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TECHNICAL NOTE

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Development of a GC-MS Method for the Simultaneous Analysis of Latent Fingerprint Components*

ABSTRACT: Latent fingerprint residue is a complex mixture of organic and inorganic compounds. A full understanding of the composition of this mixture and how it changes after deposition is lacking. Three solvent systems were compared for the simultaneous extraction and derivatization with ethyl chloroformate of selected amino and fatty acids from a nonporous substrate (Mylar®) for subsequent analysis by gas chromatography-mass spectrometry. A solvent system comprised of sodium hydroxide, ethanol, and pyridine was found to be the most effective. This method was applied to the analysis of latent fingerprint residue deposited on Mylar® and preliminary data are presented. Twelve amino acids (e.g., serine, glycine, and aspartic acid) and 10 fatty acids (e.g., tetradecanoic, hexadecanoic, and octadecanoic acids) were identified. The potential application of this method to further the understanding of latent fingerprint chemistry has been demonstrated.

KEYWORDS: forensic science, latent fingerprints, gas chromatography-mass spectrometry, ethyl chloroformate, latent fingerprint components, amino acids, fatty acids

Latent fingerprints are not readily visible and a wide variety of chemical, physical, and optical visualization techniques are used to enhance them (1). While it is understood that some visualization techniques react with specific chemical components of the fingerprint and others detect the greasy physical nature of the surface, the full extent of the mechanisms of reaction for many are not truly understood. Knowledge of the chemical composition of the latent fingerprint and of how it changes over time would aid the improvement of current techniques and the development of novel ones.

Latent fingerprints are composed of the natural secretions of the sweat glands in the skin, predominantly eccrine and sebaceous, and environmental contaminants (2,3). Eccrine sweat consists of a very high percentage of water and the remainder is a highly complex mixture of organic (e.g., amino acids, proteins, and lactate) and inorganic material (e.g., Na^+, K^+, Cl^-, and trace metal ions). Sebaceous sweat is predominantly fatty acids, glycerides, cholesterol, squalene, and a variety of lipid esters (2,3). Amino acids and lipids have been studied independently in fingerprints, using a variety of protocols (4,5). To date, no effective technique has been reported that allows the full evaluation of the natural distribution of substances in the latent fingerprint. It should be understood that the deposited latent fingerprint is likely to have a substantially different composition from samples which may be swabbed from fingers, due to the mechanics of the transfer process. The technique reported here should mimic the interaction between a finger and many nonporous surfaces.

Amino acids and lipid material have been selected for initial study, as they are some of the more common components and are targeted by many of the visualization techniques currently used. Derivatization is necessary to make these compounds more suitable for separation by gas chromatography (GC). Ethyl chloroformate (ECF) is a suitable agent for the derivatization of amino acids, fatty acids, and organic acids (6). Silylating reagents such as bis(trimethylsilyl)trifluoroacetamide (BSTFA) are also commonly used (7–9). However, ECF has several advantages compared with silylating reagents. Firstly, derivatization by silylating reagents is lengthy and usually requires elevated temperatures (7–9). Secondly, and more importantly, both silylating reagents and their derivatives are sensitive to moisture and are prone to hydrolysis. Consequently, all samples must be properly dried before derivatization and stored carefully postderivatization. ECF is a more robust reagent and derivatization is effectively instantaneous at room temperature (10). ECF can be used in media compatible with the extraction of both amino and fatty acids.

Ethyl chloroformate derivatizes amino acids to give the corresponding N(*O-S*)-ethoxycarbonyl ethyl esters. The reaction proceeds rapidly in an aqueous solution with ethanol and pyridine (11). The latter catalyzes the reaction and must be present in a molar excess of ECF (11). The alkyl groups for the amino and carboxyl group are provided by the chloroformate and alcohol, respectively (12). ECF derivatization of fatty acids results in the

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formation of monoethyl esters. This reaction proceeds most rapidly in organic solvent with ethanol and pyridine (10). Differences in the ideal conditions for extraction and derivatization for amino and fatty acids mean that it is necessary to optimize the conditions for the most favorable compromise (10). ECF has been used for the simultaneous analysis of amino and fatty acids in a variety of applications including plasma (6,13) and paint media (14,15). Three solvent systems are compared here for the analysis of amino and fatty acids deposited on a nonporous substrate, using GC with mass spectrometry (GC-MS). The sodium hydroxide method (P. Hušek, personal communication) was then applied to latent fingerprint residue.

Materials and Methods

Materials

L-aspartic acid (Asp), L-*p*-chlorophenylalanine (cPhe), L-methionine (Met), L-phenylalanine (Phe), L-valine (Val) (all $\geq$ 98%), myristic acid (MA, tetradecanoic acid), nonadecanoic acid (NA), palmitic acid (PA, hexadecanoic acid), and stearic acid (SA, octadecanoic acid) (all $\geq$ 99%), *n*-docosane ($\sim$ 99%), and pyridine (ACS reagent) were obtained from Sigma-Aldrich (Gillingham, Dorset, U.K.). Ethyl chloroformate (ECF, >98%) and iso-octane (>99.5%) were obtained from Fluka (Gillingham, Dorset, U.K.). Acetonitrile (ACN), hexane, methanol, ethanol, and chloroform were of analytical-reagent or HPLC grade and were obtained from Fisher Scientific UK Limited (Loughborough, Leicestershire, U.K.). 0.1 M hydrochloric acid and 1 M hydrochloric acid were volumetric analysis grade and also obtained from Fisher Scientific U.K. Limited. Sodium hydroxide pearls (>98%), purchased from Phillip Harris (Shenstone, Lichfield, U.K.), were used to prepare a 1% w/v solution in deionized water. Mylar® 002, polyester film, 23 μm thickness, was obtained from DuPont Teijin Films (U.K.) Limited (Middlesbrough, U.K.).

Preparation of Calibration Samples for Quantification

Amino acid calibration solutions were prepared in 0.1 M hydrochloric acid, containing Asp, Met, Phe, and Val between 0.4 and 2 μmol/mL and the internal standard cPhe at 1 μmol/mL in each. Each of these amino acids was chosen to represent some of the major amino acid types, namely acidic, sulfur-containing, aromatic, and aliphatic, respectively. Fatty acid calibration solutions were prepared in hexane, containing MA, PA, and SA between 0.2 and 1 μmol/mL and the internal standard NA at 0.5 μmol/mL in each. These fatty acids represent some of the more common acids found in latent fingerprint residue. Derivatization procedures for calibration standards have been adapted from several references (11,14).

Fifty microliters hydrochloric acid (0.1 M), 225 μL distilled water, 160 μL ethanol, and 40 μL pyridine were added to 25 μL amino acid calibration solution, followed by 25 μL ECF and vortexed. Carbon dioxide was evolved during the reaction. After 2–3 min, 500 μL 1% ECF in chloroform (v/v) was added and vortexed briefly. The upper aqueous layer was removed and discarded. 500 μL hydrochloric acid (1 M) was added and briefly vortexed. The lower organic layer was transferred to a second 2 mL vial.

Fifty microliters hexane, 54 μL pyridine, and 13.5 μL ethanol were added to 25 μL fatty acid calibration solution, followed by 13.5 μL ECF, and vortexed. Carbon dioxide evolved and in some cases a white precipitate formed. The solvent was then transferred and combined with the relevant amino acid calibration extract. The standard was blown down to dryness under a stream of nitrogen at room temperature. Fifty microliters hexane containing *n*-docosane (0.08 μmol/mL) was then added and the sample was sonicated for 1 min.

Preparation of Samples

A 1 μmol/mL amino acid solution containing equimolar concentrations of Asp, cPhe, Met, Phe, and Val in 0.1 M hydrochloric acid was prepared. Twenty-five microliters was applied to a 1 cm^2 piece of a Mylar® 002 polyester film (prewashed in hexane and methanol) and allowed to dry at room temperature overnight (maximum 15 h). A total mass of 19.12 μg amino acid was deposited. Twenty-five microliters of a 0.5 μmole/mL fatty acid solution containing equimolar concentrations of MA, NA, PA, and SA in hexane was applied to the sample on top of the amino acids and allowed to dry (5 min). A total mass of 13.35 μg fatty acids was deposited.

Extraction and Derivatization of Samples

Three extraction solutions were used that were adapted from several references (11,14), with Method 1 being provided by P. Hušek (personal communication). The solutions were made up as follows:

Method 1: 500 μL 1% aqueous sodium hydroxide–ethanol–pyridine 75:40:10;
Method 2: 200 μL hexane, 200 μL 0.1 M HCl, 100 μL ethanol; and
Method 3: 200 μL acetonitrile, 200 μL 0.1 M HCl, 100 μL ethanol.

The sample was quartered and placed in a 4 mL amber glass vial. One of the three extraction solutions was added. Five hundred microliters was a sufficient volume to ensure complete contact of solvent with the sample surfaces. The sample was placed on a shaker (200 cycles/min) for 30 min, 2, or 4 h. For Method 1, 100 μL iso-octane-ECF 3:1 (v/v) was then added and vortexed for 5–10 sec. For Methods 2 and 3, 40 μL pyridine and 25 μL ECF were added and vortexed for 5–10 sec. After 2–3 min, for all Methods, the solvent was transferred to a 2 mL vial. Five hundred microliters 1% ECF in chloroform (v/v) was added and vortexed briefly. The upper aqueous layer was removed and discarded. Five hundred microliters hydrochloric acid (1 M) was added and briefly vortexed. The lower organic layer was transferred to a second 2 mL vial and blown down to dryness under a stream of nitrogen at room temperature. Fifty microliters hexane containing *n*-docosane (0.08 μmol/mL) was then added and the sample was sonicated for 1 min.

Extraction and Derivatization of Latent Fingerprint Residue

For each donor, 10 latent fingerprints were collected on a 2 cm × 10 cm piece of a Mylar® 002 film (prewashed in hexane and methanol). It was ensured that the donors had not washed their hands within 1 h before sampling. The donors rubbed their hands together briefly and a fingerprint from each of the 10 digits was deposited, using normal pressure. Each sample was extracted and derivatized immediately after collection. Two donors were initially used to demonstrate the potential application of the method, namely a female aged 25 and a male aged 45.

The sample was cut into smaller pieces using a scalpel and placed in a 4 mL amber glass vial, and 1 mL 1% aqueous sodium hydroxide–ethanol–pyridine 75:40:10 (v/v) was added. The sample was placed on a shaker (200 cycles/min) for 1 h. Two hundred microliters iso-octane-ECF 3:1 (v/v) was then added and vortexed

for 5–10 sec. After 2–3 min, the solvent was transferred to a second 4 mL vial and 1 mL 1% ECF in chloroform (v/v) was added and vortexed briefly. The upper aqueous layer was removed and discarded. One milliliter hydrochloric acid (1 M) was added and briefly vortexed. The lower organic layer was transferred to another vial and blown down to dryness under a stream of nitrogen at room temperature. The sample was redissolved in 50 μL hexane and sonicated for 1 min.

GC-MS Analysis

A 1 μL aliquot was injected onto a 30 m × 0.25 mm internal diameter DB-17ms fused silica capillary column with a 0.15 μm film thickness of 50% phenylmethyl silicone (J&W Scientific, Folsom, CA), in splitless mode. The column was held at 100°C for 4.5 min, ramped to 190°C at 10°C/min, and then ramped at a rate of 6°C/min to 250°C and held for 1.5 min. Helium was the carrier gas (1 mL/min). The injector and transfer line temperatures were 250°C. The instrument used was a Thermo Finnigan Trace GC coupled with a Thermo Finnigan Trace MS (Mass Spec U.K. Ltd, Oldham, U.K.). The mass spectrometer was operated in scan mode, set to monitor ions 50–450 *m/z* with a scan time of 0.9 sec, following a 4.5 min solvent delay. For the fingerprint residue samples, the column temperature program was extended and held at 250°C for 5 min and the mass spectrometer was set to monitor the scan range 50–500 *m/z* with a scan time of 1 sec.

Statistical Analysis

One-way ANOVAs were performed to compare the data. All analyses were performed using Microsoft® Excel 2000, with a 95% confidence level.

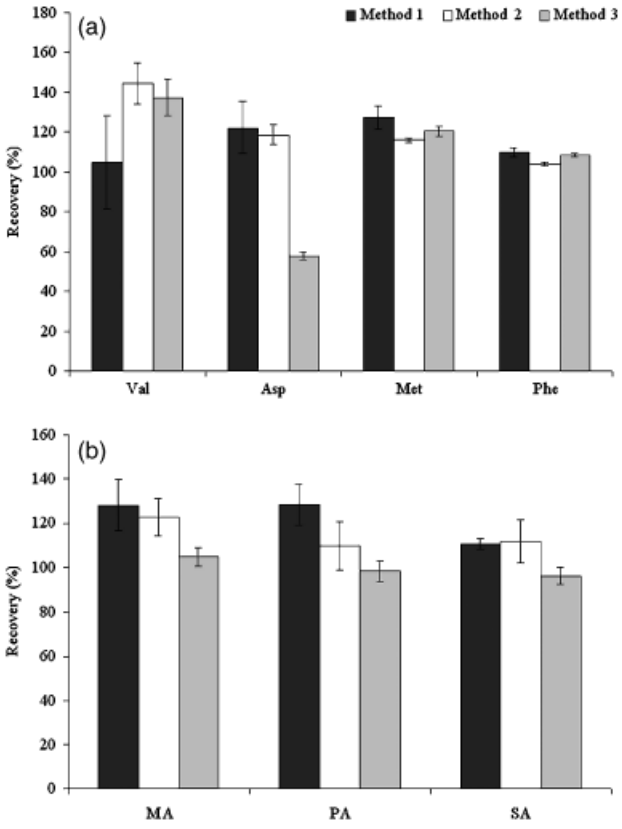

FIG. 1—*Mean recoveries of (a) amino acids and (b) fatty acids, for the three extraction methods using a 2 hour extraction time. Error bars are standard error, n = 4.*

Results and Discussion

Comparison of Solvent Systems and Length of Extraction

All five amino acids and four fatty acids deposited were successfully extracted from Mylar®, using each of the three extraction methods. NA and cPhe were subsequently used as internal standards for quantification.

For the amino acids, with the exception of valine, the highest recoveries were obtained with Method 1 (110–127%), followed by Method 3 (109–120%), and then Method 2 (104–116%) for methionine and phenylalanine. In the case of aspartic acid, Method 2 (118%) gave a higher recovery than Method 3 (58%) (2 h extraction data given in Fig. 1*a*). Similar trends were seen for all extraction times, except for 30-min extraction where Method 2 gave higher recoveries than Method 3. Significant differences in recovery between the three-solvent systems were only seen in the case of aspartic acid ($p = 0.001$). Method 3 gave a significantly lower recovery of aspartic acid, 58% compared with Methods 1 and 2 (123% and 119%, respectively). This is due to the dehydration of the derivative in the presence of acetonitrile to its parent anhydride, which is not quantified. This is also true for glutamic acid. The formation of the diethyl ester is promoted by the second addition of ECF (11). No significant differences in recovery were seen between the three extraction times for any of the amino acids. Valine repeatedly gave highly variable results compared with the other amino acids. The cause of this is yet to be determined but may be a consequence of its relative volatility.

For each solvent system, there was variation in recovery between the different amino acids (data not included). The source of this variation may include extraction efficiency, derivatization efficiency, and chromatography efficiency, as a consequence of structural differences. Derivatization efficiency is a key contributor to the variation and in turn affects the chromatography efficiency. ECF does not derivatize all active hydrogen groups, for example the hydroxyl group of threonine and serine and the free imino group of tryptophan, but in most cases this does not affect the elution of the compound from the column (11). Poor elution of threonine and serine can result as a consequence of hydrogen bonding between the unreacted group and silanol groups within the GC system (15). Both threonine and serine have been analyzed using this method but further work is being conducted to improve reproducibility. It should also be noted that arginine cannot be analyzed directly by this method. ECF does not derivatize the free imino group of arginine and there is absorption of the derivative in the GC column as a consequence (11).

For the fatty acids, overall, Method 1 gave the highest recoveries (111–128%). The relative recoveries for Methods 2 and 3 varied for each extraction time. For 2 h (Fig. 1*b*), Method 2 gave higher recoveries (110–123%) than Method 3 (96–105%). The opposite trend was seen for 30-min and 4-h extraction. Significant differences in recovery between the solvents were seen for PA for 30-min and 4-h extractions ($p = 0.049$ and 0.012, respectively), and for the stearic acid 30-min extraction ($p = 0.003$). In each case, Method 1 gave significantly higher recovery. Significant differences in the time of extraction were seen in the cases of myristic acid and Method 2 ($p = 0.043$) and stearic acid and Method 1 ($p = 0.004$). Recoveries are calculated using standards in solution that required blow down of 1 mL of solvent in comparison with 500 μL of solvent for samples. This is a stage with the potential for loss of analyte and so explains recoveries greater

than 100%. However, the results demonstrate that good recovery from plastic substrates is achievable with this protocol.

The composition of the reaction medium is key to the derivatization efficiency of fatty acids. Hušek et al (16) reported that in the case of methyl chloroformate (MCF), a 10% substitution of ACN by water resulted in a 50% reduction in yield but that by increasing the concentration of pyridine fourfold, the yield was restored to that seen for ACN alone. In the case of ECF, in a nonaqueous medium of ACN and pyridine (8%), an admixture of trace amounts of ethanol (4%) was necessary in order to esterify with yields of *c*. 95%.

The three solvent systems differ in pH. In the case of Method 1, the solvent system is alkaline (pH 13.10), while in Methods 2 and 3 it is acidic (pH $\approx$ 1.48). This difference in pH and the consequent effect on each step of the sample process may explain some of the variation seen for both amino and fatty acids. It should also be noted that hexane and hydrochloric acid (0.1 M) are immiscible. However, in the case of Method 2, due to the small volumes used here and the shaking used during extraction, the samples apparently come into contact with both phases.

Latent Fingerprint Residue Analysis

Following the demonstration that amino and fatty acids deposited on a nonporous substrate can be simultaneously extracted and analyzed, the method was applied to latent fingerprint residue. Method 1 gave the greater recoveries in general for both amino and fatty acids and consequently was applied to latent fingerprint residue to determine its potential application. Samples were collected from donors consisting of 10 combined fingerprints. Figure 2*a* shows a total ion chromatogram (TIC) of the 10 fingerprint residue sample of the female donor. Ten fatty acids and 12 amino acids have been identified in the chromatogram based on their retention time and mass spectrum. The relative intensities of characteristic ions were compared with those of standards and a range of $\pm$ 25% permitted for positive identification (17). Tables 1 and 2 outline the data obtained from the standards for those compounds identified in the sample. Ions greater than 100 m/z were sought for all compounds. In the case of serine 132 *m/z*, the base peak was used for identification but other abundant ions above 100 *m/z* were also common to threonine. Owing to peak overlap, these additional ions could not be used as unique identification ions. However, ions 60 and 86 *m/z* could be used for the identification of serine, providing consistent ratios. It is difficult to see the compound peaks in the TIC due to its complex nature and the fact that a number of compounds, particularly the amino acids, are low in abundance. Figure 2*b* shows a target compound chromatogram (TCC) of the sample. Target compound analysis is an established method for the identification of accelerant residues in fire debris approved by the American Society for Testing and Materials (ASTM) (17–19). Target compounds are identified in the chromatogram and the base ion of their mass spectrum quantified and plotted against retention time. It should be noted that the

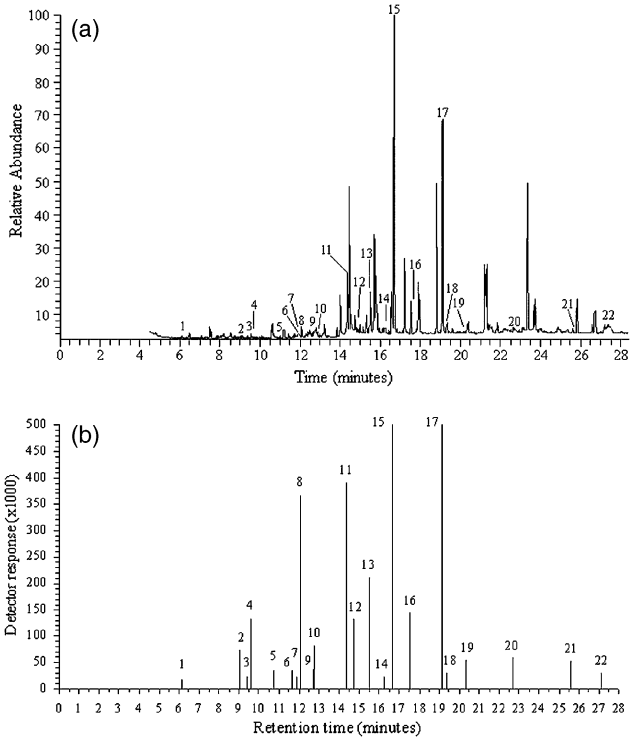

FIG. 2—*Gas chromatography-mass spectrometry data for latent fingerprint residue extraction, (a) total ion chromatogram, (b) target compound chromatogram. Numbers refer to Tables 1 and 2. In (b), hexadecanoic (15) and octadecanoic (17) acids are offscale with detector responses of 2.03 $\times$ 10^6 and 1.37 $\times$ 10^6, respectively.*

TABLE 1—*Amino acid compounds identified in latent fingerprint residue sample.*

	Target Compound	Retention Time (min)	Ion (*m/z*)	Relative Abundance (%)
2*	Alanine	9.07	116	100
			144	1.83
			189†	0.41
4	Glycine	9.57	102	100
			130	5.42
			175	4.74
5	Valine	10.73	116	28.46
			144	100
			174	2.77
6	Leucine	11.69	102	42.55
			158	100
			174	1.95
7	Isoleucine	11.92	102	36.31
			158	100
			130	15.01
9	Threonine	12.75	101	85.56
			129	100
			175	24.59
10	Serine‡	12.81	60	89.88
			86	40.45
			132	100
12	Aspartic acid	14.77	116	24.81
			142	25.88
			188	100
14	Glutamic acid	16.27	128	96.25
			156	98.31
			202	100
16	Phenylalanine	17.57	102	63.86
			176	100
			192	47.63
20	Lysine	22.72	128	14.50
			156	100
			226	8.32
21	Tyrosine	25.68	107	100
			192	52.05
			264	22.04

*Number corresponds to elution order in chromatograms in Fig. 2.

†189 *m/z* (M^+) not detected in sample and ratio could not be used for confirmation of identity but the other ratio was consistent.

‡Identification ions greater than 100 *m/z* selected with the exception of serine.

TABLE 2—*Fatty acid compounds identified in latent fingerprint residue sample.*

	Target Compound	Retention Time (min)	Ion (*m/z*)	Relative Abundance (%)
1*	Octanoic acid	6.10	88	100
			101	38.78
			129	10.54
3	Decanoic acid	9.37	88	100
			101	45.32
			157	17.04
8	Dodecanoic acid	12.07	88	100
			101	51.44
			228	3.38
11	Tetradecanoic acid	14.37	88	100
			101	56.54
			256	4.94
13	Pentadecanoic acid	15.51	88	100
			101	58.80
			270	6.01
15	Hexadecanoic acid	16.69	88	100
			101	60.45
			284	7.01
17	Octadecanoic acid	19.13	88	100
			101	63.24
			312	9.38
18	cis 9, cis 12-Octadecadienoic acid	19.35	67	100
			81	73.34
			109	24.83
19	Nonadecanoic acid	20.35	88	100
			101	64.44
			326	10.93
22	Tetracosanic acid	27.15	88	100
			101	70.40
			396	18.22

*Number corresponds to elution order in chromatograms in Fig. 2.

ordinate in the TCC is the base ion peak area and is different from the total detector response, which is the ordinate in the TIC. The TCC can then be used for visual comparison and pattern recognition. The TCC in Fig. 2*b* is clearly simpler than the TIC. The latter is complicated by several peaks, which were also present in the negative controls; for example, the peaks at 14.45 and 25.85 min are thought to be diethyl phthalate and bis(2-ethylhexyl) phthalate. The Mylar® is a probable source of these contamination peaks.

It is possible to simultaneously extract and analyze amino and fatty acids from a nonporous substrate. A similar extraction method and ECF derivatization has been used to analyze organic acids in serum (6). Compounds identified included other organic acids such as lactic, malonic, and glycolic acids, in addition to amino and fatty acids. It is therefore expected that these compounds may also be identified in fingerprint residue using this method, if present.

Conclusion

It has been demonstrated that it is possible to extract and analyze both amino and fatty acids from latent fingerprints with a single system. This has not been previously achieved in latent fingerprint chemistry studies. Further work will involve the application of this method to analyze latent fingerprints, to develop our current understanding of their composition.

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