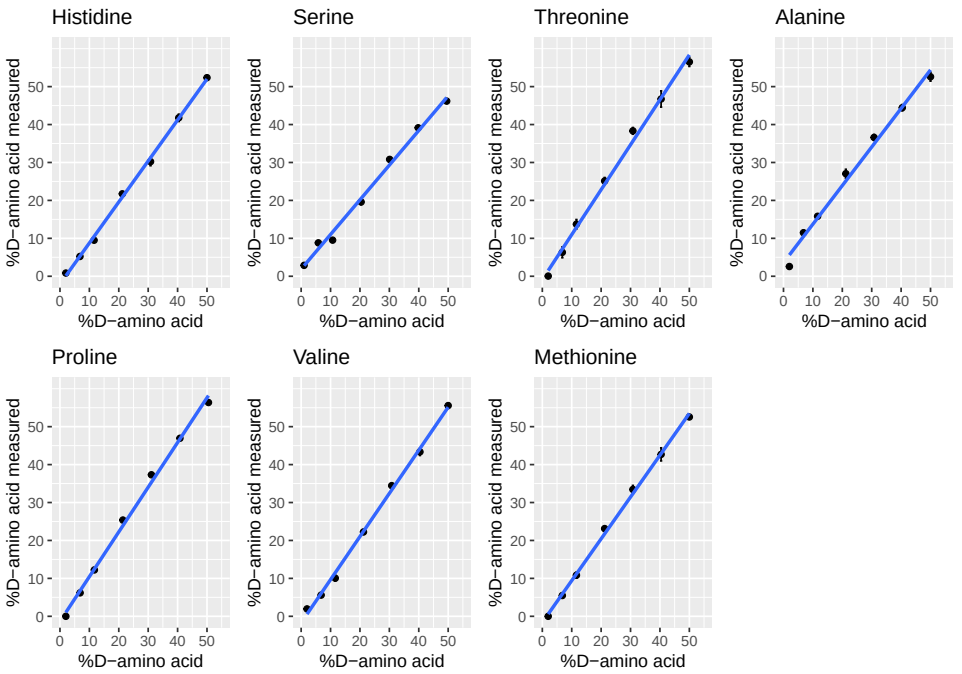

Figure 4.2: Calibration results of analysis of varying ratios (L : D = 100:0, 95:5, 90:10, 80:20, 70:30, 60:40, 50:50, adjusted for purity) of D- and L-amino acids of histidine (R^2 = 0.996), serine (R^2 = 0.996), threonine (R^2 = 0.987), alanine (R^2 = 0.992), proline (R^2 = 0.988), valine (R^2 = 0.991) and methionine (R^2 = 0.994).

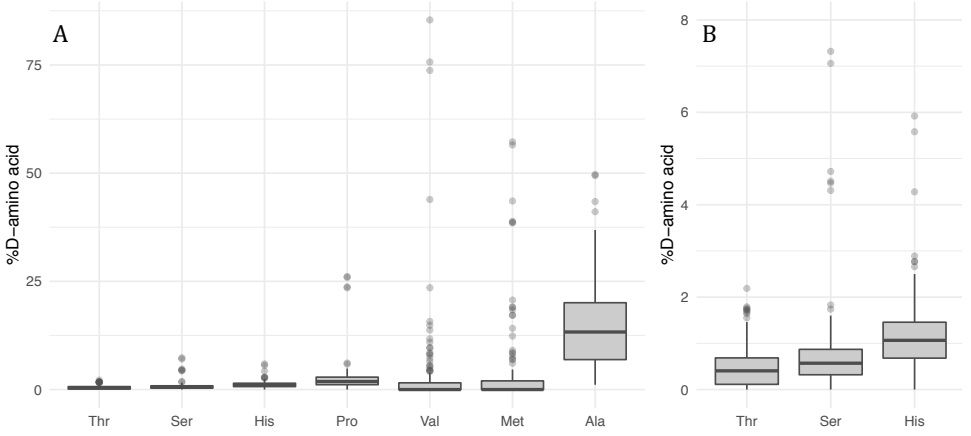

Figure 4.3: **A**: The percentage D-amino acid of threonine, serine, histidine, proline, valine, methionine and alanine in freshly deposited fingerprints of 40 donors. **B**: enlargement of the percentage D-amino acid of threonine, serine and histidine.

The average percentage of D-amino acid directly after deposition is generally low for threonine, serine and histidine, combined with a relatively low variability. Proline, valine and methionine have a slightly higher content of D-amino acid in fresh fingerprints. Lastly, alanine appeared to have the highest percentage of D-amino acid in fresh fingerprints combined with a high variability among donors. To study the effect of fingerprint ageing on the ratio of L- and D-amino acids, the enantiomer ratios of the included amino acids were determined from fingerprints aged for up to 6 months. In the 6-month period, significant changes in D/L- ratio were only observed for serine. In case of serine (Figure 4.4), a steady increase is observed for all donors with increasing fingerprint age during the first 30 days. After 30 days, D-serine has increased to over 1%, while further increasing to over 5% for 3 of the 6 donors in the 6-month period. For the other 3 donors, the %D-serine seems to eventually level-off, and even decrease after 120 days. As fingerprint age increases, variability increases as well, as can be deduced from the increasing standard deviation. The large deviation at 180 days however, is mainly caused by one donor (D4). No significant increase in D-enantiomers with time was found in case of the other 6 amino acids, resulting from problems with detectability and variability for these amino acids in aged fingerprints (data not shown).

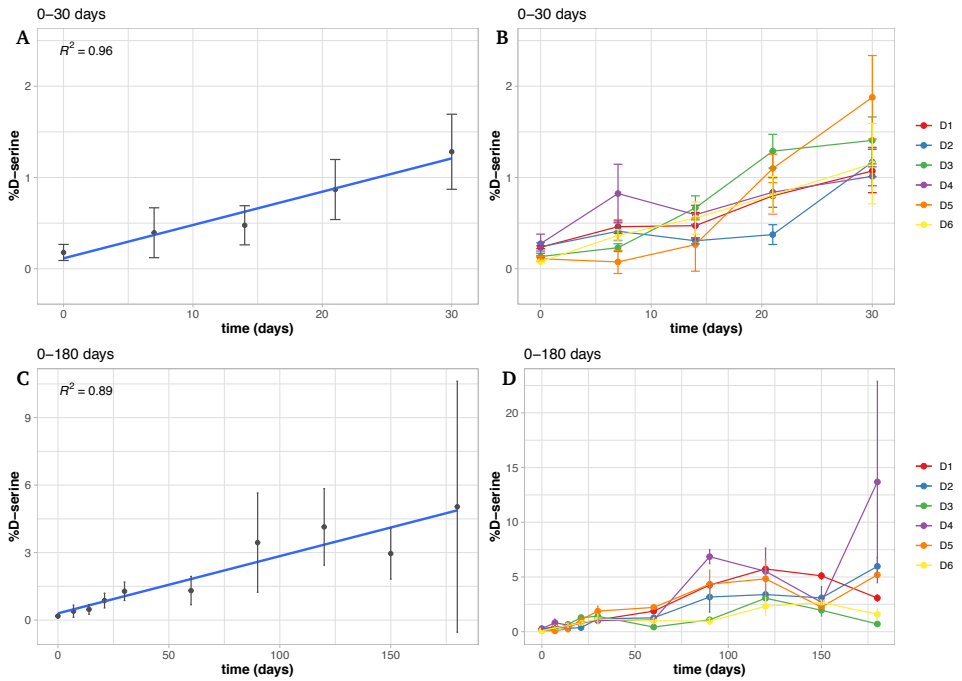

Figure 4.4: The percentage D-serine in aged fingerprints of 6 donors, aged up to 1 month (**A** and **B**) and up to 6 months old (**C** and **D**), displayed as overall for the 6 donors (**A** and **C**), and per individual donor (**B** and **D**).

4.4. Discussion

The aim of this study was to investigate the feasibility of using amino acid racemization to determine fingerprint age. In the developed method, using FLEC and UPLC-MS/MS, separation of 8 amino acid enantiomers was achieved within 46 minutes. In comparison, Einarsson and Josefsson, the first to describe the enantiomeric separation of amino acids using FLEC, achieved baseline separation of 17 pairs of D/L-amino acids in 70 minutes [22]. In the study presented here, it was possible to determine the D/L-ratio of 7 amino acids from fingerprints. When analysing the enantiomeric ratios of fresh fingerprints based on a set of 40 donors, threonine, serine and histidine showed a low variability combined with a low concentration of the D-enantiomer. After analysis of fingerprints from 6 donors up to 6 months old, only an increasing trend of the D-enantiomer was seen in case of serine for all fingerprints up to 30 days. Thereafter, for 3 donors a further increase to over 5% relative D-serine was observed, while for the other 3 donors, the initial increase is leveling off, even followed by a decrease after 150 days. Also evident is the increase in variability after the first 30 days. This shows there is potential to discriminate age across for weeks within the first 30 days. The eventual decrease, however, could be detrimental for the use of D-serine as age marker, as this would logically complicate the distinction between fingerprints with different ages. Further research is needed to develop a method which is more sensitive to the detection of the amino acids in order to accurately quantify the ratio. As each amino acid likely racemizes with a different rate, possibly different amino acids would be suitable for age estimation depending on the precise fingerprint age. In the fields of geochemistry and paleontology however, estimation is commonly done based on the enantiomers of a single amino acid, such as isoleucine or aspartic acid in quaternary science [23].

Compared to previously suggested fingerprint age determination methods, the developed method offers similar advantages as described by Van Dam et al. and Weyermann et al., by looking at ratios of potential age markers [4, 7]. Looking at the enantiomers of an amino acid however, offers the potential additional advantage of being less sensitive to the unknown starting amount and the unknown rate at which degradation has taken place. This is under the assumption that the enantiomeric ratio is independent of the starting amount and that both amino acid enantiomers are subject to the same rate of chemical degradation. When it comes to the timescale, the age estimation methods described by Hinners et al. and Van Dam et al. analyzed fingerprints up to 1 week and 3 weeks old, respectively [7, 9]. The ratio of serine enantiomers could potentially extend this timescale of fingerprint age estimation methods, possibly up to several months. It is important to note that, eventually, the concentration of amino acid enantiomers will drop below the LOQ and thus analysis of the D/L-ratio will no longer be possible.

A significantly higher amount of the D-enantiomer for alanine compared to the other amino acids in freshly deposited fingerprints was found. This was not observed when amino acid stock solutions, containing different D/L-ratios, were analysed. Interference with other fingerprint constituents could potentially influence accurate determination of the D/L-ratio. Additionally, the variability in D-alanine in fresh fingerprints was found to be large, and as such, D-alanine was not a reliable marker for age

estimation of the fingerprint deposition. This possibly is a result of environmental contamination, via consumption of food or the use of cosmetics, although it is unlikely this would only affect alanine.

Amino acid racemization in fingerprint residue is a previously unexplored area. It is well-known that the acidity plays an important role in the amino acid racemization rate [24]. The pH of fingerprint residue however, is unknown, and likely is variable both within and between donors. Additionally, fresh fingerprints consist of 20-70% water, as was recently reported by Keisar et al. [25], but will eventually dry up, since evaporation will start right after deposition. The precise mechanism of amino acid racemization in fingerprints therefore remains unknown and requires further research.

Overall, D-serine shows a promising trend for all fingerprints up to 30 days old. In older fingerprints, variability increases as for some donors a further increase is seen, whereas for others a decrease is observed. More research is needed, using larger data sets based on more donors, to elucidate the precise trend of D-serine with fingerprint age, while simultaneously investigate the behaviour of the other amino acid enantiomers. Additionally, it must be noted that the deposition pressure and time were not controlled in this study. The fact that a trend for D-serine was still observed, shows the potential of fingerprint dating based on amino acid racemization in practice. Some key parameters, such as temperature, humidity, light exposure and substrate were controlled. The influence of these factors thus remains unknown, and whereas the use of the serine enantiomer ratio in fingerprint dating could potentially overcome the issue of having unknown starting amounts, these factors likely influence the racemization rate as well. Next to confirming the potentially useful trend of D-serine, future studies should thus investigate the influence of parameters such as temperature, humidity and light exposure as well, to gain more insight in the applicability of the D/L-amino acid ratio for fingerprint dating.

4.5. Conclusion

In this chapter, the development of an UPLC-MS/MS method to determine the D/L-ratios of 7 amino acids from fingerprints, after derivatization using FLEC is presented. In order to investigate the potential of D/L-amino acid ratios for use in fingerprint age estimation, freshly deposited fingerprints from 40 donors as well as fingerprints aged up to 6 months old from 6 donors were analyzed. In case of threonine, serine and histidine, a low concentration of the D-enantiomer in freshly deposited fingerprints was found. Analysis of aged fingerprints only showed a potentially useful trend for D-serine, which increased with fingerprint age for all donors up to 30 days. Thereafter, a further a further increase was seen in case of 3 donors, while an eventual levelling off followed by a decrease was detected for the other 3 donors. Further studies are needed, using larger dataset, to confirm the potentially useful trend seen for D-serine and investigate the behavior of the other amino acid enantiomers. Additionally, analysis should also focus on investigating the influence of temperature, humidity and substrate, and extend the timescale of the study. The use of D-serine poses as an interesting target for fingerprint age determination methods, as it overcomes the issue of having an unknown amount at the time of deposition.

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5

Information from exogenous compounds in fingerprints

Science is built up of facts, as a house is built of stones;
but an accumulation of facts is no more a science
than a heap of stones is a house.

Henri Poincaré

Chapters 3 and 4 described how either donor or time-of-deposition information could be retrieved via analysis of common metabolites from fingerprints. In addition to these metabolites, fingerprints can contain exogenous compounds. These can end up in a fingerprint when an individual touches certain compounds or surfaces. Exogenous molecules can originate from a wide variety of sources, ranging from make-up and other personal care products to illicit drugs and explosives. Although interpretation of the presence of these molecules in fingerprints seems straightforward, for each class of exogenous compounds, separate studies need to be set up before this information can be implemented in casework. For instance, the ability to retrieve particular information about the condom in cases of sexual assault would be valuable for forensic investigators. This chapter aims to establish intelligence about the brand and type of condom touched by analyzing the lubricants present in a fingerprint using DESI-MS. The development of a mass spectrometry imaging method that is compatible with a common fingerprint detection method is described, combining chemical information with spatial information in the form of ridge detail.

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5.1. Introduction

Sexual assault is a major crime problem. When the identity of the perpetrator is unknown, the most important traces in sexual assault cases are often of biological origin, such as blood, semen, saliva and hair. This is due to the fact that DNA can be retrieved and analyzed from these traces, allowing for the identification or exclusion of possible criminals. Nowadays, biological material becomes increasingly harder to find in cases of sexual assault, potentially because awareness of the importance of DNA in forensic techniques and evidence in the justice system is growing among criminals [2]. Because of this awareness, as well as alertness to sexual transmitted diseases, criminals are becoming more vigilant in leaving biological traces and as a result the use of condoms in sexual assault cases has increased over the past decades [3–6]. Other types of trace evidence may thus be required to establish a link between victim and criminal. In the past, studies have shown that a condom can be a critical piece of evidence in sexual assault cases [4, 7, 8]. The analysis of condom derived traces can thus be of significant associative evidential importance. Ideally, not only confirmation of the presence of the condom but also determination of the type of condom brand used can be retrieved.

In the last decades several studies have been performed to develop methods to detect traces of condoms [9, 10]. Most of these studies focused on the lubricants that are added to the condoms by manufacturers. Indeed, primary components of lubricants such as polydimethylsiloxane (PDMS) and polyethylene glycol (PEG) were found to be detectable by desorption chemical ionization mass spectrometry [11], pyrolysis gas chromatography mass spectrometry (pyGC-MS), GC-MS [12], Raman spectroscopy [13] and Fourier transform infrared spectroscopy (FT-IR) [11, 14]. Another specific component in case of spermicide containing condoms, nonoxynol-9 (N9), could also be identified by FT-IR [11], GC-MS [15] and liquid chromatography mass spectrometry (LC-MS) [14]. Multiple studies examined the possibility to discriminate different types of condoms. Maynard et al. described a two-step method using FT-IR as a first screening tool, followed by either GC-MS, pyGC-MS or LC-MS as a confirmation method, enabling them to uniquely identify 11 types of condoms [14]. Burger et al. showed that capillary electrophoreses may also be a promising technique for classifying both condom and personal lubricants, although it remained unclear which discriminating constituents were used in the analysis [16].

However, most of these analytical techniques require sample preparation and/or extraction which may be time consuming and result in loss of the initial trace evidence. Additionally, the method itself may also limit the amount of information retrieved from the sample. For instance, analysis of silicone lubricants by GC-MS requires pyrolysis of the lubricant [12], that can cause degradation of minor components. These minor components were found to be an important differentiating factor in distinguishing sexual lubricants and personal hygiene products, which contain similar major components such as PDMS and PEG [17]. Also, preservation of the original evidential trace can be of great interest in forensic science. In this respect, the use of ambient ionization mass spectrometry techniques is more favorable. A popular technique that has been used in recent years is direct analysis in real time (DART) MS, that has been shown to be a very effective tool for the detection of both the major and minor com-

ponents of condom lubricants, without the need to extensively prepare the sample or potential loss of evidence [18–22]. Furthermore, DART analysis is highly effective in discriminating lubricants. Baumgarten et al. and Maric et al. successfully discriminated condom and personal lubricants using DART-Time-of-Flight (TOF) MS analysis [20, 21]. Using a DART-High Resolution MS (HR-MS) analysis technique, Coon et al. could rapidly generate diagnostic chemical fingerprint signatures of 110 condoms, enabling them to discriminate condoms of 16 different brands [22]. However, a disadvantage of DART analysis is that this technique is unable to retrieve spatial chemical information from the samples, such as fingerprints containing lubricants.

Lubricated fingerprints are likely to be found at a crime scene of sexual assault, as handling of a condom will transfer the outer coating of the condom onto the perpetrator's fingerprints, potentially leaving condom lubricant contaminated fingerprints behind [23, 24]. Detection of a lubricant from a fingerprint found at a sexual assault scene, would greatly increase the strength of the evidence, as it not only establishes contact with a condom but also indicates the presence of the criminal at the crime scene [23]. Bradshaw et al. developed a method for the visualization of condom lubricant within a fingerprint by mapping the fingerprint ridge pattern using Matrix Assisted Laser Desorption/Ionization Mass Spectrometry Imaging (MALDI-MSI) [23]. In a follow-up study they showed that differentiation of 6 different condoms in lubricated fingerprints was possible using MALDI-MSI, Raman spectroscopy and ATR-FTIR imaging in a synergistic manner [24]. Besides MALDI-MSI, desorption electrospray ionization (DESI) MSI has also been shown to be a powerful analytical tool. Whereas MALDI offers excellent spatial resolution ~20 µm, one of the disadvantages is the necessity to apply matrix solution to the sample [25]. This lengthens the process and modifies the samples in some respects. One of the advantages of DESI-MSI is that samples require no sample preparation and can be readily analyzed. Using DESI, typically spatial resolutions of ~100 µm can be achieved [25]. However, the choice of the electrospray solvent composition is essential, as the interaction between the electrospray and the surface greatly influences the sensitivity and spatial resolution [26]. Mirabelli et al. were able to generate chemical images of latent lubricated fingerprints deposited on different surfaces and of different ages using DESI-MS [27]. The data acquired from DESI-MS analysis of lubricated fingerprints, combined with supervised pattern recognition statistical analysis (linear discriminant analysis (LDA) and soft independent modeling of class analogy (SIMCA)), enabled Mirabelli et al. to distinguish 10 different condom types with a 94% prediction ability for both LDA and SIMCA [28].

However, fingerprints in practice often need to be detected first, as they appear latent. One of the most used visualization techniques for latent fingerprints on non-porous substrates is cyanoacrylate (CA) fuming [29, 30]. Fingerprints from a sexual assault scene that have been analyzed at a forensic lab, are potentially treated with CA. In this regard, the aim of our study was to develop a method to analyze lubricated fingerprints, which was compatible with CA fuming and able to differentiate between different types of condoms. The developed method, based on DESI-MS analysis, is capable of generating chemical images, mapping common lubricant components in a lubricated fingerprint. Additionally, using this method, combined with a statistical

approach, principal component analysis (PCA) followed by linear discriminant analysis (LDA), differentiation between 32 types of condoms from 21 different brands was possible.

5.2. Materials and methods

5.2.1. Materials

UPLC-grade acetonitrile and formic acid were purchased from Biosolve (Valkenswaard, Netherlands). UPLC-grade methanol was purchased at Merck (Darmstadt, Germany). Cyanoacrylate was purchased from BVDA (Haarlem, Netherlands). Microscope glass slides were purchased from Thermo Fischer Scientific (Breda, Netherlands). 24-wells slides were purchased from Prosolia (Indianapolis, USA). Reference masses Purine (5 mM) and hexakis(1H, 1H, 3H-tetrafluoropropoxy)phosphazine) (HP-0921, 2.5 mM) were purchased from Agilent Technologies (Santa Clara, USA). Mitra microsampler tips (10 µL) were purchased from Neoteryx (Torrance, USA). A range of 32 different condoms were purchased at online pharmacy and condom websites (Table 5.1).

5.2.2. Lubricant samples

Lubricant of each condom was collected by swiping the interior of the condom package and both sides of the condom with a 10 µL microsampler until saturation. The microsampler is a volumetric absorption microsampling device (VAMS), that only absorbs 10 µl of sample. Hence, it provides more control over the amount of lubricant to be sampled, in comparison to cotton swabs, that absorbed too much lubricant. The condom lubricant was carefully transferred onto the 24-wells slide by slightly touching each well once with the lubricated microsampler tip. Each lubricant was spotted 12 times (n=12) on separate wells. As a control, each sample well was followed by a blank well. Slides were left to dry for at least 1 hour at room temperature (RT). Next, cyanoacrylate (CA, 0.5 gram heated to 120°C) fuming was performed on all slides, in a MVC1000 fuming system (Foster and Freeman LTD, Worcestershire, UK) for 10 minutes at 80% humidity. Slides were then left to dry overnight at RT before analysis.

5.2.3. Fingerprint samples

Fingerprints were donated voluntarily by a female and male donor, after giving informed consent. No ethical approval was obtained as the material was gathered in a noninvasive manner and did not infringe on any privacy of the donors. All experiments were carried out following institutional guidelines and according to relevant laws. All condoms (n=32) were handled by each donor (n=2). After touching the condom and the inside of the packaging, the lubricant was distributed over the finger. After 5 minutes of drying, fingerprints of each condom lubricant were deposited on microscope slides. From each donor, a blank fingerprint was used as a control (n=2). Slides were left to dry for at least 1 hour at RT followed by CA fuming, as described above. Fingerprints were then left to dry overnight at RT.

Table 5.1: The 32 condoms used in this study, the abbreviation used, the manufacturer and their country of origin.

Condom	Abbreviation	Manufacturer	Country
Billy Boy Extra Lubricated	BBEL	Mapa Health Care	Germany
Balance Condoom	BC	Condoom-anoniem	Netherlands
Beppy Soft Comfort	BSC	Beppy	Netherlands
Durex Classical Natural	DCN	Durex	UK
Durex Extra Safe	DES	Durex	UK
Durex Feeling Sensitive	DFS	Durex	UK
Durex Orgasmic	DO	Durex	UK
Durex Performa	DP	Durex	UK
Durex Real Feeling	DRF	Durex	UK
Durex XL Power	DXLP	Durex	UK
Euroglider	EU	Asha International / Euroglider	Netherlands
EXS Regular	EXS	LTC Health Care	UK
Fair Squared Original	FSO	Fair Squared GMBH	Germany
Glyde Ultra Naturelle	GUN	Glyde Health	Australia
Just Safe Standaard	JSS	Safe	Netherlands
Kruidvat Classic	KC	Kruidvat	Netherlands
Kruidvat Extra	KE	Kruidvat	Netherlands
Kruidvat Sensation Banana	KSb	Kruidvat	Netherlands
Kruidvat Sensation Chocolate	KSc	Kruidvat	Netherlands
Kruidvat Sensation Strawberry	KSs	Kruidvat	Netherlands
Kruidvat Ultra	KU	Kruidvat	Netherlands
LELO HEX Condooms	LH	Lelo	Sweden
Level Popular	LP	Your Levels BV	Netherlands
MoreAmore Soft Skin	MASS	Bizzy Diamond BV	Netherlands
MySize	MS	R&S Germany	Germany
Mates SKYN Original	MSO	Lifestyle Healthcare	Australia
ON Natural Feeling	ON	R&S Germany	Germany
Playboy Lubricated	PL	Playboy	USA
Pasante Naturelle	PN	Pasante Healthcare Ltd / Karex	UK
Startex	ST	ForeSee line	Belgium
Uniq Pull	UP	Uniq International	Colombia
Wingman	WI	Wingman	Netherlands

5.2.4. DESI-Q-TOF MS

Desorption electrospray ionization mass spectrometry (DESI-MS) data were acquired using an Agilent technologies (Santa Clara, USA) 6530 quadrupole time-of–flight (Q-TOF) MS equipped with a Prosolia (Indianapolis, USA) 2D-DESI. 24-wells sample slides were analyzed in dwell mode using positive polarity with the following parameters: spray voltage, 5 kV; nitrogen sheath gas pressure, 6.0 bar; drying gas flow, 8 L/minute, source gas temperature, 300°C; acquisition time, 200 ms; mass range, *m/z* 100-1200; inlet-to-surface distance, ~1 mm and tip-to-surface distance, ~3 mm. Combinations of several spray incident angles (52°, 45° and 35°) and tip-to-inlet distances (4, 5, 6, and 8 mm) were tested. Best results were achieved with an angle of 45° and a tip-to-inlet distance of 6 mm. A larger tip-inlet distance led to decreased carry-over, also described by Mirabelli et al. [28]. A dwell time of 20 seconds was used, with a post-acquire-delay time of 30 seconds in between the wells. To further avoid carry-over after analysis of each sample well, the next blank well was dwelled for 5 minutes before measuring the next sample. Additionally, the MS-inlet was cleaned after analysis of 3-4 slides to avoid carry-over, as also indicated by Mirabelli et al. [28]. Different spraying solvents were tested, namely a mixture of acetonitrile and water (90:10 v/v), acetonitrile, methanol and a mixture of methanol with water (90:10 v/v). All solvents contained 0.4% formic acid, 0.02 mM Purine and 0.025 mM HP-0921. Best result were achieved with a mixture of acetonitrile and water (90:10 v/v) when sprayed at a constant volumetric flow rate of 3 µL/minute, delivered by a syringe pump (Fusion 100, Chemyx, Stafford, USA). The 64 lubricated and 2 blank fingerprints were analyzed using the same settings, but instead of dwelling, 5 scans of 3 mm were measured within each fingerprint, using a 150 µm/second scan rate (totaling to 20 seconds) and a step size of 1 mm. MS full scan data were acquired with Agilent MassHunter Data Acquisition software (version B.08.00). Before data analysis, the first line of each analyzed fingerprint was removed from the results, as these often contained spectra with low intensities. This was likely a result of sample wetting, as described by Bodzon-Kulakowska et al. [31]. Lubricant components were putatively annotated using the online METLIN mass spectral metabolite database [32] and comparison with previously obtained results from literature.

5.2.5. Fingerprint imaging

Chemical images (12 x 20 mm) of a cyanoacrylated blank and EXS lubricated fingerprint were acquired using the same parameters as described above. The MS-inlet was cleaned after acquisition of each chemical image. Images were acquired using a 150 µm/second scan rate, resulting in a 30 µm pixel width and step size (totaling to 400 rows). Data were converted to imzML, using FireFly (v. 3.0.1.1, Prosolia, Indianapolis, USA), and subsequently analyzed using MSIReader (v1.01) [33].

5.2.6. Statistics

Data were converted to mzXML and the 5000 most abundant peaks were filtered using msConvert [34]. Data were subsequently processed with R (version 3.4.2) using R studio (Version 1.1.456) and the MALDIquant package [35]. Mass spectra were square-root transformed and normalized using the total ion current (TIC). Af-

ter aligning and averaging the spectra, peaks were detected using the corresponding MALDIquant functions. Principal component analysis (PCA) was then executed to reduce the data dimensionality. After splitting the data in a 75% training and 25% test set, the first 12 PCs (explaining 90% of the cumulative proportion of variance) were used to generate a linear discriminant analysis (LDA) model, using the MASS package [36]. Classification accuracy was evaluated by generating confusion matrices using the caret package [37].

5.3. Results

5.3.1. Detection of condom lubricants

As a first screening of the chemical components of condom lubricants, a detection method was developed based on the analyses of the 24-wells cyanoacrylated sample slides. Typical scans of lubricants from 4 different condoms are shown in Figures 5.1, 5.2, 5.3, 5.4. As can be deduced from these spectra, distinctive patterns, originating from polymers that make up a large part of the lubricants, were found. Closer analysis of the detected *m/z* values that form these ion series lead to the putative annotation of the major components of the condom lubricants (Table 5.2). A large component of many lubricants appeared to be poly(ethylene glycol) (PEG), whereas *m/z* values corresponding to polydimethylsiloxane (PDMS) were detected as well. In some lubricants, the polyethoxylated phenol nonionic surfactants octoxynol-9 or nonoxynol-9, serving as spermicides, were observed (Table 5.2). Next to these chemical components, multiple ion series corresponding to the fatty alcohol ethoxylates PEG decyl ether and PEG dodecyl ether were found, often used as non-ionic surfactants (Table 5.2) [38]. Poly(propylene glycol) (PPG) was putatively annotated in some of the lubricants as well. In addition to the ion series resulting from the polymers largely present in condom lubricants, a few molecular ion species were also detected and putatively annotated. An example is the detection of benzocaine, a local anesthetic used in two of the Durex condoms (Performa and Orgasmic) (Table 5.2). Furthermore, masses corresponding to undecylamine and dodecylamine were observed. As all samples were subjected to cyanoacrylate fuming, a commonly used detection technique for latent fingerprints within the forensic setting, a mass corresponding to a cyanoacrylate (CA) fragment was also found (Table 5.2). Finally, in controls, except CA, none of the above mentioned chemical components were found (data not shown).

5.3.2. Differentiation of condom lubricants

To differentiate between the 32 condoms, principal component analysis (PCA) and linear discriminant analysis (LDA) were performed, as both are shown to be effective in discriminating condom lubricants based on mass spectra [20, 21, 28]. PCA was performed to reduce data dimensionality, using the mass spectra acquired from the 32 different condom lubricants (Figure 5.5). Using only the first two principal components (PCs), a distinction between major lubricant classes could already be made. To gain more insight in which components can be used to differentiate condom lubricants, the loadings of the first 5 PCs were analyzed (Figure 5.6). The first PC contains *m/z* values corresponding to the $[M+Na]^+$ and $[M+K]^+$ ion series of PEG, whereas in

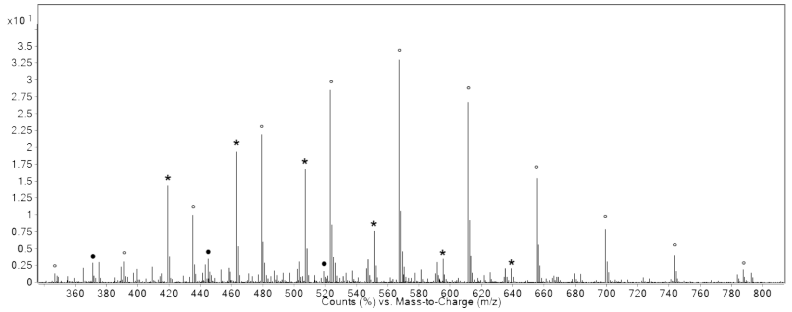

Figure 5.1: Spectrum obtained from DESI-MS analysis of spots of lubricant from BSC condom, showing ion series corresponding to PDMS (•) and nonoxynol-9 $[M+Na]^+$ (*) and $[M+K]^+$ (°).

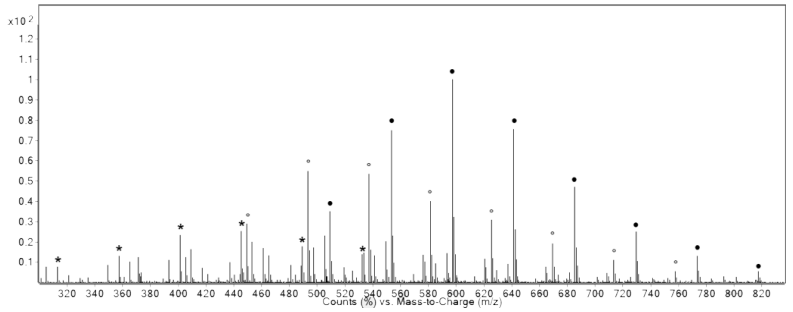

Figure 5.2: Spectrum obtained from DESI-MS analysis of spots of lubricant from MSO condom, showing ion series corresponding to poly(ethylene glycol) decyl ether (*), octoxynol-9 $[M+K]^+$ (•) and $[M+Na]^+$ (°).

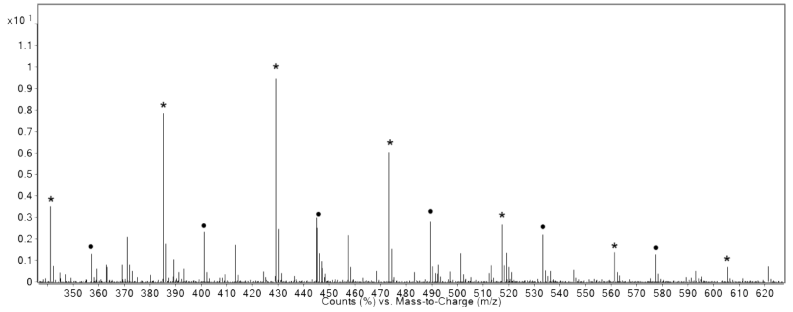

Figure 5.3: Spectrum obtained from DESI-MS analysis of spots of lubricant from KC condom, showing ion series corresponding to poly(ethylene glycol) dodecyl ether $[M+Na]^+$ (*) and poly(ethylene glycol) decyl ether $[M+Na]^+$ (•).

Table 5.2: Detected *m/z* values, their putative annotation and corresponding formula.

Putative annotation	Experimental *m/z* values	Formula	n	Ref
Benzocaine	166.0862	$C_9H_{11}NO_2$ $[M+H]^+$	-	[20, 32, 39]
Undecylamine	172.2058	$C_{11}H_{25}N$ $[M+H]^+$	-	[32]
Dodecylamine	186.2217	$C_{12}H_{27}N$ $[M+H]^+$	-	[32]
Ethyl cyanoacrylate	556.1794	$(C_6H_7NO_2)_n$ $[M+H\text{-}C_4H_8N]^+$	n=5	-
Poly(ethylene glycol)	195.1226, 239.1489, 283.1753, 327.1017, 371.2279, 415.2540, 459.2808, 503.3059	$H(C_2H_4O)_nOH$ $[M+H]^+$	n=4...11	[14, 40]
	217.1046, 261.1308, 305.1572, 349.1836, 393.2098, 437.2359, 481.2622, 525.2883, 569.3160, 613.3404, 701.4077	$H(C_2H_4O)_nOH$ $[M+Na]^+$	n=4...14	
	233.0785, 277.1041, 321.1298, 365.1567, 409.1832, 453.2097, 497.2360, 541.2620, 585.2889, 629.3150	$H(C_2H_4O)_nOH$ $[M+K]^+$	n=4...13	
Poly(ethylene glycol) decyl ether	313.2348, 357.2611, 401.2873, 445.3135, 489.3393, 533.3649, 577.3712	$C_{10}H_{21}(C_2H_4O)_nOH$ $[M+Na]^+$	n=3...9	-
Poly(ethylene glycol) dodecyl ether	341.2662, 385.2923, 429.3183, 473.3441, 517.3681, 561.3913, 605.4167	$C_{12}H_{25}(C_2H_4O)_nOH$ $[M+Na]^+$	n=3...9	[41]
Poly(propylene glycol)	273.1674, 331.2093, 389.2514	$H(C_3H_6O)_nOH$ $[M+Na]^+$	n=4...6	[40]
	347.1857	$H(C_3H_6O)_nOH$ $[M+K]^+$	n=5	
Poly(dimethylsiloxane)	371.1013, 445.1200, 519.1382	$(C_2H_6SiO)_n$ $[M+H]^+$	n=5...7	[40]
	429.0882	$(C_2H_6SiO)_n$ $[M+H\text{-}CH_4]^+$	n=6	
Octoxynol-9	449.2877, 493.3136, 537.3400, 581.3661, 625.3919, 669.4184, 713.4439, 757.4698	$C_{14}H_{21}(C_2H_4O)_nOH$ $[M+Na]^+$	n=5...12	[40, 42]
	509.2876, 553.3138, 597.3399, 641.3662, 685.3925, 729.4182, 773.4447, 817.4707	$C_{14}H_{21}(C_2H_4O)_nOH$ $[M+K]^+$	n=6...13	
Nonoxynol-9	419.2772, 463.3031, 507.3291, 551.3552, 595.3811, 639.4076	$C_{15}H_{23}(C_2H_4O)_nOH$ $[M+Na]^+$	n=4...9	[14, 23, 40, 42]
	347.1982, 391.2244, 435.2509, 479.2771, 523.3033, 567.3295, 611.3555, 655.3815, 699.4078, 743.4352, 787.4599	$C_{15}H_{23}(C_2H_4O)_nOH$ $[M+K]^+$	n=2...12	

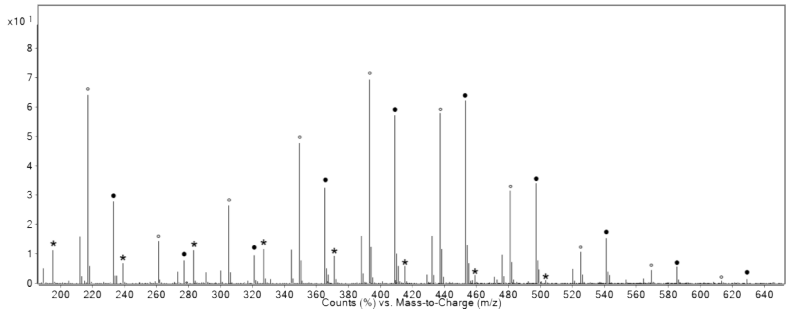

Figure 5.4: Spectrum obtained from DESI-MS analysis of spots of lubricant from DO condom, showing ion series corresponding to poly(ethylene glycol) $[M+H]^+$ (*), $[M+K]^+$ (•) and $[M+Na]^+$ (°).

the second PC *m/z* values of nonoxynol-9 (both $[M+Na]^+$ and $[M+K]^+$) are incorporated. In the third PC, putatively, octoxynol-9 (both $[M+Na]^+$ and $[M+K]^+$) was found to be the major component. Undecylamine and an unidentified *m/z* of 848.6672 are the major contributors to the fourth PC. In the fifth PC, dodecylamine, PEG, PPG and an unidentified *m/z* of 125.9863 were the strongest differentiating factors.

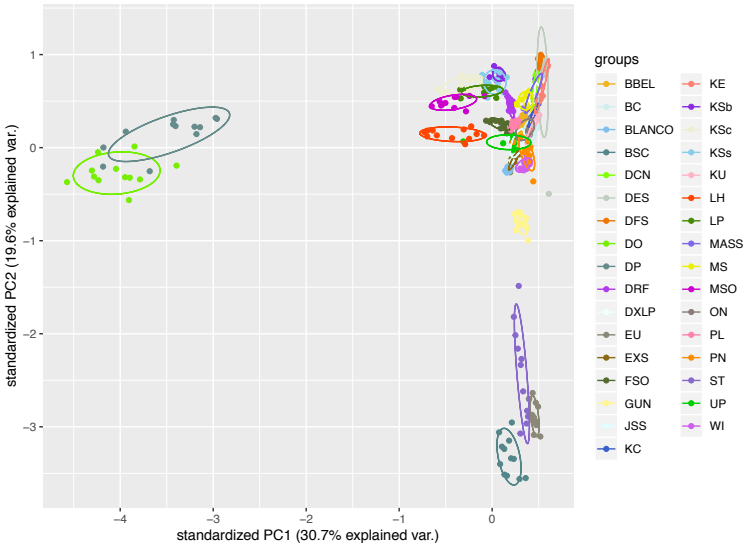

Figure 5.5: Principal component analysis score plot based on the analysis of 32 condoms and blank (n=12) using the first two principal components. Separate clustering of several lubricant groups is observed.

Next, linear discriminant analysis (LDA) was used to generate a classification model using the first 12 PCs (explaining 90% of the cumulative proportion of variance (Figure D.1)), based on the training data (75% of lubricant data obtained from analysis of the 24-wells slides). The generated model was subsequently evaluated by classification of the test data (25% of lubricant data obtained from analysis of the 24-wells slides).

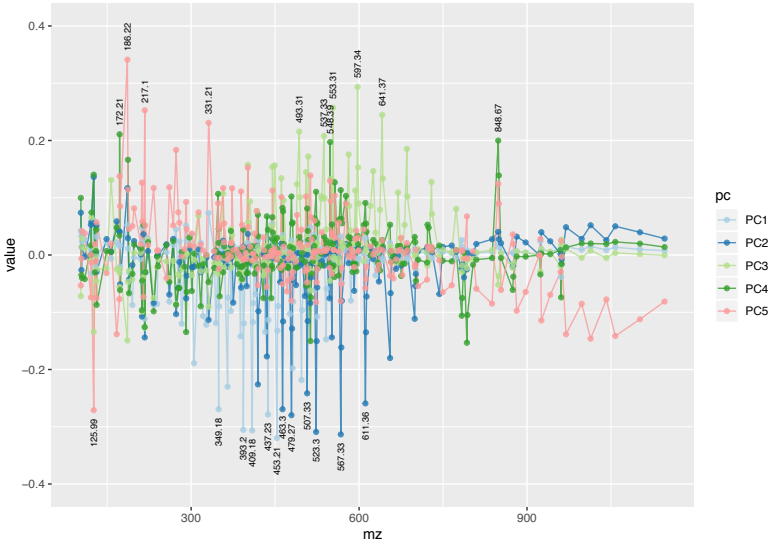

Figure 5.6: Plot of the absolute values of components loadings of the DESI-MS spectra from condom lubricant for the first 5 principal components.

Analysis of the resulting confusion matrix shows that the model is able to classify condom lubricants with high accuracy (99.0%)(Table D.1). Only 1 sample was predicted incorrectly; a sample containing KSb was predicted by the PCA-LDA model as KSs (Table D.1), which both originate from the same brand of flavored condoms (Kruidvat Sensations). These lubricants likely contain the same basis, while different colorants and flavorings are added. Subsequently, the PCA-LDA classification model was generated based on the data from the lubricated cyanoacrylated fingerprints in the same manner. Using this data, an overall accuracy of 90.9% was achieved (Table D.2). A few missclassification were present but seem to be explicable, such as the prediction of DES as DCN (both Durex condoms) and the prediction of DP as DO (both Durex condoms that contain benzocaine). However, the model performed poor for one specific condom lubricant, namely PL (sensitivity of 40%). Further analysis of the PL data revealed the low intensity of many of the chemical components, possibly explaining the poor performance of the model in this case.

5.3.3. Imaging of condom lubricants in fingerprints

Visualization of the presence of condom traces within fingerprints would greatly enhance the strength of the evidence, as it both establishes the presence of the suspect at the crime scene and contact with a condom. Therefore, full chemical images were obtained from fingerprints that handled an EXS condom and a blank (natural) fingerprint, both treated with CA fuming (Figure 5.7). As expected, CA (*m/z* 556.1794) was present in both fingerprints, and reveals the friction ridge pattern of the fingerprint in both cases (Figure 5.7C and 5.7D). When rendering the chemical distribution of PDMS (using the hexamer ion signal at *m/z* 445.1200) for both fingerprints, only in

the lubricated fingerprint a distinctive image was acquired, that was absent in the blank (Figure 5.7A and 5.7B). A similar result was obtained for the major lubricant component PEG (using the dodecamer ion signal at *m/z* 585.2889, Figure 5.7E and 5.7F). PEG was found to be highly abundant in the lubricated fingerprint, while only minor abundance was found in the blank. Importantly, in case of the lubricated fingerprint, classification using a subset of the data (to get the same number of averaged scans), correctly predicts the source of the lubricant as EXS (Table D.3).

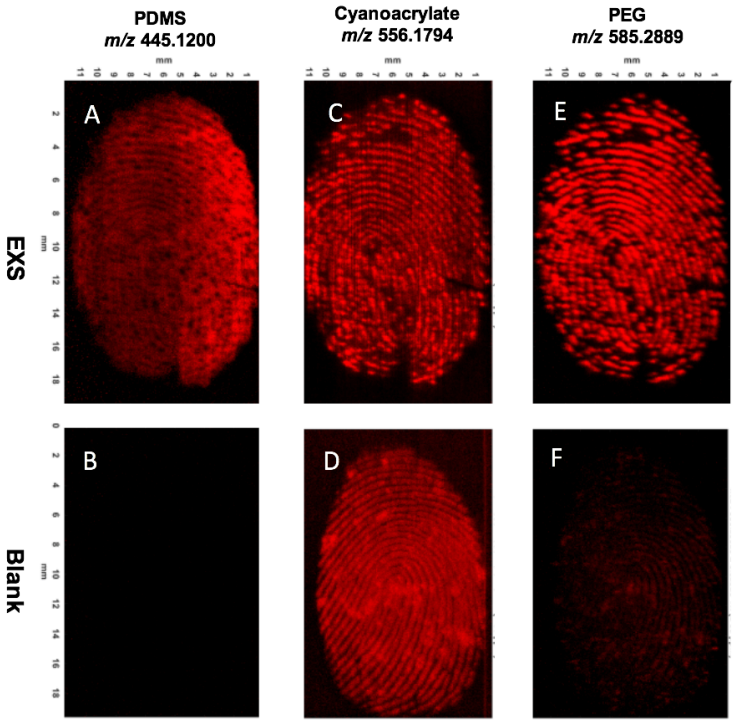

Figure 5.7: Chemical images (12 x 20 mm) showing the distrubution of PDMS (m/z 445.1200, A and B), cyanoacrylate (m/z 556.1794, C and D) and PEG (m/z 585.2889, E and F) throughout a fingerprint containing EXS lubricant (A, C and E) and a natural blank (B, D and F) fingerprint.

5.4. Discussion

To the best of our knowledge, this is the first study describing a method for the differentiation of condom lubricants from CA treated fingerprints using DESI-MS combined with a PCA-LDA classification model. The generated model showed high accuracy for both direct analysis of condom lubricant spots (99.0%), as well as lubricated fingerprints (90.9%). Moreover, since a large range of different brands and types of condoms, commonly sold in the Netherlands, were analyzed, a representative database was collected.

The detection, discrimination and visualization of condom derived traces from fin-

gerprints is of significant evidential importance in sexual assault cases, as it provides crucial information on the presence of a criminal at a crime scene as well as contact with a condom and type of condom used, thereby greatly increasing the strength of the evidence. In previous studies, it has already been shown that condom lubricants can be detected and discriminated, solely or within fingerprints, using several MS techniques, including DART-MS [20–22], MALDI-MS [23, 24] and DESI-MS [27, 28]. However, in these studies the effect of cyanoacrylate (CA) was not examined, while in forensics CA fuming is frequently performed to visualize fingerprints as they often appear latent. The current described method is shown to be compatible with cyanoacrylate fuming, rendering it more suitable for application to forensic casework. Additionally, full chemical images could be acquired from CA treated lubricated fingerprints, showing the spatial distribution of lubricant components such as PEG and PDMS throughout the fingerprint, which can be combined with classification of condom lubricants. The spatial information provided by chemical imaging, confirms that the lubricant was transferred by fingerprint contact as it links the presence of condom lubricant to the fingerprint ridge detail, making it of more evidential value than the sole analysis and comparison of condom components.

MALDI-MS was previously shown to have the potential to discriminate between different condom brands or types, combined with chemical imaging in a multidisciplinary analytical approach, by Bradshaw et al. [24]. However, the advantage of using DESI-MS as compared to MALDI-MS techniques, is that no matrix or sample preparation is needed and analysis can be performed at ambient pressure. DART-MS analysis offers straightforward analysis without the need for sample preparation, and was shown to be able to achieve high classification accuracies based on condom lubricant spectra [20–22], but lacks the capability to generate chemical images. DESI-MS was found to combine the easy and direct analysis of condom lubricant samples with the ability to perform chemical imaging resulting in high accuracy detection and discrimination of condom traces. Although MALDI-MS is capable of achieving higher spatial resolutions, the chemical images generated using DESI-MS show ridge flow and general pattern, which was found to be sufficient for the purpose of this method.

Using the developed DESI-MS method, multiple condom lubricant components were found. Among the most commonly encountered compounds were ion series corresponding to PEG, PDMS, nonoxynol-9, octoxynol-9 and PEG dodecyl ether. Based on the loadings of the first PCs, PEG, nonoxynol-9 and octoxynol-9 seem to be the most discriminatory lubricant components. Being an essential part of many lubricant bases, PDMS, PEG and nonoxynol-9 have been analyzed from condom lubricant traces using various analytical techniques, and have, not surprisingly, been included in many recent condom lubricant classification studies [20–22, 27, 28]. The detection of octoxynol-9 in condom lubricants is less commonly encountered, but has been described by Thomas et al. [42] and Bradshaw et al. [23]. The putative annotation of two fatty alcohol ethoxylates (PEG decyl ether and PEG dodecyl ether), that possible serve as ethoxylate lubricants, are in agreement with findings by Mirabelli et al., who already mentioned the possible presence of ethoxylate lubricant in certain types of condoms [28]. The m/z values used for the putative annotation of poly(ethylene glycol) dodecyl ether in our study, correspond to previously described

polymer fragments from an unknown ethoxylated polymer species by Mirabelli et al. [28]. Additionally, Musah et al. reported the detection of octyl alcohol ethoxylate from Skyn condoms, after DART-MS analysis [16]. The *m/z* values corresponding to undecylamine and dodecylamine were also detected, which, to our knowledge, are not commonly detected in condom lubricants, although octylamine was identified in many of the previous studies, mainly used as emulsifier, dispersant or lubricant [18, 20–22, 27].

There are numerous alternative approaches available to generate classification models based on analytical data. In our approach, PCA was used as a first step, to reduce data dimensionality, making the data easier to perceive. LDA was subsequently chosen as classification method as it showed to be an easy and fast classification method, which had already proven to be effective in discriminating condom lubricants based on mass spectra in previous studies by Maric et al., Baumgarten et al., and Mirabelli et al. [20, 21, 28]. In terms of classification accuracy based on lubricant spectra using DART-MS, Maric et al. achieved a 98.7% accuracy based on classification of 90 lubricants to one of 12 distinctive groups [20], Baumgarten et al. acquired a 88.9% accuracy when classifying 18 different lubricants [21], while Coon et al. discriminated 110 condom types from 16 different brands with a 97.4% accuracy [22]. Classification of lubricants from 10 different condoms using DESI-MS by Mirabelli et al. resulted in a 94% accuracy [28]. Our results are largely in line with these previous studies, as a 99.0% accuracy when analyzing condom lubricant spots, and a 90.9% accuracy based on analysis of lubricant containing fingerprints was obtained. Additionally, these results show that the presence of CA does not interfere with the detection and discrimination of condom lubricants, and high accuracy classification of CA fumed lubricant traces using DESI-MS and PCA-LDA analysis is attainable.

Some of the misclassifications in our study seem to be caused due to lubricants originating from the same condom brand. When analyzing lubricant spots, a sample containing KSb was predicted as KSs (both Kruidvat condoms), while in fingerprints containing lubricants, DES was predicted as DCN (both Durex condoms) and DP was predicted as DO (both Durex condoms that contain benzocaine). The misclassification of condom lubricant originating from two different Durex sources was also experienced in one occasion by Mirabelli et al. [28], likely being the result of similarities between condom lubricants originating from the same brand. This was shown by Maric et al. and Coon et al., who classified condom lubricants to a major lubricant group/brand with high accuracy [20, 22]. Predicting the condom lubricant traces by brand only, instead of brand and type, would presumably lead to an increased classification accuracy in our study as well. For one particular condom (PL), a low sensitivity (40%) in lubricated fingerprints was found, which seemed to be the result of low ion intensities, possibly explaining the poor performance of the statistical model in this case.

As the major components of condom lubricants are known contaminants in mass spectrometry [40], carry-over problems during method development and optimization were encountered, that were similar to the effects described by Mirabelli et al. [28]. In their study, it was found that the most relevant parameters determining the 'memory

effect' were the distance between the spray tip and ion transfer line and between the ion transfer line and sample. Too short distances resulted in contamination of the ion transfer line, as sample material could be sucked into the MS inlet [28]. Indeed, we also found that increasing the ion transfer line-to-surface distance and spray tip-to-ion transfer line distance, together with cleaning the MS inlet after 3-4 samples, resulted in avoidance of sample carry-over, indicating that these are crucial settings and actions for reliable results when analyzing condom lubricant traces with DESI-MS. Also, when imaging lubricated fingerprints using DESI-MS, we found that high amounts of condom lubricant in the fingerprints did not generate high quality chemical images, due to a decrease in clear ridge detail as a consequence of high abundances of PDMS and PEG ion signals. However, the classification model still predicted the source of the lubricant correctly, indicating that a discrimination could still be made.

In this chapter, the sole focus on the analysis of condom lubricant traces in CA treated fingerprints. However, the main components of these condom lubricants, such as PEG and PDMS, can also be found in many personal care products [17]. As a result, analysis of fingerprints that possibly contain traces of any of these personal care products, may lead to misclassifications. Although the ability to discriminate between personal care products and condom lubricants in fingerprints was not analyzed in the present study, a recent study performed by Moustafa and Bridge showed that discrimination between these classes of products is possible using DART-MS and LDA [17]. The addition of discriminating factors from other classes of personal care products to the current developed model would further increase the forensic applicability of the generated method. Furthermore, fingerprints with condom lubricant traces were only measured from glass substrates, while in practice, fingerprints can be found on all available substrates. Further optimization of the analysis of fingerprints containing condom lubricant traces on several different substrates would be beneficial for the applicability of the developed method. Indeed, Mirabelli et al. showed that chemical analysis and imaging of fingerprints containing condom lubricant is possible on metal and paper surfaces [27, 28]. However, spectra obtained from paper surfaces had lower signal intensities due to sorption effects, and a wash-out effect was encountered when analyzing on metal surfaces [27, 28].

5.5. Conclusion

In this chapter, the development of a DESI-MS method for the detection and discrimination of condom lubricant traces from fingerprints is described that, combined with a PCA-LDA classification model, has an overall accuracy of 90.9% and is compatible with CA fuming, making it more applicable for forensic casework. Additionally, full chemical images of fingerprints containing condom lubricant traces could be acquired, visualizing the spatial distribution of condom lubricant compounds, such as PDMS and PEG. This confirms that the condom lubricant is originating from the fingerprint and not the substrate, thereby increasing evidential strength. These results are promising leads for further development of DESI-MS methods to qualitatively analyze exogenous compounds from fingerprints for use in forensic science.

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6

Collection of analytes from fingerprints in a non-marking way

If we knew what it was we were doing,
it would not be called research, would it?

Albert Einstein

In Chapters 3 and 4 of this dissertation, the chemical composition of fingerprints was used to gain additional information about the donor and fingerprint age. These analyses are often destructive to the fingerprint ridge detail and thus are not compatible with the current fingerprint processes. The method described in Chapter 5 maps the distribution of compounds of interest in a chemical image, and therefore combines chemical information with the information enclosed in the ridge detail. However, the analytes of interest in Chapters 3 and 4 require chromatographic separation before subsequent MS detection and quantification. Therefore, a method that could retrieve analytes of interest from fingerprints in a non-marking way (i.e. extracting chemical compounds without physically marking the fingerprint ridge detail) would greatly broaden the applicability of the analytical methods developed in this dissertation. The aim of this chapter is to achieve the non-marking retrieval of amino acids and DNA from fingerprints using hydrogels. In the first part of this chapter, a brief introduction to amino acid profiling as well as the use of hydrogels is provided. Then, the development of a method to extract polar compounds from fingerprints while subsequently lifting the hydrogel in a non-marking manner is described. Finally, the performance of the hydrogel to that of current sampling methods, such as using a cotton swab and completely dissolving the trace is compared.

Parts of this chapter have been published in *Analyst* **143**, 4 (2018) [1].

6.1. Introduction

The amino acid profile of an individual may provide information such as the gender, health and age of a donor [2, 3]. Common methods of amino acid profile analysis require access to either the blood, urine, saliva, faeces or cerebrospinal fluid to generate a donor profile [4]. Interest in methods using sweat deposits in fingerprints for the analysis of amino acid levels has increased in recent years as analytical methods have been developed that allow for the rapid and accurate quantification of amino acid concentrations using GC, CE and (U)HPLC-MS [5–10]. Sweat as a diagnostic biofluid has several advantages as it can be easily collected from patients without the need for invasive procedures and is safer for diagnosticians to handle as saliva or blood may contain viruses and must be treated with extra care [11, 12]. Previous studies, where sweat has been analyzed for amino acid profiling, have relied on the use of sweat collection devices that need to be attached to an individual's skin in order to collect adequate quantities of sweat for analysis, or via fingerprints by completely dissolving the fingerprint [13, 14]. For the sweat collection studies large volumes of sweat (~50 µL) are required alongside specialist equipment to produce an amino acid profile [14].

In the field of forensic science, an investigator will primarily use swabs or lifting tape to collect evidence from a crime scene. Swabs are a destructive method of analyte collection and while they excel at absorbing analytes, they show poor release of the trapped analytes that are intended for analysis [15]. Lifting tape or gel lifters are a common way to recover fingerprints from surfaces, but these techniques do not facilitate the extraction of chemical components without destruction of the ridge detail. Ideally a collection material is non-marking (i.e. it does not physically mark the evidence although chemical components are extracted) to the surface it is applied on and shows rapid adsorption and release of collected analytes.

A method which collects amino acids from a surface or complex matrix like a fingerprint could be of interest for forensic investigators, but also for diagnostic clinical purposes. While methods exist for the direct chemical analysis of fingerprint residue, they rely on the introduction of the entire fingerprint into either a solvent or an analytical instrument in order to detect the presence of certain analytes on a fingerprint [16]. This is not ideal for forensic investigators as it generally requires destruction of the fingerprint ridge detail, or specialised equipment to perform surface analysis at a crime scene. Hydrogels would provide a flexible solution to sample collection as they can be applied to non-porous surfaces. Hydrogels are 3D networks of hydrophilic polymers in which water is the dispersion medium [17]. They have been found to be useful in a variety of areas from drug delivery and tissue engineering to cell culturing [18–20]. Their capacity to absorb and hold water potentially makes them suitable as extraction media for water- soluble analytes from a surface.

In this chapter, the possibility of using hydrogels as a means of collecting amino acids from fingerprints is demonstrated, as well as their comparative performance for the use of direct extraction with a solvent for amino acid analysis. Both the collection of amino acid deposits on a glass surface and from fingerprints using hydrogels will be described. Additionally, since the collection of DNA from fingerprints is increasingly adapted in forensic casework, the use of hydrogels to collect DNA from fingerprints was investigated.

6.2. Materials and methods

6.2.1. Pre-hydrogel and amino acid solutions

Dextran-methacrylate was chosen as a hydrophilic polymer as it is conveniently cross-linked by photo-initiated radical polymerization forming a hydrogel. The method described by De Smedt et al. was followed to prepare dextran-methacrylate with a substitution degree of 2.5 [21]. The structure of the material was confirmed using ^{1}H-NMR. The photo-initiator used, lithium phenyl(2,4,6-trimethylbenzoyl)phosphinate (LAP), was prepared according to the method described by Fairbanks et al. [22]. The structure of the material was confirmed using ^{1}H-, ^{13}C-, and ^{31}P-NMR. Pre-hydrogel solutions were prepared by dissolving 1% w/v LAP and 10% w/v dextran-methacrylate in distilled water (1 mL, pH 6.5) under sonication for 20 minutes at room temperature. Amino acid and isotopically labelled internal standard (IS) solutions of glycine, L-alanine, L-serine, L-proline, L-valine, L-threonine, L-cysteine, hydroxy-L-proline, L-isoleucine, L-leucine, L-asparagine, L-ornithine, L-aspartic acid, L-glutamine, L-lysine, L-glutamic acid, L-methionine, L-histidine, L-phenylalanine, L-arginine, L-tyrosine, L-tryptophan and L-cystine of 2.0 mg/L were prepared as described previously [10].

6.2.2. Glass cover slip functionalization

Borosilicate microscope cover slips of dimensions 25 × 25 mm were purchased from Fisher Scientific. The slides were activated prior to functionalization using a Harrick's plasma cleaner for 240 seconds. They were transferred to a vacuum chamber containing 100 µL of 3-(trichlorosilyl)-propyl-methacrylate and left under dynamic vacuum for 4 hours to functionalize the surface.

6.2.3. Fingerprint deposition

Fingerprints were deposited using a custom made fingerprinting device (Figure 6.1) based on a similar device reported by Fieldhouse [23]. This device maximizes a uniform application of force on the fingerprint and only allows a certain area of the fingerprint to be exposed to the substrate surface. Fingerprints were produced on glass slides. The hands of three males and two females, all ranging between 20 and 26 years old, were washed before returning to normal work activities. After one hour two fingerprints per donor were deposited on glass slides. After drying in air for one hour, the fingerprints were extracted either by hydrogel extraction or direct solvent extraction. Fingerprints for DNA extraction and quantification were produced as follows: 3 donors (2 male and 1 female, aged 21 to 28) were asked to rub their fingers against their forehead and hair for 3 seconds to generate more shed skin cells and subsequently donate 3 fingerprints on glass slides.

6.2.4. Amino acid standard samples

To determine the hydrogel extraction efficiency, amino acid working solution (25 µL) was deposited on a glass slide and then dried in air for 30 minutes. The amino acids were then extracted using hydrogels, or were extracted directly with a solvent.

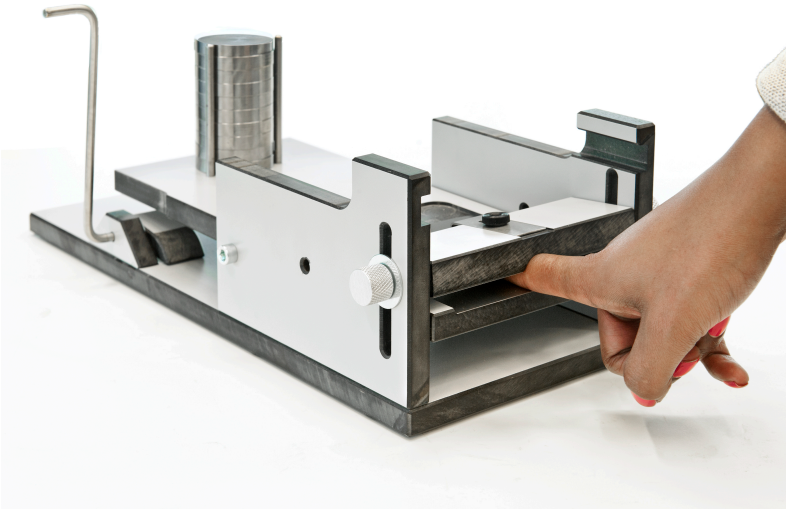

Figure 6.1: Custom made fingerprinting device applied to maximize reproducibility in fingerprint deposition.

6.2.5. Analyte absorption experiments

In a typical extraction experiment, a freshly prepared solution (20 µL) of dextran-methacrylate (10% w/v) and LAP (1% w/v) is applied to a surface containing analytes of interest (Figure 6.2A and B). Thereafter, a methacrylate-functionalized glass cover slip is placed over the solution to be able to collect the hydrogel (Figure 6.2C). After 3 minutes, the sample is irradiated for 30 seconds, using a simple 405 nm laser pen (1 mW), generating the hydrogel (Figure 6.2D). During cross-linking the methacrylate groups of the dextran-methacrylate and the cover slide will react, hereby covalently binding the hydrogel to the glass cover. By careful removal of the cover slide, the hydrogel containing the available hydrophilic analytes is taken away, leaving nonabsorbed components behind (Figure 6.2E).

6.2.6. Amino acid extraction

Hydrogels were applied onto a surface of interest as described above and subsequently transferred to a beaker and the analytes were extracted with MeOH/H_2O (3 mL, 1 : 1) under sonication for 30 min. IS solution (10 µL) was added and the extract was then transferred to a 15 mL polypropylene conical tube. The sample solution was then evaporated under nitrogen flow, and the material was dissolved in MeOH containing 5% v/v formic acid (50 µL) and transferred into an injection vial. Solvent extracted samples, which were prepared for comparison, were processed similarly, except that the glass slides containing the amino acids were transferred to a beaker containing MeOH/H_2O directly.

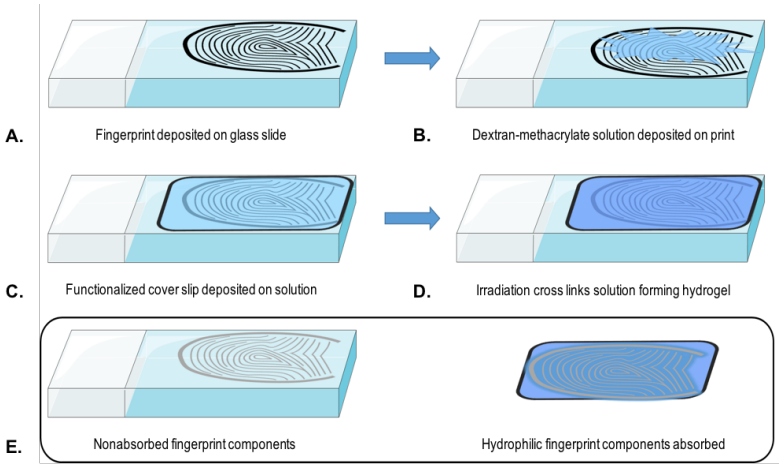

Figure 6.2: Schematic representation of water-soluble analyte extraction from a fingerprint by hydrogel formation, including, fingerprint deposition on a glass slide (A), deposition of the dextran-methacrylate solution on the fingerprint (B), deposition of a functionalized cover slip on the fingerprint with solution (C), irradiation of the fingerprint sample (D) and lastly the removal of the cover slip and hydrogel from the glass slide (E).

6.2.7. Amino acid analysis by UPLC-MS

Amino acids were quantified using UPLC-MS, as described previously [10]. In brief, for each amino acid a calibration curve ranging from 0.10 to 1.60 mg/L was produced, corresponding to 5–80 ng of a single amino acid in the sample volume (50 µL) for a fingerprint. These solutions were prepared by adding respectively 2.5, 5.0, 10, 15, 30, and 40 µL of 2.0 mg/L amino acid working solution into a vial. IS solution (10 µL), composed of isotopically labeled amino acids, was added to each vial, and MeOH containing 5% v/v formic acid was added to make a total volume of 50 µL. The samples were then analysed using UPLC-MS using a 150 mm UPLC amide BEH column (Waters, Milford, MA, USA). A Waters Acquity I-class UPLC autosampler and a binary solvent pump were used to inject and separate the sample solution. The effluent of the column was analysed by means of electrospray ionisation time-of-flight MS (ESI-TOF-MS) using an accurate mass TOF with a dual electrospray source (6220 Agilent, Santa Clara, CA, USA). Data were processed and quantified using Agilent Mass Hunter Qualitative Analysis software (version B.05.00) and Quantitative Analysis software (version B.05.00).

6.2.8. DNA extraction and quantification

DNA was collected from fingerprints using either a cotton swab or via the hydrogel method. When using the cotton swab, the complete fingerprint was swabbed with a dry cotton swab, which was subsequently transferred to a 1.5 mL aliquot containing 300 µL ATL buffer (Qiagen, Inc.). Hydrogel lifting of the fingerprints was performed as described above. After lifting, the hydrogels were transferred to a 1.5 mL aliquot containing 300 µL ATL buffer. The concentration of human DNA in the samples was

determined using real-time PCR as described by Nicklas and Buel [24].

6.3. Results and discussion

6.3.1. Amino acid standard solution

Initial experiments were performed using the standard amino acid solution containing 22 amino acids. After absorption and subsequent extraction from the hydrogel, 19 amino acids out of 22 (all but L-cysteine, L-lysine and L-methionine) were successfully detected and quantified while 20 were found in the samples directly extracted with solvent (all but L-cysteine and L-methionine). Figure 6.3 shows the extraction efficiency (IS corrected) of each amino acid for both methods. The extraction efficiency varied between amino acids and extraction methods and it was in general below 100%. For all the detected and quantified amino acids, the extraction efficiency was similar using either extraction method, except for L-lysine, L-histidine, L-tryptophan and L-cystine. Figure 6.3 shows that L-methionine and L-cysteine were not found in any samples collected using either extraction method. The lack of L-cysteine may be caused by the formation of cysteic acid, which is indicated by the detection of an ion signal at m/z 170.012, corresponding to the monoprotonated ion of this species. L-Methionine may undergo oxidation to yield methionine sulfoxide, as its monoprotonated ion at m/z 166.052 is detected. L-Tryptophan is extracted with 50% efficiency when solvent extraction is used while it is extracted with 10% efficiency when the hydrogel is applied. As reported earlier, the analysis of L-tryptophan, when extracted from fingerprints, can be problematic [13]. In this case, it is possible that the radical photoinitiator, used in the gel, attacks the conjugated ring system of L-tryptophan reducing its concentration. However, it is clear from Figure 6.3 that the hydrogel is not only capable of collecting the amino acids from a surface, but that subsequent extraction and detection from the hydrogel is also possible. While values are relatively similar across both methods it must be noted that the use of a hydrogel includes the extra step of collecting the analytes from the surface.

6.3.2. Amino acids recovered from fingerprints

After successfully applying the hydrogel system to absorb amino acids from a glass surface, the possibility to absorb these species from fingerprints in a non-marking way was investigated (Figure 6.4, amino acids reported in relative abundances to serine). The application of the hydrogel to an actual fingerprint led to the positive identification of 15 different amino acids. This indicates that at least 15 amino acids were absorbed into the hydrogel, and could subsequently be identified and quantified (Figure 6.4, red bars). Importantly, visualization of the fingerprints after hydrogel treatment was still successful, as can be seen in Figure 6.5. In comparison, 17 amino acids were detected from fingerprints that were extracted with a solvent and completely dissolved for analysis, see Figure 6.4 (blue bars). As can be seen in Figure 6.4, the hydrogel extracted fingerprints yielded largely similar amino acid profiles to the solvent extracted fingerprints, except for L-histidine and L-tryptophan abundances. L-Histidine and L-tryptophan were not detected using the hydrogel, which can be explained by the lower extraction efficiency of these amino acids using the hydrogels, as described

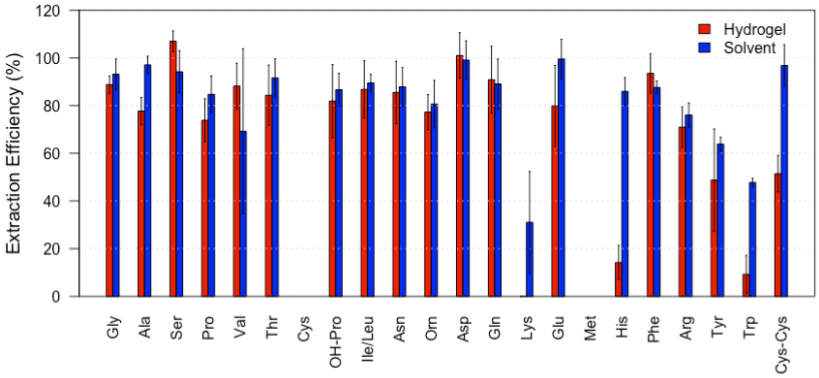

Figure 6.3: Comparison of extraction efficiencies (IS corrected) of amino acids extracted with hydrogel (red) compared to solvent (blue) (N = 6). Extraction efficiency is calculated for every amino acid as the detected amount relative to the amount deposited on the glass slide.

above. The large variance in the recovered amino acid profiles likely originates from the natural variation of amino acid concentration in fingerprints and the relatively low amount of fingerprints included in this proof of principle. While a significant effort was made to create reproducible fingerprints, variation between donors (inter-variability) cannot be avoided [25]. In terms of absolute amino acid extraction, the hydrogel lifted fingerprints on average yielded 532 ng of amino acid compared to 807 ng in the solvent extracted fingerprints. Despite this, the relative amino acid concentrations obtained with the hydrogels coincided with the relative concentrations collected via direct solvent extraction. L-Tryptophan is not detected likely due to its low abundances in fingerprints [2], and/or the potential radical degradation. L-Cysteine and L-methionine are not detected, which concurs with the results obtained when extracted from amino acid standard samples as described above.

6.3.3. Visualization of lifted fingerprints

Central to this method is its non-marking nature. As the primary solvent is water the hydrophilic constituents of the fingerprint are collected selectively, leaving enough hydrophobic material to visualize the fingerprint using cyanoacrylation followed by dyeing with basic yellow. Figure 6.5 is a split fingerprint example of the comparison in quality between a standard cyanoacrylate and basic yellow treatment and lifting using the hydrogel prior to cyanoacrylate and basic yellow treatment. It is clear that after lifting the hydrogel, including the absorbed hydrophilic solutes, there is sufficient material for further development using standard fingerprint visualization techniques. The majority of the ridge detail is still present with slight degradation of the fine details. The likely cause of the “smudges” is localized variances in the absorption of analytes. As fingerprints vary in their composition the likelihood for one area to be slightly more hydrophobic and therefore repel the penetration effects of the hydrogel is higher.

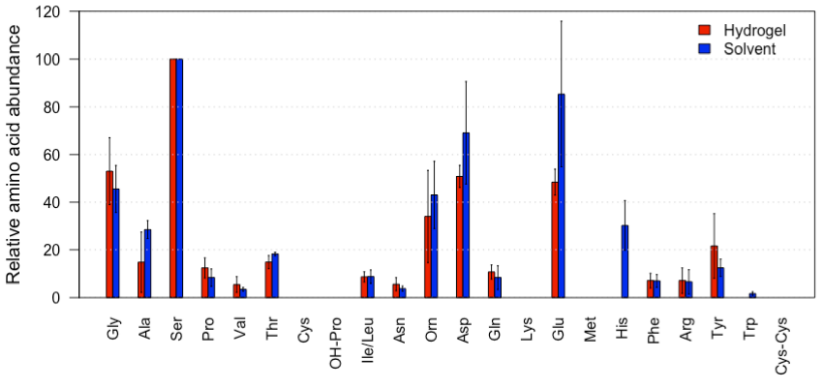

Figure 6.4: Comparison of amino acid profiles (i.e. the relative abundance to serine) extracted from hydrogel (red) compared to solvent (blue) collected from fingerprints (N = 5). Amino acid profiles per extraction method are calculated as an average of the amino acid profiles from the different donors.

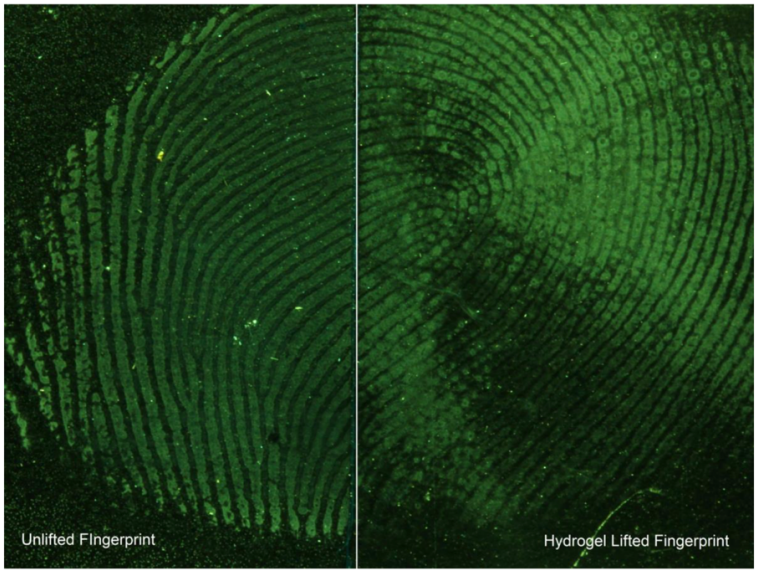

Figure 6.5: Comparison of ridge detail from a split fingerprint which was partially extracted using a hydrogel. Left: Treated with cyanoacrylate and basic yellow. Right: Hydrogel extracted and subsequently treated using cyanoacrylate and basic yellow dyeing.

Alternatively, this could potentially be due to the mechanical action of lifting or due to pressure differences during deposition. However, the method presented here is unique in that it can successfully collect analytes of a hydrophilic nature of interest from a fingerprint surface in similar quantities as traditional methods and allow for the subsequent visualization of the fingerprint.

6.3.4. DNA recovered from fingerprints

After applying the hydrogel to extract amino acids from fingerprints, the ability of the hydrogels to absorb DNA from fingerprints was investigated. Compared to conventional DNA sampling using a cotton swab, the hydrogels roughly yielded between 20–60% of the DNA quantity (Figure 6.6). Although this is significantly lower than the swab, the hydrogels could still be an addition to the current workflow, for instance when sampling unequal surfaces such as gun grips. Moreover, DNA could be recovered from fingerprints while leaving sufficient material behind for visualization. In this experiment, since it is likely impossible to exactly reproduce the quantity of touch DNA in fingerprints, a large variation in DNA quantity was observed. The larger variation in DNA yielded by the hydrogels is most likely due to the extra steps in the sample preparation, especially the transfer of the lifted hydrogel to an aliquot, indicating that further optimization of the sample preparation is necessary.

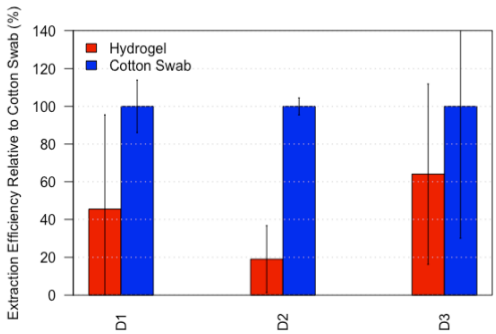

Figure 6.6: Comparison of DNA quantified using hydrogel (red) relative to cotton swab (blue) collected from fingerprints (N = 3) from three donors. As the DNA quantity in fingerprints varies between donors, results are reported as extraction efficiency relative to cotton swab per donor (i.e. the average DNA quantity recovered with the hydrogel relative to the average DNA quantity recovered with a cotton swab for each donor).

6.4. Conclusion

It was shown that cross-linked hydrogels can be applied to absorb amino acids from both fingerprints and amino acid solution deposits on glass, and extracted the amino acids from the hydrogel for their qualitative and quantitative detection using UPLC-MS. These results show the selective uptake of the water solubles, leaving sufficient

material for further investigations of the fingerprint. Post-collection visualization of the fingerprints is also shown to be possible using cyanoacrylate and immersion in a basic yellow dye. Moreover, the hydrogels were able to collect DNA from fingerprints with a 20–60% yield compared to the conventional cotton swab. Together, these results show that hydrogels are promising materials for evidence collection as they combine both the dissolving power of a solution with the physical lifting capacity of a gel. Combined with the rapid release of analytes when immersed in common solvents they present themselves as a possible replacement or complementary method for analyte collection.

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7

Conclusion

Nothing in life is to be feared,
it is only to be understood.
Now is the time to understand more,
so that we may fear less.

Marie Curie

The aim of this dissertation was to find and investigate new markers in fingerprint residue that can be used to retrieve donor information, or additional information about the trace or crime, from the chemical composition of fingerprints. This could potentially facilitate the extraction of forensically relevant information from fingermarks which are currently of no use for a criminal trial, either due to the lack of ridge detail, or the absence of the reference fingerprint. To achieve this aim, a method to analyze amino acids and other polar compounds from fingerprints, in a fast and reliable way, needed to be developed, in order to be able to extract and quantify these compounds from fingerprints in large data sets. Then, it was required to investigate how the fingerprint composition varies both between and within donors, to study the relation between fingerprint composition and donor traits and discover potential markers in fingerprint residue. The focus was not only on the retrieval of information of donor traits, but also on the potential to derive information about the trace or crime, such as the derivation of information from the presence of exogenous compounds and the estimation of the age of a fingerprint. To render the chemical analysis of fingerprints compatible with the current fingerprint process, investigations into methods to collect compounds of interest from fingerprint residue without marking the ridge detail were also needed. The research described in this dissertation has shown that both donor information as well as information about the trace or crime can potentially be deduced from the chemical analyses of fingerprints. Additionally, it was shown that chemical analyses of polar compounds can be performed without destructing the fingerprint ridge detail in such a way that traditional fingerprint comparison is no longer possible.

The developed UPLC-MS method, based on an amide stationary phase, was suitable for the analysis of common polar metabolites. Using this method, amino acid profiles could be obtained from fingerprints without derivatization using a 25 minute analytical method. Moreover, since the TOF-MS acquires full-scan data, the method allows for the screening of new potential markers as well. A limitation of this method is that as a result of the sample preparation and amide stationary phase, which was specifically chosen to analyze amino acids, the non-polar analytes, which will have no affinity with the chosen column, will be largely missed in the analysis. As a consequence, the found markers based on the UPLC-MS data, were all relatively polar compounds. To get a more complete view of the composition of fingerprint residue, extracts should be splitted and analyzed using both a hydrophilic and hydrophobic stationary phase, as well as both positive and negative ionization modes using mass spectrometry. This will also bring forward new potential markers, which were missed in the current research, either due to the sample preparation, a lack of affinity with the stationary phase, or a low ionization efficiency. Moreover, the markers that were found, could only be putatively annotated, as a TOF-MS was used as mass analyzer. Additionally, no retention times were available due to the absence of reference standards. This was a result of the experimental design, as the aim was to investigate and discover new markers, and corresponding reference standards could thus not be analyzed simultaneously with the samples. This allowed identification of the found markers only based on accurate mass, resulting in putative annotations. Tandem MS would allow for a higher level of identification as defined by the 2007 metabolomics

standards initiative [1].

It was investigated how the chemical composition differs both between and within fingerprint donors, and which donor traits potentially influence this composition. To analyze how fingerprint chemical composition differed between donors (inter-variability), a data set comprising of 1852 fingerprints from 463 donors, collected at the Dutch music festival Lowlands was analyzed. Amino acids and lipid components were quantified using LC and GC based mass spectrometric methods. For the determination of donor gender, 3 metabolites were found to be higher in male donors compared to female donors: L-phenylalanine, L-(iso)leucine and palmitoleic acid. In fingerprints of donors who indicated to have used cannabis in the past 24 hours, a relative higher concentration of L-alanine was found. Lastly, L-proline was found to be relatively higher in fingerprints of donors who indicated to have consumed alcohol in the past 24 hours. Since both the UPLC-MS and GC-MS method used yielded more data than the specifically quantified metabolites, a further untargeted data analysis was conducted as well. Based on this approach, is was possible to distinguish fingerprint types for both the UPLC-MS data (natural vs eccrine, 95.3% accuracy) as well as the GC-MS data (natural vs sebaceous, 86.8% accuracy). Compounds that appeared to play a role in these classifications were putatively annotated as urea and amino acid degradation products, among others, for the UPLC-MS data and, among others, squalene and mono-unsaturated fatty acids for the GC-MS data. Donor gender was best classified based on the data obtained from natural fingerprints analyzed with UPLC-MS, resulting in a 77.9% accuracy. Compounds used in the prediction were putatively annotated as products from the GMP degradation pathway. A donor trait that could be predicted with high accuracy based on both UPLC-MS data from natural and eccrine fingerprints was smoking habit (e.g. prediction of everyday smoking habit with accuracy of 90.4% based on natural fingerprints). The classifiers used in these models were putatively annotated as nicotine and cotinine, a degradation product of nicotine. These findings are promising leads for the future development of classification of donor usage of other stimulants. Although this sort of information may not be useful for individualization purposes, it could help forensic investigators in narrowing down the suspect population, or potentially exclude suspects as donor. However, there are several limitations that need to be overcome before application. All fingerprints were collected on the same type of non-porous surface and fingerprint residue was immediately dissolved after deposition. This means they were not subjected to ageing, potentially causing found markers to degrade, or fingerprint visualization techniques, potentially lowering a markers concentration by consuming it or washing it away. Additionally, porous substrates are generally known to absorb the fingerprint residue quickly, thereby potentially hindering extraction of analytes of interest from them [2]. More research is thus needed, not only to confirm the identity of found markers, but also to investigate the persistence of these markers in more realistic fingerprints (i.e. aged and subjected to visualization) on different substrates.

To study the degree of variability of chemical composition within a donor (intra-variability), fingerprints from 4 different donors were collected three times a day during one week (Monday to Friday). Amino acid content was determined using the same UPLC-MS method, and showed to range between 270 and 630 ng per fingerprint.

Variation within donors was found to be lower and ranged between 39.1% to 66.9% (RSD) during 5 days, based on total amino acid content (15.0-100% relative range). In comparison, Dorakumbura et al. found the intra-variability to range between 4 and 100% when deposition was not controlled, based on the abundance of squalene [3]. Using a similar untargeted approach, based on the full-scan MS-data, a classification model was developed to distinguish between the 4 fingerprint donors, using the fingerprints collected on 5 separate days. This indicated that although intra-variability is large, there seem to be certain distinguishing components that facilitate the discrimination between donors based on the fingerprint composition. Even without being able to link this composition to a reference, this could potentially be used on a crime scene to determine how many different donors left fingerprints behind, as was also proposed by Hair et al., based on analysis sweat samples [4]. Our results indeed showed that overall variability within donors is large, but the presence of potential exogenous compounds can still be used to discriminate between fingerprints from different donors. We hypothesize these markers could be a result of certain habits in the daily routine of the donor. This implies that a change in behavior could potentially hinder correct classification. More research is needed, including larger numbers of donors and an extended time frame, to identify important markers and investigate their stability and persistence. For example, to further investigate if the markers found for donor smoking habit, putitvely annotated as nicotine and cotinine, can validly and reliably be applied in practice, fingerprints from a selected donors' set of smokers and non-smokers should be analyzed over an extended period of time to determine the variability of these markers. Additionally, information on the persistence of these markers after a donor stops smoking should be obtained too, as well as information on the influence of environmental factors such as passive smoking. It must be noted that for the study into the intra-variability, the same limitations as previously described in case of the analysis of the inter-variability apply; fingerprints were collected on one type of non-porous surface and analyzed without being subjected to ageing or visualization techniques.

Another thesis aim was to investigate if, next to donor information, the fingerprint chemical composition could be used to derive fingerprint age information. This is potentially crucial for fingerprint evidence, as this could link a suspect to the time frame around the crime. D-amino acids were investigated as potential targets for fingerprint age estimation. A method was developed to extract and relatively quantify D/L-amino acid ratios from fingerprints. This was successful for the amino acids histidine, serine, threonine, alanine, proline, methionine and valine. After the analysis of fresh fingerprints from 40 donors, serine, threonine and histidine showed the lowest percentage of D-enantiomer combined with a low variability. After analyzing fingerprints up to 6 months old, only a significant increase was seem in case of D-serine, which thereby poses as an interesting target for fingerprint age estimation methods. In general, D-serine showed a promising trend for fingerprints up to 30 days old. The proposed target offers similar advantages as methods developed by Weyerman et al. and Van Dam et al., where the relative ratios correct for an unknown starting amount [5, 6]. The use of enantiomers of the same compound however, corrects for the unknown starting amount as well as the unknown rate at which degradation of the compound

has taken place so far, under the assumption that D- and L-enantiomers degrade at identical rates. The influence of temperature, humidity, light exposure and substrate have not been studied in the current research. Even though the method has the potential to correct for the unknown degradation that has occurred since deposition, these parameters are likely to influence the racemization rate as well. Further research is thus needed to confirm the trend found for D-serine, and investigate the behavior of other fingerprint amino acids. This likely requires further optimization of the analytical methods used, to increase sensitivity. If the trend for D-serine in aged fingerprints is validated, it seems likely that other amino acids may be subject to racemization as well. Since every amino acid possibly has a different racemization rate, the enantiomeric ratios of different amino acids can be taken into account, to increase the accuracy of age estimation and extend the time frame.

Whereas the focus so far had mostly been on compounds present in donor excretion, a subsequent thesis aim was to determine if the presence of exogenous compounds in fingerprints, resulting from contact with certain items or products, could be used to derive information. A DESI-MS method was developed to analyze condom lubricants in fingerprints. It was investigated if the developed method was compatible with cyanoacrylate fuming, a commonly used detection technique for fingerprints on non-porous surfaces. Common lubricant components such as PDMS, PEG and nonoxynol were readily detected from cyanoacrylated fingerprints, donated after contact with a condom. Based on the analysis of fingerprints containing condom lubricant, a classification accuracy of 90.9% was achieved. Moreover, full chemical images of the cyanoacrylated fingerprints could be obtained, showing the distribution of the lubricant components throughout the fingerprint. These results should serve as an example for the development of other methods for the analysis of other exogenous compounds from fingerprints, such as personal care and related products (i.e. hairgel, make-up, sunscreen etc.), using chemical imaging approaches. Additionally, as shown by previous studies, there is potential for the detection of illicit compounds such as drugs and explosives in fingerprints as well [7–11]. For each class of analytes however, methods need to be optimized and validated before application in practice is possible. This includes the analytical method itself, but parameters such as the sample surface could play an essential role as well.

With the exception of the aforementioned DESI-MS method, which was shown to be compatible with cyanoacrylate fuming, many of the used methods in this dissertation are destructive to the fingerprint ridge detail, and were not tested for compatibility with the current fingerprint detection processes. Therefore, the next thesis aim focused on the development of a method which collects analytes from fingerprints in a non-marking manner. To be compatible with the current process, a generic method was developed that is capable of retrieving polar analytes from fingerprints, without altering the fingerprint ridge detail. In a proof of principle, the hydrogel-based method showed to be capable of extracting amino acids and DNA from fingerprints in a non-marking way. The fingerprint residue could still be subsequently visualized using cyanoacrylate fuming followed by basic yellow dyeing. The development of methods such as these for different substrates and fingerprint visualization techniques, is essential for the implementation of chemical analyses in the fingerprint domain. Hence,

more work is needed to investigate and develop methods which either leave the fingerprint ridge detail unmarked while analyzing the chemical composition, or facilitate the compositional analysis after common fingerprint detection techniques have been applied. As fingerprints in practice often appear latent, compositional analysis before common detection techniques may prove challenging, as it is difficult to distinguish where to sample and analyze. Vice versa, compositional analysis after a fingerprint detection technique has been applied, may hinder the chemical profiling because fingerprint constituents might have reacted with the detection techniques, have been washed away, or are not easily extracted.

The results presented in this dissertation show there is potential for the compositional analysis of fingerprints. Whereas the quantitative analysis of common metabolites such as amino acids and lipids from fingerprints did not yield particular donor information, the detection of exogenous compounds showed to be useful, as was demonstrated by the finding of nicotine and cotinine in fingerprints of smokers. Moreover, the detection of lubricant components in fingerprints that had been in touch with condoms, combined with a high classification accuracy for brand and type, showed a practical example of how information can be extracted from exogenous compounds in fingerprints. In addition to what had been demonstrated before by Mirabelli et al. using DESI to analyze fingerprints containing lubricants, compatibility with a development process was demonstrated[12, 13]]. These approaches build on the work of Francese et al., Hinners et al. and Bouslimani et al., although care should be taken before the detection of an exogenous compound is contributed to a specific activity or donor trait [14–16]. This type of information, on certain donor habit or activity, is complementary with the donor information that can be retrieved from DNA. Forensic DNA phenotyping encompasses the prediction of appearance traits of unknown donors, based on DNA found on a scene. In addition to the determination of donor gender from DNA, forensic DNA phenotyping can be used to predict donor eye, hair, and skin color with reasonably high accuracies [17]. If donor information, such as smoking habit can be derived from the same trace, these techniques combined, could render a more specific donor profile. Future work should focus on the development of methods to analyze and classify the use of multiple other exogenous compounds and stimulants from fingerprints. Additionally, further development of methods that render the chemical analysis compatible with the current fingerprint detection processes is needed, to be able to facilitate compositional analysis of fingerprints in practice. The chemical analysis of fingerprints shows to be a promising tool to infer specific donor information, which can be used to narrow down the suspect population or, alternatively, exclude suspects as donor. Moreover, the chemical profiling approach based on full-scan MS-data described in this dissertation can be extended to other fields, both within and outside forensic science. Chemical profiling in forensic science is potentially interesting in multiple areas of expertise, which use similar mass spectrometry-based methods, such as, among others, explosives, toxicology and illicit drugs investigations. In general, the approach may be applied in any field using full-scan MS-data to discover new markers, potentially answering more fundamental (bio)chemical research questions.

Overall, the research presented in this dissertation shows that the compositional

analysis of fingerprints is a promising tool and could be a valuable contribution to the continuously developing field of forensic science.

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List of Abbreviations

AFIS	Automated fingerprint identification system
CA	Cyanoacrylate
CE	Capillary electrophoresis
CI	Confidence interval
DART	Direct analysis in real time
DESI	Desorption electrospray ionization
DMEA	Dimethylethanolamine
ESI	Electrospray ionization
FLEC	1-(9-fluorenyl) ethyl chloroformate
FT-IR	Fourier-transform infrared spectroscopy
GC	Gas chromatography
GMP	Guanosine monophosphate
HILIC	Hydrophilic interaction chromatography
HPLC	High performance liquid chromatography
IS	Internal standard
LAP	Lithium phenyl-2,4,6-trimethylbenzoylphosphinate
LC	Liquid chromatography
LDA	Linear discriminant analysis
LESA	Liquid extraction surface analysis
LOD	Limit of detection
LOQ	Limit of quantification
MALDI	Matrix assisted laser desorption ionization
MRM	Multiple reaction monitoring
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
MSI	Mass spectrometry imaging
PC	Principal component
PCA	Principal component analysis
PDMS	Polydimethylsiloxane
PEG	Polyethylene glycol
pyGC	Pyrolysis gas chromatography
QqQ	Triple quadrupole
RSD	Relative standard deviation
RT	Room temperature
SIMCA	Soft independent modeling of class analogy
SIMS	Secondary ion mass spectrometry
TOF	Time-of-flight
U(H)PLC	Ultra-high-performance liquid chromatography

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A

Supplementary information Chapter 2

Table A.1: Gender and age of the 19 donors who donated their fingerprints in this study.

Donor	Gender	Age
1	F	21
2	F	20
3	F	26
4	M	27
5	F	21
6	F	25
7	F	22
8	M	28
9	F	28
10	M	66
11	M	32
12	M	25
13	M	21
14	M	45
15	M	42
16	M	52
17	F	20
18	F	42
19	F	21

A

Table A.2: MS/MS conditions (MicroMass Quattro Premier XE) for multiple reaction monitoring in ESI^+.

Compound	Parent ion (*m/z*)	Daughter ion (*m/z*)	Dwell time (s)	Cone voltage (V)	Collision energy (eV)
Cystine	240.90	119.70	0.040	10.00	18.00
Cystine	240.90	151.90	0.040	16.00	10.00
Cystine-D6	247.10	155.00	0.040	16.00	10.00
Ornithine	133.00	43.20	0.040	12.00	26.00
Ornithine	133.00	70.10	0.040	12.00	16.00
Ornithine	133.00	115.90	0.040	12.00	10.00
Ornithine-D6	139.20	76.20	0.040	12.00	14.00
Lysine	147.00	56.10	0.040	14.00	30.00
Lysine	147.00	84.00	0.040	14.00	14.00
Lysine	147.00	130.00	0.040	14.00	8.00
Lysine-D4	151.10	88.00	0.040	14.00	14.00
Arginine	175.01	115.90	0.040	20.00	14.00
Arginine	175.01	130.00	0.040	20.00	14.00
Arginine	175.01	157.90	0.040	20.00	12.00
Arginine-D7 15N4	186.00	78.00	0.040	30.00	22.00
Aspartic acid	134.00	74.00	0.040	12.00	12.00
Aspartic acid	134.00	88.00	0.040	12.00	10.00
Aspartic acid	134.00	116.00	0.040	12.00	8.00
Aspartic acid-D3	137.00	75.00	0.040	14.00	14.00
Histidine	156.00	82.90	0.040	16.00	20.00
Histidine	156.00	93.00	0.040	16.00	24.00
Histidine	156.00	110.00	0.040	16.00	12.00
Histidine-D3	159.00	113.00	0.040	16.00	12.00
Glutamic acid	148.00	83.90	0.040	20.00	16.00
Glutamic acid	148.00	101.90	0.040	20.00	12.00
Glutamic acid	148.00	129.90	0.040	20.00	8.00
Glutamic acid-D5	153.00	88.20	0.040	16.00	18.00
Asparagine	133.00	74.00	0.040	14.00	14.00
Asparagine	133.00	87.00	0.040	14.00	10.00
Asparagine	133.00	116.00	0.040	14.00	12.00
Asparagine-D3	135.90	90.00	0.040	14.00	10.00
Serine	105.80	60.00	0.040	20.00	10.00
Serine	105.80	70.00	0.040	20.00	12.00
Serine	105.80	87.90	0.040	20.00	12.00
Serine-D3	109.10	91.00	0.040	20.00	12.00
Threonine	120.00	56.20	0.040	16.00	14.00
Threonine	120.00	74.10	0.040	16.00	10.00
Threonine	120.00	102.00	0.040	16.00	8.00
Threonine-D2	122.10	76.10	0.040	16.00	10.00
Hydroxyproline	132.00	41.30	0.040	20.00	22.00
Hydroxyproline	132.00	68.00	0.040	20.00	18.00
Hydroxyproline	132.00	86.00	0.040	20.00	14.00
Hydroxyproline-D3	135.10	71.10	0.040	20.00	18.00
Alanine	90.00	44.20	0.040	16.00	8.00
Alanine	90.00	62.10	0.040	16.00	4.00
Alanine	90.00	72.30	0.040	16.00	16.00
Alanine-D4 13C3 15N 1	98.00	51.00	0.040	18.00	10.00
Tyrosine	182.00	91.00	0.040	14.00	26.00
Tyrosine	182.00	136.00	0.040	14.00	12.00
Tyrosine	182.00	165.00	0.040	14.00	10.00
Tyrosine-D2	184.00	167.00	0.040	14.00	10.00
Glutamine	147.00	56.20	0.040	14.00	26.00
Glutamine	147.00	84.00	0.040	14.00	16.00
Glutamine	147.00	129.90	0.040	14.00	10.00
Glutamine-D5	152.10	135.00	0.040	14.00	10.00
Valine	118.00	55.20	0.040	14.00	20.00
Valine	118.00	72.00	0.040	14.00	10.00
Valine-D8	126.00	80.00	0.040	16.00	10.00
Proline	116.00	43.00	0.040	22.00	22.00
Proline	116.00	70.10	0.040	22.00	12.00
Proline-13C5 15N1	121.90	75.00	0.040	22.00	12.00
Methionine	150.00	56.20	0.040	16.00	16.00
Methionine	150.00	104.00	0.040	16.00	10.00
Methionine	150.00	133.00	0.040	16.00	10.00
Methionine-D3	153.10	107.10	0.040	16.00	10.00
Tryptophan	205.10	118.00	0.040	14.00	28.00
Tryptophan	205.10	145.90	0.040	14.00	14.00
Tryptophan	205.10	188.10	0.040	14.00	8.00
Tryptophan-D5	210.20	150.10	0.040	18.00	18.00
Isoleucine	132.00	44.30	0.040	16.00	22.00
Isoleucine	132.00	69.10	0.040	16.00	16.00
Isoleucine	132.00	86.10	0.040	16.00	10.00
Isoleucine-13C6 15N1	139.00	92.00	0.040	18.00	10.00
Phenylalanine	166.00	77.00	0.040	14.00	34.00
Phenylalanine	166.00	103.00	0.040	14.00	28.00
Phenylalanine	166.00	120.00	0.040	14.00	14.00
Phenylalanine-D5	171.10	125.00	0.040	14.00	14.00
Leucine	132.00	44.00	0.040	16.00	16.00
Leucine	132.00	86.00	0.040	16.00	10.00
Leucine-D10	142.20	96.00	0.040	16.00	10.00

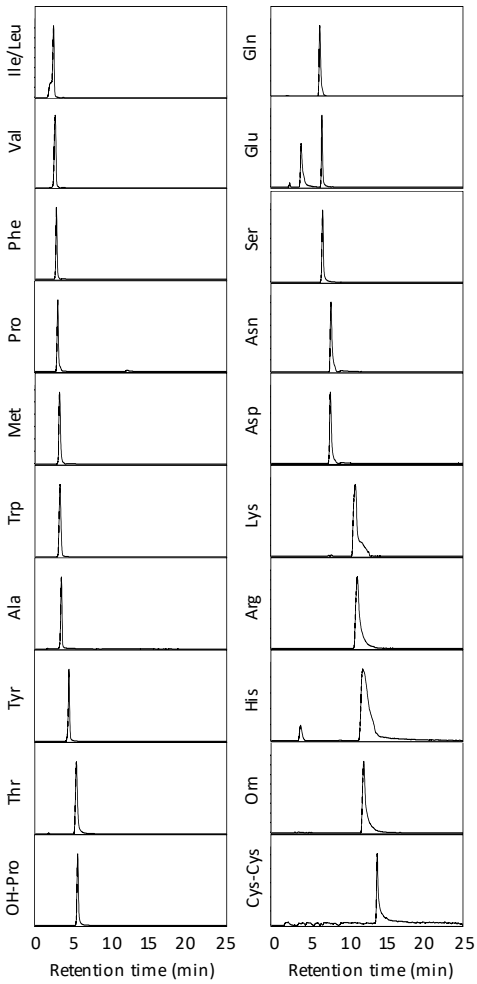

Figure A.1: Figure 1: Extracted ion chromatograms (XICs) of a typical separation of 21 amino acids on the time-of-flight MS.

Table A.3: Calculated extraction efficiency of the 21 amino acids, based on a positive control, determined by UPLC-TOF-MS

Compound	Extraction efficiency (%)
L-alanine	105.0
L-arginine	97.3
L-asparagine	104.9
L-aspartic acid	109.3
L-cystine	117.7
L-glutamic acid	112.9
L-glutamine	110.7
L-histidine	112.2
hydroxy-L-proline	110.0
L-iso-/leucine	113.9
L-lysine	98.6
L-methionine	89.1
L-ornithine	102.5
L-phenylalanine	113.0
L-proline	110.5
L-serine	111.0
L-threonine	111.7
L-tryptophan	109.6
L-tyrosine	96.0
L-valine	109.5

B

Supplementary information Chapter 3

Questionnaire

Q1. What is your gender?
1 = Male
2 = Female

Q2. What is your age?

Q3. Are you left- or righthanded?
1 = Left
2 = Right

Q4. How many alcohol consumptions have you had in the past 24 hours?
1 = 0
2 = 1-5
3 = 6-10
4 = 11-15
5 = >15

Q5. Have you used drugs in the past 24 hours? If so, please indicate in the list:
1 = Cannabis
2 = XTC (MDMA)
3 = Cocaine
4 = Heroine
5 = Speed (amphetamine)
6 = Mushrooms
7 = Ketamine
8 = GHB
9 = LSD
10 = Methadone
11 = 4FMP
12 = Other

Q6 Have you used painkillers in the past 24 hours? If so, please indicate in the list:
1 = Diclofenac (as well as fixed dose combinations) (Voltaren, Arthrotec)
2 = Ibuprofen (Advil, Brufen, Nurofen, Sarixell, Spidifen, Zafen)
3 = Naproxen (Aleve, Naprovite)
4 = Paracetamol (as well as fixed dose combinations) (Panadol, Sinaspril, APC, Finimal)
5 = Meloxicam (Movicox)
6 = Etoricoxib (Arcoxia)
7 = Acetylsalicylic acid (Aspirin, Aspro)
8 = Tramadol (Tramagetic)

Q7. Have you used antidepressants in the past 24 hours? If so, please indicate in the list:
1 = Paroxetine (Seroxat)
2 = Amitriptyline (Sarotex)
3 = Citalopram (Cipramil)
4 = Fluoxetine (Prozac)
5 = Sertraline (Zoloft)

Q8. Have you used hormonal contraceptive in the past 24 hours? If so, please indicate in the list:
1 = Estrogen with levonorgestrel (Microgynon, Lovette, Stediril)
2 = Estrogen with cyproterone (Diane)
3 = Estrogen with drospirenon (Yasmin, Yaz)

Q9. Have you used medicinal drugs to treat allergies in the past 24 hours? If so, please indicate in the list:
1 = Desloratadine (Aerius)
2 = Levocetirizine (Xyzal, Zyrtec)

Q10. Have you used medicinal drugs to treat ulcer disease in the past 24 hours? If so, please indicate in the list:
1 = Omeprazole (Losec)
2 = Pantoprazole (Pantozol)
3 = Esomeprazole (Nexium)
4 = Ranitidine (Zantac)

Q11. Have you used sleep- or calming aids in the past 24 hours? If so, please indicate in the list:
1 = Oxazepam (Seresta)
2 = Temazepam (Normison)
3 = Diazepam

Q12. Have you used ointments in the past 24 hours? If so, please indicate in the list:
1 = Hydrocortisone
2 = Triamcinolone (Kenacort)
3 = Fusidic acid (Fucidin)
4 = Ketoconazole (Nizoral)
5 = Miconazole (Daktarin)
6 = Betamethasone (Diprosone)
7 = Clobetasol (Dermovate)

Q13. Have you used blood pressure regulators in the past 24 hours? If so, please indicate in the list:
1 = Acetylsalicylic acid (Aspirin, Aspro)
2 = Carbasalaatcalcium (Ascal)
3 = Acenocoumarol
4 = Metoprolol (Selokeen, Lopressor)
5 = Atenolol (Tenormin)
6 = Lisinopril (Zestril)
7 = Amlodipine (Norvasc)
8 = Enalapril (Renitec)
9 = Losartan (Cozaar)
10 = Perindopril (Coversyl)
11 = Nifedipine (Adalat)

Q14. Have you used other medicinal drugs in the past 24 hours? If so, please indicate in the list:
1 = Methylphenidate (Ritalin, Concerta, Medikinet, Equasym)
2 = Macrogol (Movicolon, Molaxole, Forlax)
3 = Lactulose (Legendal)
4 = Insulin (short- and long-acting)
5 = Calcium (+Vit D) (Calci-chew, effervescent tablets)
6 = Metformin (Glucophage)
7 = Levothyroxine (Thyrax)
8 = Codeine
9 = Ferrofumaraat
10 = Antibiotics
11 = Loperamide (Imodium, Diacure)

Q15. When did you last wash your hands (washing in the experiment not included)?
1 = <1
2 = <2
3 = <3
4 = <6
5 = >6

Q16. Indicate your stress level on a scale from 1 to 5, 1 = no stress, 5 = very stressed
1 = 1
2 = 2
3 = 3
4 = 4
5 = 5

Q17. What is part of your normal diet?
1 = omnivorous
2 = vegetarian
3 = vegan

Q18. What did you eat last night?
1 = omnivorous
2 = vegetarian
3 = vegan

Q19. Do you have any food allergies?
1 = None
2 = Gluten
3 = Lactose
4 = Peanuts
5 = Nuts
6 = Shellfish
7 = Eggs
8 = Fish
9 = Soy
10 = Other

Q20. How many cups of coffee do you drink on an average day?
1 = 0
2 = 1-2
3 = 3-4
4 = 5 or more

Q21. How many cups of coffee did you drink in the past 24 hours?
1 = 0
2 = 1-2
3 = 3-4
4 = 5 or more

Q22. How many packs of cigarettes do you smoke on an average day?
1 = 0
2 = < 1
3 = 1
4 = 2
5 = >2

Q23. How many packs of cigarettes did you smoke in the past 24 hours?
1 = 0
2 = < 1
3 = 1
4 = 2
5 = >2

Q24. How many consumptions of soda do you drink on an average day?
1 = 0
2 = 0-5
3 = 6-10
4 = 11-15
5 = > 15

Q25. Did you indicate >0, please indicate if you drink regular soda, diet soda or both:
1 = regular
2 = diet
3 = both

Q26. How many consumptions of soda did you drink in the past 24 hours?
1 = 0
2 = 0-5
3 = 6-10
4 = 11-15

Q27. Did you indicate >0, please indicate if you drink regular soda, diet soda or both:
1 = regular
2 = diet
3 = both

Q28. Have you touched food since you last washed your hands (not including washing of hands in the experiment)?
1 = Yes
2 = No

Q29. Have you touched cosmetics (make-up, sunscreen, hair gel etc.) since you last washed your hands (not including washing of hands in the experiment)?
1 = Yes
2 = No

Q30. Have you touched drugs since you last washed your hands (not including washing of hands in the experiment)?
1 = Yes
2 = No

Q31. If so, please indicate in the list:
1 = Cannabis
2 = XTC (MDMA)
3 = Cocaine
4 = Heroine
5 = Speed (amphetamine)
6 = Mushrooms
7 = Ketamine
8 = GHB
9 = LSD
10 = Methadone
11 = 4FMP
12 = Other

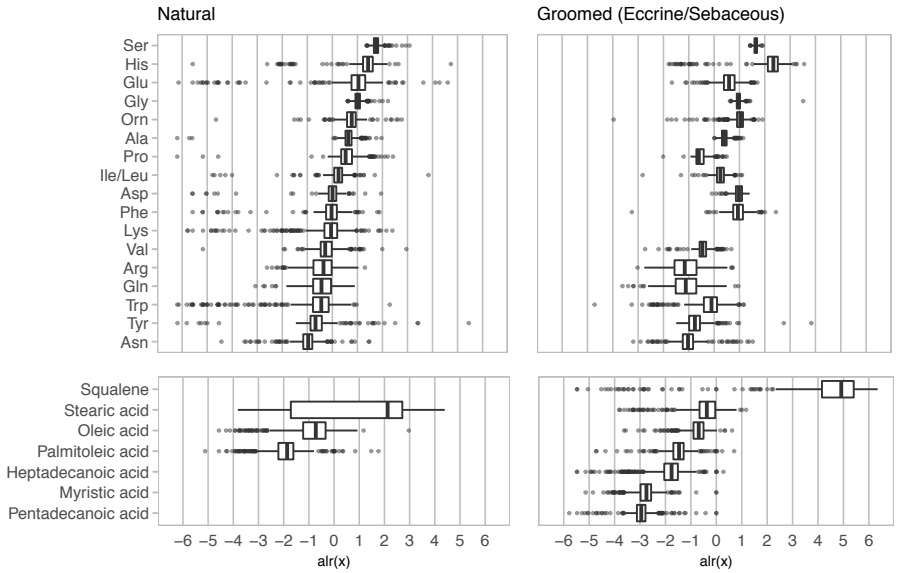

Figure B.1: Additive log-ratio (alr) transformed metabolite levels of the natural, eccrine and sebaceous fingerprints.

Table B.1: Mass over charge values of the 30 preselected features using the mRMRe package.

LCMS print type	GCMS print type	LCMS Gender Natural	LCMS Gender Eccrine	GCMS Gender Natural	GCMS Gender Sebaceous	LCMS Smoking Natural	LCMS Smoking Eccrine	LCMS Smoking 24h Natural	LCMS Smoking 24h Eccrine
61.04	36.07	85.00	70.51	36.07	36.07	74.06	68.98	74.06	68.98
84.91	39.16	85.96	83.02	40.06	41.20	89.99	81.52	80.05	84.96
124.92	42.20	111.06	85.96	41.19	41.20	96.04	90.06	84.00	90.06
130.05	43.19	124.54	90.06	42.20	41.20	102.97	92.07	90.09	92.07
131.05	44.19	127.07	92.95	43.19	44.18	110.02	96.99	95.54	96.99
137.06	60.09	129.06	111.52	44.19	59.10	122.08	98.99	96.04	98.99
139.05	60.09	130.96	114.89	59.10	60.10	131.96	102.97	96.92	102.97
147.08	66.11	132.24	119.05	60.09	60.10	132.10	122.92	102.97	111.01
152.03	71.20	137.05	123.05	60.09	65.11	150.11	123.05	122.08	116.01
159.08	76.10	145.11	127.07	65.10	71.20	155.05	130.96	131.96	122.08
166.95	79.10	150.11	130.96	66.11	74.10	161.03	131.96	132.10	123.05
169.99	79.10	152.06	131.96	71.20	74.10	163.12	132.10	134.12	131.96
175.12	79.10	155.05	137.05	76.10	75.10	164.13	137.06	136.08	138.12
264.84	81.12	163.12	139.05	81.11	76.10	172.13	138.12	139.91	139.05
271.04	87.10	169.04	150.11	86.20	91.10	177.10	139.05	146.12	155.05
277.92	93.10	171.96	152.06	93.10	93.10	184.98	140.96	150.11	161.03
315.26	96.10	203.05	154.10	94.11	98.10	205.07	140.99	163.12	163.12
325.11	103.01	206.14	172.13	95.12	119.10	233.08	163.12	164.13	166.10
343.29	117.05	241.71	183.08	98.20	125.19	258.17	166.10	172.13	172.12
362.16	128.19	275.05	206.10	115.10	168.20	275.05	172.12	177.10	179.13
365.28	137.20	284.10	206.14	117.05	191.03	300.29	175.12	205.07	206.14
366.11	153.18	300.29	229.89	125.19	191.19	325.11	179.13	275.05	225.12
366.28	168.20	306.17	263.03	130.10	191.20	343.29	203.18	300.29	258.17
423.06	191.19	316.28	303.20	139.18	193.03	381.08	206.14	325.11	296.29
428.39	203.10	343.29	352.15	142.20	203.10	412.19	208.04	325.11	327.10
449.37	203.20	393.10	360.15	168.20	203.20	490.18	296.29	343.29	352.15
461.30	211.30	490.17	393.31	203.10	211.30	512.16	352.15	393.10	365.11
465.25	213.20	522.34	403.11	211.30	213.20	522.20	360.15	448.17	389.10
663.33	222.30	541.35	428.39	267.30	253.31	554.19	412.19	450.15	585.37
723.33	253.31	786.60	486.36	331.09	267.30	758.57	585.37	758.57	663.33

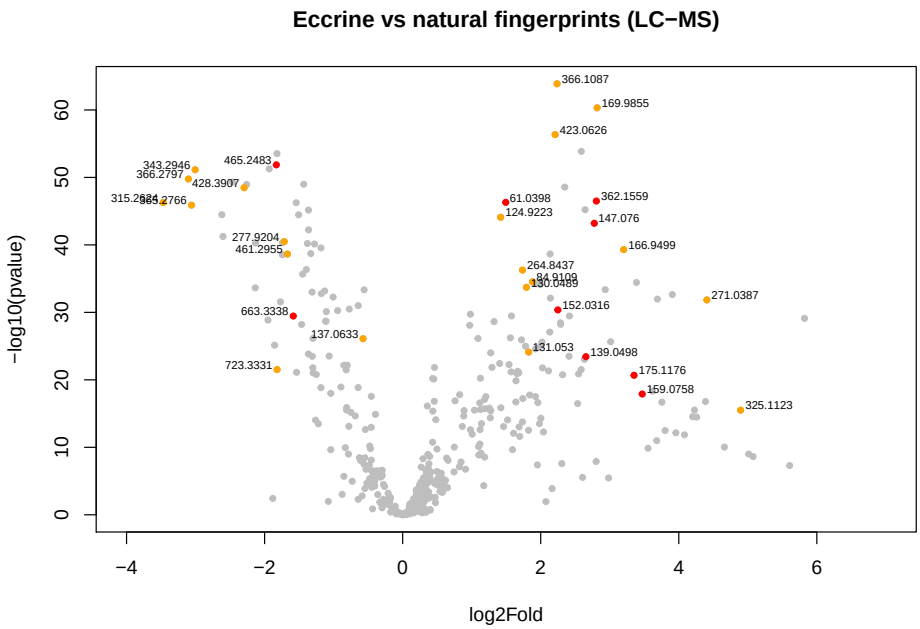

Figure B.2: Volcano plot of the features used in the classification of eccrine and natural fingerprints using the LC-MS data. In orange, the 30 features that were preselected. In red, the most important predictors used.

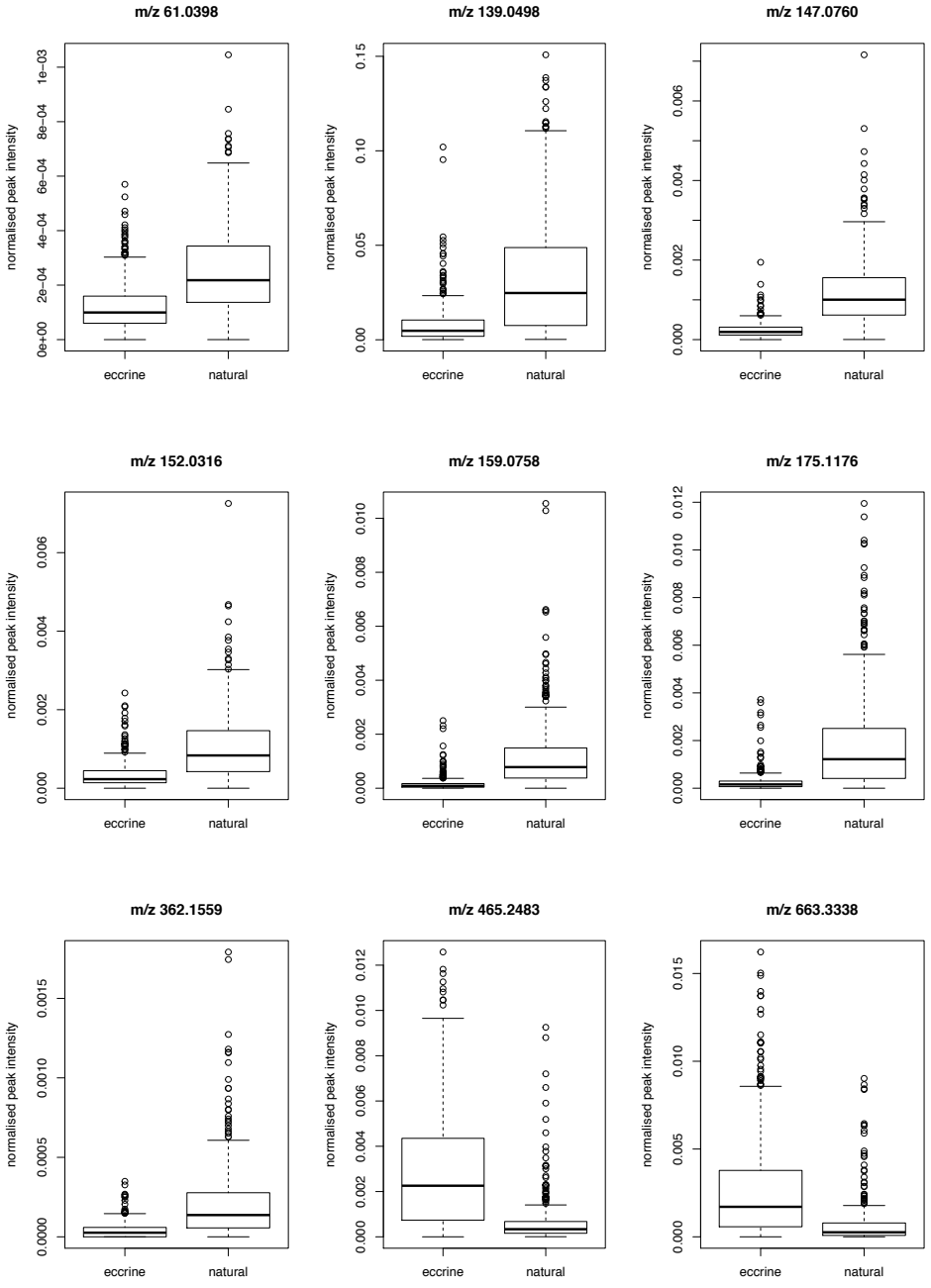

Figure B.3: Boxplots of the features used in the model to predict fingerprint type (eccrine vs natural) based on LC-MS data. From left to right putative compounds: urea (*m/z* 61.0398), urocanic acid (*m/z* 139.0498), L-glutamine (*m/z* 147.0760), pyroglutamic acid (*m/z* 152.0316), 4-methylene-L-glutamine (*m/z* 159.0758), L-arginine (*m/z* 175.1176), tripeptide Val-Glu-Asp or Asp-Leu/Ile-Asp (*m/z* 362.1559), tetrapeptide: Lys-Lys-Ser-Cys (*m/z* 465.2483), tetrapeptide: Arg-Arg-Phe-Tyr [M+Na]+ (*m/z* 663.3338).

Table B.2: Putative peptides found in LC-MS fingerprint data and the relative mass error (in ppm).

Experimental *m/z*	Putative annotation	Theoretical *m/z*	Δppm
284.0998	Trp-Gly $[M+Na]^+$	284.1006	2.81
361.1531	Pro-Met-Asn or Met-Gly-Gly-Pro $[M+H]^+$	361.1540	2.49
362.1559	Val-Glu-Asp or Asp-Ile/Leu-Asp $[M+H]^+$	362.1558	0.28
377.1956	Cys-Val-Arg or Ala-Arg-Met $[M+H]^+$	377.1966	2.65
384.1594	Phe-Ser-Met or Tyr-Ala-Met $[M+H]^+$	384.1588	1.56
398.1731	Cys-Tyr-Ile/Leu or Met-Thr-Phe $[M+H]^+$	398.1744	3.26
412.1884	Val-Tyr-Met $[M+H]^+$	412.1901	4.12
448.1655	Asp-Gln-Ala-Asp or Gly-Gln-Asp-Glu $[M+H]^+$	448.1674	6.47
449.1482	Asn-Met-Tyr or Tyr-Cys-Ala-Ala or Cys-Ala-Ser-Phe or Tyr-Met-Gly-Gly or Cys-Gly-Phe-Thr $[M+Na]^+$	449.1465	3.78
450.1515	Phe-Met-Met $[M+Na]^+$	450.1492	5.11
465.2483	Lys-Lys-Ser-Cys $[M+H]^+$	465.2490	1.50
466.1742	Cys-Pro-Pro-Gln $[M+Na]^+$	466.1731	2.36
467.1777	Ala-Asp-Phe-Asp or Glu-Gly-Asp-Phe or Met-Trp-Met $[M+H]^+$	467.1773	0.86
472.1306	Cys-Pro-Cys-Gln $[M+Na]^+$	472.1295	2.33
473.3424	Lys-Val-Lys-Val $[M+H]^+$	473.3414	2.11
476.1609	Thr-Cys-Cys-Lys $[M+Na]^+$	476.1608	0.21
504.3184	Lys-Ile/Leu-Pro-Phe $[M+H]^+$	504.3180	0.79
521.3343	Ile/Leu-Ile/Leu-Ile/Leu-Tyr $[M+H]^+$	521.3334	1.73
522.2008	Val-Cys-Trp or Glu-Gly-Trp-Met or Ala-Asp-Met-Trp $[M+H]^+$	522.2017	1.72
527.1568	Cys-Tyr-Tyr-Gly $[M+Na]^+$	527.1571	0.57
548.3437	Gly-Phe-Ile/Leu-Ile/Leu-Val $[M+H]^+$	548.3443	1.09
554.1902	Trp-Asp-Asp-Pro $[M+Na]^+$	554.1857	8.12
619.3083	His-Trp-Val-Arg $[M+Na]^+$	619.3075	1.29
620.3133	Ile/Leu-Arg-Tyr-Phe $[M+Na]^+$	620.3167	5.48
647.3375	Phe-Arg-Phe-Arg $[M+Na]^+$	647.3388	2.01
663.3338	Arg-Arg-Phe-Tyr $[M+Na]^+$	663.3337	0.15

Table B.3: Confusion matrix of cforest classification model for fingerprint type based on GC-MS data with an overall 86.8% accuracy (CI: 81.8% - 90.9%).

		Reference	
		Natural	Sebaceous
Prediction	Natural	106	19
	Sebaceous	12	98

Table B.4: Confusion matrix of cforest classification model for donor gender based on LC-MS data of the eccrine fingerprints with an overall 71.7% accuracy (CI: 62.4% - 79.8%).

		Reference	
		Male	Female
Prediction	Male	28	16
	Female	16	53

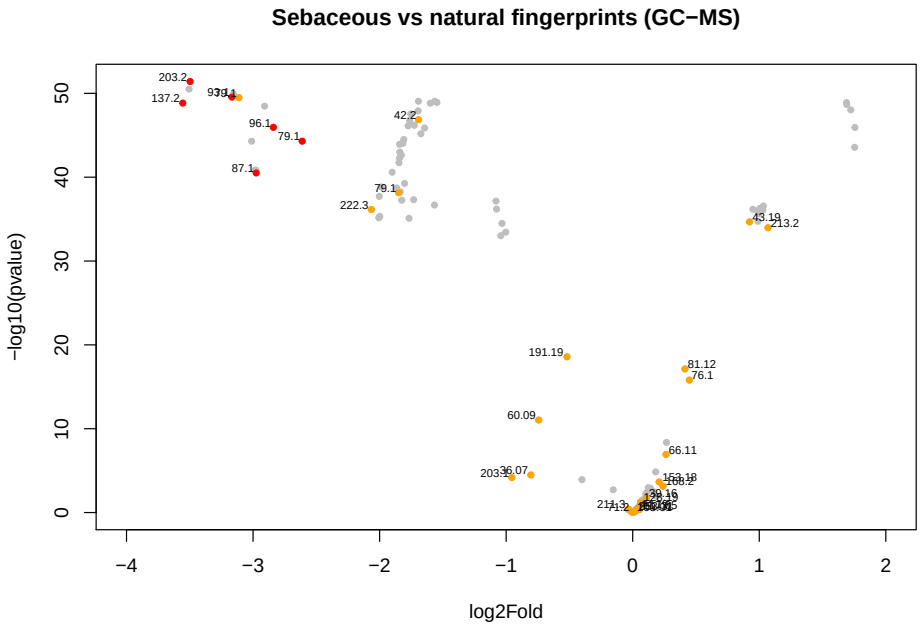

Figure B.4: Volcano plot of the features used in the classification of sebaceous and natural fingerprints using the GC-MS data. In orange, the 30 features that were preselected. In red, the most important predictors used.

Table B.5: Confusion matrix of cforest classification model for donor gender based on GC-MS data of the natural fingerprints with an overall 68.1% accuracy (CI: 58.7% - 76.6%).

		Reference	
		Male	Female
Prediction	Male	24	16
	Female	20	53

Table B.6: Confusion matrix of cforest classification model for donor gender based on GC-MS data of the sebaceous fingerprints with an overall 64.6% accuracy (CI: 55.0% - 73.4%)).

		Reference	
		Male	Female
Prediction	Male	26	22
	Female	18	47

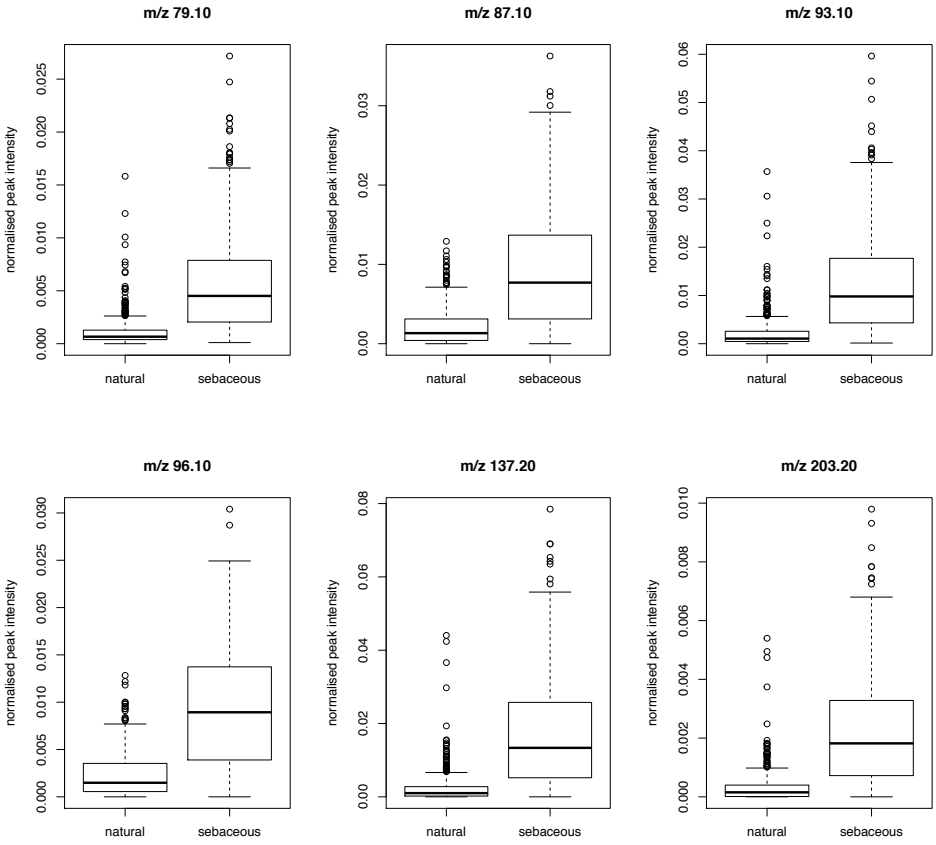

Figure B.5: Boxplots of the features used in the model to predict fingerprint type (natural vs sebaceous) based on GC-MS data. From left to right putative fragments from: squalene/cholesterol (*m/z* 79.10), FAMES (*m/z* 87.10), squalene/cholesterol (*m/z* 93.10), FAMES (mono-unsaturated) (*m/z* 96.10), squalene (*m/z* 137.20) and squalene (*m/z* 203.20).

Table B.7: Confusion matrix of cforest classification model for everyday smoking habit of donors, based on LC-MS data of the eccrine fingerprints with an overall 90.2% accuracy (CI: 83.1% - 95.0%).

		Reference	
		Non-smoker	Smoker
Prediction	Non-smoker	75	6
	Smoker	5	26

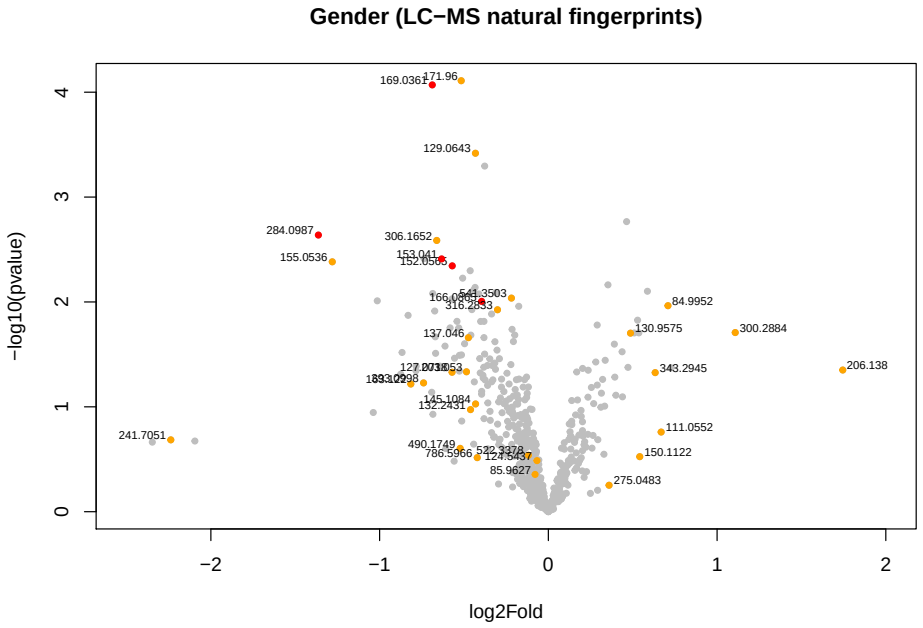

Figure B.6: Volcano plot of the features used in the classification of donor gender based on the LC-MS data of natural fingerprints. In orange, the 30 features that were preselected. In red, the most important predictors used, as well as *m/z* 153.0410 and 166.0865, putatively xanthine and L-phenylalanine, respectively.

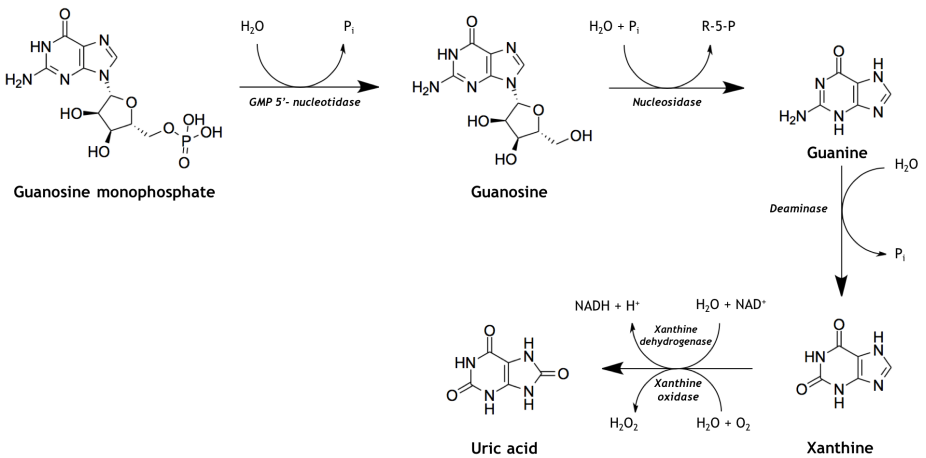

Figure B.7: Guanosine monophosphate (GMP) degradation pathway. GMP is catabolized to uric acid via guanosine, guanine and xanthine, respectively.

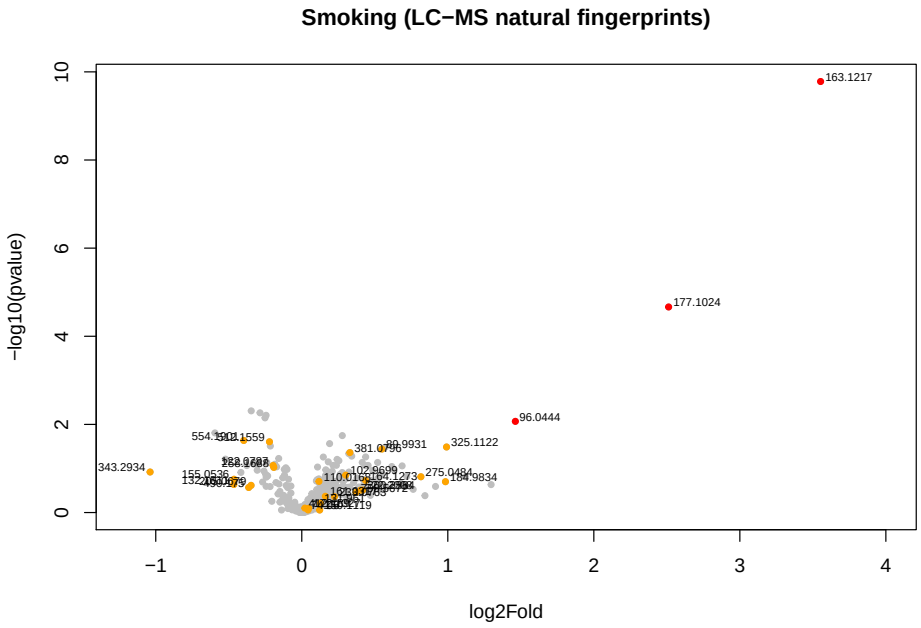

Figure B.8: Volcano plot of the features used in the classification of smokers based on the LC-MS data of natural fingerprints. In orange, the 30 features that were preselected. In red, the most important predictors used.

Table B.8: Confusion matrix of cforest classification model for past 24 hour smoking of donors, based on LC-MS data of the natural fingerprints with an overall 87.7% accuracy (CI: 80.3% - 93.1%).

		Reference	
		Non-smoker	Smoker
Prediction	Non-smoker	69	10
	Smoker	4	31

Table B.9: Confusion matrix of cforest classification model for past 24 hour smoking of donors, based on LC-MS data of the eccrine fingerprints with an overall 87.6% accuracy (CI: 80.1% - 93.1%).

		Reference	
		Non-smoker	Smoker
Prediction	Non-smoker	69	11
	Smoker	3	30

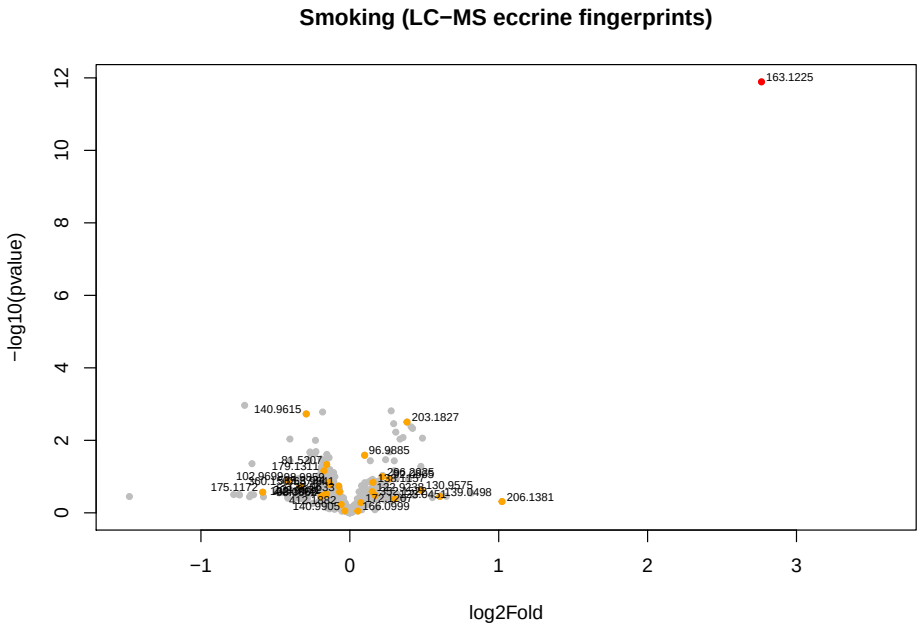

Figure B.9: Volcano plot of the features used in the classification of smokers based on the LC-MS data of eccrine fingerprints. In orange, the 30 features that were preselected. In red, the most important predictor used.

Table B.10: Features used in the classification model for past 24-hour smoking based on LC-MS data, their putative annotation, relative mass error (in ppm) and fold change. P-values were calculated using *t*-tests.

Experimental *m/z*	Putative annotation	Δppm	Higher in	Fold change natural	p-value	eccrine	
96.0444	Hydroxypyridine $[M+H]^+$	0	Smoker	2.50	7.01E-03	n.f.	-
163.1225	Nicotine $[M+H]^+$	3.07	Smoker	11.07	5.64E-11	6.33	3.91E-13
177.1024	Cotinine $[M+H]^+$	1.13	Smoker	4.77	2.06E-05	n.f.	-

Table B.11: Features found in natural fingerprints of participants that indicated to have used cosmetic or personal care products such as make-up, sunscreen or hair gel compared to those who did not based on LC-MS data of natural fingerprints. Their putative annotation, relative mass error (in ppm) and fold change. P-values were calculated using *t*-tests.

Experimental *m/z*	Putative annotation	Δppm	Higher in	Fold change	p-value
90.0907	DMEA $[M+H]^+$	6.66	Touched	1.59	3.52E-01
104.1069	Choline $[M]^+$	0.96	Touched	1.77	1.33E-02
115.0365	Glycerol $[M+Na]^+$	0.87	Touched	2.65	2.59E-07
206.1380	Panthenol $[M+H]^+$	3.40	Touched	4.74	2.78E-02
228.1208	Panthenol $[M+Na]^+$	0.88	Touched	3.73	1.87E-03
275.0484	Ensulizole $[M+H]^+$	0.36	Touched	1.44	3.54E-01

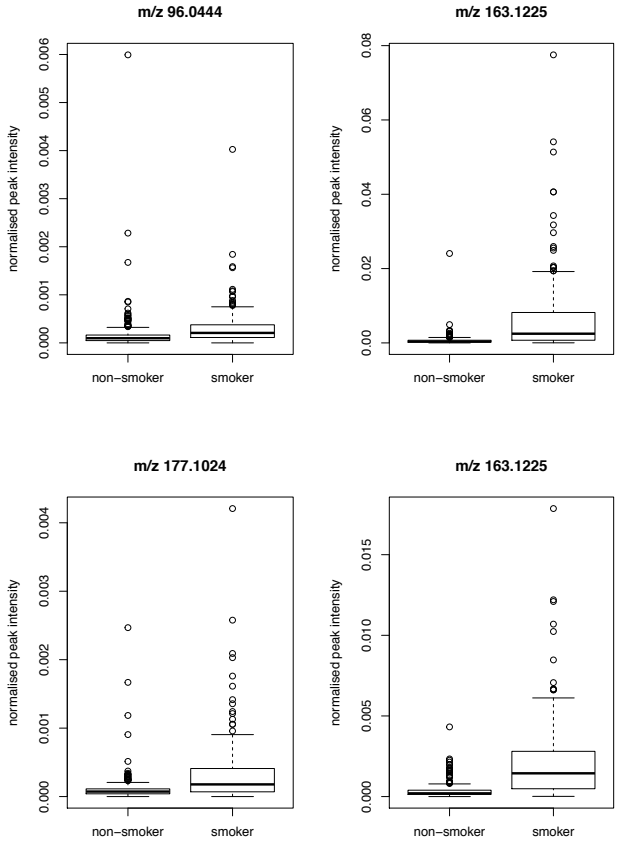

Figure B.10: Boxplots of the features used in the model to predict past 24-hour smoking based on LC-MS data. From left to right putative compounds: hydroxypyridine (*m/z* 96.0444) in natural fingerprints, nicotine (*m/z* 163.1225) in natural fingerprints, cotinine (*m/z* 177.1024) in natural fingerprints and nicotine (*m/z* 163.1225) in eccrine fingerprints.

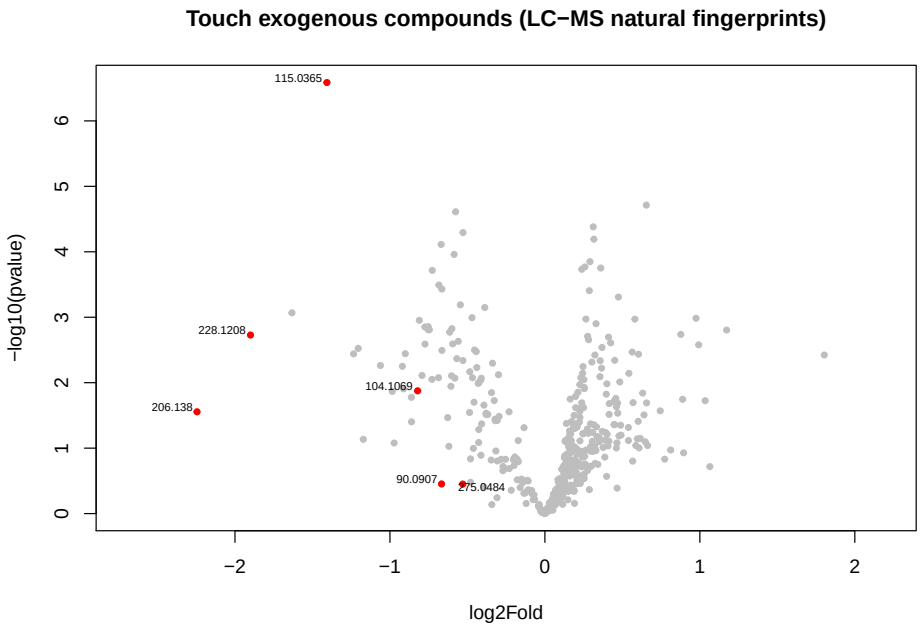

Figure B.11: Volcano plot of the features found in natural fingerprints of participants that indicated to have used cosmetic or personal care products such as make-up, sunscreen or hair gel compared to those who did not, based on LC-MS data. In red, the features highlighted in the main text, putatively annotated to: glycerol (*m/z* 115.0365), panthenol (*m/z* 206.138 and *m/z* 228.1208), ensulizole (*m/z* 275.0484), DMEA (*m/z* 90.0907) and choline (*m/z* 104.1069).

C

Supplementary information Chapter 4

Table C.1: The optimized MRM parameters.

Compound	Precursor (*m/z*)	Product (*m/z*)	Collision Energy (V)
FLEC-Alanine	326.15	178.111	33.663
FLEC-Alanine	326.15	193.111	10.253
FLEC-Serine	342.16	178.125	34.472
FLEC-Serine	342.16	193.111	12.124
FLEC-Proline	352.12	178.071	35.888
FLEC-Proline	352.12	193.054	11.416
FLEC-Valine	354.18	178.125	34.978
FLEC-Valine	354.18	193.054	10.253
FLEC-Threonine	356.15	178.054	35.534
FLEC-Threonine	356.15	193.125	10.253
FLEC-Hydroxy-d-proline	368.12	178.111	36.596
FLEC-Hydroxy-d-proline	368.12	193.111	13.388
FLEC-Methionine	386.15	178.125	37.303
FLEC-Methionine	386.15	193.125	12.933
FLEC-Histidine	392.15	178.111	39.882
FLEC-Histidine	392.15	193.111	19.607
FLEC-Phenylalanine	402.18	178.125	38.416
FLEC-Phenylalanine	402.18	193.071	13.185
FLEC-Tyrosine	418.18	178.111	42.916
FLEC-Tyrosine	418.18	193.111	17.483

Table C.2: Limit of detection and limit of quantification of the amino acids of interest.

	LoD (µg/L)	LoQ (µg/L)
D-alanine	0.8	2.6
L-alanine	0.7	2.4
D-serine	0.2	0.6
L-serine	0.2	0.6
D-proline	0.4	1.3
L-proline	0.4	1.4
D-valine	0.3	1.0
L-valine	0.3	1.1
D-threonine	0.8	2.6
L-threonine	0.7	2.5
D-methionine	0.3	1.1
L-methionine	0.3	1.0
D-histidine	1.8	6.1
L-histidine	1.6	5.2

D

Supplementary information Chapter 5

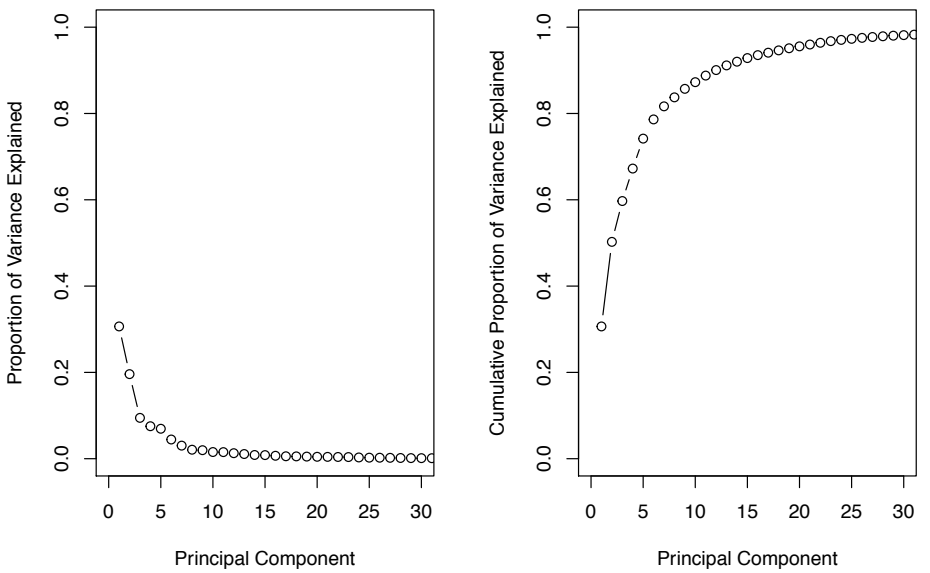

Figure D.1: Plot showing the variance explained by the principal components (both proportion and cumulative proportion) against the number of principal components.

Table D.1: Confusion matrix of the PCA-LDA classification model based on the DESI-MS analysis of condom lubricant sample spots, with an overall accuracy of 99.0%.

	BBEL	BC	BLANCO	BSC	DCN	DES	DFS	DO	DP	DRF	DXLP	EU	EXS	FSO	GUN	JSS	KC	KE	KSb	KSc	KSs	KU	LH	LP	MASS	MS	MSO	ON	PL	PN	ST	UP	WI
BBEL	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BC	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BLANCO	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BSC	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DCN	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DES	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DFS	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DO	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DP	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DRF	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DXLP	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
EU	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
EXS	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FSO	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GUN	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
JSS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
KC	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
KE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
KSb	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
KSc	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0
KSs	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
KU	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0
LH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
LP	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0
MASS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0
MS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0
MSO	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
ON	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0
PL	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0
PN	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0
ST	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0
UP	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0
WI	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2

Table D.2: Confusion matrix of the PCA-LDA classification model based on the DESI-MS analysis of fingerprints containing condom lubricant traces, with an overall accuracy of 90.9%.

	BBEL	BC	BLANCO	BSC	DCN	DES	DFS	DO	DP	DRF	DXLP	EU	EXS	FSO	GUN	JSS	KC	KE	KSb	KSc	KSs	KU	LH	LP	MASS	MS	MSO	ON	PL	PN	ST	UP	WI
BBEL	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BC	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BLANCO	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BSC	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DCN	0	0	0	0	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DES	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DFS	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DO	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DP	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DRF	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DXLP	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
EU	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
EXS	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
FSO	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GUN	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
JSS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
KC	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
KE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
KSb	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
KSc	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
KSs	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
KU	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
LH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0
LP	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
MASS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0
MS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0
MSO	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0
ON	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
PL	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0
PN	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
ST	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
UP	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0
WI	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2

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Table D.3: Confusion matrix of the PCA-LDA classification of DESI-MS analysis of EXS lubricated fingerprint (rows 50, 100, 150, 200, 250 and 300 of the chemical image was used as input data), showing their correct prediction.

	BBEL	BC	BLANCO	BSC	DCN	DES	DFS	DO	DP	DRF	DXLP	EU	EXS	FSO	GUN	JSS	KC	KE	KSb	KSc	KSs	KU	LH	LP	MASS	MS	MSO	ON	PL	PN	ST	UP	WI
BBEL	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BC	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BLANCO	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BSC	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DCN	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DES	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DFS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DO	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DP	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DRF	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DXLP	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
EU	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
EXS	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FSO	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GUN	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
JSS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
KC	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
KE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
KSb	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
KSc	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
KSs	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
KU	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LP	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MASS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MSO	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ON	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PL	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PN	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ST	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
UP	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
WI	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Curriculum Vitæ

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Education

2004–2010 Gymnasium NT+ NG
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2010–2014 Bachelor Life Science & Technology
Delft University of Technology
University of Leiden

2014–2016 Master Life Science & Technology
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Thesis: Towards absolute protein quantification in *S.cerevisiae* (baker's yeast) using mass spectrometry and ^{13}C- protein internal standards.
Supervisor: Dr. S.A. Wahl

2016–2020 PhD Forensic Chemistry
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Promotor: Prof. Dr. C.J. de Poot
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List of Publications

5. **W. van Helmond**, M. Weening, V. Vleer & M. de Puit, *Analysis of amino acid enantiomers from aged fingerprints*, Analytical Methods **12**, 2052-2057 (2020).

4. **W. van Helmond**, M.P.V. Begieneman, R. Kniest & M. de Puit, *Classification of condom lubricants in cyanoacrylate treated fingerprints by desorption electrospray ionization mass spectrometry*, Forensic Science International **305** (2019).

3. **W. van Helmond**, A.W. van Herwijnen, J.J. van Riemsdijk, M.A. van Bochove, C.J. de Poot & M. de Puit, *Chemical profiling of fingerprints using mass spectrometry*, Forensic Chemistry **16** (2019).

2. **W. van Helmond**, V. O'Brien, R. de Jong, J.H. van Esch, S. Oldenhof & M. de Puit, *Collection of amino acids and DNA from fingerprints using hydrogels*, Analyst **143**, 900-905 (2018).

1. **W. van Helmond**, C.J. Kuijpers, E. van Diejen, J. Spiering, B. Maagdelijn & M. de Puit, *Amino acid profiling from fingerprints, a novel methodology using UPLC-MS*, Analytical Methods **9**, 5697-5702 (2017).