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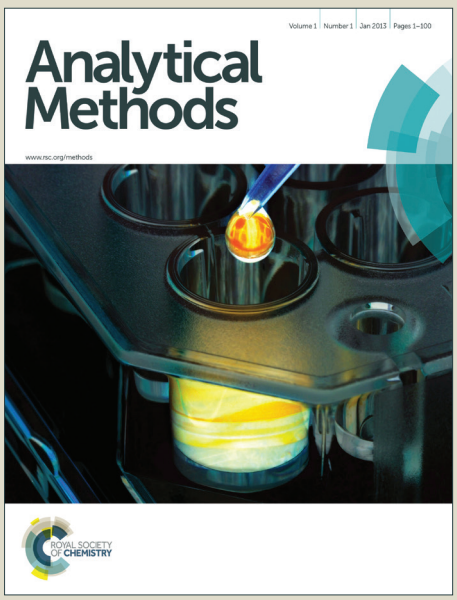

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Extraction of Fatty Compounds from Fingerprints for GCMS Analysis

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Abstract

The composition of fingerprints can contain a wealth of information with regards to the donor of the fingerprint. Fatty acids and other related sebaceous material can be used to classify donor groups, as previously reported. The extraction of these particular materials from the fingerprint entities has proven to be rather tedious and difficult to reproduce on standardised samples. We present a two step method to obtain a broad spectrum of sebaceous materials from fingerprints in high yields with good reproducibility. By dissolving fingerprint material in MeOH in the presence of TMSCl the fatty acids are esterified to their corresponding fatty acid methyl esters. During this extraction some of the other sebaceous material is extracted as well. Only in a consecutive extraction with $CHCl_3$ is an optimal extraction of the fatty content of a fingerprint achieved.

Introduction

The chemical composition of fingerprints has been investigated numerous times,[1] for different purposes, such as the age estimation of a fingerprint,[2, 3] determining the inter- and intravariability[1, 4] and the determination of the efficacy of fingerprint reagents.[5] In our earlier studies we had already noticed there were several different methods described for the isolation of fingerprint constituents from different substrates. As we focused on several particular applications of these analytical methods,[5] we did not further explore these differences. In this paper we describe the development of a robust and reproducible extraction method for the analysis of fatty components (fatty acids, wax esters, squalene and cholesterol amongst others). Ultimately with any form of extraction of an unknown sample, it is important to know what the efficiency is for known samples.

We also perform a comparative study of different methods as described in the literature. All GCMS methods mentioned in the literature make use of similar columns; DB-5MS (30 m x 0,25 mm, J&W Scientific), ZB-5 (30 m x 0,25 mm, Phenomenex), DB-17ms (30m x 0,25 mm, J&W Scientific), HP-5MS (30 m x 0,25 mm, Agilent) and Intercap-17MS (30m x 0,25 mm, GLScience). The time and temperature programs used may differ between the methods described, which will have an effect on the actual separation of the particular components of interest. We have chosen a commonly used GC column for the separation in order to investigate the differences between the methods for the extraction of the material from the original matrix.

The following summary of extraction methods found in the literature is not exhaustive, but gives a representative overview of known methods for the extraction of fingerprint constituents from a surface, for analytical purposes.

Asano reported the chemical composition of fingerprints for gender determination purposes in 2002.[6] Fingerprint excretions were deposited on glass beads and extracted with chloroform ($CHCl_3$) and analysed by GCMS without any derivatisation. A total of eleven compounds were identified in the fingerprints of 10 males and 10 females. These compounds included fatty acids, fatty acid methyl esters, cholesterol and squalene. Ultimately no statistically significant gender difference could be identified from these results. One of the limitations in this study was that the samples used consisted of fingertip excretions on glass beads, rather than actual fingerprints.

Archer described the changes in lipid composition of latent fingerprints as a function of time in 2005 for 5 males.[2] Fingerprints were deposited on glass fibre filter paper and extracted using a solution of *N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide (MSTFA) in dichloromethane (CH_2Cl_2), with chlorononane (in hexane) as an internal standard. MSTFA generates a trimethylsilyl derivative of

carboxylic acids, which are easier to separate by GCMS. There appeared to be a significant difference in the amount of substance deposited by the subjects and also differences in the composition of the fingerprints.

Croxton et al. described the use of ethyl chloroformate (ECF) in $CHCl_3$ for the derivatisation of amino acids and fatty acids extracted by a 1% aqueous mixture of sodium hydroxide-ethanol-pyridine 75:40:10 (v/v), from Mylar film.[7] A total of 10 fatty acids were identified with this method. The method was later applied by Croxton to determine the variation in amino acid and lipid composition of fingerprints.[4] A total of 18 donors deposited on Mylar film and a total of 7 derivatives of fatty acids were identified using GCMS, besides squalene. From the results Croxton and co-workers concluded that using groomed fingerprints for testing novel visualisation methods is not advised, as there are significant differences between the composition of natural and groomed fingerprints.

In 2007 Morgan et al. described the use of bis(trimethylsilyl)trifluoroacetamide (BSTFA) for the isolation of predominantly fatty acids from fingerprints.[8] A total of 7 fatty acids, squalene and cholesterol were identified in the fingerprints of an unknown number of donors. The method described was not intended to obtain information on the variabilities in the fingerprint composition, hence the use of glass beads for fingertip deposition.

Weyermann et al. reported the use of CH_2Cl_2 for the extraction of a wide range of materials from fingerprints.[9, 10] The variety of materials could be assigned as exogenous materials, as well as endogenous. The isolation of certain fatty acids that were present in fingerprints and cosmetic products was achieved in one extraction. The wax esters in particular were a new addition to the spectrum of materials to be identified in fingerprints. A total of 29 wax esters were identified in the fingerprints of 7 donors.

When looking at the methods reported for the use of the materials for dating purposes, the inter- and intravariability are a major drawback. It appears that the reproducibility of a given application is rather low. A method proposed by Weyermann et al. is generating the relative ratios between the different compounds to limit this variability effect.

In addition Weyermann tested some latent fingerprint development techniques to establish the effect on the composition of the depositions. It was found that there was little difference when surfaces were treated with cyanoacrylate, 1,2-indanedione (HFE7100 and CH_2Cl_2 formulation) and powders, apart from the additional contamination from the actual reagent formulation.

In 2013 we described the use of propyl chloroformate for the extraction and derivatisation of amino acids from fingerprints and analysis by GCMS.[5] This method was based on the earlier development by Croxton et al.[7] As we have focused on the isolation of amino acids at that time, we have not taken the

extraction and analysis of fatty acids into account. Moreover, we have not used the PCF method to describe the inter- or intravariability of fingerprint composition. We have only used the method to determine the efficacy of fingerprint enhancement reagents for amino acids. The PCF mediated extraction and derivatisation of fatty acids from a fingerprint matrix could potentially be used for the analysis envisaged in the study described in this paper.

The method described by Dorman et al. for the extraction of fatty acids from fingerprints deposited on a glass slide, using a Whatman filter paper, appears to give reasonable results in terms of the reproducibility of the analytical method.[11] Dorman compared the chromatogram and mass spectra of the free fatty acids from fingerprints against the standard solution of fatty acid methyl esters. Dorman also found the methyl ester derivatives of fatty acids already present in fingerprints.[12]

Most recently Weyermann and Girod described the lipid composition of fingermark residue and donor classification,[10] using their earlier reported method.[9] In this study the fingerprints of 25 donors where extracted and analysed with the purpose of classification of the donors. A total of 104 lipids were detected, with a relatively low intra-variability compared to the inter-variability.

Overall there are several communications on the extraction of sebaceous compounds from fingerprints and subsequent GCMS analysis. Noteworthy is the rather large difference in the approaches for extraction. Some protocols use solely non-polar solvents, such as CH_2Cl_2 or $CHCl_3$, where as others describe the necessity of derivatisation reagents. These reagents are introduced to change the polarity of the more polar materials in a fingerprint, which increases the solubility of the materials and makes them more viable for GCMS separation.

Sha and Li described the esterification of various amino acids using trimethylchlorosilane (TMSCl) in MeOH (MeOH) at room temperature.[13] In their communication Sha and Li present the derivatisation of several carboxylic acids, containing an amino moiety in other positions as the a-position, as is the case with natural amino acids. In particular the esterification of 6-aminohexanoic acid, 4-aminobutanoic acid and 3-aminopropanoic acid, in high yields, shows that the derivatisation of carboxylic acids is readily achieved under very mild conditions. This method could potentially be used for the derivatisation, extraction and reproducible analysis of carboxylic compounds in fingerprints.

The previous methods for the extraction and analysis of fatty component described in the literature, as summarised above, all appear to have benefits and also downsides. What we find significantly missing is a clear description of the efficiency of the extraction methods and the presentation of the statistical variability in the results when used on real fingermarks.

In this paper we investigate the differences between the reported extraction and separation methods. Table 1 gives an overview of the extraction methods used in this study for comparison. To determine extraction efficiency we used a solution containing known concentrations of squalene and 3 fatty acids, that have previously been identified in fingerprints.[1] Furthermore we describe the full analytical specification of the most efficient method in respect to the extraction, derivatisation and analysis of several designated sebaceous materials. The chemical profile of fingerprints provides a better insight into the donor variability and donor classification. Also the experiments can be used to gain a better understanding of the efficacy of visualisation reagents used in current and future practice.

[Insert Table 1]

Table 1 - Seven solvent methods used for preliminary extraction

Materials and Methods

Docosane, Squalene (99%), stearic acid (98.5%), dodecanoic acid (99.5%), nonanoic acid, $CHCl_3$ (99.9%), trimethylsilyl chloride (TMSCl) (99%) were purchased from Sigma Aldrich (Zwijndrecht, the Netherlands). CH_2Cl_2 (>99%, HPLC grade) was obtained from Fluka (Zwijndrecht, the Netherlands). MeOH (98.8%) obtained from Merck (Darmstadt, Germany). Copier paper (Fastprint, 80 g/m² A4) was obtained from Buhrmannubbens (Zutphen, the Netherlands). The cover glass (24x32 mm thickness no. 1) and 50 µL vial (27.5x4 mm) were purchased from VWR International (Amsterdam, the Netherlands). The 1.5 mL vial (crimp neck vial 32x11mm) and the spring (36x5mm) were purchased from Grace (Zoetermeer, the Netherlands).

Stock solution

A stock solution was prepared by dissolving squalene (20 mg), nonanoic acid [C9:0], dodecanoic acid [C12:0] and stearic acid [C18:0] (20 mg each) in CH_2Cl_2/MeOH (10 mL, 1:1 v/v).

From this stock solution 1, 2, 3, 4 and 5 mL quantities were taken, docosane (0.25 mg) was added and the volume was brought to 10 mL (CH_2Cl_2/MeOH (1:1 v/v)). A small amount of the stock solution (10 µL) was deposited onto the paper and glass cover slips for extraction with different solvent systems.

Sebaceous marks

Preliminary work explored sebaceous marks from 1 male donor, aged 25, with each extraction run in triplicate. The main study explored the effect of extraction on marks from 10 donors, ages 21 to 64, 7 male and 3 female. Each donor deposited 6 marks for duplicate extractions with the three solvents. Information regarding dietary habits and cosmetics for all donors was obtained in order to successfully identify all the compounds detected in the extracted marks, as shown in Table 2.

[insert table 2]

Table 2 - Donor details for sebaceous marks

All donors were requested to not wash hands for 30 minutes prior to deposition, following standard CAST guidelines.[14] Donors rubbed their fingers on their face for 10 seconds and then rubbed their fingers together to produce a homogeneous deposit. The marks were then deposited onto the paper and glass cover slip substrates with approximately 1Kg of pressure for a duration of 5 seconds.

Eccrine marks

The effect of extraction on eccrine marks was also explored for 2 male donors, ages 22 to 25. Each donor deposited 6 marks for duplicate extractions with the three solvents. Information regarding dietary habits and cosmetics for all donors was again obtained in order to successfully identify all the compounds detected in the extracted marks, as shown in Table 3.

[insert table 3]

Table 3 - Donor details for eccrine marks

All donors were requested to wash their hands and then wear gloves for 30 minutes prior to deposition, following standard CAST guidelines.[14] Donors then rubbed their fingers together to produce a homogeneous deposit. The marks were then deposited onto the glass cover slip substrate with approximately 1Kg of pressure for a duration of 5 seconds.

Solvents

Seven solvent systems previously identified in the literature were explored to determine their extraction success, as shown in Table 1.

Preliminary research using deposited marks excluded MeOH and involved solvent methods one to six. This was narrowed down to the three most successful solvent methods for the main study exploring 600 sebaceous marks from ten donors, as shown in Table 4.

[Insert Table 4]

Table 4 - Most successful methods for extraction

The 12 eccrine marks were also extracted with the three most successful solvent methods, as shown in Table 4. The deposited stock solution was extracted using these three solvent methods six times so as to determine the overall extraction efficiency of each method.

Extraction from glass

The deposited stock solution and marks were extracted from glass using 2mL of the chosen solvent, covered with aluminium foil and placed in an ultrasonic bath (Brandson 3210) for 10 minutes to facilitate the extraction. The extraction solution was evaporated under nitrogen flux. 80µL of the solvent was added to re-dissolve the precipitate and the sample mixed using a vortex for 20 seconds. The solution was re-evaporated under nitrogen flux and the dry extract was diluted in 20 µL of solvent with 0.05 mg/mL docosane as internal standard and mixed with a vortex for 20 seconds.

Solvent extraction efficiency

The extraction efficiency of the three best solvent methods was determined using 6 extractions per solvent of stock solutions of known concentrations of squalene and fatty acids.

The extraction efficiency for squalene was determined by depositing 10 µL of a solution of squalene (19.9 mg, 0.048mmol) in CH_2Cl_2, (50 mL) onto the glass cover slip. It was left to dry for 15 minutes. The dried deposition was taken up in CH_2Cl_2 or $CHCl_3$ and analysed by GCMS.

A solution of three fatty acids was prepared by dissolving nonanoic acid [C9:0] (43.6 mg, 0.276 mmol), dodecanoic acid [C12:0] (44.0 mg, 0.220 mmol) and stearic acid [C18:0] (36.0g, 0.127 mmol) in MeOH (100 mL). 10 µL of the stock solution was deposited on glass cover slips and left to dry for 15 minutes.

Both depositions were then extracted following the same method as described earlier, with docosane (0.052mg/mL) as an internal standard. The ratio of the peak areas of the sample compounds (squalene, nonanoic, dodecanoic and stearic acids) to the internal standard docosane was then used to produce a calibration curve. The quantitative GCMS results for each of the 18 extractions were then used to calculate the efficiency of the three solvent methods as a percentage of the expected value.

GCMS analysis

Analyses were carried out on a GCMS (6890N/ 5973 inert; Agilent 276 Technologies Schweiz AG, Basel, Switzerland). Separation was carried out on a HP-5MS capillary column (30 m x 0.25 mm, Agilent Technologies Schweiz AG). The chromatographic elution was temperature programmed following the method detailed in (Weyermann et al., 2011), with a starting temperature of 80°C for 1 min, then increased to 230°C at a rate of 10°C/ min, then from 230° to 310°C at a rate of 4°C/min, and

held at 310°C for 8 minutes. The carrier gas was helium with a constant flow of 1 mL⁄ min. The sample was injected in split mode with a solvent delay of 4 min by auto sampler. The injector temperature was maintained at 250°C. For MS detection, ions were formed by electron impact at 230°C using a mass selective detector. Masses were scanned in the quadrupole at 150°C from m⁄z 30 to 650 u. The obtained mass spectra were further evaluated employing the NIST database (MS Search; NIST, MSS Ltd. Manchester, England).

Results

Extraction of stock deposits

In several preliminary experiments we found that the separation of free fatty acids, predominantly the smaller ones, on the GC column (HP-5MS) was not reproducible, even though earlier reports have described the elution under the exact same conditions.

In these early experiments we found an enormous variation in the abundance as measured from the total ion count (TIC) in the GCMS analyses, when using standard solutions of fatty acids.

We deduced that the polarity of these particular compounds were hindering extraction from the substrate and not eluting properly from the column. It appeared that the problem decreased with an increasing length of the fatty acids. An explanation for this is that with an increase in the amount of carbons, the molecule becomes less polar and as a result will dissolve more readily in a non-polar solvent, such as CH_2Cl_2. Not discouraged by these results, we have carried out a comparison of other suggested extraction systems.

We carried out some initial esterification experiments on pure fatty acids with the described system,[13] and established that this would be a potentially good manner to extract and analyse fatty acids from fingerprints, with the objective to isolate this entity and other materials from fingerprints.

The experiments for the determination of the efficiency of extraction using each of the 7 solvents were run in triplicate, resulting in 21 samples for the stock solution. The analyses for each solvent method were evaluated with an extraction score for the 3 fatty acids and 3 potential FAMEs (6 compounds) expected to be extracted out of 18 (given as a percentage) , and whether squalene was detected or not, as shown in Figure 1.

[Insert Figure 1]

Figure 1- Extraction of stock solution using 7 solvent methods

Figure 1 uses a frustum plot to represent the data, where the size of the cone dictates the success of extraction. Complete cones and discs are used to represent quantitative detection or no detection of squalene respectively. Fatty acids and FAMEs are shown as discs for no detection and as frustrated or incomplete cones (frustums) for quantitative detection equating to the percentage of 18 compounds quantitatively detected. The percentage is also presented as a data label.

Squalene was quantitatively identified in the samples extracted using solvents 1-3; MeOH/CH_2Cl_2, CH_2Cl_2 and $CHCl_3$, as shown by the cone in Figure 1. Squalene was not detected in the samples extracted using solvents 4-7; MeOH/TMSCl, CH_2Cl_2/MeOH/TMSCl, $CHCl_3$/MeOH/ TMSCl or MeOH, as shown by the disc present in Figure 1. The three fatty acids and their respective fatty acids methyl esters (FAMEs) were not detected in solvents 1 and 2; MeOH/CH_2Cl_2 and CH_2Cl_2, as shown by the disc present in Figure 1, but were quantitatively identified for solvents 3-7; $CHCl_3$, MeOH/TMSCl, CH_2Cl_2/ MeOH/TMSCl, $CHCl_3$/MeOH/TMSCl and MeOH represented by frustums in Figure 1. Under the mild conditions of esterification with MeOH/TMSCl we were able to produce the methyl ester derivatives of the starting materials in good yields. One point of concern was the potential transesterification of wax esters and mono-, di- and triglycerides. A further literature search revealed that Brandi et al. reported on the conversion of triglycerides by using TMSCl in MeOH to deliver the corresponding FAME products.[Brandi and Salvini] The fact that a mixture of TMSCl in MeOH would not only derivatise fatty acids, but also transesterify triglycerides or wax esters, could potentially disturb the extraction of compounds from fingerprints. Or at least, one would not only find the FAME's obtained from the esterification of fatty acids, but also the FAME's as a product from transesterification from triglycerides, and potentially diglycerides, monoglycerides and wax esters. We have treated several solutions of pure triglycerides with TMSCl in MeOH under the same conditions as the esterification of fatty acids. GCMS analysis of the reaction mixture showed no presence of the corresponding fatty acids from either triglycerides or wax esters.

There was a difference between solvents 4-6, with 83% of the expected compounds being detected for the stock extracted using MeOH/ TMSCl compared to 56% and 61% for CH_2Cl_2/ MeOH/ TMSCl, $CHCl_3$/ MeOH/ TMSCl respectively, as shown in Figure 1. Overall the best extraction was obtained from solvent methods 3 and 4, as the stock extracted using $CHCl_3$ yielded squalene in quantifiable concentrations, as shown in Figure 1, as well as one of the fatty acids equating to a 6% extraction success. The stock extracted using MeOH/ TMSCl resulted in the highest percentage extraction for the fatty acids and FAMEs of 83%, as shown in Figure 1. Extraction using MeOH was unsuccessful as squalene was not quantitatively detected and MeOH had an extraction success for fatty acids and FAMEs of only 6%. MeOH was therefore not explored any further as neither squalene nor any significant number of fatty acids were detected, represented by a disc and small frustum respectively in Figure 1. Solvent 7 was therefore discounted as a viable extraction method for further study.

From the extraction of the stock solution, it can be recommended that the identification of the target for extraction can be of significant assistance to extraction, as some solvents result in more successful extraction of specific components.

Sebaceous fingerprints initial experiments

Deposited marks were extracted using 6 solvent systems and compounds were quantitatively identified with each method, as shown in Figure 2.

[Insert Figure 2]

Figure 2 - Preliminary extraction success with 6 solvents for sebaceous marks

Example chromatograms for the extraction of sebaceous marks using each solvent method are shown in Figure 3.

[Insert figure 3]

Figure 3 - Example chromatograms of sebaceous marks extracted using 6 solvent methods

Figure 2 uses discs, frustums and complete cones to represent the data. A disc equates to no detection of the compound and a complete cone indicates quantitative detection of the compound. The incomplete cone or frustum indicates the compound was detected but below the limit of quantitation.
Squalene and cholesterol were quantitatively identified in the samples extracted using solvents 1-3; MeOH/CH_2Cl_2, CH_2Cl_2 and $CHCl_3$, as shown by complete cones in Figure 2. Neither squalene nor cholesterol were quantitatively detected in the samples extracted using solvents 4-6; MeOH/TMSCl, CH_2Cl_2/MeOH/TMSCl, $CHCl_3$/ MeOH/ TMSCl or MeOH, represented by a flat disc in Figure 2. Both compounds were detected below the limit of quantitation in the samples extracted using CH_2Cl_2/MeOH/TMSCl, represented by a frustum in Figure 2. Fatty acids were quantitatively detected in almost all extractions apart from the MeOH/CH_2Cl_2 mixture, which gave an amount below the limit of quantitation, show by a frustum in Figure 2. Both fatty acids and FAMEs were detected in solvent methods 4-6 containing TMSCl, shown as complete cones, which is as expected, as TMSCl acts as a methylating agent to the fatty acids. Wax esters were quantitatively detected only in the samples extracted using $CHCl_3$, although peaks below the limit of quantitation were detected in the samples extracted using CH_2Cl_2, shown by a frustum in Figure 2. No wax esters were observed for the other solvent extraction methods, shown by flat discs.
From the number of compounds quantitatively detected for each solvent method, CH_2Cl_2, $CHCl_3$ and MeOH/ TMSCl were determined to be more successful at extraction, as shown in Table 5. These three

solvent methods were then explored in more detail for the main study exploring marks from a large donor set.

Eccrine fingerprints

No significant differences were found between the marks extracted with the three different solvent methods, most likely due to the small donor set. There were significantly fewer compounds quantitatively detected in the extracted eccrine marks, resulting in little overall difference between the three solvent methods, as shown in table 5.

[insert table 5 Average and total number of compounds quantitatively identified in eccrine marks

Marks composed of purely eccrine sweat should contain no compounds present in sebaceous sweat (such as wax esters, squalene, sterols, fatty acids or FAMEs, hydrocarbons, alcohols), although a small number of FAMEs and alcohols were detected using all three extraction methods, also shown in table 7, possibly due to the compounds remaining on the fingers even after washing. This indicates that a more thorough method for the washing of the hands is required to completely remove all sebaceous material from the fingers for analysis of purely eccrine marks.

Extraction efficiency of the 3 best solvent systems

Squalene was most successfully extracted from deposited marks using $CHCl_3$. The calculated extraction efficiency was determined to be 54% for dichloromethane and 58% for $CHCl_3$, as shown in table 6. The average relative standard deviations (RSD) for DCM and $CHCl_3$ are 24% and 18% respectively. A comparison of the RSD for the extraction of squalene from actual fingerprints using DCM and $CHCl_3$ is shown in table 7. The values for DCM and $CHCl_3$ are much higher at 80% and 95% respectively.

[Insert table 6]

Table 6 - Extraction efficiencies for squalene using dichloromethane and chloroform calculated from standard solutions of known concentrations of squalene

[Insert table 7]

Table 7: Peak areas and relative standard deviation for squalene using dichloromethane and chloroform calculated from fingerprints

Fatty acids and FAMEs were most successfully extracted from deposited marks using MeOH/TMSCl. The calculated extraction efficiency was determined for the three fatty acids and was 45% for dodecanoic acid, 69% for stearic acid, and 69% for nonanoic acid, as shown in table 8. The relative standard deviations were also calculated for dodecanoic, stearic and nonanoic acids and equate to 10%, 7% and 8% respectively. The overall average extraction efficiency for MeOH/ TMSCl for all three fatty acids was 61%, with an RSD of 23%.

[Insert table 8]

Table 8 -Extraction efficiencies for nonanoic, dodecanoic and stearic acids using methanol/ trimethyl silyl chloride calculated from standard solutions of known concentrations of amino acids

These extraction efficiencies demonstrate the success of each solvent system with prepared solutions. Efficiencies for real sebaceous and eccrine marks would be particularly beneficial for establishing an optimum solvent for extraction. To determine extraction efficiency however, a known initial concentration of compounds prior to extraction is required, which is currently not possible to establish for real fingerprints. Additionally variation in the concentration of marks after extraction may only be due to variability in the amount of material actually deposited.

Discussion and conclusion

It is clear that the efficiency of extraction of fatty components in fingerprints varies greatly when comparing the different solvent systems. This research has identified that different solvents are more successful at extracting specific components from deposited marks, indicating the optimal extraction methodology is a combination of solvent methods. These findings allow the design of a robust and reproducible analytical method, which can successfully extract and quantify a number of compounds from a fingerprint residue. Further research is required to perfect this method, so as to explore potential interactions between additional variables.

Preliminary work exploring a two-step process using MeOH/ TMSCl followed by $CHCl_3$ was successful in yielding both FAMEs and squalene in quantitative amounts. The chromatogram showing both FAMEs and squalene is shown in Figure 4. Further work is necessary to improve the extraction efficiency of the method, so as to match that of the individual solvent methods.

[Insert figure 4]

Figure 4 - Chromatogram of two-step extraction process using methanol/ trimethyl silyl chloride followed by chloroform

From the results described above, it is clear that the derivatisation of fatty acids by GCMS is essential for good reproducibility of the extraction and analysis by GCMS. In addition to this finding we have also found that extraction of more polar material, such as smaller fatty acids, is not reproducible when using non-polar solvents such as CH_2Cl_2 or $CHCl_3$. The proposed method in this paper is not transforming triglycerides or wax esters to the corresponding fatty acids methyl esters through a transesterification mechanism.

The efficiency of extraction of deposited marks using seven solvent methods was explored using a stock solution containing known concentrations of three fatty acids and squalene. MeOH/ TMSCl was the most successful for fatty acid extraction, with 83% of the expected fatty acids and FAMEs determined. Importantly we found no effect of these conditions on the potentially present triglycerides. Extraction using MeOH was unsuccessful compared to the other solvent methods as neither squalene nor any significant number of fatty acids were detected. MeOH was therefore discounted as a viable extraction method. Deposited sebaceous marks were extracted using six solvents and dichloromethane, $CHCl_3$, and MeOH/ TMSCl yielded the greatest number of compounds quantitatively identified. Extraction using these three solvent methods on a large number of deposited sebaceous marks established that the optimum solvent for extraction is dependent on the target compounds. $CHCl_3$ was most successful for the extraction of squalene, cholesterol and wax esters, while MeOH/ TMSCl was determined as most successful for the extraction of fatty acids and FAMEs. Variations with donors were also observable, with differences between donors of different genders and ages being observable with all three solvent methods.

Although there were no significant differences observed in the total number of compounds extracted for eccrine marks with the three different solvent methods, the amount of material present in a fingermark leaves us to conclude that when one is attempting to produce eccrine marks, just washing the hands might not be sufficient to achieve this goal..

Extraction efficiencies were calculated using stock solutions containing known concentrations of squalene and fatty acids. Squalene was most successfully extracted from deposited marks using $CHCl_3$, with an efficiency of 58%, compared to 54% for dichloromethane. The extraction efficiency for MeOH/TMSCl was determined for the three fatty acids as 69% for nonanoic acid, 45% for dodecanoic acid, and 69% for stearic acid, equating to an overall average extraction efficiency of 61%. The RSD in the extraction efficiencies for the various solvent systems were between 7% and 24% indicating reasonably good reproducibility in quantification of known concentrations. The extraction efficiencies for real fingerprints, although useful, could not be determined as the starting concentration of substances was unknown. However, the RSD for the detection of squalene in real fingerprints could be calculated. It was found to be in the range 80% - 95% and is most likely much larger than the RSD

for the known concentrations because for real fingerprints it is not possible to deposit the same mass of material in each fingerprint from each donor reproducibly and so this increases the variability in the results. Additionally, the RSD may increase with the smaller sample quantities present in real fingerprints.

The outcome of this study therefore recommends the use of MeOH/TMSCl for extraction and derivatisation of fatty acids followed by the use of $CHCl_3$ for the extraction of squalene, cholesterol, FAME's and wax esters. Further research is required to gain insight into the effects of contaminants on the proposed method, or whether additional extraction steps would be required.

Ethical statement

The fingerprints used in this study were donated by volunteers with prior consent. Before donating the fingerprints the volunteers learned of the aims of the experiments and they were given the possibility to withdraw their consent at any point in time during the study.
For evaluation purposes the names of the individuals were retained together with the chemical profile of their fingerprint. During the experiments no attempts were made to visualise the latent fingerprints or to take images of the fingerprints used in this study.

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Method	Solvent	Abbreviation
1	Methanol/Dichloromethane [1:1]	MeOH/DCM
2	Dichloromethane	DCM
3	Chloroform	$CHCl_3$
4	Methanol/Trimethyl silyl chloride [1:40µl]	MeOH/TMSCl
5	Dichloromethane/ Methanol/Trimethyl silyl chloride [1:1:40µl]	DCM/MeOH/TMSCl
6	Chloroform/ Methanol/Trimethyl silyl chloride [1:1:40µl]	$CHCl_3$/MeOH/TMSCl
7	Methanol	MeOH

Number	Age	Gender	Cosmetics	Diet
1	25	M	Aftershave	Omnivore
2	26	M	Body oil	Omnivore
3	27	F	Make up	Omnivore
4	21	F	Hand cream	Omnivore
5	22	M	Hair gel	Omnivore
6	36	F	-	Omnivore
7	43	M	Cream	Omnivore
8	47	M	-	Omnivore
9	64	M	Aftershave	Omnivore
10	64	M	-	Omnivore

Number	Age	Gender	Cosmetics	Diet
1	25	M	Aftershave	Omnivore
2	22	M	Hair gel	Omnivore

Method	Solvent	Abbreviation
2	Dichloromethane	CH_2Cl_2
3	Chloroform	$CHCl_3$
4	Methanol/Trimethyl silylchloride (1:40 ul)	MeOH/TMSCl

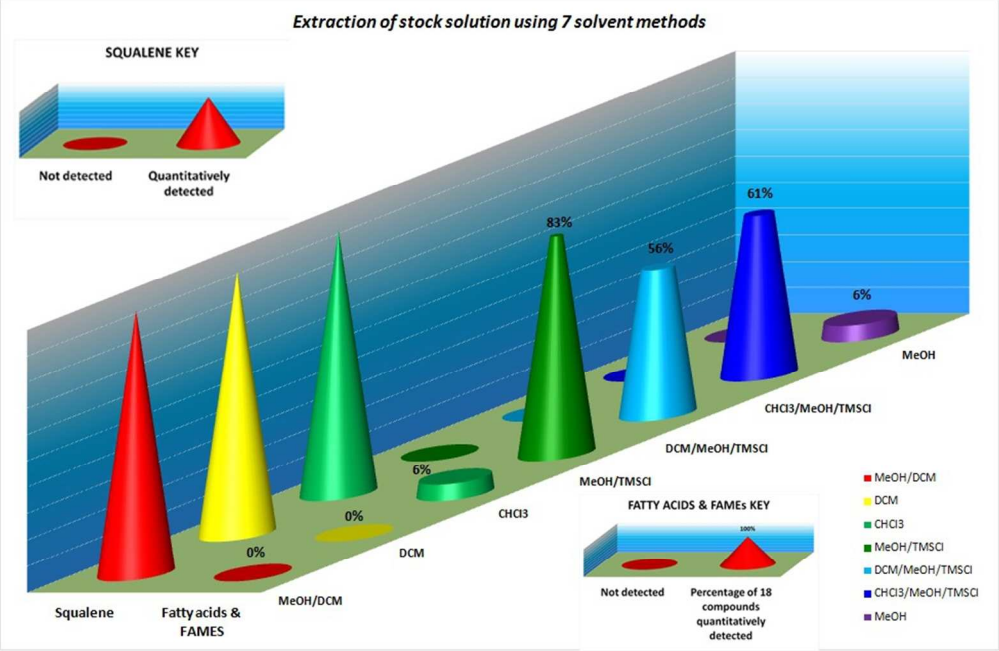
Extraction of stock solution of squalene & 3 fatty acids					Fatty acids & FAMEs	
Number	**Solvent**	**Squalene**	**Fatty acids**	**FAMEs**	**Score /18**	**% detected**
1	MeOH/DCM	Quantitatively detected	Not detected	Not detected	0/18	0%
2	DCM	Quantitatively detected	Not detected	Not detected	0/18	0%
3	$CHCl_3$	Quantitatively detected	Quantitatively detected	Not detected	1/18	6%
4	MeOH/TMSCl	Not detected	Quantitatively detected	Quantitatively detected	15/18	83%
5	DCM/MeOH/TMSCl	Not detected	Quantitatively detected	Quantitatively detected	10/18	56%
6	$CHCl_3$/MeOH/TMSCl	Not detected	Quantitatively detected	Quantitatively detected	11/18	61%
7	MeOH	Not detected	Not detected	Quantitatively detected	1/18	6%

No.	Solvent	Fatty acids	FAMEs	Squalene	Cholesterol	Wax esters
1	MeOH/DCM	Detected	Not detected	Quantitatively detected	Quantitatively detected	Not detected
2	DCM	Quantitatively detected	Not detected	Quantitatively detected	Quantitatively detected	Detected
3	$CHCl_3$	Quantitatively detected	Not detected	Quantitatively detected	Quantitatively detected	Quantitatively detected
4	MeOH/TMSCl	Quantitatively detected	Quantitatively detected	Not detected	Not detected	Not detected
5	DCM/MeOH/TMSCl	Quantitatively detected	Quantitatively detected	Detected	Detected	Not detected
6	$CHCl_3$/MeOH/TMSCl	Quantitatively detected	Quantitatively detected	Not detected	Not detected	Not detected

Solvent	Fatty acids	FAMEs	Squalene	Cholesterol	Wax esters	Other	TOTAL	STDEV
DCM	0.0	2.0	0.0	0.0	0.0	2.5	4.5	2.1
CHCl3	0.0	1.3	0.0	0.0	0.0	2.5	3.8	3.2
MeOH/TMSCl	0.0	2.3	0.0	0.0	0.0	2.0	4.3	2.5

Solvent Method	Peak Areas		Ratio	C (mg/ml)	Efficiency	Average Efficiency (%)	Standard deviation	Relative standard deviation
	Docosane	**Squalene**						
DCM (5 samples)	1.04×10^7	2.56×10^7	2.46	0.22	0.38	**54%**	**13%**	**24%**
	4.47×10^6	1.24×10^7	2.78	0.22	0.43			
	1.30×10^7	5.36×10^7	4.11	0.22	0.63			
	1.46×10^7	5.58×10^7	3.82	0.22	0.59			
	1.11×10^7	4.89×10^7	4.42	0.22	0.68			
$CHCl_3$ (6 samples)	4.65×10^6	1.58×10^7	3.40	0.22	0.46	**58%**	**11%**	**18%**
	1.07×10^7	4.01×10^7	3.75	0.22	0.51			
	1.13×10^7	4.05×10^7	3.57	0.22	0.48			
	9.32×10^6	4.50×10^7	4.82	0.22	0.65			
	1.00×10^7	5.08×10^7	5.06	0.22	0.69			
	1.21×10^7	6.04×10^7	5.01	0.22	0.68			

Docosane	Dodecanoic acid	Ratio	C (mg/ml)	Efficiency	Average Efficiency (%)	Standard Deviation	Relative standard deviation
5.38×10^6	8.96×10^6	1.67	0.22	0.50			
8.74×10^6	1.28×10^7	1.47	0.22	0.44			
4.55×10^6	7.28×10^6	1.60	0.22	0.48	**45%**	**5%**	**10%**
7.61×10^6	9.53×10^6	1.25	0.22	0.38			
5.68×10^6	7.70×10^6	1.36	0.22	0.41			
5.72×10^6	8.87×10^6	1.55	0.22	0.47			
Docosane	**Stearic acid**						
5.38×10^6	1.97×10^7	3.65	0.22	0.72			
8.74×10^6	2.96×10^7	3.38	0.22	0.66			
4.55×10^6	1.69×10^7	3.71	0.22	0.73	**69%**	**5%**	**7%**
7.61×10^6	2.38×10^7	3.13	0.22	0.61			
5.68×10^6	1.95×10^7	3.43	0.22	0.67			
5.72×10^6	2.13×10^7	3.72	0.22	0.73			
Docosane	**Nonanoic acid**						
5.38×10^6	1.49×10^7	2.77	0.18	0.72			
8.74×10^6	2.27×10^7	2.59	0.18	0.67			
4.55×10^6	1.29×10^7	2.83	0.18	0.73	**69%**	**5%**	**8%**
7.61×10^6	1.75×10^7	2.30	0.18	0.59			
5.68×10^6	1.47×10^7	2.59	0.18	0.67			
5.72×10^6	1.63×10^7	2.84	0.18	0.74			



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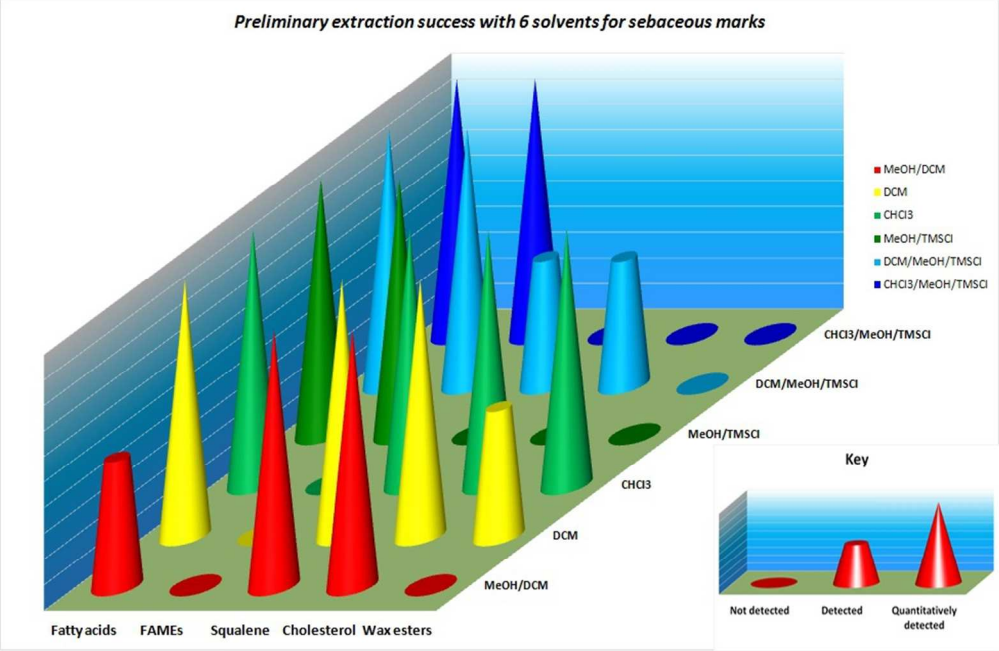

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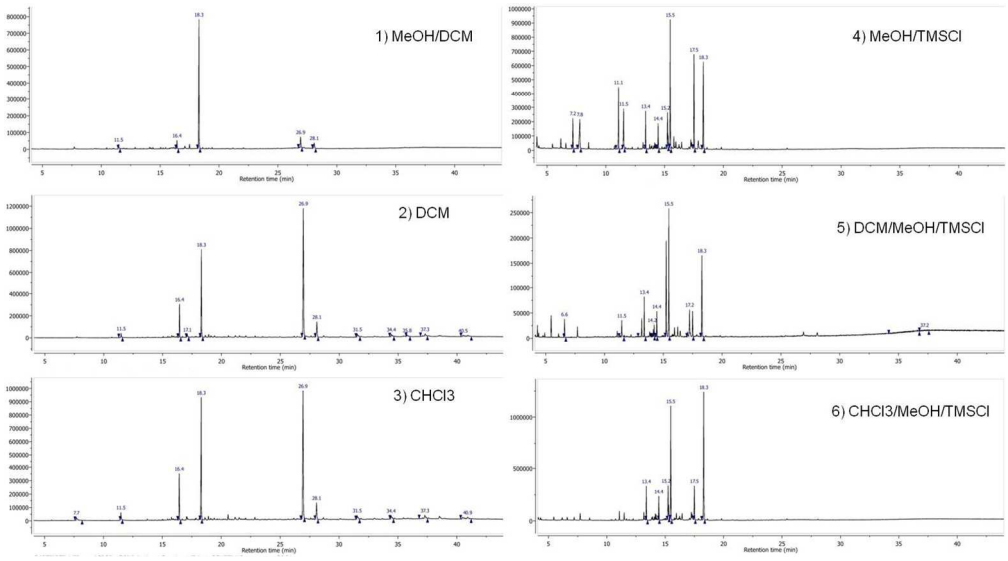

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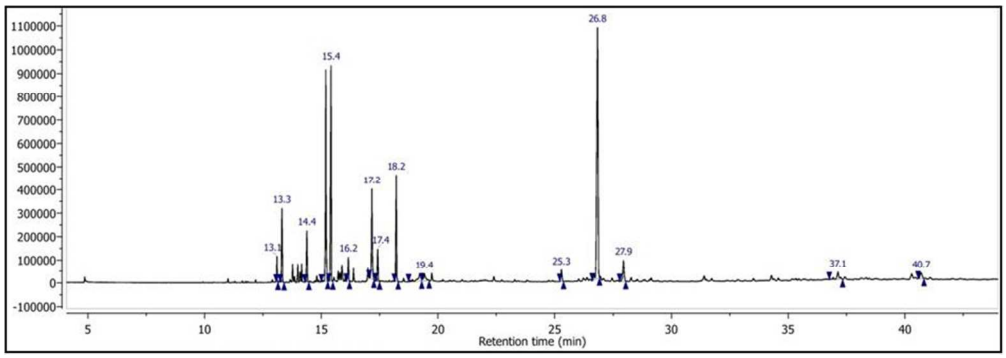

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