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

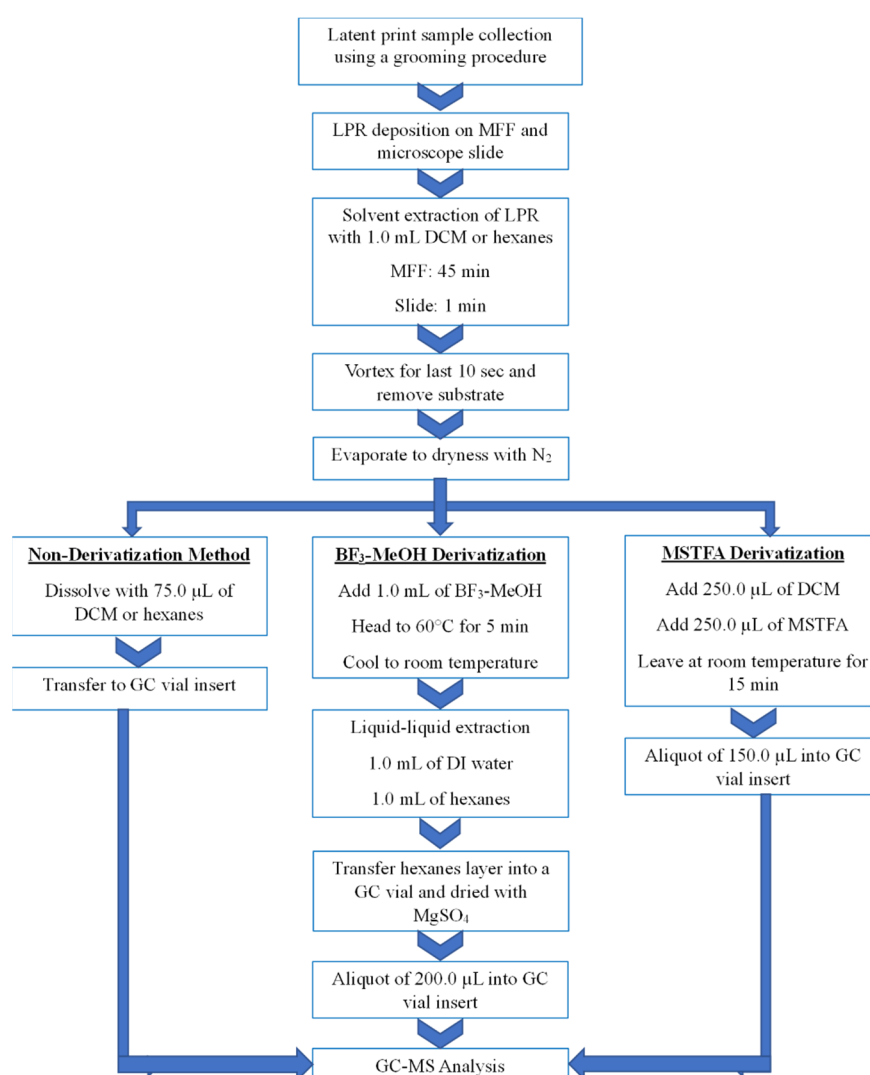**Jessica Kindell [1] and Candice Bridge [1,2,*]**

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Supplemental Materials

Figure S1. Flowchart of the sample preparation and derivatization methods for this manuscript.

Latent Print Chemistry

     Latent prints are one of the three major fingerprint evidence left at crime scenes and are comprised of sweat oils, dirt, and other exogenous materials collected on the fingers and transferred to a surface creating a translucent print. LP chemistry is composed of a combination of organic and inorganic components and elements secreted from the body

through glands found in the dermis layer of the skin in addition to the transference of external sources of contamination. The skin has three types of glands: the eccrine, sebaceous, and apocrine glands [1–4]. The eccrine sweat glands are distributed all over the body but are mainly concentrated on the volar surfaces [5,6]. The sweat produced is composed of 99% water with trace amounts of organic and inorganic components such as amino acids, vitamins, urea, chloride, sodium, salts, phosphates, and ammonia [1–3,7]. The amino acids in sweat have been a research focus for characterizing latent prints with the use of various instrumental analytical techniques such as GC-MS, LC-MS, and UV-spectrophotometry. Amino acids found in sweat are serine, glycine, alanine, ornithine, ornithine-lysine, aspartic acid, threonine, histidine, valine, leucine, glutamic acid, isoleucine, phenylalanine, lysine, and tyrosine [2,7,8].

Secretions from the sebaceous glands, which are located throughout the body, and are associated with hair follicles, but are heavily concentrated on the head with the primary function of lubricating and moisturizing the skin and hair [2]. Sebum is generally composed of fatty acids and triglycerides (57.5%), wax esters (26.0%), squalene (12.0%), cholesterol (1.5%), and sterols (3.0%) [2,9,10]. The fatty acids and any mono- and diglycerides present in sebum are a result of the hydrolysis of triglycerides by bacterial lipases when exposed to the skin surface [2,11]. Since the sebaceous glands are not located on the volar surfaces, the oils can only be transferred to these regions typically by touching the face or hair. Traces of metabolites from drugs, medications, and diet-specific foods could be part of the composition of the eccrine and sebaceous gland secretions.

Lastly, the bodily secretion from the apocrine glands, which is located around the thicker and courser hair found in the genital and armpit regions [1,2]. The gland is larger than the eccrine gland and is located above the sebaceous gland duct [2]. Apocrine glands secrete thicker fluid that is often mixed with sebum, but components isolated from these secretions are proteins, carbohydrates, cholesterol, iron, sulfates, and steroids [2]. Table S1 lists some of the components identified in literature that uses non-derivatized samples.

Table S1. Components identified in literature of latent print residues analyzed using a non-derivatization method.

Component name	MW (g/mol)	Type	Reference	Component name	MW (g/mol)	Type	Reference
Nonanoic acid	158.23	FA	[12,13]	Cholesterol acetate and isomers	428.6962	Sterol ester	[14]
Diglycerides	176.12	Glyceride	[12,13,15]	Myristyl hexadecenoate	450.8	WE	[16]
Dodecanoic acid	200.3178	FA	[12,13,15,17,18]	9-hexadecenoic acid tetradecyl ester	452.7962	WE	[13,14,17]
Tridecanoic acid	214.348	FA	[12,13,16–18]	Myristyl palmitate	452.8	WE	[13,16]
Tetradecenoic acid	226.360	FA	[13,16,18,19]	Palmityl myristate	452.8	WE	[13,16]
Hpetadecanoic acid	270.45	FA	[12,13,16,19]	9-hexadecenoic acid hexadecyl ester	476.8	WE	[17]
Eisocanoic acid methyl ester	312.5304	FAME	[12,13,16,19]	Palmityl palmitoleate	478.8	WE	[13,18]
Docosanoic acid methyl ester	354.6101	FAME	[13]	Palmityl palmitate	480.86	WE	[13,18]
Tricosanoic acid methyl ester	368.6	FAME	[13]	9-hexadecenoic acid octadecyl ester	506.9	WE	[17]
Tetracosanoic acid methyl ester	382.7	FAME	[13]	9-hexadecanoic acid octadecyl ester	508.9	WE	[17]
Cholesterol	386.6535	Sterol	[13,14,17,18,20]	9-hexadecenoic acid eicosyl ester	534.9398	WE	[17]
Myristyl myristate	424.7	WE	[13,16–18]	Cholesterol esters	637.1	Sterol ester	[13,14,17,18,20]
Lauryl palmitate	424.7	WE	[13,16,18]	Mixed di- and monoglycerides	---	Glycerides	[15]

| Stearyl decanoate | 424.743 | WE | [16] | Monoglycerides and phospholipids | --- | Glycerides | [15] |

Since most of the components found in these secretions are amino acids and lipids, they typically contain either carboxyl, amino, or hydroxy functional groups which induce dipole-dipole moments and can form hydrogen bonds, making them polar or extremely polar. If analyzing samples using a more nonpolar GC column, which is the common general-use column, the amino acids and lipids would need to be derivatized. As previously mentioned, derivatization would make polar components more compatible to a nonpolar stationary phase by removing the active hydrogen on polar functional groups and replacing them with a more nonpolar functional group; thereby decreasing the overall polarity of the target component. The three commonly used general derivation reaction types are alkylation, acylation, and silylation [21–23]; however, only alkylation with BF₃-MeOH and silylation with MSTFA are be discussed herein.

BF₃ Derivatization

Polar components in latent prints, e.g., fatty acids and amino acids, underwent a Fischer esterification with BF₃-MeOH. This type of derivatization requires a carboxyl group, found in both lipids and amino acids, an alcohol (i.e., methanol), and a Lewis acid catalyst (Figure S2) [24]. The oxygen on the carbonyl is protonated by the catalyst, i.e., boron trifluoride (BF₃), creating an electrophile. Then the nucleophilic methanol attacks the electrophilic carbon. Deprotonation of the methanol by the hydroxyl group of the intermediate removes the positive charge on the oxygen of the nucleophile resulting in a loss of water. Then the BF₃ leaves resulting in a methyl ester. The reverse is true due to BF₃-MeOH's ability to transesterify esters into methyl esters. Any wax esters present are susceptible to be transformed into methyl esters and alcohols which would contribute to the overall abundance of the fatty acids already present within the residue.
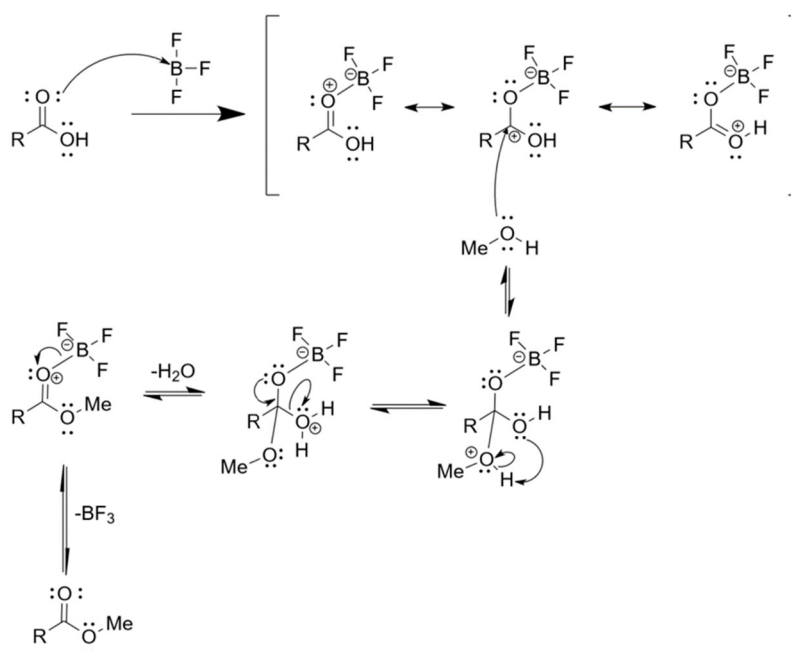
Figure S2. Proposed Fischer esterification of a carboxylic acid, i.e., free fatty acids in the LPR, with a Lewis ester, boron trifluoride (BF₃,) in the presence of an alcohol, methanol (MeOH).

When fatty acids are esterified, the resulting methyl esters will elute faster than the fatty acids (Figure S3). Even though the methyl esters are heavier than the fatty acids by a mass of 14 a.u., they have lower boiling points because of the weak London dispersion and dipole-dipole forces, causing vaporization to occur at lower temperatures [24].

Meanwhile, non-derivatized fatty acids exhibit stronger intramolecular forces, primarily due to hydrogen bonding from both oxygens on the hydroxyl and carbonyl groups. This would result in more energy needed, i.e., higher temperatures, to break those bonds, thus eluting after methyl esters.
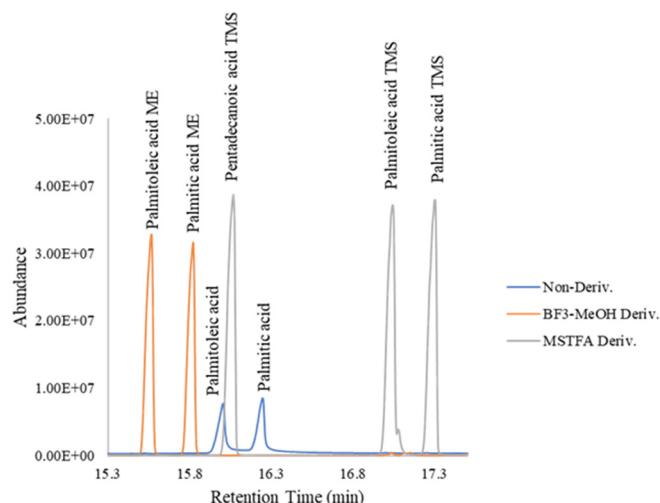
Figure S3. Example chromatogram of the chemical shifts between non-derivatized fatty acids, BF₃-MeOH derivatives, and MSTFA derivatives.
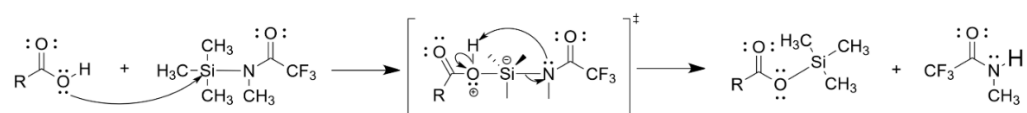
Table S2. Component identification list for the less abundant peaks observed in the BF₃-MeOH/slide (left) and BF₃-MeOH/MFF (right) TICs for the forehead LPRs of volunteer 4. * identifies components observed in derivatized and non-derivatized samples.

BF₃-MeOH/Slide		BF₃-MeOH/Slide	
Component Identification	Retention Time (min)	Component Identification	Retention Time (min)
Decane	5.40	Decane	5.73
Undecane	6.52	Undecane	6.87
Dodecanoic acid, methyl ester*	10.49	2,4-di-tert-butylphenol	10.73
Methyl tetradecanoate	11.87	Dodecanoic acid methyl ester*	10.86
Methyl 13-methyltetradecanoate	12.45	Dodecanoic acid, 4-methyl, methyl ester	11.27
Tetradecanoic acid, 12-methyl, methyl ester	12.57	Methyl 11-methyl dodecanoate	11.37
9-octadecenoic acid (Z)-, methyl ester	12.63	Dodecanoic acid, 10-methyl, methyl ester	11.43
Methyl 9-heptadecenoate (E or Z) cis-10-heptadecenoic acid, methyl ester	14.71	Tridecanoic acid, methyl ester*	11.66
Hexadecanoic acid, 14-methyl, methyl ester Heptadecanoic acid, methyl ester*	14.99	Methyl tetradecanoate	12.16
Hexanoic acid, 2-ethyl, hexadecyl ester Octanoic acid, hexadecyl ester	19.90	7-hexadecenoic acid, methyl ester (Z)-Methyl Z-11-tetradecanoate	12.29
Tris(tert-butyldimethylsiloxy)arsane	26.5	Pentadecanoic acid, methyl ester Methyl-13-methyltetradecanoate	13.02
		Myristic acid, 12-methyl, methyl ester	13.11
		cis-10-heptadecenoic acid, methyl ester	15.53
		Heptadecanoic acid, methyl ester*	15.83

MSTFA Derivatization

Silylation undergoes nucleophilic substituion to replace the hydroxyl group or an active hydrogen of an amino or fatty acid to create a trimethylsilyl (TMS) derivative (Figure S4). The oxygen on the hydroxyl group acts as a nucleophile to attack the silicon on the TMS group of MSTFA. An intermediate forms while simultaneously protonating the nitrogen on the MSTFA from the hydroxyl group of the of the acid creating a leaving group. Out of the two nucleophilc substitution reaction, Sₙ2 is more favored due to the use of polar aprotic solvents since silylation reagents and TMS derivatives are moisture

senstive and can react with polar protic solvents instead. Chromatographiclly, the TMS derivative elutes after the fatty acid because it has more mass and is more non-polar than their respective fatty acids (Figure S3). Squalene, wax esters, and possibly triglycerides would be unaffected by the derivatization reagents due to the lack of an active hydrogen on their structure.

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 derivatized and non-derivatized samples.

MSTFA/Slide		MSTFA/MFF	
Component Identification	Retention Time (min)	Component Identification	Retention Time (min)
Ethanamine, 2TMS derivative	4.74	Ethanamine, 2TMS derivative	4.74
Methyltris(trimethylsiloxy)silane	5.74	Methyltris(trimethylsiloxy)silane	5.69
Silanol, trimethyl-phosphate (3:1)	8.21	Silanol, trimethyl-, phosphate (3:1)	8.13
Dodecanoic acid, TMS derivative*	11.42	Dodecanoic acid, TMS derivative	11.34
9-tetradecenoic acid, (E)-, TMS derivative	12.75	Tridecanoic acid, TMS derivative	11.64, 11.85, 12.05
Pentadecanoic acid, TMS derivative	13.53, 13.62	9-tetradecenoic acid, (E)-, TMS derivative	12.66
Heptadecanoic acid, TMS derivative*	15.55, 16.26	Pentadecanoic acid, TMS derivative	13.18, 13.41, 13.50
Cholesterol, TMS derivative*	25.79	Palmitic acid, TMS derivative	14.24, 15.40, 15.53, 16.11
		9, 12-octadecadienoic acid, (Z, Z)-, TMS derivative	16.72
		Cholesterol, TMS derivative*	25.63

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