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Comparison of Derivatization Methods for Groomed Latent Print Residues Analysis via Gas Chromatography

Jessica Kindell [1] and Candice Bridge [1,2,*]

[1] Department of Chemistry, University of Central Florida, Orlando, FL 32816, USA
[2] National Center for Forensic Science, University of Central Florida, Orlando, FL 32816, USA
* Correspondence: cbridge@ucf.edu; Tel.: +1-407-823-1263

Abstract: The practice of latent print analysis is comprised of a visual examination and the comparison of the fingerprint pattern from a questioned print to an exemplar(s). When a questioned print is either smudged or contains little pattern detail, the print comparison would be considered an inconclusive determination. However, in these scenarios, the latent print residues (LPRs) could provide associative information to supplement the current ACE-V (Analysis, Comparison, Examination-Verification) process. Advancements using analytical techniques allow for the analysis of LPR chemistry; however, derivatization is generally required to increase the abundance of components not traditionally observed in gas chromatography. This study aimed to determine whether two derivatization reagents, boron trifluoride in methanol (BF_3-MeOH) and *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA), provide a better recovery of LPR components from a porous or non-porous substrate. Five volunteers deposited groomed latent print samples onto two substrates: a microfiber filter (porous) and a microscope slide (non-porous). The residues were derivatized or evaporated prior to the gas chromatography-mass spectrometry (GC-MS) analysis. The percent recoveries were higher, >83%, in the DCM extracted samples for both substrates compared to those samples prepared in hexanes. DCM/MSTFA derivatization provided the recovery of fatty acids that ranged from 20 to 30% for both substrates and a recovery of squalene at a rate of 2.37% for the filter sample and 4.2% for the slide sample. These rates were higher than the recovery rates obtained for the hexanes/BF_3-MeOH-derivatized samples, with a range of 1–8% for the fatty acids recovery rates and 0.6–0.85% for squalene from both substrates. Overall, the MSTFA derivatization reagent produced higher recoveries for LPR on porous and non-porous substrates while providing a LPR chromatographic profile similar to that of a non-derivatized sample. The use of DCM as a solvent provided a wider range of LPR components recovered than hexanes and, thus, should be used as the extraction solvent when derivatizing samples, regardless of the substrate.

Keywords: latent print residues; derivatization; non-derivatization; gas chromatography; mass spectrometry

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1. Introduction

Latent prints (LPs) are composed of sebum, sweat, and dirt collected on the friction ridges from the hands or palms and transferred onto a surface creating an "invisible" mark. When found at a crime scene, latent prints are developed and examined by fingerprint analysts to determine whether a questioned print has similar or dissimilar features to known exemplars based on the ACE-V (Analysis, Comparison, Examination-Verification) protocol. However, during the analysis step, if the questioned LP exhibits poor quality in Levels 1, 2, and 3 details; is smudged; or is an unsuitable partial print, the analyst could deem the print unusable for the ACE-V process and thus discard it from future evaluation. Instead of discarding the print(s), they could be analyzed chemically to obtain more information to supplement the visual results. However, there are several components that are not readily observed via traditional instruments, and thus, derivatization of the

components is necessary. Additional information on latent print chemical makeup is provided in the Supplementary Materials.

Gas chromatography-mass spectrometry (GC-MS) is a universal instrument used to separate and identify the components of complex mixtures based on volatility, polarity, and the mass fragmentation pattern. Since LPRs are complex mixtures composed of sebaceous and eccrine secretions, GC-MS studies have been performed for component identification with samples prepared via non-derivatization [1–4] and derivatization [5–8] methods. While non-derivatized samples can be analyzed directly with a short sample preparation time, some components, such as underivatized fatty acids, may not be observed in the resulting spectra on nonpolar GC columns. Peaks of polar LPR analytes tend to tail in the GC-MS chromatograms on a nonpolar column, and fronting may occur if the samples are too concentrated, resulting in poor peak resolution affecting accurate retention time determination.

Derivatization is used to prepare polar components, i.e., fatty acids, for chromatographic analysis, and it provides the advantage of better resolution, separation, peak detection, and reduced peak tailing [9–11]. Additionally, derivatization decreases the boiling point of components capable of hydrogen bonding, resulting in changing the component's volatility and boiling point, making them more susceptible to detection within the temperature range of a gas chromatograph [9–11]. If all LPR components can be detected with derivatization via a GC-MS analysis, then it could be included in the ACE-V protocol to analyze poor-quality or poor condition LPs during or after the analysis step.

In the current literature, several manuscripts have investigated the effects of either non-derivatized or derivatized methods on LPR; however, there have been few investigations comparing derivatization methods and their effects on the residue. Several derivatized reagents have been evaluated in the literature to investigate the LPR profile and recovery, including *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA) [8,12], N, O-bistrifluoroacetamide (BSTFA) [13,14], trimethylsilyl chloride (TMSCl) [6], ethyl chloroformate (ECF) [5,7], and boron trifluoride-methanol (BF_3-MeOH) [15–17], though one study by Cadd et al. [6] compared non-derivatized solvents and derivatized reagents for LP analysis. That research group determined that the non-derivatizing solvents alone were able to extract the components with higher concentrations, such as squalene and a few fatty acids; however, after adding a derivatizing reagent, the fatty acids and other secretion components were more apparent. They determined that a multistep extraction process using a derivatization reagent followed by a common non-derivatization solvent would yield a more complete LPR profile. However, multistep protocols may not be possible given the time constraints and the case backlog experienced by many forensic crime laboratories; therefore, one-step extractions might be more beneficial and easier to incorporate into laboratory protocols.

This study aimed to compare and determine which derivatization method works best on a porous or non-porous surface to yield a representative profile of the LPR in comparison to its non-derivatized sample. The reagents chosen for this study were BF_3-MeOH, an alkylation reagent, and MSTFA, a silylation reagent, since they are common reagents used in both fatty acid and latent print research. The reagents, BF_3-MeOH and MSTFA, underwent Fischer esterification and nucleophilic substitution, converting the active hydrogens into polar moieties (OH, SH, and NH) to either a methyl ester or trimethylsilyl (TMS) derivative, respectively [9,10] (Figure 1). The advantages of using BF_3-MeOH are that it is cost-efficient and has a short derivatization time due to its ability to esterify and transesterify in one step; however, it is harmful to humans and has a limited shelf life [17]. Meanwhile, MSTFA is also cost-efficient, compared to other silylating reagents; it can derivatize components at room or high temperatures and is safe for many GC columns, though it is moisture-sensitive. Considering that both derivatization methods can be easily introduced into the ACE-V protocol, they were both evaluated in this study. The goal of this study was to investigate how each derivatization reagent affects the peaks observed in the resulting chromatogram and which one works better at recovering LPRs from porous or non-porous substrates. This

study explored if both derivatization methods operated on the same LPR components and if they increased the abundance of lipids, amino acids, and fatty acids to the same extent so that they could be observed in the resulting chromatogram.

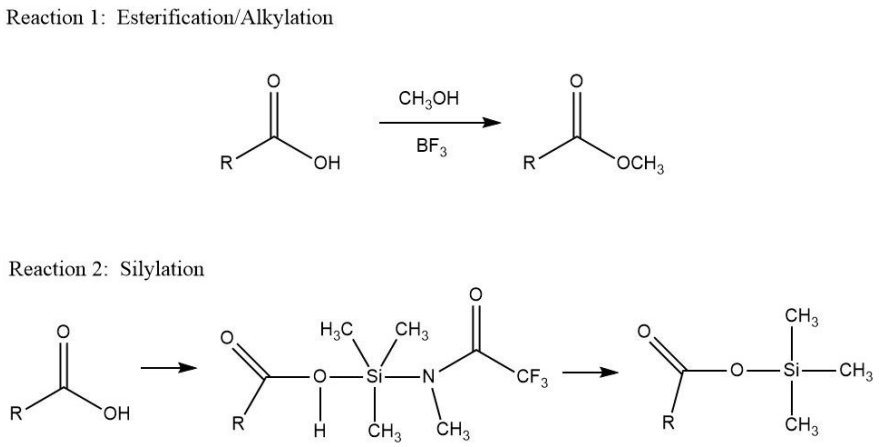

Figure 1. Reaction schemes of the esterification/alkylation and the silylation of a carboxylic acid (e.g., fatty acid) into a methyl ester and trimethyl silyl ester [18–20].

2. Materials and Methods

2.1. Materials

Dichloromethane (DCM, optima grade), hexanes (branched and linear, HPLC grade), and octadecanoic acid (stearic acid, laboratory grade) were purchased from Fisher Scientific (Fair Lawn, NJ, USA). MSTFA (98–100%) was purchased from ThermoFisher Scientific (Waltham, MA, USA). Methyl pentadecanoate and BF_3-MeOH (1.3M, Supelco) were purchased from Millipore Sigma (St. Louis, MO, USA). Tetradecanoic acid (myristic acid, >99.0%), methyl tetradecanoate (methyl myristate, >98.0%), hexadecanoic acid methyl ester (methyl palmitate, >97.0%), octadecanoic acid methyl ester (methyl stearate, >97.0%), 10-octadecenoic acid (oleic acid, >99.0%), 10-octadecenoic acid methyl ester (methyl oleate, >99.0%), and squalene (>98.0%) were purchased from Tokyo Chemical Industry Co. (TCI, Portland, OR, USA). Hexadecanoic acid (palmitic acid, 99.0%) was purchased from Aldrich Chemical (Milwaukee, WI, USA). (Z)-7-hexadecenoic acid (palmitoleic acid, 99.0%) was purchased from Acros Organics (Fair Lawn, NJ, USA). Pentadecanoic acid (99.0%) was purchased from Alfa Aesar (Ward Hill, MA, USA). Dodecane was purchased from Fluka®® (St. Louis, MO, USA) and used as an internal standard (IS) for the extraction recovery rates.

Fisherbrand®microscope slides (Fisher Scientific, Fair Lawn, NJ, USA), Whatman glass microfiber filters GF/F (GE Healthcare, Hatfield, UK), and KimTech Science* kimwipes (Kimber-ly-Clark Professional, Roswell, GA, USA) were purchased and used for fingerprint deposition and subsequent extraction. Nitrogen gas (Airgas, Radnor, PA, USA) evaporated the solvents and reduced the atmospheric moisture from interacting with the derivatization reagents and samples. Anhydrous magnesium sulfate (99+%), purchased from Strem Chemicals, Inc. (Newburyport, MA, USA), was used for water removal after BF_3 derivatization and extraction.

Solvent Safety: DCM is classified as a hazardous airborne pollutant and carcinogen, and the United States Environmental Protections Agency has proposed to prohibit most industrial and commercial uses, as well as limit the manufacturing and distribution of the solvent [21]. Considering these safety concerns, potential alternatives that can be used instead of DCM for extraction purposes are 2-methyltetrahydrofuran, toluene, ethyl acetate, and methyl tert-butyl ether [22].

2.2. Methods

2.2.1. Latent Print Residue Collection

This study was approved by the ethical standards of the University of Central Florida Institution Review Board (IRB SBE-18-14639). Five volunteers (one male and four females) were asked to donate 20 groomed latent fingerprints each for a total of 100 prints for the derivatization analysis. Each print was assigned a unique sample number to minimize the use of personal information. It was noted that none of the volunteers wore any skincare products before sample collection. Additionally, 20 groomed latent prints from four of the five volunteers were collected for the non-derivatized analysis only, for a total of 80 prints.

Each volunteer washed their hands before depositing a groomed print. To deposit a print, the index finger was rubbed back and forth ten times across their forehead and then placed on the target surface. This process was repeated for the right side of the nose, left side of the nose, right side of the chin, and left side of the chin, creating one set. For this manuscript, only the groomed LPRs collected from the forehead and nose will be discussed herein. Each volunteer deposited four sets of latent prints on four different substrates (two microscope slides and two microfiber filters (MFF)). The LPRs collected from each location were individually analyzed in triplicate. Since it has been illustrated that there is little variation in LPRs between facial locations, nine of the fifteen replicates were combined together to describe how the derivatization process worked on a substrate for each volunteer [3,23,24].

Prints deposited on the filter were directly solubilized in their respective solvents. However, the prints deposited on the microscope slides were collected for derivatization using a kimwipe that was dampened with either DCM for the samples derivatized with MSTFA or hexanes for BF_3-MeOH derivatization [23,25]. The latent prints for each derivatization/substrate combination were collected on different days to allow all samples to be derivatized and analyzed on the same day as collection.

2.2.2. Derivatization Sample Preparation Protocols

All three derivatization processes started with an extraction process, where the groomed prints that were deposited on the MFF were extracted in 1.0 mL of the solvent for 45 min [14,23]. Since solvent rinsing the slides left behind a visible residue, a solvent-dampened kimwipe was used to collect the slide LPRs and was then extracted in 1.0 mL of the solvent for 1.0 min, since it was a thinner material than the MFFs. The extraction and evaporation methods were based on the methods from Girod [1] and Koenig [3]. Figure S1 contains the sample preparation and derivatization flowchart of the following experimental design.

Esterification Derivatization Process

The sampled MFF or slide kimwipe was placed in a 5.0 mL reaction vial with 1.0 mL of hexanes for 45.0 min or 1.0 min, respectively, at room temperature. The samples were vortexed before removing the substrate from the vial, and then, the residual solvent was evaporated with nitrogen gas to dryness. The following derivatization method was slightly modified from the method suggested by Sigma-Aldrich [26], Ichihara et al. [17], and Nordstrom et al. [16]. One milliliter of 1.3M BF_3-MeOH was pipetted onto the evaporated residue, and the solution was heated to 60.0 °C for 5.0 min and then cooled to room temperature. One milliliter of deionized water and one milliliter of hexanes were added to the reaction vial and vortexed for sample extraction. The hexanes layer was transferred to a GC vial and dried with magnesium sulfate to remove the residual water. A 200.0 µL aliquot of the anhydrous hexanes solution was analyzed via GC-MS. Only hexanes were used as the solvent for this derivatization process.

Silylation Derivatization Process

The adapted sample extraction process for the MSTFA derivatization [18] was similar to the BF_3 derivatization method but used DCM as the solvent instead of hexanes. The

samples were evaporated to dryness with nitrogen gas. Subsequently, the sample was dissolved in 250.0 µL of DCM and 250.0 µL of MSTFA under nitrogen gas, capped, and left at room temperature for 15.0 min to complete the derivatization process. Finally, a 150.0 µL aliquot of the solution was analyzed via GC-MS. Only DCM was used as the solvent for this derivatization process.

Non-Derivatization Sample Preparation

The print samples from the four volunteers were prepared using the same derivatization method sans the derivatization reagents. These prints were collected from the forehead and nose locations only. The solvents used for non-derivatized latent print extraction were DCM and hexanes, since they were the same solvents used with the derivatization reagents.

Groomed latent prints deposited onto the MFF were extracted in 1.0 mL of either solvent for 45.0 min and 1 min for the slide/kimwipe samples. The samples were vortexed, and after removing the substrate from the 5.0 mL reaction vials, the LPR solutions were evaporated to dryness with nitrogen gas. The samples were then reconstituted to 75.0 µL in hexanes or DCM for GC analysis. If the samples were reconstituted in any higher volume, squalene was the only analyte observed.

Two types of contaminants were observed at 23.74–24.1 min for volunteer 2. One contaminant was bis(2-ethylhexyl) ester-1,3-benzenedicarboxylic acid, and the second was a coelution of bis(8-methylnonyl) ester-1,2-benzenedicarboxylic acid and didecan-2-yl phthalate. These contaminants may have come from the gloves used during the extraction process or cross-contamination originating from one of the volunteer samples. Since these contaminants were found in the non-derivatized samples, volunteer 2′s hexane slide sample and all the DCM/slide samples were not included in the results.

2.2.3. Extraction Recovery

Two stock solutions of 200 ppm of myristic acid, pentadecanoic acid, palmitoleic acid, palmitic acid, oleic acid, stearic acid, and squalene solubilized in DCM and hexanes were prepared to calculate the percent recovery of the extraction and derivatization methods. Separate solutions of 200 ppm of squalene in DCM and hexanes were also made for extraction and derivatization efficiency. To determine the extraction efficiency, a 100 µL aliquot of the stock solution was deposited onto a MFF or microscope slide and was allowed to dry naturally. The samples were prepared for analysis using the non-derivatization, BF_3-MeOH derivatization, and the MSTFA derivatization protocols. Aliquots were removed during the derivatization process for the GC-MS analysis to monitor and calculate the percent recovery throughout the extraction and derivatization steps (refer to Table 1).

Table 1. List of the extraction recovery tests.

Test	Experiment Description
Test 1	time trials of 1 min and 45 min extractions using DCM and hexanes
Test 2	squalene extraction and derivatization
Test 3	extraction test post-evaporation
Test 4	step-by-step extraction and derivatization test for BF_3-MeOH and MSTFA A. pre-evaporation with the 1.0 mL solvent extraction (compared against underivatized neat stock solution) B. post-evaporation just before dryness (compared against underivatized neat stock solution) C. reconstitution of stock solution for the DCM samples only (compared against underivatized neat stock solution) D. post-derivatization (compared against derivatized neat stock solution)

The percent recoveries of the components during the extraction and derivatization steps were calculated using the percent recovery equation (Equation (1)):

$$\% \text{ extraction recovery} = \frac{\text{experimental } \left(\frac{A}{IS}\right)}{\text{initial } \left(\frac{A}{IS}\right)} \quad (1)$$

where A is the peak height of the target analyte and IS the internal standard peak height. All non-derivatized aliquots were compared to the neat stock solution, and the derivatized aliquots were compared to the derivatized neat stock solution. The extraction recovery samples were analyzed in duplicate, and the derivatization recovery samples were analyzed in triplicate. The timed trials and squalene were analyzed once.

2.2.4. Instrumental Parameters

GC-MS analysis was performed with an Agilent 7890B gas chromatograph interfaced with a 5977B mass spectrometer detector (MSD) and an autosampler (Agilent, Santa Clara, CA, USA). One microliter of the sample was injected into the injection port that was set at a temperature of 250 °C, and a splitless injection mode was used. The column was a DB-5ms Ultra Inert capillary column with a length, diameter, and film thickness of 30 m × 250 µm × 0.25 µm. Helium was the carrier gas, with a 1.0 mL/min flow rate and an average velocity of 23.684 cm/s. The initial oven temperature of 50 °C was held for 2.0 min, followed by a temperature ramp of 15 °C/min to 200 °C, which was held for 2.0 min, then with a final temperature ramp of 8 °C/min to 320 °C and was held for 7.0 min. The mass spectrometer had a source temperature of 230 °C, a quadrupole temperature of 150 °C, and a transfer line temperature of 250 °C. The mass spectra were scanned between the mass-to-charge ratios (m/z) of 30 to 550, and a solvent delay of 4.0 min was used. Samples were analyzed in triplicate, with a solvent blank performed in between each sample.

2.2.5. Data Analysis

Seven components were chosen to represent the LPR due to their prominent peak heights, as compared to the other components present in the samples, and are listed in Table 2. They were used to compare the derivatization methods for each substrate.

Table 2. Seven prominent components identified in the LPR profiles.

Component Identification
Myristic acid
Pentadecanoic acid
Palmitoleic acid
Palmitic acid
Oleic acid
Stearic acid
Squalene

These components, except for squalene, will be referred to as fatty acids in this manuscript and not by the methyl ester or trimethylsilyl derivatives to eliminate confusion. MassHunter Qualitative Analysis B.07.00 (Agilent, Santa Clara, CA, USA) was used for peak integration, peak identification, and background subtraction of the mass spectral data. MSD ChemStation E.02.02 (Agilent, Santa Clara, CA, USA) and the NIST14 mass spectral library were used to verify peak identification. Additionally, the seven major components were also identified and verified by comparing their retention times and mass spectral fragmentation patterns to known reference standards. These peak heights were normalized by the base peak and subsequently used for statistical analysis. All averages, standard deviations, and relative standard deviations were calculated in Microsoft Excel.

3. Results and Discussion

Latent print residues were deposited onto a porous and non-porous substrate and subsequently derivatized using two methods: BF_3-MeOH and MSTFA. The derivatized LPRs were compared to non-derivatized samples to determine if one derivatization method works better on porous or non-porous surfaces. The Supplementary Materials contains information about latent print chemistry (Table S1) and the derivatization mechanisms for both BF_3 and MSTFA (Figures S2–S4).

3.1. BF_3 Derivatization

3.1.1. Solid Surfaces

The non-porous surfaces found at crime scenes were represented as a glass microscope slide for this study. A dampened kimwipe was used to ensure all the residue was removed instead of multiple solvent rinses. The percent recovery was 100% for the non-derivatized squalene and approximately 1% for the derivatized method. Meanwhile, >72% of the non-derivatized fatty acids were recovered compared to the 6–9% calculated for the derivatized FAMEs. Hexanes were chosen as the extracting solvent for BF_3-MeOH, because hexanes should extract nonpolar components and solubilize fatty acids due to the long hydrocarbon tail, which would contribute to a decrease in the overall polarity of the molecule. Hexanes were also used with the BF_3-MeOH process, because they are immiscible with water and MeOH and would be able to extract the methyl ester derivatives.

Four volunteers (1, 2, 3, and 5) donated groomed latent print samples to compare non-derivatized and derivatized methods (Figure 2) in the recovery of LPRs from a solid substrate. Squalene was the dominant peak in the non-derivatized sample chromatograms (Figure 2). The fatty acids were mostly present in the 12.0–18.0 min region for all volunteers, with palmitic acid and stearic acid identified in all samples. Cholesterol was identified in the non-derivatized hexanes slide samples of volunteers 1 and 3 at low abundances. The wax esters were identified as 9-hexadecenoic acid heptyl ester (Rt = 27.16 min), myristic acid hexadecyl ester (Rt = 27.34 min), palmitic acid hexadecyl ester (Rt = 28.862 min), 9-hexadecenioc acid eicosyl ester (Rt = 30.298 min), and 9-hexadecenoic acid octadecyl ester (Rt = 27.941). These wax esters and other components were corroborated in the literature (Table S1) [1,4]. Amino acids were not detected nor identified in the non-derivatized non-porous samples.

Compared to the non-derivatized samples, the base peak for the BF_3-MeOH derivatize slide samples was palmitic acid (Figure 3A,F,K,P,U). The fatty acids were easily observed due to the decrease in squalene abundance. The derivatized FAME profile had a similar gaussian-like curve to the non-derivatized FAME profile. Cholesterol was not identified in all the LPRs collected. The wax esters seen in the non-derivatized hexanes/slide samples were not present in the BF_3-MeOH-derivatized samples. This was likely due to the wax esters being transesterified and esterified into a methyl ester, which would also contribute to the increased abundance of FAME peaks from the FAs, which was not a desired outcome.

The chromatographic profile of the derivatized samples varied between volunteers, as seen in Figure 3A–Y. Table S2 contains a list of the components identified in the BF_3-MeOH/slide samples via the NIST14 library. Stearic acid was the second-most abundant peak in all of volunteer 3's LPRs, with squalene, myristic acid, pentadecanoic acid, palmitoleic acid, and oleic acid being, at most, 9–15% of the base peak (Figure 3K). Volunteers 1 (Figure 3A) and 5 (Figure 3U) had similar spectra, where palmitoleic acid was the second-most abundant peak, followed by squalene and/or a combination of squalene, myristic acid, and stearic acid. Finally, pentadecanoic acid and oleic acid were the least abundant FAMEs in the chromatogram for volunteers 1 and 5. Same as the non-derivatized samples, amino acids were absent in the identification of the peaks detected. This was likely due to the volunteers not producing any sweat or the amino acids not being derivatized.

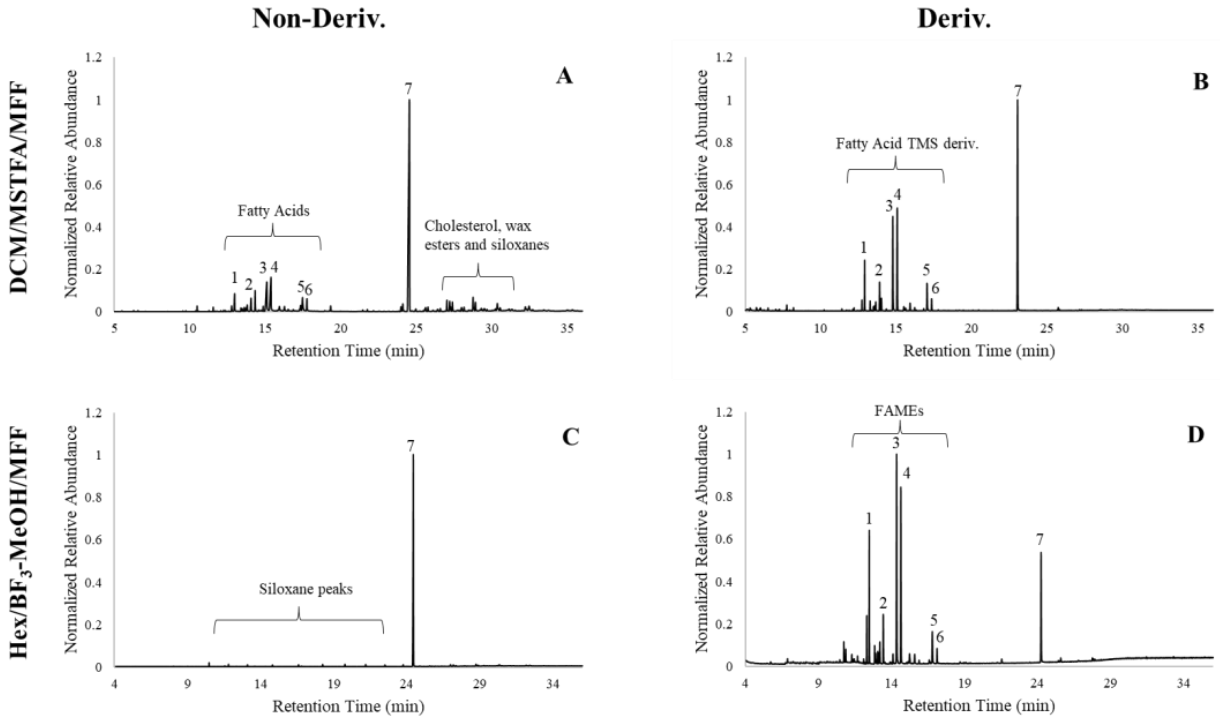

Figure 2. A representation of the total ion chromatograms (TICs) of the normalized non-derivatized (**left**) and derivatized (**right**) LPRs analyzed. (**A**) Non-derivatized DCM extracted LPR from MFF substrate. (**B**) DCM/MSTFA derivatized LPR from MFF substrate. (**C**) Non-derivatized hexanes extracted LPR from MFF substrate. (**D**) BF_3-MeOH derivatized LPR from MFF substrate. The seven predominant peaks chosen for this study are as follows: (1) myristic acid, (2) pentadecanoic acid, (3) palmitoleic acid, (4) palmitic acid, (5) oleic acid, (6) stearic acid, and (7) squalene.

3.1.2. Porous Surfaces

Porous surfaces were represented as MFFs for this study. The percent recovery was >86% for the 45 min extraction time limit for the fatty acids and squalene with DCM. To ensure that DCM was the most appropriate solvent, the recovery was conducted with hexanes as well. When hexanes were used as the extraction solvent, the fatty acid recovery rate was significantly lower at 30–40% for the 1 min extractions, and there was a 14–22% extraction rate with the 45 min time limit. It was also noted that the extraction recovery was less than the recovery of LPRs from the non-porous substrate. One proposition for the higher extraction recoveries for the slide kimwipes was that the kimwipe was a thinner material, with fewer layers of cellulose material that were less compacted, in comparison to the borosilicate glass microfibers in the MFF. The derivatization recovery was similar to the non-porous slide samples, with a low recovery of derivatized fatty acids between 1 and 4% and squalene with a <1.0% recovery.

Squalene was also the most abundant peak in the non-derivatized LPRs (Figure 2), as supported by the literature. Similar chemical profiles were observed for the porous substrates as seen for the non-porous ones in volunteers 2 and 5. The intensity of the fatty acids was less in volunteers 1 and 3, and although they were present, the siloxane peaks from the column were readily observed and identified in the fatty acid region (Figure 2C). This was likely because not enough sebum transferred to the substrate from the groomed fingers. Cholesterol was identified in all the non-derivatized volunteer samples at Rt = 26.980 min. Similar wax esters were identified in the porous samples as observed in the non-porous with the addition of tetradecenoic acid hexadecyl ester (Rt = 27.342 min) and hexadecenoic acid dodecyl ester (Rt = 25.751 min).

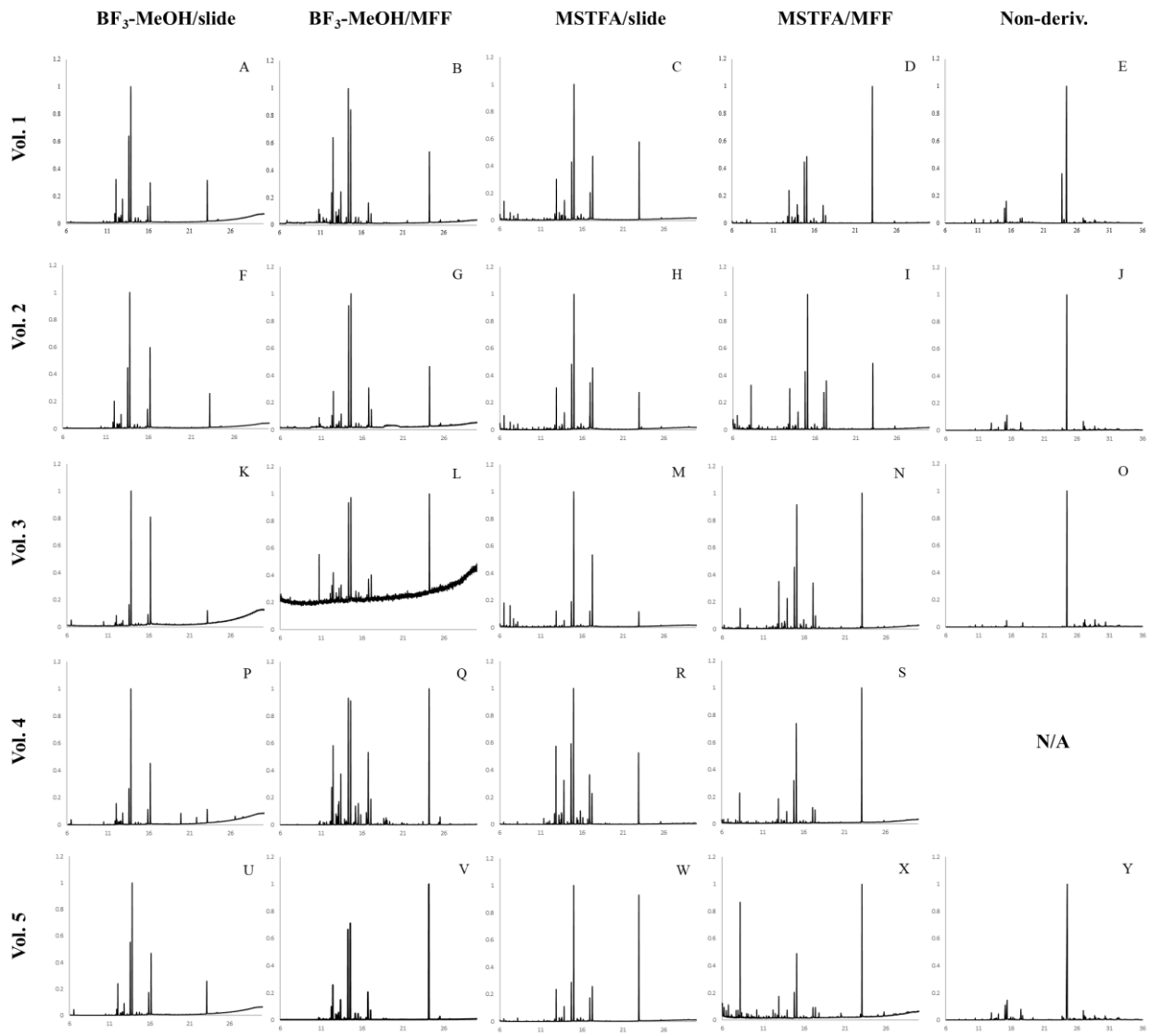

Figure 3. Representative LPR TICs of all five volunteers for the BF_3-MeOH/slide, BF_3-MeOH/MFF, MSTFA/slide, MSTFA/MFF, and non-derivatization. The TICs for volunteer 1 are displayed in panels (**A**–**E**), volunteer 2 in (**F**–**J**), volunteer 3 in (**K**–**O**), volunteer 4 in (**P**–**S**), and volunteer 5 in (**U**–**Y**). Volunteer 4 did not donate prints for non-derivatization but is present to show how variable the prints are per person. The *y*-axis is the normalized relative abundance (0–1.2), and the *x*-axis is the retention time in minutes.

The LPR profiles of the derivatized porous samples looked similar to the non-derivatized chromatograms for volunteers 1 and 5 (Figure 3B,V). The base peak was either squalene, palmitic acid, or palmitoleic acid, and the fatty acids were slightly lower in abundance. Volunteer 5 consistently had myristic acid, oleic acid, pentadecanoic acid, and stearic acid as the fourth- through seventh-most abundant peaks in all the replicates. Volunteer 3 showed the same trend as volunteer 5 but with a lower overall abundance of the LPR profile (Figure 3L). Myristic acid and oleic acid were about 20–30% of the base peak for volunteer 2 (Figure 3G), with pentadecanoic and stearic acids having the lowest intensities of the seven major analytes, with between 8 and 16% the relative abundance of palmitic acid of the base peak. Finally, volunteer 1 was different than the other volunteers, because myristic acid was consistently their fourth-most abundant peak. Cholesterol was not identified in the recovered BF_3-MeOH samples.

The primary difference between the derivatized MFF and slide profiles was the higher abundances of squalene and palmitoleic acid in the porous samples. Stearic acid was one of the less abundant peaks for all volunteers in the porous samples, as opposed to one of the most abundant peaks after palmitic acid in the non-porous samples. It was hypothesized that, besides the inherent variability of sebum, the difference between the two samples was the substrate. The cellulose material in the kimwipe is a polar molecule that likely interacted with the polar heads of stearic acid and palmitic acid through intermolecular forces, thus providing a good removal of these molecules from the non-porous surface. With a higher concentration in the kimwipe material, once derivatized, it has more molecules that can subsequently be detected. This is similar to the interaction of squalene and the borosilicate glass fibers, which are nonpolar molecules. Thus, squalene interacts with the substrate material and has higher concentrations than more polar molecules and, thus, yields higher concentrations in the resulting chromatogram. However, this theory needs further investigation, since it could not be determined why there was a high abundance of palmitoleic acid in the MFF samples. Since there was an inconsistency in the recovery between the non-derivatized and derivatized samples, Table S2 contains some of the other peaks identified in the derivatized MFFs. However, there were few components, such as dodecanoic acid, which were observed in both the derivatized and non-derivatized samples.

3.2. MSTFA Derivatization

3.2.1. Solid Surfaces

Dichloromethane provided the highest percent recovery (100%) for extracting squalene and the six major fatty acids at the evaporation stage (Test 4B). The percent derivatization recovery for non-porous samples was calculated to be about 4% for squalene and between 24 and 30% for the fatty acids. The higher percent recovery for both the derivatized and evaporation stages was likely due to the intermediate polarity DCM has, where it can solubilize both the polar carboxylic moiety and the nonpolar carbon chain of the fatty acids, allowing them to interact with the derivatization process [9,12]. Dichloromethane could also be disrupting any interactions the target analytes may have with the borosilicate glass and cellulose fibers compared to the hexanes, yielding better solubilization.

The non-derivatized DCM-extracted samples were not included in this paper due to a major contamination of phthalates within the LPR profile. However, the MSTFA-derivatized samples were compared to the components identified in the literature. The chromatograms had a similar profile to the BF_3-MeOH-derivatized non-porous samples, where palmitic acid was the most abundant fatty acid and the base peak for all the volunteers. Stearic acid was the second-most abundant peak in volunteer 3 (Figure 3M). The MSTFA-derivatized profiles for volunteers 1 and 5 (Figure 3C,W) were similar to the BF_3-MeOH-derivatized chromatogram, but squalene was noted to be the second-most abundant peak in most of the samples instead of stearic acid. Volunteer 2 had a higher abundance of oleic acid (about 30% of the base peak in the MSTFA samples) than seen in their BF_3-MeOH-derivatized samples, which was approximately 20% of the base peak (Figure 3H). MSTFA was able to derivatize cholesterol, when present, and other components; however, no wax esters were detected, leading to the conclusion that they were absent during the time of collection or were diluted to undetectable levels, because they should have been derivatized as well (Table S3).

3.2.2. Porous Surfaces

The percent recovery of LPR from the MFF using DCM/MSTFA was 2.4% for squalene and 20–34% for the fatty acids. It was slightly lower than the kimwipe/slide recovery but was better overall than the BF_3-MeOH-derivatized recovery rates. The difference between the non-derivatized DCM/MFF samples compared to the derivatized MSTFA/MFF samples was that squalene was the dominant peak in the non-derivatized LPRs, which agreed with other studies [1,4,6], cholesterol, and wax esters (Figure 2).

However, the base peak in the derivatized MSTFA LPRs was either squalene or palmitic acid (Figure 3D,I,N,S,X). Squalene decreased in abundance compared to the fatty acids due to diluting the LPRs with the reagent and DCM for the analyses. It was also observed that squalene was one of the highest peaks in the MFF samples. The relative abundance of the squalene peak with respect to the other analytes was dependent upon the substrate and the volunteer, which has also been observed elsewhere [13]. The non-derivatized DCM-extracted LPR profiles for the volunteers held similarities to the BF_3-MeOH-derivatized porous samples, though a noticeable difference was that palmitoleic acid was about 40–70% of the palmitic acid in volunteers 1–3 compared to the 60–100% observed in the BF_3-MeOH derivatization. Additionally, all seven targeted fatty acids were present and identified in all derivatized MSTFA samples, as opposed to the non-derivatized samples, with either all or a combination of the seven fatty acids present. Additional MSTFA-derivatized peaks observed are presented in Table S3.

3.3. Best Derivatization Method for Each Substrate

Several questions were used to determine which derivatization method was best for recovering LPRs from the porous and non-porous surfaces: (1) Were the same peaks observed in the non-derivatized samples seen in the derivatized samples? (2) Did the raw abundance of the fatty acids change between the non-derivatized and derivatized methods? (3) Did each derivatization method recover the same peaks? (4) Which method provided higher recovery for each substrate/derivatization combination?

Question 1: Squalene and the fatty acids were identified in the non-derivatized and both derivatization methods. There were several components that were only observed between the non-derivatized method and one of the derivatization methods, such as cholesterol, which was only present in the non-derivatized and the MSTFA-derivatized LPRs. The only exception was the wax esters, which were identified in the non-derivatized samples but were observed in their derivatized form (i.e., fatty acids and alcohols) with BF_3-MeOH, or they were severely diluted during the MSTFA derivatization process and, thus, were not observed. Amino acids were not present in any of the derivatized or non-derivatized samples. Since amino acids are usually found in sweat, they were not expected to be observed, because none of the volunteers were sweating at the time of sample collection, along with the prints being sebaceous-rich through grooming. Some amino acids had boiling points higher than the column, and others could have eluted too quickly through the nonpolar column, which also could have explained their absence, though further investigation is needed.

Question 2: Comparing the derivatized and non-derivatized abundances, squalene was the dominant peak for all the non-derivatized hexane MFF and hexane slide LPRs, and their abundance was even higher in the derivatized samples. The fatty acids in the non-derivatized samples were similar in abundance. The abundance of the fatty acids in volunteer 2's BF_3-MeOH/MFF-derivatized samples was approximately half the abundance observed in the non-derivatized hexane MFF samples, whereas the fatty acids in volunteer 5 were approximately the same height as those in the non-derivatized samples. Meanwhile, the BF_3-MeOH/slide fatty acids were also comparable to the non-derivatized peaks, although the ratios of stearic acid to oleic acid were different. Stearic acid in the BF_3-MeOH/slide was found to be about 66–75% more abundant than oleic acid (example in Figure 3E) in the non-derivatized residues. The significant decrease in squalene between the derivatized samples and the abundance observed in the non-derivatized samples was due to the dilution of the sample when performing liquid–liquid extraction. Squalene is an unsaturated hydrocarbon with no active hydrogens, so it would not participate in the esterification process, and thus, it was affected by the increased volume of the solution. For the DCM/MFF and MSTFA/MFF samples, squalene in volunteer 1 was about 50% more abundant than in the MSTFA/MFF samples, while the MSTFA/MFF fatty acids were about 50–75% more abundant than in the DCM/MFF samples. Volunteer 2 exhibited almost equal amounts of squalene in the non-derivatized and derivatized samples; however, derivatized

fatty acids were significantly more abundant, about 10 times more, than the non-derivatized samples. Both squalene and the fatty acids were more abundant in the derivatized than the non-derivatized samples for volunteer 3. Squalene was more abundant in the DCM/MFF than the MSTFA/MFF samples; however, the opposite was seen with the fatty acids in MSTFA/MFF being about 50–60% more abundant than in the DCM/MFF samples.

Question 3: Both reagent systems were able to recover the derivatives of the primary seven fatty acids that were monitored throughout the study. Although the chromatographic profiles looked similar between the BF_3-MeOH- and MSTFA-derivatized samples, there were several components that were only observed in one sample or the other. Considering that the same samples were derivatized by the two different reagent systems, it was interesting that some minor components were not shared between the two sets of derivatized samples. The porous samples derivatized by MSTFA and BF_3-MeOH shared at least three fatty acids: dodecanoic acid, tridecanoic acid, and pentadecanoic acid (Tables S2 and S3). Based on the porous samples, there were some molecules that were observed for one derivatization reagent but not the other, while the MSFTA process yielded the presence of cholesterol, ethanamine, and some more fatty acids than were monitored. Considering the same samples were also derivatized by BF_3-MeOH, more components could be identified, including straight-chain alkanes (i.e., decane and undecane), alcohols, and more fatty acids than observed in the MSTFA derivatization. This indicates that the BF_3-MeOH process extracted more components than initially anticipated based on the use of a nonpolar solvent. Additionally, the observation of an alcohol in the BF_3-MeOH samples indicated that there was an interaction with alcohols during the process, despite the fact that a nonpolar extraction solvent was used. A similar comparison could not be accomplished with the non-porous samples due to contamination issues.

Question 4: Even though the chemistry of the LPRs differed (Figure 3) for each volunteer that were collected on different days [4], as supported by the literature [1], higher recovery of the LPRs was observed with DCM as the extraction solvent and MSTFA as the derivatization solvent. This was primarily due to the recovery of cholesterol from both substrates. DCM provided >83% recovery for all stock solution samples at the 1 and 45 min extraction times for the slide/kimwipe and MFF substrates. Conversely, hexanes extracted <83% of squalene at both extraction times for both substrates. The fatty acids extracted from hexanes yielded a 14–21% recovery rate from the MFF substrate at the 45 min extraction time and >100% for the 1 min extraction with the kimwipe. DCM also consistently recovered the fatty acids in the non-derivatized LPRs as opposed to the hexanes, where there were only siloxanes or low abundances of recovered fatty acids. Additionally, during the percent recovery of the stock solution through each derivatization process (Table 1), DCM consistently yielded higher recovery rates. The final recovery rate percentages from the MFF substrate via the MSTFA process were approximately 2% and between 20 and 35% for squalene and the fatty acids, respectively, compared to 0.85% for squalene and 1–3% for the fatty acids recovered via the BF_3-MeOH derivatization process. Similar observations were seen for the slide/kimwipe samples derivatized by MSTFA, with a recovery rate percentage of 4.2% for squalene and 20–30% for the fatty acids. The recovery rates were also lower for the samples derivatized by BF_3-MOH at a rate of 0.6% for squalene and 6–9% for the fatty acids.

4. Conclusions

The aim of study was to determine which of two common derivatization reagents would recover the most LPRs from porous and non-porous surfaces considering that all crime scenes have these types of surfaces. Both the non-derivatized and derivatized methods produced chromatographic results similar to those in the literature, which have used a combination of one- or two-step derivatization processes. The derivatization reagent that recovered the most LPRs from both porous and non-porous surfaces was MSTFA. While both processes targeted acidic hydrogens, especially the extraction and derivatization of fatty acids and amino acids, the MSTFA process also recovered squalene and cholesterol

without derivatizing the wax esters. BF_3-MeOH is a good derivatizing reagent for porous substrates; however, it only produced methyl esters during derivatization while retaining squalene. Although hexane and DCM were both used as extraction solvents, DCM should be considered for the extraction solvent for both methods instead of using hexanes, because it can solubilize a much wider range of components and, thus, aid in the recovery of LPRs for subsequent instrumental analyses.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/forensicsci3020023/s1: Figure S1: Flowchart of the sample preparation and derivatization methods for this manuscript. Table S1: Components identified in the literature of latent print residues analyzed using a non-derivatization method. Figure S2: Proposed Fischer esterification of a carboxylic acid, i.e., free fatty acids in the LPR, with a Lewis ester, boron trifluoride (BF3,), in the presence of an alcohol, methanol (MeOH). Figure S3: Example chromatogram of the chemical shifts between non-derivatized fatty acids, BF3-MeOH derivatives, and MSTFA derivatives. Table S2: Component identification list for the less abundant peaks observed in the BF3-MeOH/slide (left) and BF3-MeOH/MFF (right) TICs for the forehead LPRs of volunteer 4. * Identifies components observed in the derivatized and non-derivatized samples. Figure S4: Proposed nucleophilic substitution reaction of a carboxylic acid, with MSTFA producing a trimethylsilyl derivative. Table S3: Component identification list for the less abundant peaks observed in the MSTFA/slide (left) and MSTFA/MFF (right) TICs for the forehead LPRs of volunteer 4. * Identifies components observed in the derivatized and non-derivatized samples.

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