TECHNICAL NOTE

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Separation of Visibly-Excited Fluorescent Components in Fingerprint Residue by Thin-Layer Chromatography

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ABSTRACT: The use of lasers for the detection of fingermarks is widespread in the forensic field. Despite this, and the fact that many studies have been conducted into the composition of fingermark residue, the components responsible for the inherent visible fluorescence remain unidentified.

Traditionally compositional studies have been performed on sweat, sebum, or skin surface washes, none of which are truly representative of the situation when a fingerprint is deposited on a surface. In this paper thin-layer chromatography (TLC) has been performed on sebum-rich fingermarks laid directly onto TLC plates and an argon ion laser used to visualize the separated components. It has been found to be a robust and reproducible method for studying the fluorescent components in fingermark residue and is considered to be more realistic than other methods of sample preparation as it eliminates the chances of extraneous matter being extracted from the skin surface. Investigations into the nature of the separated compounds have also been made and the results are reported.

KEYWORDS: forensic science, laser, thin-layer chromatography, fingerprints, visible fluorescence

In 1977, Dalrymple, Duff, and Menzel (1) advocated the use of lasers to supplement the fingerprint detection techniques available at that time. Since then, the use of lasers to search crime scenes and exhibits for potential marks has become commonplace. Typically, laser examination is one of the first stages in the fingerprint detection process. However, despite the continued successful detection of fingerprints by this method, the chemical components responsible for the inherent visible fluorescence have not been identified.[2] One reason for the slow progress in this respect has been the lack of a systematic process for observing the chemical components in fingermark residue (fluorescent or otherwise).

There have been several studies separating fingerprint residue chromatographically using a variety of systems, but comparison of the results is made difficult due to inconsistency in the sampling, extraction, and analytical methods used (2–7). Invariably, little attention is paid to the sample population, for example, the age, sex, and racial origin of the donors, even though studies by Nazzaro-Porro et al. (2) in 1979 showed variations in skin surface lipids with age and sex of donor. More recently and significantly, Buchanan et al. (3) identified differences in the lipid composition on the fingertips of adults and children.

Although researchers have not been slow to take advantage of the latest analytical techniques, for example, gas chromatography–mass spectrometry (GC-MS) (3), there has been a lack of fundamental research to establish a robust protocol which can routinely be used to analyze "real" fingerprints, that is, fingermarks laid on a surface, rather than washings or swabbings from the skin.

Bramble, in 1995 (4), showed that it was possible to extract material from fingermarks deposited directly onto thin-layer chromatography (TLC) plates. This method of sample collection is preferable to washing or swabbing because it more realistically represents the natural deposition of residue and eliminates the chances of extraneous matter being extracted from the epidermis.

Reported here is a robust TLC system that was devised for the separation of the inherently visible fluorescent components of latent fingerprint residue using Bramble's method of sample deposition. An early attempt was made by Duff and Menzel (5) to identify the fluorophores by separating swabbed fingermark material on a thin-layer chromatography plate. The luminescent characteristics of some of the separated components caused them to suggest that riboflavin (and its derivatives) or other B vitamins may be responsible. Although spectroscopic evidence was provided to support their suggestions, the vitamins were not chromatographed and no further work has been carried out to substantiate this theory.

The system developed and reported in this paper allows the routine separation of three or more fluorescent components directly from fingermarks. Some preliminary investigations have been made into the identity of these components and the results are also reported.

Experimental Procedure

The findings of this paper are as a result of two separate experimental procedures. The first involved the development of a robust

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[3] For the purposes of this study, no distinction has been made between components secreted onto the skin surface and those of external origin.

system for the separation of the fluorescent components in fingermark residue by thin-layer chromatography. The second experiment investigates the nature of the separated compounds through visualization treatments.

Experiment 1—Separation of Fluorescent Components from Fingerprint Residue by Thin-Layer Chromatography (TLC)

Many solvent systems are cited in the literature for separating classes of compounds (8). As the chemical nature of the fluorescent compounds under investigation in this study was unknown, numerous solvent systems were tested to assess resolving power. A chloroform/methanol (4:1) system was found to produce the best separation of the fluorescent components in terms of the number of bands observed and their resolution as seen by fluorescence detection. Consequently, this system was tested with a total of six donors, one of whom was sampled over a period of time to assess the reproducibility of the results.

The separation of fingerprint residue was performed on Merck aluminum-backed high-performance thin-layer chromatography (HPTLC) plates (silica gel 60, pre-coated, non-fluorescent, 20 cm $\times$ 20 cm). The plates were prepared according to the method used by Bramble (4), which involved conditioning the TLC plates to ensure there was no extraneous matter present before starting the analyses. This required the plates to be run in a solvent system until the solvent front reached the top. This conditioning was repeated twice for each plate and was carried out in a TLC tank lined with a polythene bag. A new bag and fresh solvent were used for each wash. The conditioning solvents were methanol for the first wash and chloroform/methanol (4:1) for the second. After conditioning, the plates were allowed to dry before laying down samples.

A sebum-rich fingermark was deposited in a sample box marked approximately 1 cm from the bottom of the plate. Samples were obtained only from male subjects of Caucausian origin (aged 25–35 years) who wiped the fingertip on their nose and forehead before deposition onto the plate. The fingers were not washed or treated in any way prior to this. There was no control over the pressure applied to the TLC plate by the donors other than asking them not to press unduly hard.

The prepared plates were introduced into a sealed TLC tank lined with a polythene bag containing the mobile phase of chloroform/methanol (4:1) and allowed to develop until the solvent had run approximately 15 cm. All plates were developed within 1 h of depositing the samples.

Developed plates were illuminated with an argon ion laser (Spectra Physics, model 2580) tuned to 514 nm and at an irradiance of approximately 250 Wm^{-2}. Fluorescence was observed through 550 nm long-pass goggles (Lase-R Shield Inc, model GU1), the transmission spectrum of which is shown in Fig. 1.

Experiment 2—Investigation into the Nature of the Compounds Fluorescing

This section describes the preliminary investigations into the identity of the fluorescent compounds separated by the thin-layer chromatography system described in Experiment 1.

The experiment is split into three stages; the first two describe the treatment of developed plates to reveal lipid material and primary amino acid groups, respectively. The third stage uses TLC to compare the chromatographic properties of the separated bands with a number of chemicals reported to be present in sweat, sebum, and/or fingermark residue.

Stage 1: Charring Treatment to Reveal Lipid Material

Lipid material was visualized using a two-stage charring procedure. Firstly the plates were dipped into an aqueous solution of

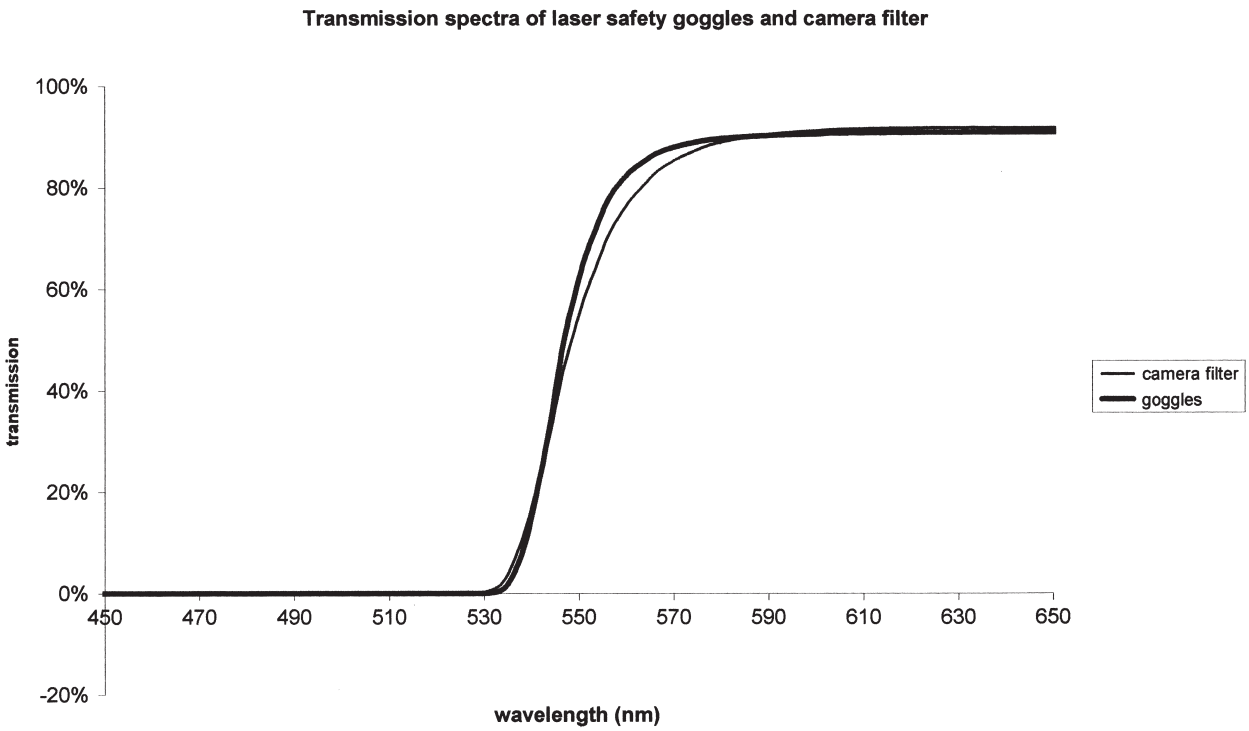

FIG. 1—*Transmission spectra for laser safety goggles and camera filter.*

10% copper sulfate, 8% phosphoric acid, then heated on a hotplate at 200°C for 2–4 min. Lipids appeared pink/brown. For this step, the TLC separation was performed on Merck glass-backed HPTLC plates (silica gel 60, pre-coated, non-fluorescent, 10 cm × 20 cm).

Stage 2: Treatment with Ninhydrin to Reveal Primary Amino Groups

Ninhydrin is used to identify amino acids and primary amines, which react to produce a compound called Ruhemann's purple (9,10). Although it is known that no naturally occurring amino acids fluoresce in the visible region of the spectrum (11) this reagent was used to show if any primary amino groups were present on the fluorophores. The plates were treated with ninhydrin solution (0.5% in CFC 113) and then heated in a humidity cabinet (Vindon Scientific Ltd.) at 75°C, 75% relative humidity for 10 min. Any aliphatic primary amino groups present would result in Ruhemann's purple.

Stage 3: Comparison with Control Chemicals

A series of TLC plates were run using the protocol in Experiment 1 and with control chemicals spotted at equal intervals along an origin level with the center of the fingermark sample.

Control chemicals were chosen from a range of compounds reported to be present on the skin surface or in sweat or sebum (12–18). These were lipids (squalene, cholesterol, oleic acid, palmitic acid, monopalmitin, dipalmitin, tristearin, and palmitic acid palmityl ester), vitamins (vitamin B2—riboflavin, lumiflavine—a decomposition product of riboflavin, vitamin B6—pyridoxine, vitamin E—tocopherol, vitamin K1), porphyrins (haematoporphyrin, protoporphyrin), bilirubin, urobilin, creatine, uric acid, and urocanic acid. All chemicals were 0.1% in methanol or chloroform and were spotted onto the plate using a glass capillary (80 × 0.1 × 0.2 mm, Bilbate Ltd., Daventry, UK).

Reagents

All control chemicals were purchased from Sigma Chemical Company and used without further purification. Chemicals for the visualization stage were general-purpose grade (GPR) and purchased from BDH. Analytical reagent grade solvents were used throughout.

Image Capture

Images of fluorescence were captured using a Kodak DCS 460D digital camera. The exposure was 30 s at f11. The plates were "painted" with the laser illumination to expose the entire area to the irradiation. The camera was fitted with a 550-nm orange long pass filter, the transmission spectrum of which is shown in Fig. 1.

Visible images were captured using an Epsom GT-9000 scanner and Adobe Photoshop (version 4.0.1) software.

Results

Experiment 1

Figures 2 and 3 illustrate the typical separation of the fluorescent components present in a fingermark when it is subjected to TLC using a chloroform/methanol (4:1) solvent system. Three bands are observed which fluoresce orange, green, and yellow/green (in order of decreasing Rf) but some fluorescence always remains in the sample box at the origin. The top orange band (Band 1) sometimes appears to split, with the upper portion being more yellow in color. It is possible that this could be incomplete resolution of two bands. The upper portion, however, does not seem to be a continuous band; it is disjointed and speckled in appearance.

Table 1 and Fig. 4 detail the TLC results achieved with one donor sampled 20 times over a period of eight months (July to

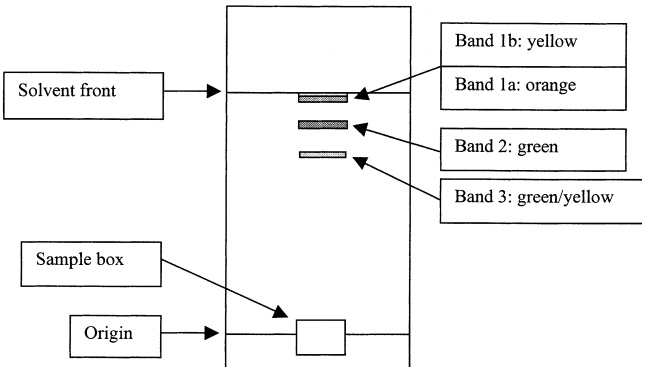

FIG. 2—*Schematic to illustrate the typical TLC separation of visible fluorescent components from fingermark residue when chloroform/methanol (4:1) is used as the mobile phase, and silica gel 60 as the stationary phase. Positioning of bands is approximate.*

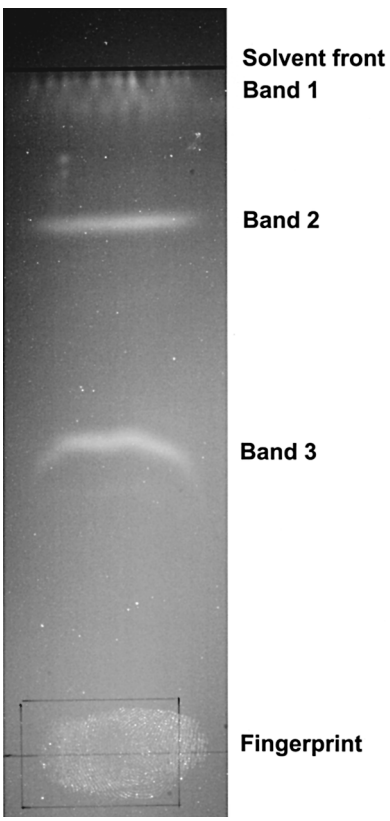

FIG. 3—*Typical TLC separation of fluorescent components from fingermark residue when chloroform/methanol (4:1) is used as the mobile phase, and silica gel 60 as the stationary phase, and an argon ion laser (514 nm) as the source of illumination.*

TABLE 1—*TLC separation of fluorescent components from the fingerprints of one male donor sampled 20 times over a period of eight months (July-March).*

		Experiment No.									
Band*	Color	1	2	3	4	5	6	7	8	9	10
1b	Y	†	†	†	†	99	99	99	99	†	†
1a	O	99	98	94	96	96	97	98	96	98	97
2	G	84	86	80	79	90	88	81	83	87	83
3	Y/G	55	58	72	69	88	68	‡	‡	82	78
		Experiment No. 2									
Band*	Color	11	12	13	14	15	16	17	18	19	20
1b	Y	†	†	†	†	†	†	†	98	98	†
1a	O	98	96	95	98	98	98	99	95	95	99
2	G	90	91	90	90	89	89	93	‡	‡	93
3	Y/G	82	82	80	84	83	84	87	68	70	68

NOTE: Experiments performed on aluminum-backed HPTLC plates (silica gel 60) using a solvent system of chloroform/methanol (4:1). Values given are Rf × 100.

Y = yellow; O = orange; G = green.

* Refer to Fig. 2

† Bands 1a and 1b are indistinguishable.

‡ Band is not observed.

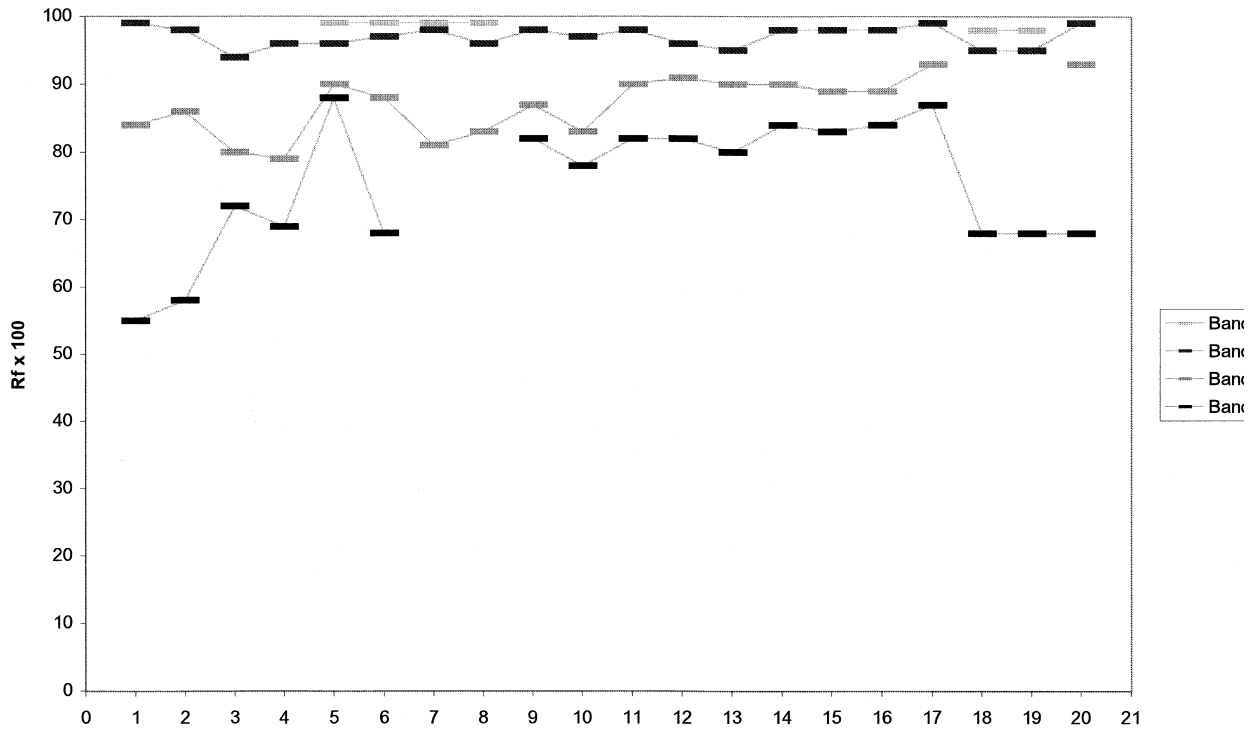

FIG. 4—*Graph of Rf × 100 values achieved from the TLC separation of fingermark residue from one male donor sampled 20 times over a period of eight months (July-March). Chloroform/methanol (4:1) was used as the mobile phase, silica gel 60 as the stationary phase, and an argon ion laser (514 nm) as the source of illumination.*

March). This shows the pattern of separation to be reproducible over a period of time, although occasionally only one of Bands 2 and 3 is observed and the Rf value of Band 3 is variable.

The same three fluorescent bands were also observed with other fingermark donors. A random selection is illustrated in Fig. 5. Occasionally additional bands have been seen, but an extensive survey has not been done to assess whether these bands are donor dependent. In particular, a very weak band has been noticed below Band 3 (see Fig. 5). This was difficult to see by eye, although in some cases it was detected by the camera.

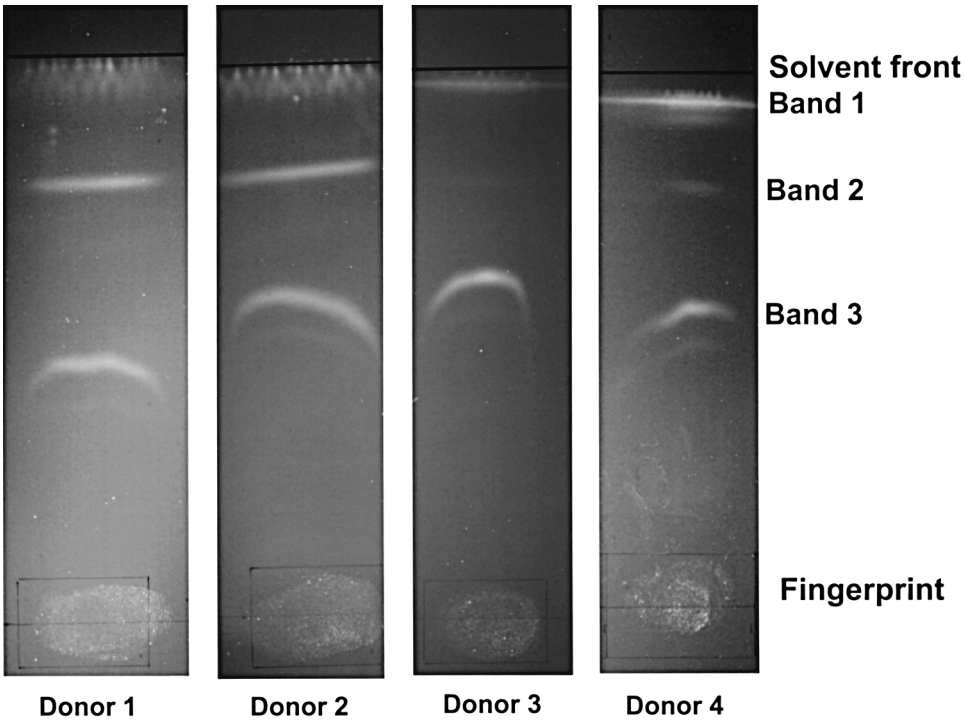

FIG. 5—*TLC separation of the fluorescent components in the fingermark residue of four male, Caucasian donors (aged 25–35 years). Chloroform/methanol (4:1) was used as the mobile phase, silica gel 60 as the stationary phase and an argon ion laser (514 nm) as the source of illumination.*

No measurements of the fluorescence intensities of the separated bands were taken.

Experiment 2

Stage 1: Charring Treatment to Reveal Lipid Material

The charring visualization method revealed that the chloroform/methanol solvent system separated the lipid material into two bands (Bands A and B). The top band (A) was positioned next to the solvent front, overlapping with the orange fluorescent band (Band 1). The size and shape of the charred band, however, was not consistent with Band 1. Band B was slightly below Band 1. This is illustrated in Fig. 6.

Stage 2: Treatment with Ninhydrin to Reveal Amino Acids

Fingerprint material remaining at the origin reacted with ninhydrin to produce a purple color (see Fig. 7). The fluorescent bands did not immediately react, but after 24 h purple coloration was observed in the region of fluorescent Band 1. This was too weak to image successfully.

Stage 3: Comparison with Control Chemicals

Only bilirubin of all the control chemicals was found to resemble the fluorescence characteristics and Rf values of any of the bands separated from fingerprint material. Table 2 shows how the Rf value correlates with Band 1 separated from a fingermark run on the same TLC plate. All experiments involved the same fingermark donor and were carried out over a period of six months (September to March).

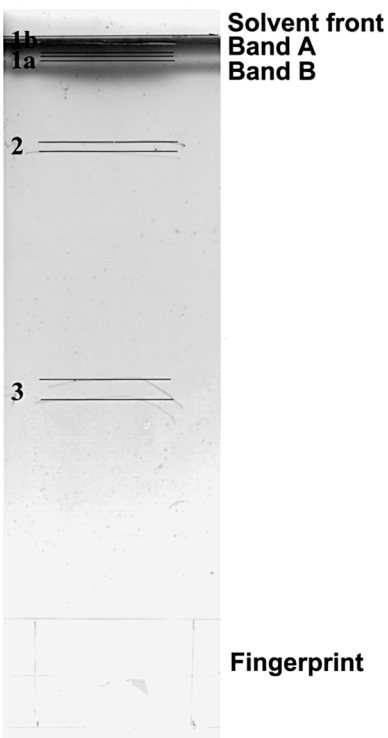

FIG. 6—*Fingermark from a male donor separated on a silica gel HPTLC plate using chloroform/methanol (4:1) as the mobile phase, an argon ion laser (514 nm) for visualization of fluorescent material, and a charring treatment to reveal lipid material.*

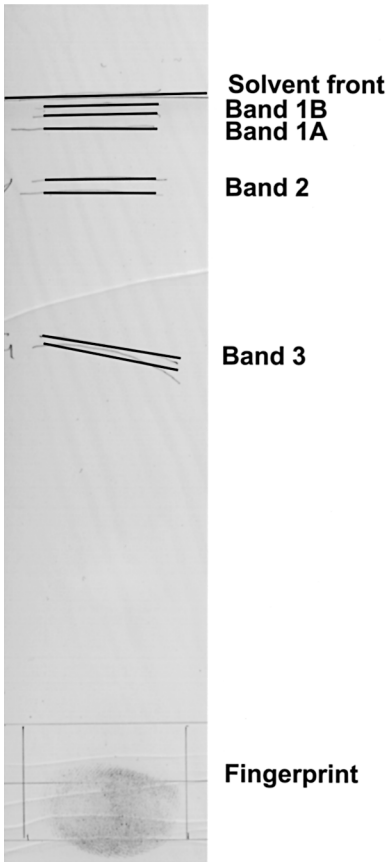

FIG. 7—*Fingermark from a male donor separated on a silica gel HPTLC plate using chloroform/methanol (4:1) as the mobile phase, an argon ion laser (514 nm), and ninhydrin for visualization.*

Discussion

This paper documents a robust, reproducible method for separating three components from fingermark residue, which fluoresce in the visible region of the spectrum. The method involves direct deposition of fingermarks onto TLC plates and elution in a chloroform/methanol (4:1) solvent system. Sample preparation does not involve any washing or swabbing stages that could extract additional components from the skin surface. This provides a more realistic starting point for the analysis of fingermarks. The separated components fluoresce orange, green, and yellow/green (in order of decreasing Rf). This pattern of separation is reproducible for a range of male donors and for one donor over a period of time. Elution of material may, however, be incomplete as some fluorescence remains at the origin and Band 1 sometimes appears to be two bands that have not been fully resolved (Bands 1a and 1b).

An acknowledged difficulty with any TLC method is in obtaining consistent Rf values between runs (19,20). Each step in the experimental procedure can introduce imprecision into the technique. Although errors are minimized through careful and consistent working practices, there are variables in the experiments over which we have limited control. For example, ideally the resolution of bands should be independent of the amount of material applied to the plate, but in practice this is not necessarily the case. The amount of residue on a person's finger is likely to vary on a daily basis, and there is no way of controlling the amount of material deposited onto the TLC plate. The only way to monitor any effect would be to weigh the amount of residue deposited and control the pressure applied to the plate when the print is laid. This was not, however, deemed necessary for this study.

Errors can also arise through the chromatographic process even though a strict protocol was followed for each plate. For example, the temperature and relative humidity at which the experiments were performed could affect the saturation of the TLC chamber and lead to variations in the Rf values.

For maximum reproducibility of Rf values the distance between the origin and the solvent front should be kept constant. This is not entirely possible in practice, but the development was monitored and stopped at approximately the same distance for each plate.

Marking of the solvent front and the bands themselves (under laser illumination) was done as carefully as possible. Distances were measured with a ruler to 0.5 mm. When calculating Rf values, any errors in the marking and measuring of bands may cause variation.

The occasional variations seen in the pattern of separation from fingerprints could be due to numerous factors. For example, there was no experimental control over the diet and ambient conditions, etc. to which a donor was subjected. Without an extensive survey it is not possible to assess whether any variations are donor dependent, the result of some contaminant, or materials of topical origin. The TLC protocol reported in this paper may allow this to be determined in the future.

Overall, the method of depositing samples, as pioneered by Bramble (4), seems to be preferable to the traditional methods of swabbing and washing. Duff and Menzel (5) observed far more fluorescent components than we have been able to achieve. They acknowledged, however, that their method of sample preparation could have extracted extraneous matter from the epidermis.

Duff and Menzel also suggested that riboflavin may be respon-

TABLE 2—*Correlation of Rf × 100 values for fingerprint Bands 1 and 2 with control of bilirubin run on the sample plate.*

		Experiment No.											
	Color	1	2	3	4	5	6	7	8	9	10	11	12
Band 1 b*	Y	99	99	99	99	†	†	†	†	†	†	†	†
Band 1 a*	O	96	97	98	96	98	97	98	96	95	98	99	99
Bilirubin	O	97	‡	99	‡	98	97	97	96	97	98	99	99

NOTE: Experiments performed on aluminum backed HPTLC plates (silica gel 60) using a solvent system of chloroform/methanol (4:1).
Y = yellow; O = orange.
* Refer to Fig. 2.
† Bands 1a and 1b are indistinguishable.
‡ Bilirubin absent from plate.

sible for the inherent fluorescence of fingerprints. Riboflavin is insoluble in ether and acetone, so it is unlikely that they would have isolated it through their washings. In this study riboflavin did not match the bands extracted directly from fingermarks.

Attempts to identify the fluorophores in this paper have led to the conclusion that the separated material is not lipid. None of the naturally occurring amino acids fluoresce in the visible region of the spectrum (11), and as on reaction with ninhydrin no immediate formation of Ruhemann's purple is evident with any of the fluorescent bands, the fluorophores do not appear to contain any primary amino groups. Twenty-four hours after reaction with ninhydrin, however, a faint purple coloration is seen in the region of Band 1. Although formation of Ruhemann's purple is generally taken as a sign of primary amino groups, other functionalities have been found to react with ninhydrin (9,10,21,22). These may produce a purple product or some other interfering compound, possibly at a slower rate. Porphyrin moieties of cytochromes and hemoglobin have been reported as causing interference (9), certain secondary amines, e.g., pyrrolidine produce purple-red pigments (10), and a range of non-nitrogenous compounds (aldehydes, ketones, keto acids, and monosaccharides) also react positively with ninhydrin (21,22).

The separated components do not match a range of pure control chemicals, for example, various vitamins, lipids, porphyrins, and other compounds that are purported to be present in sweat or sebum (12–18). There were, however, similarities between Band 1 and the control of bilirubin. Bilirubin is a pigment of bile and the major end product of the biological breakdown of haem.

No matches with control chemicals were found for the other separated bands, but it must be remembered that the controls were purchased in their pure form and it is unknown how these compare to the "real" components on the skin surface. It is possible that the separated bands consist of a number of compounds of one class, or the fluorophore could be trapped within a non-fluorescent matrix that could be altering the chromatographic properties.

There is also the possibility that the fluorescent material is an oxidation product or decomposition product of one or more of the compounds present in the fingerprint residue. For example, porphyrins are reported to be responsible for the yellow/red autofluorescence (i.e., intrinsic fluorescence) of human skin (18). Porphyrins are photosensitizers and are thought to induce lipid oxidation through a reactive singlet oxygen species. Initially hydroperoxides are produced; these decompose into free radicals which initiate fatty acid autoxidation (23,24).

It is still unknown if the visibly-excited fluorescence is due to some component(s) secreted onto the skin surface or due to material(s) of external origin, but in the future it should be possible to identify the separated compounds through an analytical method such as LC-MS with fluorescence detection. Alternatively, if the components are volatile then it may be possible to use direct probe GC-MS and analyze the compounds directly off the TLC plates.

The robustness of the TLC system as demonstrated by this paper leads to numerous opportunities for studying and analyzing the fluorescence of fingermarks. Apart from the extensive surveys to determine the variations that occur between individuals, it may be possible to observe changes in the fluorescent material over time. This could be achieved by depositing fingermarks onto TLC plates and storing for varying periods prior to development. An experiment of this type was attempted by Menzel (25) but the study was deemed a failure and the results were not formally published even though the worth of such a study was recognized. It is known, however, that some compounds exhibit increased stability to autoxidation on silica gel (26). It would need to be established that the fluorescent components are not affected by this phenomenon before a study of this type is attempted using the current protocol.

The TLC method reported has already been used to successfully investigate the action of an electrical discharge as a fluorescence enhancement technique (27). In the future it may be possible to use it to investigate other existing techniques, and once the fluorophores have been identified it may be possible to develop new fingermark reagents to target these compounds.

Conclusions

This paper reports a robust thin-layer chromatography method that can reproducibly be used to separate at least three fluorescent components from fingermarks deposited directly onto TLC plates.

This separation has been shown to be reproducible for one donor over a period of time, and a number of other donors have also been shown to produce a similar pattern of separation.

Although the fluorescent compounds have not been identified, there is evidence to suggest that one of them may be bilirubin, the biological breakdown product of haem. The thin-layer chromatography separation has been used to investigate a modified electrical discharge method for the fluorescent enhancement of fingerprints. Previously such studies were not possible because of the lack of a robust and systematic method for investigating the fluorescent components in fingermark residue.

Acknowledgments

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