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General forensics

Amino acid sensitive latent fingermark detection formulations based upon improvised carrier solvents for resource-limited environments: A proof-of-concept study

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ABSTRACT

HFE-7100 and petroleum ether (bp 40–60 °C) are routine carrier solvents used in amino acid sensitive fingermark reagents such as 1,2-indanedione/zinc chloride (IND-Zn) and ninhydrin. However, limited resource jurisdictions face major challenges in sourcing laboratory-grade reagents due to budgetary and geographical restrictions. Common hydrocarbon solvents, available from hardware stores and similar outlets, may offer a more readily available and inexpensive alternative for such jurisdictions. This study assessed the range of improvised carrier solvents for their suitability in the IND-Zn formulation and found that eight different hydrocarbon solvents were able to develop fingermarks when substituted into the formulation. The formulation based on shellite solvent outperformed all other improvised formulations tested, providing high-quality fingermarks and similar sensitivity to a petroleum ether (bp 40–60 °C) based formulation across six porous substrates using six donors. A similar approach was applied to ninhydrin, where it was discovered that formulations using kerosene, white spirits and shellite as carrier solvents performed comparably to a formulation based on petroleum ether (bp 40–60 °C). By chemically characterising each solvent using gas chromatography-mass spectrometry, this study also provided a greater understanding of how the chemical composition of a carrier solvent impacts the efficacy of the resulting IND-Zn formulation, emphasising that hydrocarbon solvents should contain short-chain alkanes and minimal aromatic content to give a more volatile formulation. Additionally, the identity of the solvent was found to cause slight differences in the colour and luminescence intensity of ink diffusion on porous substrates. These results indicate that improvised hydrocarbon solvents are suitable alternatives to common carrier solvents with their operational use offering major benefits for limited resource jurisdictions by reducing costs and supply chain risks. A thorough risk assessment must be conducted by each jurisdiction that intends to use this method as health and safety considerations are concerns with these improvised solvents.

Introduction

While forensic science is a global practice, there are regions of the world that experience fundamental challenges to forensic service provision, including supply chain disruptions, limited resources and lack of training [1]. Recognising this challenge, Bouzin et al. [2] proposed the concept of 'frugal forensics', which is defined [2] as "the development of resilient and economical forensic science provision that meets the needs of society without compromising quality and safety". Sustainability serves as the foundation of frugal forensics as the concept was developed in line with the United Nations Sustainable Development Goals (SDGs), a set of goals designed to foster peace and prosperity among people and the environment [3]. Forming a key part of these goals is the development and maintenance of peace and justice within nations, as addressed by 'SDG 16 – Peace, Justice and Strong Institutions' [3]. The SDGs imply that investigatory forensic science is essential for developing contemporary forensic practices that target the vulnerabilities and strengths of a jurisdiction. Such frugal practices may include exploiting chemical analogues of typical forensic reagents or sourcing reagents from more local providers. To aid in assessing the suitability of these practices, Bouzin *et al* [2]. developed an evaluation tool to assess a method's suitability to a particular jurisdiction.

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Timor-Leste is a low-income, fragile nation, considered to be part of the Global South. It should be made clear that "Global South" refers to regions of lower socio-economic status, limited global impact and societal inequity, rather than a geographic location [2]. Major issues facing Timor-Leste include geopolitical conflict, health crises and fiscal instability [4]. Additionally, the nation is extremely vulnerable to crises like COVID-19 and natural disasters [4]. As a result, forensic service provision in Timor-Leste faces major challenges including budgetary restrictions and geographic isolation. The Policia Nacional de Timor-Leste (PNTL) forensic budget is on average around $2000 USD whereas expenditure in the United Kingdom, as an example of a highly developed nation, typically amounts to hundreds of millions of dollars [5]. Furthermore, because Timor-Leste is part of a small, isolated island, it faces difficulties accessing forensic resources and services, as highlighted in a recent study [6]. Frugal practices that can be readily adopted are therefore crucial in nations like Timor-Leste, where country development often requires structural reform that can take between months and years to come into effect.

One of the main problems facing Timor-Leste is the availability of resources. For geographically remote regions, access to forensics consumables and services can be a major concern [1,2]. Although importation is possible, it comes with high freight costs and the risk of supply chain disruptions, exacerbated by global crises like COVID-19. Another option is to outsource forensic services; however, this incurs costs of the service, transport, personnel and potentially the expert witness, making the process uneconomical. For these reasons, various forensic services are provided in-house, in particular fingermark processing. By examining the Timor-Leste situation, it becomes obvious that budgetary and geographic restrictions present difficulties in purchasing expensive chemical reagents for fingermark techniques. Therefore, it is clear there is a need to develop fingermark techniques that are fit-for-purpose, robust and economical for jurisdictions such as Timor-Leste.

Typically used for fingermark development on porous substrates, amino acid sensitive reagents are popular due to their ability to produce highly coloured and/or photoluminescent fingermarks [7]. Ninhydrin remains an effective fingermark method used across many international jurisdictions. A key feature of ninhydrin formulations is the use of a non-polar carrier solvent, such as petroleum ether (bp 40–60 °C), to prevent the dissolution of inks on documents, thus preserving evidence integrity [8]. With time, HFE-7100 became the favoured carrier solvent in modern formulations, including those from the United Kingdom Defence Science and Technology Laboratory (DSTL) [9] and the Australian Federal Police (AFP), due to its solvent properties and non-flammability [10]. The low cost of ninhydrin and lack of additional specialist equipment, such as wavelength specific light sources, make it a suitable reagent for use in resource limited jurisdictions. However, a similar amino acid sensitive reagent exists, 1,2-indanedione-zinc (IND-Zn), which has superior sensitivity and contrast when viewing fingermarks under luminescent conditions [7]. A variety of operational formulations exist for IND-Zn, including the AFP [10], UK Centre for Applied Science and Technology (CAST) [11], and German Bundeskriminalamt (BKA) [12]. The carrier solvents used in the operational formulas (typically HFE-7100) are neither economical nor readily available in the Global South. A particular problem is that the supply of HFE-7100 will be discontinued by 2025 [2,13]. It is also a single-source proprietary product produced by 3 M™ Novec, making it susceptible to supply chain disruptions [13]. Bouzin [14] developed an IND-Zn formulation for the Seychelles (referred to from this point as the SPF formulation), which maximises performance and minimises cost, based on petroleum ether (bp 40–60 °C) as the carrier solvent [15]. Studies found that the use of such laboratory-grade solvents as the carrier solvent has minimal impact on the efficacy of IND-Zn for fingermark visualisation [15–17].

In a frugal context, employing laboratory-grade solvents remains difficult. In addition to supply chain disruptions, the carrier solvent is the major contributor to the cost of IND-Zn and ninhydrin formulations, and so there is reason to investigate alternative carrier solvents for the Global South. Bouzin et al [15]. stated that ideal carrier solvents should be volatile, non-toxic, cost-effective, readily available and have minimal effect on ink diffusion. In particular, the solvent should be available from multiple sources, minimising the effect of supply chain disruptions. For a low-budget laboratory concerned with the availability of resources, improvised hydrocarbon solvents may be a solution. In Timor-Leste, hydrocarbon solvents such as kerosene, a commonly used cooking fuel, as well as lighter fuel are readily available and inexpensive. The use of improvised solvents for fingermark reagents would offer major benefits by potentially reducing costs and supply chain risks.

This study aimed to determine whether a variety of improvised solvents could be used as alternative carrier solvents for amino acid sensitive fingermark reagents for laboratory based processing of paper evidence. Fingermarks treated using ninhydrin and IND-Zn formulations containing improvised solvents were directly compared to those treated with a petroleum ether (bp 40–60 °C) formulation. The overall performance and sensitivity of IND-Zn formulations were thoroughly investigated as well as the applicability of these solvents to determination of latent fingermarks on patterned (printed) paper and cardboard surfaces. Finally, a recommendation score for sustainable application in Global South jurisdictions such as Timor-Leste was determined using the comparative score assessment tool developed by Bouzin et al [2].

Materials and methods

Substrates

The substrates used in this study are representative of the types of substrates commonly encountered in casework by the Timor-Leste Police Development Program (TLPDP). In total, this study used six types of porous substrates that were sourced from Australian providers as outlined in Table 1.

Chemicals

1,2-indanedione (Reddy Chemtech), ethyl acetate (≥99.7 %, UNIVAR-APS), acetic acid (≥99.7 %, Lab-Scan), ethanol (≥99.5 %, UNIVAR-Ajax Finechem), zinc chloride (≥98 %, Sigma Aldrich), phenylalanine (Fluka BioChemika), alanine (BDH Chemicals), leucine (≥98 %, Sigma Aldrich), toluene (>99.8 %, Honeywell-Burdick & Jackson), 1-butanol (99.8 %, Sigma Aldrich), dichloromethane (>99.9 % Honeywell-Burdick & Jackson) and diphenylmethane (99 %, Sigma Aldrich), ninhydrin (>98.0 %, TCI), ethanol (99.9 % HPLC grade, Scharlau). A range of hydrocarbon solvents were also used in this research as outlined in Table 2. All reagents were used as received without further purification.

Fingermark collection and storage

To first benchmark donor properties, natural fingermarks were

Table 1
List of substrates used in this study.

Description	Colour	Weight (gsm), thickness
Winc. Carbon Neutral Copy Paper	White	80
J. Burrows, A4 100 % Recycled White Copy Paper	White	80
J.Burrows, Stick-It Notes 101x152mm Yellow	Yellow (with blue printed lines)	70
COS Book9033 A4 notepaper	White (with blue printed lines)	60
PPS Cardboard Box	Brown	2 mm
Ink printed paper	White (with black printed text)	-
Recycled cardboard packaging	Brown (with black printed text)	-

Table 2
List of carrier solvents used in this study. Solvent cost analysis for one litre of solvent (in AUD), sourced from at least two regular suppliers based in Australia.

Carrier Solvent	Average Cost (in Australian $, September 2023)	Price Source
White spirits	$7.50	Bunnings/Mitre 10
Mineral turpentine	$5.55	Bunnings/Mitre 10
Low odour turpentine	$10.30	Bunnings/Mitre 10
Kerosene	$6.25	Bunnings/Mitre 10
Low odour kerosene	$14.80	Bunnings/Mitre 10
Shellite	$12.40	Bunnings/Mitre 10
Zippo lighter fluid	$71.96	BCF/Cloud 9 Smoke Shop
Enamel thinner	$20.00	Bunnings/Mitre 10
Petroleum ether (bp 40–60 °C)	$106.95	Sigma Aldrich/Fisher Scientific

collected from ten donors (details outlined in Table 3, additional donor D11 added for ninhydrin sub-study) where donors are referred to using an alphanumeric code, for example, D6 is donor 6. This study was approved by the Curtin University Human Research Ethics Committee (Approval Number HRE2023-0090).

For subsequent experiments, natural fingermarks from a subset of six donors were collected, including split marks (a fingermark divided to give two equal halves, where each half is developed with a different technique) and depletion series (sequential impressions from the same finger to give progressively weaker marks) [18]. The number of fingermarks developed for each of the IND-Zn experiments are provided in Table 4.

To address intra-donor variation, strict fingermark collection procedures were followed. To prevent contamination, donors were instructed to not wash their hands for 30 minutes prior to collection and to avoid contact with chemicals, water, or food. For natural fingermarks, donors were asked to rub their hands together to ensure a homogenous sample and touch the substrate for five seconds with constant light-medium pressure. All samples were stored in identical conditions with fingermark samples stored in a dark file cabinet under office conditions (around 25 °C) for one week prior to treatment. The temperature and relative humidity of this environment were previously established as 20 – 23 °C and 28 – 67 %, respectively, using a Digitech QP-6013 data logger [19].

Instrumentation

Fluorescence spectrophotometry

To investigate the fluorescence intensity produced by different IND-Zn formulations, amino acid samples were prepared by depositing 1 µL of a mixed amino acid stock solution (10 mg/mL leucine, alanine and phenylalanine) onto copy paper, spreading the solution into a thin layer and allowing it to dry for 24 hours prior to treatment. The samples were treated with IND-Zn formulations and photographed under the photoluminescent conditions as described in the following sections. The emission spectra of the developed amino acid samples were acquired using the Cary Eclipse Fluorescence Spectrophotometer (Agilent) combined with a fibre optic probe. The probe was positioned over the sample, with a consistent distance maintained between substrate and probe during measurements. The instrument was operated in fluorescence mode using an excitation wavelength of 505 nm, and emission spectra were collected between 530 nm and 640 nm with a 5 nm excitation and emission slit width. A background spectrum of each undeveloped substrate was collected prior to sample analysis. The 'simple reads' mode of analysis was also used to directly measure the intensity of the fluorescence emission at 560 nm. Data acquisition was carried out using the Agilent Cary Eclipse Scan Application version 1.2 software and exported as CSV files for analysis in Excel.

Table 3
Details of the fingermark donors used in this study. Biological sex identified by the individual and cosmetic use refers to the regular use of a wide range of products such as make-up, sunscreen, and moisturisers.

Donor No.	Age	Biological Sex	Cosmetic Use
1	25	Male	No
2	39	Male	Yes
3	23	Male	Yes
4	21	Male	Yes
5	41	Male	No
6	34	Female	Yes
7	21	Female	Yes
8	20	Female	Yes
9	24	Female	Yes
10	21	Female	Yes
11	20	Female	Yes

Gas chromatography – mass spectrometry (GC-MS)

An Agilent (Santa Clara, CA, USA) 7890B gas chromatograph fitted with an Agilent J&W DB-1701 column (30 m x 0.25 mm ID capillary column chemically bonded with 14 % Cyanopropylphenyl / 86 % Dimethyl polysiloxane at 0.25 µm film thickness) coupled to an Agilent 5977B mass spectrometer was used in electron ionisation mode. Two GC-MS instrument methods were used to analyse the two different elution solvents: toluene and dichloromethane (DCM), the parameter of which are detailed below. GC-MS data was processed using Agilent MassHunter Version 10.

Toluene with 0.5 µL/mL 1-butanol internal standard and dichloromethane (DCM) with 0.025 µL/mL diphenylmethane internal standard were used as dilution solvents. To prepare samples, selected carrier solvents from Table 2 were diluted in the DCM and toluene solvents at a concentration of 5 µL/mL.

Each elution solvent was used for the respective syringe washing. On the auto-injection system: wash bottles, waste bottles and syringes solely used for each elution solvent were employed to minimise the possibility of contamination from other solvents. The mass spectrometer (MS) was operated in positive full scan mode with the ion source set to 70 eV and current emission of 34.6 µA.

Toluene solvent GC-MS: The injection port temperature was set to 110 °C using splitless mode, with the inlet set to purge to the split vent at 0.025 minutes after injection. The oven was set initially at 45 °C, held for 2 minutes, then raised to 150℃ at a rate of 20 ℃/minute. The method was retention time locked to 1-butanol at 3.7 minutes and the MS was stopped at 4.1 minutes as the elution of toluene occurs shortly after this time. The MS collected a mass range of *m/z* 25–150 amu.

DCM solvent GC-MS: The injection port temperature was set at 270 °C using splitless mode, with the inlet set to purge to the split vent at 0.5 minutes after injection. The oven was set initially at 45 °C, held for 2 minutes, then raised to 150 °C at a rate of 8 ℃/minute, then 270 °C at a rate of 25 °C/minute, then held for 3 minutes. The method was retention time locked to diphenylmethane at 16.8 minutes. The MS collected a mass range of *m/z* 30–500 amu.

Reagent preparation

IND-Zn

The IND-Zn working solutions were prepared following the formulation outlined by Bouzin [14]. 1,2-indanedione (600 mg) was dissolved in ethyl acetate (45 mL) and acetic acid (10 mL). The mixture was diluted with petroleum ether (bp 40–60 °C, 900 mL) before adding the zinc chloride (10 mL) stock solution (200 mg zinc chloride in 30 mL ethanol). Eight other formulations were prepared, each containing a different carrier solvent as outlined in Table 2. For the purposes of this study, each formulation is referred to according to the carrier solvent, for example, the zippo formulation contains the Zippo lighter fluid.

Ninhydrin

The ninhydrin-petroleum ether formulation was prepared according

Table 4
Summary of fingermark impressions developed and assessed for IND-Zn experiments.

Description of experiment	Donors	Replicates	Depletions	Substrates	Solvents	Total number of fingermarks assessed
Benchmarking of donor pool to assess general donor performance with IND-Zn (petroleum ether)	10	6	0	1	1	60
Relative performance of improvised solvents in IND-Zn formulations	10	2	0	2	8	320
Head-to-head comparison of IND-Zn formulations	6	2	0	4	4	288[a]
Sensitivity assessment of IND-Zn formulations	6	2	10	2	4	288[b]
Performance assessments of IND-Zn formulations on challenging substrates	1	5	0	2	4	40

[a] Fingermark halves
[b] Only the 1st, 5th and 10th depletions were assessed

to the method adapted from the BKA [20]. Ninhydrin (2.484 g) was dissolved in ethanol (20 mL) with a stirrer bar and upon dissolution, petroleum ether (bp 40–60 °C, 480 mL) was added. Alternative solvent solutions were prepared similarly, with ninhydrin (1.050 g, 1.016 g, 1.033 g) dissolved in ethanol (8 mL) before white spirits, shellite and kerosene (192 mL) were added respectively.

Fingermark development

IND-Zn

For each formulation, samples were treated by submersion in the appropriate IND-Zn working solution for a few seconds and allowing the excess solution to drip back before air drying on paper towels. Samples were dried in ambient laboratory conditions on paper towels. Once completely dry, IND-Zn treated samples were processed using an ironing press for 10 seconds at 160 ̊C.

Ninhydrin

Fingermark samples were processed according to Stoilovic et al. [10] For each formulation, samples were treated by submersion in the appropriate ninhydrin solution for a few seconds and allowing the excess solution to drip back before air drying on paper towels. After solvent evaporation, the treated samples were allowed to develop in the dark over 3–4 days at room temperature. A humidity oven was not used as may be the norm in more developed jurisdictions due to lack of access; this reflects the usual operational practice in limited-resource jurisdictions such as Timor-Leste.

Fingermark visualisation

All fingermarks were digitally recorded using a Nikon D300 camera, mounted on a Firenze Mini Repro stand, set on manual exposure mode. The camera was equipped with an AF-S Micro NIKKOR 60 mm lens and a Tamron 62 mm SKYLIGHT 1 A filter. The Nikon camera was connected to a desktop computer running Nikon Camera Control (version 2.8.0).

IND-Zn samples were photographed 24 hours after development under photoluminescent conditions using a Rofin Polilight® PL500 (Rofin Australia Pty. Ltd., Australia) 505 nm light source with a Schott OG 550 nm (long pass) filter while in a dark room. All samples were photographed using ISO 200 with the light source positioned ~30 cm from the sample. To increase visibility, the aperture and shutter speed were modified for each IND-Zn formulation as indicated in the presented Results & Discussion. Photographed samples were stored in paper envelopes in an office cupboard (~25 °C).

Ninhydrin treated samples were illuminated with four Mirabella cool white 4000 K everyday globes and the ambient light of the room. Photographs were taken at a camera height of 35 cm, with a shutter speed of 1/160 seconds and an aperture of f/11 using the Nikon Camera Control Program (version 2.31.0). After grading, the captured images were post-processed (+40 % brightness, −20 % contrast) using Microsoft Word for reporting.

Fingermark grading

Fingermarks developed with IND-Zn were graded using the UK CAST assessment scale [18] which assesses the overall quality of a fingermark by the proportion of clear ridge detail (see Table 5). Fingermarks were further classified as useful fingermarks which would be suitable for comparison (graded a 3 or 4), detectable but not useful fingermarks (graded 1 or 2) or not detectable (graded 0) [15,21]. Ninhydrin fingermarks were not graded.

Statistical analyses

Following the recommended best practices by Hockey et al [22]. non-parametric tests were performed on the CAST graded datasets (ordinal data) which are concerned with the ranking of the grades rather than the grades themselves [22]. To perform statistical analyses, the data was first converted to vectors in Excel which were then input as R codes to compute the statistical analysis using R version 4.3.0 software. All statistical tests, outlined in Table 6, were performed at a 95 % confidence level ($p < 0.05$). Statistical data analysis including fingermark grade frequency tables, R codes and results are provided in the Supplementary Information (See Table S1 and subsequent outputs).

Ink diffusion study

Eight different pens of various types and colours (see Table 7) were used to investigate the effect of carrier solvent on ink diffusion. Using pencil, a grid was created on copy paper, filled with writing samples from each pen. Samples were treated with IND-Zn formulations and photographed as outlined previously. It is important to note that care was taken when removing samples from the working solution so that any excess solution would run horizontally rather than vertically across other inks. Three drying periods were investigated including 24 hrs, 1 week and 1 month.

Frugal forensics assessment

Bouzin *et al.* [2] stated that frugal forensics is based on three core principles; Resilient, Economical and Quality, as well as six attributes; Performance, Accessibility, Availability, Cost, Simplicity and Safety (PAACSS). An evaluation tool was proposed to assess a method's suitability to a particular jurisdiction which involves the assessment of each of the six PAACSS attributes (see Table 8). Evaluations were performed by comparing alternative methods (improvised solvent formulations) with a nominal method (petroleum ether (bp 40–60 °C) formulation), resulting in a recommendation score.

Results and discussion

Preliminary considerations

The aim of this study was to investigate the suitability of improvised

Table 5
CAST grading system for the assessment of developed fingermarks [18]. Photographs of fingermarks developed using SPF IND-Zn from this work.

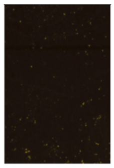
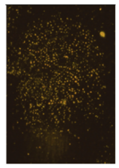
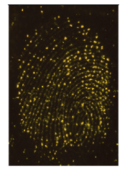
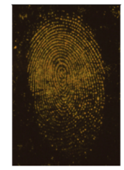
Grade	0	1	2	3	4
Friction ridge development	No evidence of a fingermark	Some evidence of a fingermark	Less than 1/3 clear ridge detail	Between 1/3 and 2/3 clear ridge detail	Over 2/3 clear ridge detail
Classification	Not detectable	Detectable but not suitable for comparison	Detectable but not suitable for comparison	Suitable for comparison	Suitable for comparison
Photographic representation					

Table 6
Summary of the non-parametric tests used in this study.

Statistical Test	Purpose
Mann-Whitney U Test	Assesses the differences between only two treatments.
Kruskal-Wallis	Assesses the differences between two or more treatments.
Chi-squared Test	Assesses whether two or more treatments have a different distribution of frequencies.

Table 7
List of pens used in this study.

Pen type	Brand/model	Colour	Sample No.
Permanent marker	Sharpie Fine Point	Pink	1
		Blue	2
	Staedtler Lumocolour	Black	6
Ballpoint pen	Artline Smoove	Blue	3
	Gel pen	Red	4
Gel pen	BIC Gel-ocity	Red	5
	Felt tip liner	Yellow	7
Felt tip liner	Artline 200	Black	8

Table 8
Comparative score assessment tool [2].

Comparative Score for each PAACSS		Recommendation Score (combined total)	
Score	Description	Total score	
+2	Major benefit of alternative method	≥2	Strongly Recommend
+1	Significant benefit of alternative method	1	Recommend
+0.5	Minor benefit of alternative method	0.5	Maybe recommend
0	No difference between methods	<0	Not recommend
−0.5	Minor benefit of nominal method		
−1	Significant benefit of nominal method		
−2	Major benefit of nominal method		

solvents as alternative carrier solvents for selected amino acid sensitive reagents. The initial goal was to investigate IND-Zn, due the high performance of the SPF formulation developed by Bouzin [14] for a limited resource jurisdiction. A secondary goal was to assess whether improvised solvents could also be used with ninydrin. As such, IND-Zn was first used to assess donor performance within a wider donor pool of 10 subjects (D1 to D10, D11 was an additional donor used solely for the ninhydrin sub-study). According to the International Fingerprint Research Group (IFRG) guidelines [18], this research falls under a Phase 1 study and is recommended to use three to five donors that represent a range in donorship including weak, medium and strong donors (accounting for inter-donor variation) [18]. To ensure this study met these donor requirements, fingermarks from ten donors were developed using the IND-Zn petroleum ether formulation to assess the donor pool. Fig. 1 demonstrates that there is substantial variety within the donor pool. For example, fingermarks from D1 generally show almost completely continuous ridge detail, whilst those from D5 exhibit overdevelopment with substantial ridge diffusion. A subset of six donors (D1, D4, D5, D6, D8 and D10) that covered the range were selected and used for all subsequent Phase 1 experiments.

The relative performance of the eight improvised solvents was assessed by developing fingermarks on copy paper and cardboard. As seen in Fig. 2, all solvents were able to achieve development of fingermarks. All solvents were able to achieve grades of 3 and 4 on copy paper, yet on cardboard, the most frequent grade across all solvents was 0, and only the shellite, enamel thinner and zippo formulations were able to achieve grades greater than 0. Across all solvents, the trend of poorer performance on cardboard compared to copy paper is not unexpected, as studies previously reported the decreased sensitivity of 1,2-indanedione formulations on cardboard substrates [16,17]. This is likely due to the increased porosity of cardboard substrates, causing the fingermark residue to absorb further into the substrate, therefore, reducing the availability of amino acids for reaction with 1,2-indanedione [15,16,23]. Zhao et al [17]. and Bouzin et al [16]. have reported that IND-Zn formulations with stronger luminescence achieve greater contrast on substrates like brown paper and cardboard.

This is consistent with the results of this study, where the shellite formulation achieved the highest grade (a grade 3) on cardboard, owing to the superior luminescence intensity compared to the other improvised solvents (see Fig. 2).

A key difference between the physical properties of the solvents used in this study is their volatility. It was observed that when using solvents with higher boiling points, such as kerosene (145–300 °C) and mineral turpentine (148–200 °C), the fingermark samples retained an oily layer after immersion and had substantially long drying times (~30 mins for paper and several hours for cardboard). Solvents with lower boiling points such as petroleum ether (40–60 °C) and shellite (50–135 °C) were observed to evaporate much quicker when poured into the glass trays and consequently, a greater volume of working solution was consumed to process samples. The increased evaporation rate may affect the composition of the working solution as well as the efficacy of the reagent in operational work due to the greater volumes required. Throughout this study, substantial amounts of background staining (dark brown/green stains) were observed for samples developed with the petroleum ether formulation. This is due to the introduction of contaminating substances and increased water content during solution usage. To minimise this effect, the working solution must be replaced after every three or four samples. It should also be noted that large quantities of vapour were produced when using kerosene, mineral turpentine, and enamel thinner, emphasising that care must be taken to work in well-ventilated areas.

Of the eight solvents tested, three were chosen for further investigation for both ninhydrin and IND-Zn formulations including shellite

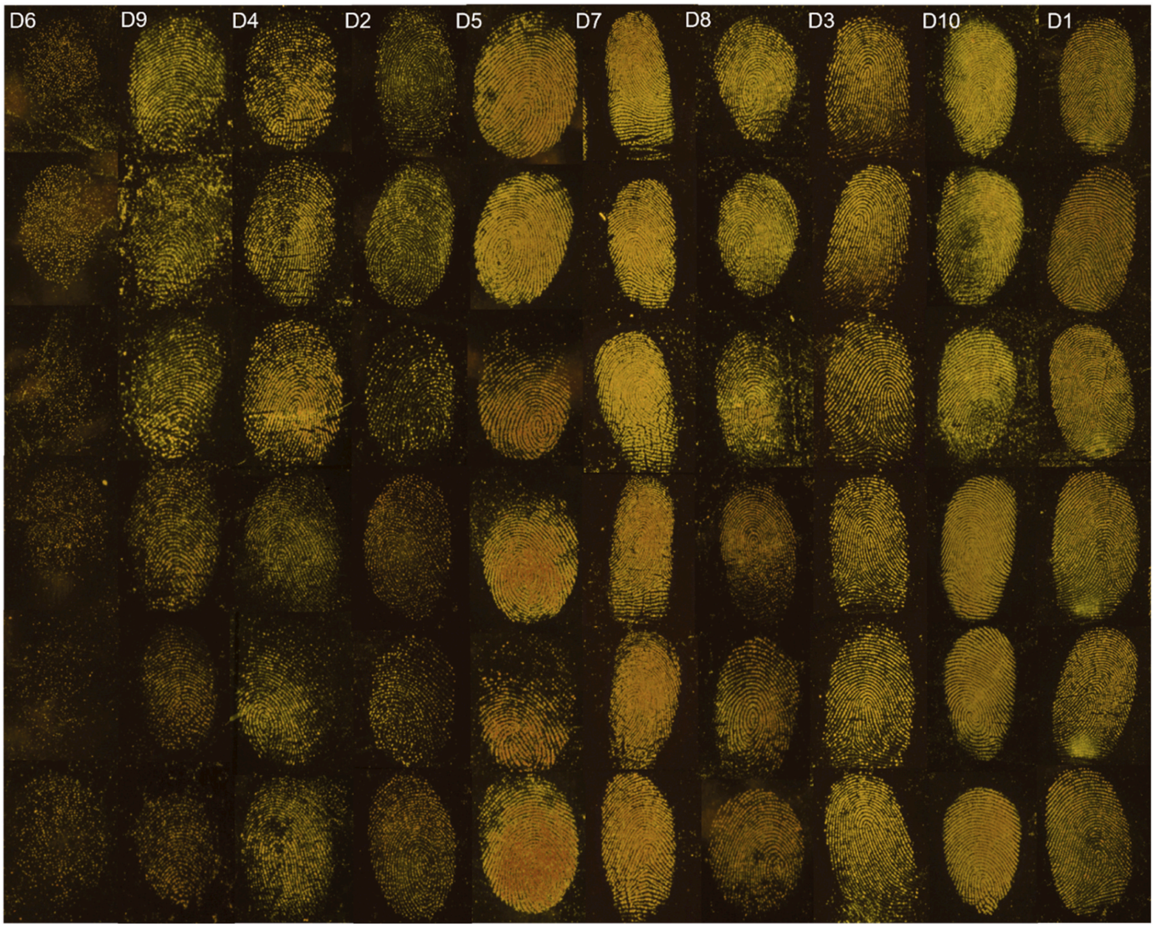


Fig. 1. Six individual natural fingermarks from ten donors developed using the IND-Zn petroleum ether formulation ranging from weak donors (left) to strong donors (right). Imaged using Rofin Polilight® PL500 (Rofin Australia Pty. Ltd., Australia) 505 nm light source with a Schott OG 550 nm (long pass) filter, photographic conditions were ISO 200, ½ sec shutter speed and f/11 aperture.

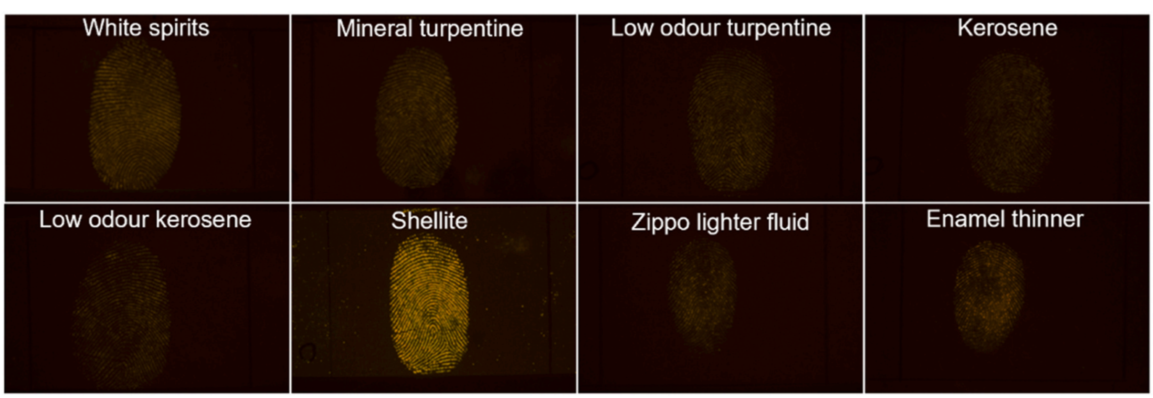


Fig. 2. Natural fingermarks from Donor 1 developed on copy paper using IND-Zn formulations with different improvised solvents. Imaged using Rofin Polilight® PL500 (Rofin Australia Pty. Ltd., Australia) 505 nm light source with a Schott OG 550 nm (long pass) filter, photographic conditions were ISO 200, ½ sec shutter speed and f/11 aperture.

(due to its high initial performance), white spirits (due to its reduced cost) and kerosene (due to its widespread availability in limited resource jurisdictions).

Comparison of improvised solvents with petroleum ether (40–60 °C)

Performance of IND-Zn

The selected improvised solvents were assessed by a head-to-head comparison, using split prints, to directly compare the performance of the improvised solvent formulations against the petroleum ether (40–60 °C) formulation. The comparison was conducted across four substrates with six donors, generating a total of 288 fingermark halves, which were each graded and grouped according to Table 4. Across all substrates, it was observed that the shellite formulation produced considerably higher quality fingermarks than the other improvised solvent formulations with greater luminescence intensity and good contrast (Fig. 3). To ensure no

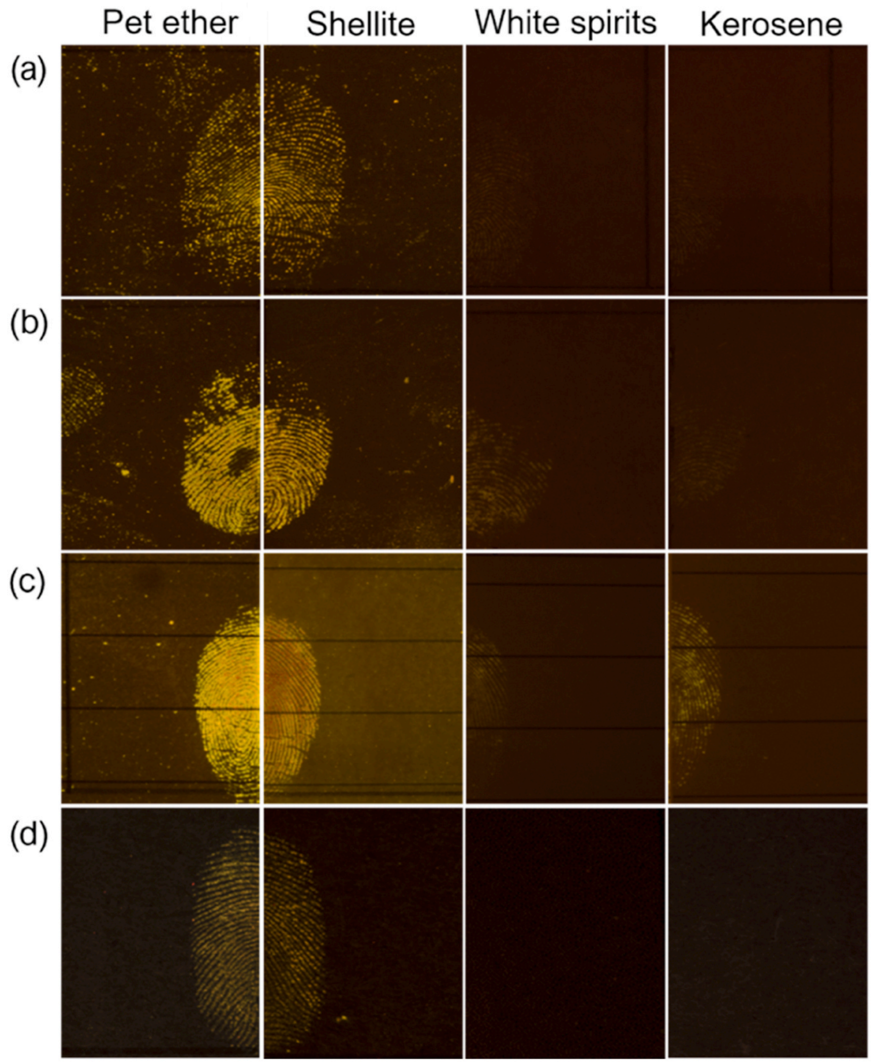


Fig. 3. Fingermark halves from Donor 1 treated with petroleum ether (40–60 °C, pet ether), shellite, white spirits and kerosene formulations on copy paper (a), recycled paper (b), notepaper (c) and cardboard (d), showing the fluorescence and background intensity. Imaged using Rofin Polilight® PL500 (Rofin Australia Pty. Ltd., Australia) 505 nm light source with a Schott OG 550 nm (long pass) filter, photographic conditions were ISO 200, ½ sec shutter speed and f/11 aperture.

bias to one side of the fingermark, a replicate fingermark was taken from each donor whereby the opposing half of the fingermark was treated with the alternative formula than the first fingermark. For example, if the left half was first treated with petroleum ether and right half Shellite, the replicate treated the left half with Shellite and right half with petroleum ether (see supporting information Figures S1 to S4 for images of all assessed fingermarks). This was reflected in the distribution of the grades (Fig. 4) as 54 % of the total marks developed with the shellite formulation were considered suitable for comparison (graded 3 or 4). Conversely, the proportion of such marks for the white spirits and kerosene formulations was only 6 %, and on average, 44 % of the total marks developed with these formulations were not detected.

The aim of a head-to-head comparison is to compare the performance of new techniques to that of an existing technique [18]. However, within a frugal forensic context, it may not be necessary for a new technique to perform as well as the existing technique; rather, the goal is that it can operate at all. In light of this, it was hypothesised that the performance of the improvised solvents could be improved by changing photographic conditions. Previously, samples were photographed under conditions that are best suited to the petroleum ether (40–60 °C) formulation. Aperture and shutter speed, both photographic parameters that control exposure, were adjusted, and the best combination of these parameters for each formulation and substrate were identified (outlined in Table 9).

As shown in Fig. 5, fingermark halves were re-photographed using the improved photographic conditions, and subsequently re-graded as summarised in Fig. 6. By improving photographic conditions, more ridges and greater ridge detail were observed, as well as a general increase in contrast. Overall, it was found that 46 % of identifiable marks (those not originally graded 0) had an increase in grade when using improved photographic conditions. The shellite formulation, again, produced the highest proportion of marks suitable for comparison (56 %), and the proportion of such marks for the kerosene and white spirits formulations improved considerably from 6 % to 21 % and 27 %, respectively.

Mann-Whitney U tests were performed on the fingermark halves to determine whether there were significant differences in the performance of the formulations. For the white spirits and kerosene formulations, a significant difference in performance (p-values of 0.00817 and 0.0104, respectively) was observed between each improvised solvent and the petroleum ether formulation. However, the test showed no statistical difference in the performance between the shellite and petroleum ether formulations, with a p-value of 0.153. Additionally, Chi-squared tests were performed to determine whether two formulations have different distributions of CAST grade frequencies. The test showed conclusively that there was no statistical difference in the distribution of grade

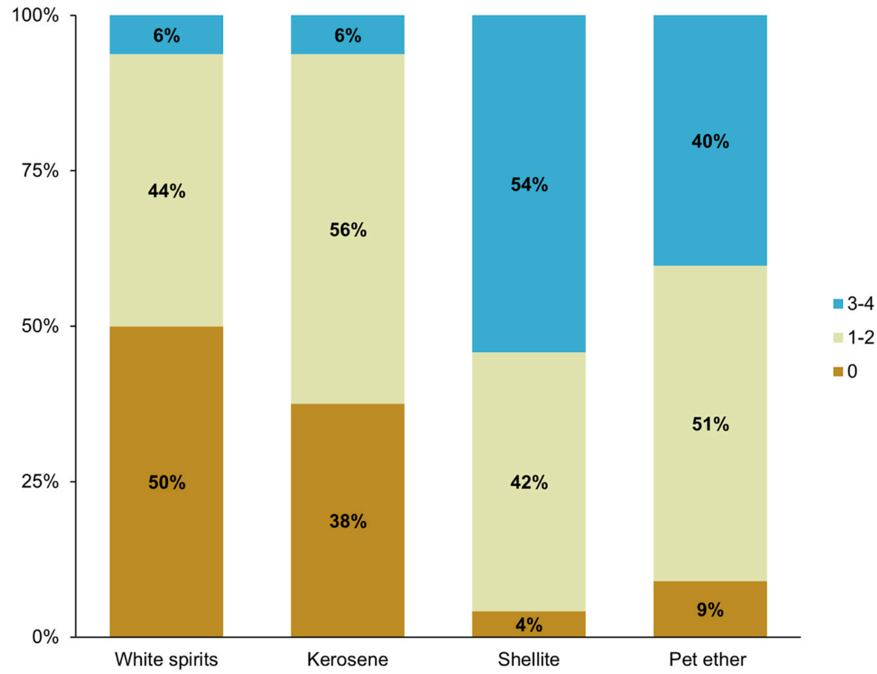


Fig. 4. Summary of results per formulation for all donors and substrates categorised into fingermarks that are undetectable (0), detectable but not suitable for comparison (1–2) and suitable for comparison (3–4). Pet ether = petroleum ether (40–60 °C).

Table 9
Photographic conditions suitable for each IND-Zn formulation and substrate.

Substrate	Parameter	IND-Zn Formulation			
		Petroleum ether	Shellite	White spirits	Kerosene
Copy paper	Shutter speed	1/2 sec	1/2 sec	1/2 sec	1/3 sec
	Aperture	f/11	f/11	f/8	f/8
Recycled paper	Shutter speed	1/2 sec	1/2 sec	1/2 sec	1/3 sec
	Aperture	f/11	f/11	f/8	f/8
Notepaper	Shutter speed	1/2 sec	1/2 sec	1/2 sec	1/3 sec
	Aperture	f/11	f/11	f/10	f/8
Cardboard	Shutter speed	1/2 sec	1/2 sec	1 sec	1 sec
	Aperture	f/11	f/8	f/10	f/10

frequencies between the shellite and petroleum ether formulations with a p-value of 0.641. This indicates that there is no dependency between grades and treatments, supporting the observed similarities in performance of these two formulations.

The Chi-squared test found significant differences in the distribution of CAST grades for the white spirits and kerosene formulations (p-values 0.00184 and 0.00351) when compared to the petroleum ether formulation. Evidently, the improvised solvents, particularly shellite, show promise for their applicability as carrier solvents in the IND-Zn formulation within a frugal context.

Variation in fluorescence intensity for IND-Zn developed marks

Fluorescence spectrophotometry

Fluorescence spectrophotometry was used to make a quantitative assessment of the fluorescence intensity of samples developed using different carrier solvents in the IND-Zn formulation. A mixed amino acid solution was dispersed onto copy paper, allowed to dry overnight and developed using the different IND-Zn formulations.

When excited with 505 nm light, the emission spectra of amino acid samples (Fig. 7) exhibited a broad emission band from approximately 545 nm to 620 nm. The main emission occurs at 560 nm and there is a second, less intense emission at approximately 595 nm, agreeing with literature values [24]. The intensity of these emissions differ for each solvent, corresponding with the observed variation in the brightness of developed fingermarks. A control of “zero concentration” amino acid (water) samples were also developed with the petroleum ether formulation, and the lack of emission peaks for these samples demonstrates the dependence of the IND-Zn technique on the presence of amino acids within the fingermark residue [7].

To further investigate the difference in fluorescence intensity of samples, the fluorescence emission at 560 nm was measured for amino acid samples (n = 10) treated with each carrier solvent. The distribution of intensities is shown in Fig. 8, where the greatest average intensity of samples was produced by the petroleum ether formulation, followed by the shellite, kerosene and white spirits formulations. These results provide a quantitative confirmation of the observed trends whereby samples treated with petroleum ether and shellite formulations exhibit much stronger fluorescence than those treated with white spirits and kerosene.

Sensitivity

The first, fifth and tenth depletions were compared to assess the sensitivity of the different carrier solvent formulations, as summarised in Fig. 9. As expected, the proportion of fingermarks detected, as well as the proportion of useful fingermarks, decreases down the depletion series. The shellite formulation was the most sensitive, with 25 % of the tenth depletion fingermarks graded a 3 or 4. The shellite and petroleum ether formulations were relatively more sensitive, with 92 % of the tenth depletion fingermarks being detected, whereas the proportion of such marks was 67 % for the white spirits and kerosene formulations. A Kruskal-Wallis test was performed to determine whether there were significant differences in the sensitivity of the solvents. For depletion fingermarks (n = 120), a significant difference was found (p-value 0.000214) between the solvents, indicating that the shellite formulation significantly outperformed the other solvents for weak fingermarks. Additionally, a Chi-squared test showed a significant difference in the distribution of CAST grade frequencies (p-value 0.000000499) between the solvents, indicating that the performance of a formulation for weak

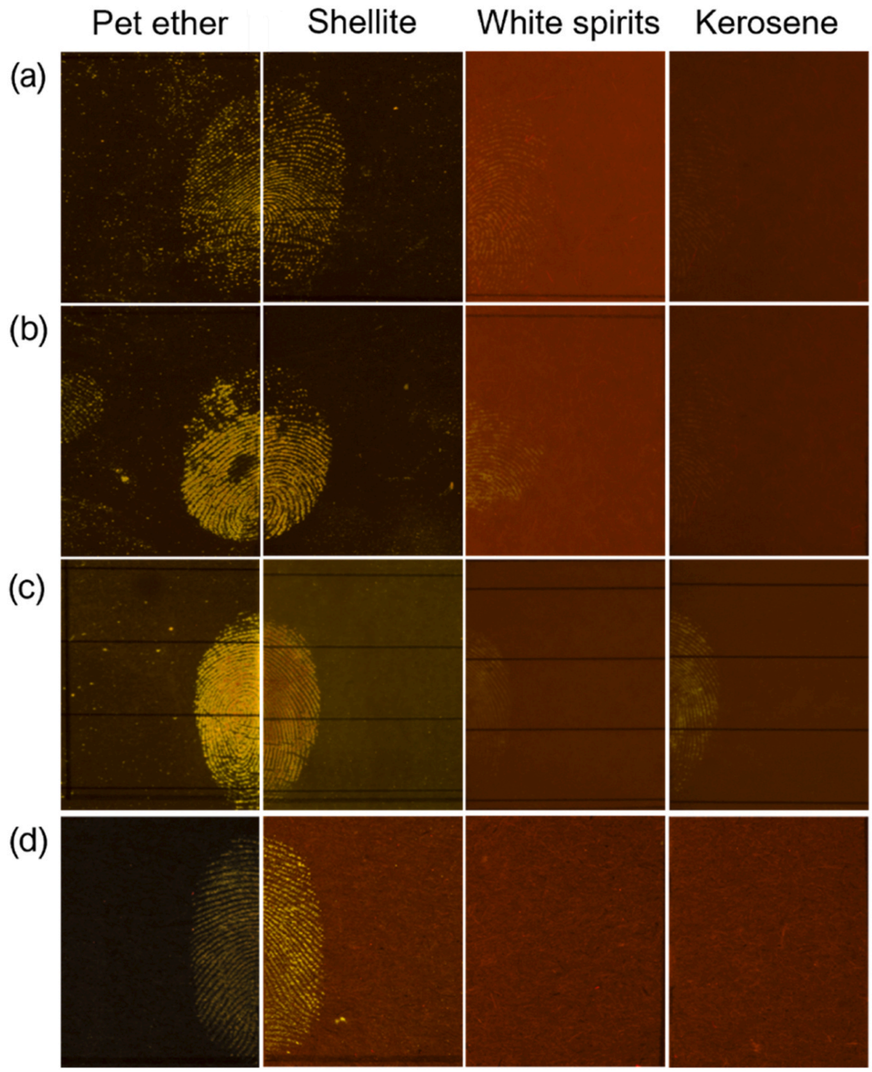


Fig. 5. Fingermarks from Donor 1 treated with petroleum ether (40–60 °C, pet ether), shellite, white spirits and kerosene formulations on copy paper (a), recycled paper (b), notepaper (c) and cardboard (d), showing the fluorescence and background intensity. Imaged using Rofin Polilight® PL500 (Rofin Australia Pty. Ltd., Australia) 505 nm light source with a Schott OG 550 nm (long pass) filter, photographic conditions specific to each solvent and substrate, outlined in Table 9.

fingermarks depends on the carrier solvent.

Perfomance of IND-Zn formulations on challenging substrates

Although operational use of improvised solvents would require significant validation under local conditions (i.e. in Timor-Leste), a preliminary trial was conducted using substrates similar to that seen in casework, in particular, copy paper and recycled cardboard packaging both with printed black ink. These were chosen as coloured and patterned substrates are inherently more challenging for fingermark detection [25]. Fingermarks from D3, considered to be a 'medium' donor, were developed on these substrates to establish the general performance of the formulations. The photographic conditions for both copy paper and cardboard (Table 9) were found to be suitable for the printed paper and recycled cardboard packaging respectively.

Fig. 10 shows fingermarks developed on the printed paper with high-quality fingermarks obtained using the shellite and petroleum ether (40–60 °C) formulations, whilst the white spirits and kerosene formulations provided only some detectable marks. Fig. 11 shows fingermarks developed on the recycled cardboard packaging following the same trend with a much poorer performance by the white spirits and kerosene formulations. The petroleum ether and shellite formulations, known to have greater luminescence intensity, have considerable background staining on the cardboard substrate, preventing clear visualisation of fingermarks. It must be mentioned that some of the background development may be due to handling prior to use in this study. Due to the lack of high-quality fingermarks achieved on the cardboard packaging substrate, only the printed paper was further investigated in this limited study.

Fingermarks from four donors (D 3, 4, 7 and 10) were developed on the printed paper, generating a total of 160 fingermarks. The results, summarised in Fig. 12, demonstrate the successful performance of the improvised solvents on a patterned substrate, with all solvents able to achieve a grade of 3 or 4 for at least 50 % of the developed samples. Once again, the shellite formulation outperformed the other improvised solvents with the highest proportion (76 %) of fingermarks classified as useful, followed by kerosene (63 %) and white spirits (50 %). An unexpected result was that the petroleum ether formulation performed slightly worse than the shellite and kerosene formulations. This is thought to be due to overdevelopment frequently observed for samples developed with this formulation, resulting in low CAST grades.

These results indicate that IND-Zn formulations with improvised solvents may perform highly on patterned paper substrates, suggesting they may be successfully applied to casework.

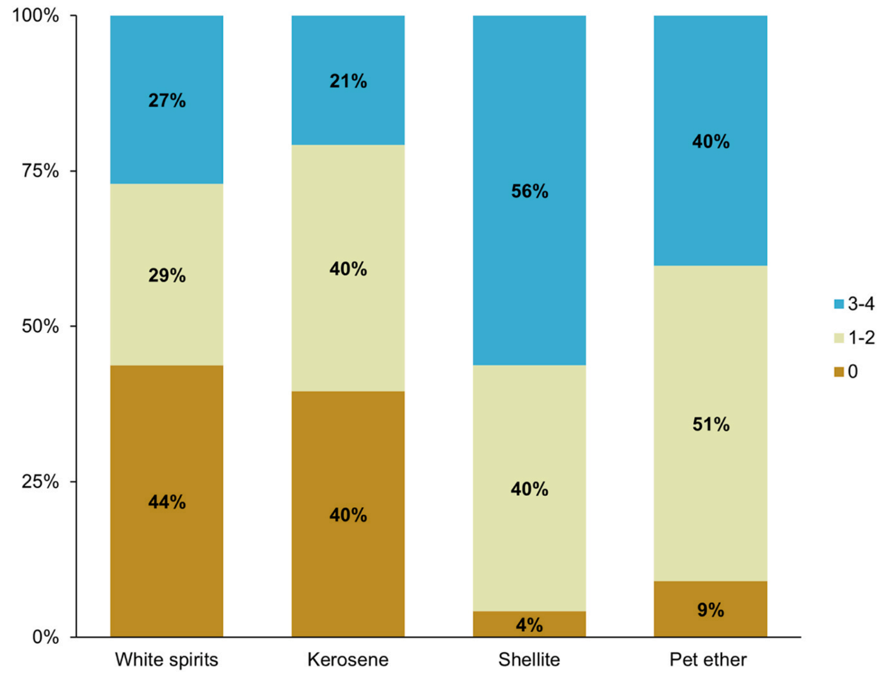


Fig. 6. Summary of results per formulation for all donors and substrates re-photographed and categorised into fingermarks that are undetectable (0), detectable but not suitable for comparison (1–2) and suitable for comparison (3–4). Pet ether = petroleum ether (40–60 °C).

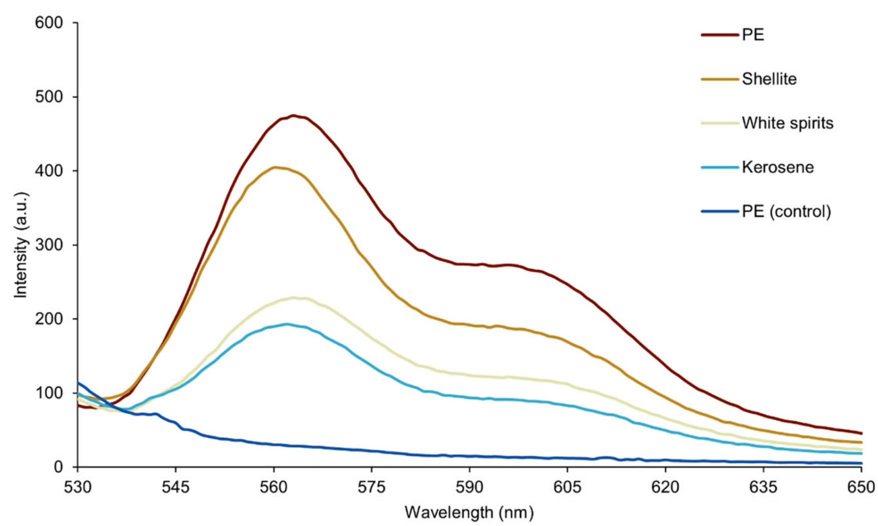


Fig. 7. Emission spectra of amino acid spot samples treated with petroleum ether (40–60 °C) PE), shellite, white spirits and kerosene formulations (excitation wavelength 505 nm). PE (control) refers to a water sample with no amino acids present.

Chemical composition of improvised solvents

This study demonstrated that the shellite formulation unambiguously outperforms the other improvised solvents, producing brighter, better-quality fingermarks when used in IND-Zn formulations. Naturally, the question to be asked is why do the improvised solvents perform differently? The variation in performance between the solvents is attributed to their differing chemical composition. These solvents are petroleum distillates, meaning they are hydrocarbon solvents obtained from crude oil with their composition reflecting the fraction they were derived from [26]. Petroleum distillates are predominantly aliphatic (alkanes, branched alkanes and cycloalkanes) with a distinct aromatic component dependent on the boiling point range. Based on international standards, the solvents were characterised using GC-MS (see Supplementary Information Figures S5-S8) and classified based on carbon (n-alkane) range and aromatic (C1-C4) range as outlined in Table 10 [26]. A summary of the chemical composition of each solvent is found in Table 11.

It is worth noting that despite the use of various chemicals as carrier solvents over time, the literature fails to provide information regarding the effect of the chemical composition of a solvent on the efficacy of IND-Zn formulations [27].

Regarding the performance of the improvised solvents, the obvious trend is that as the length of the hydrocarbon chain and thus boiling point increases, the performance of the solvent decreases. An ideal carrier solvent should be volatile as its quick evaporation depresses diffusion of writing ink and ridge detail [28]. Of the improvised solvents, shellite contains the shortest n-alkanes (C4-C7) and has the lowest BP (50–135 °C), making it the most volatile. A second trend observed was that as the aromatic content of the solvents increases, the performance of

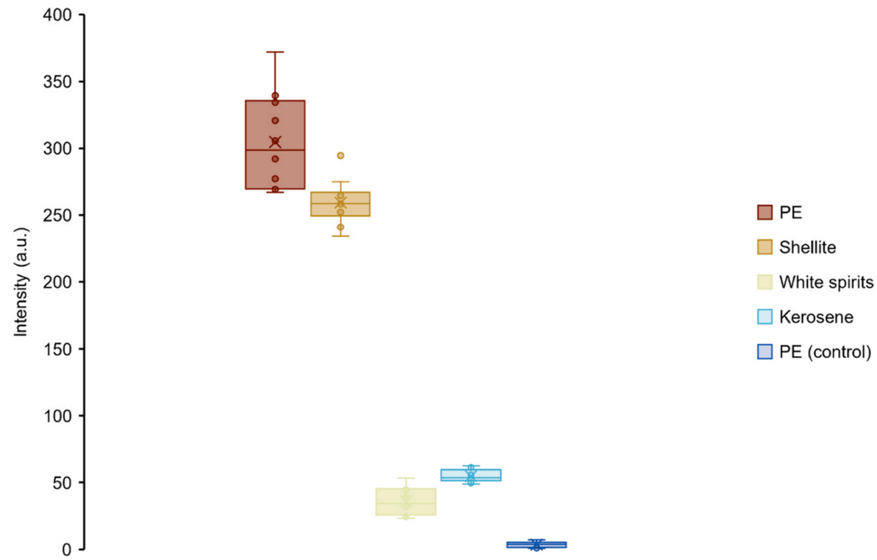


Fig. 8. Comparison of the distribution of fluorescence emission intensity at 560 nm from amino acid spot samples treated with the petroleum ether (PE), shellite, white spirits and kerosene formulations (excitation wavelength 505 nm). PE (control) refers to a water sample with no amino acids present.

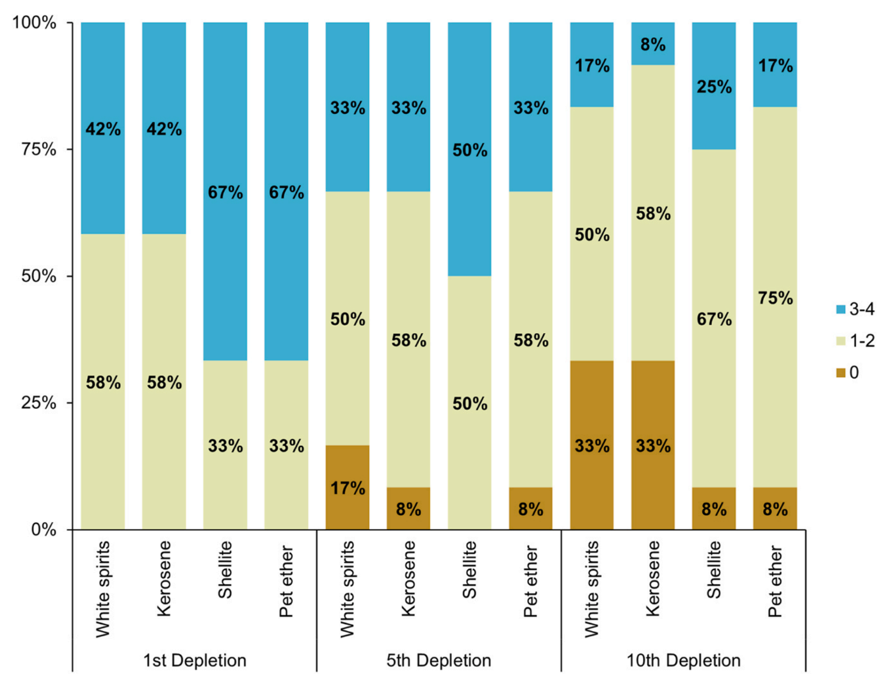


Fig. 9. Percentage grade distribution across first, fifth and tenth depletions of fingermarks treated with the white spirits, kerosene, shellite and petroleum ether formulations on copy paper and notepaper. Pet ether = petroleum ether (40–60 °C).

the formulation decreases. This may be due to interactions with latent fingermark constituents, but is more likely a result of involvement of the solvent in the reaction between the amino acids and the 1,2-indanedione that impacts on the production of the luminescent product. The longer chain alkanes and presence of aromatics in kerosene and white spirits also explain the oily residue on developed samples as well as the extended drying time. It was concluded that the ideal carrier solvent should be volatile and contain short-chain hydrocarbons with minimal aromatic components.

Ink diffusion study

As the carrier solvent plays an important role in minimising ink diffusion [16,28], following the approach taken by Bouzin et al [16]. a series of comparisons were performed to evaluate the effect of substituting improvised solvents in the IND-Zn formulation on ink diffusion. Ink formulations are typically a mixture of a colourant (a dye or pigment) and resins carried in a glycol-based solvent or water [29]. Exposure of inks to highly polar solvents may cause the dyes and resin components to run and diffuse [29]. To investigate this, a range of eight pens including gel pens, ballpoint pens, and permanent markers (see Table 7) were sampled on copy paper, treated using the different formulations and viewed under white light and photoluminescent conditions to assess ink diffusion. This was repeated for three time periods including 24 hours, 1 week and 1 month. Visible and/or luminescent ink diffusion was observed for a number of inks both immediately after immersion in the working solution as well as following heat treatment (see Fig. 13). The occurrence and extent of ink diffusion is due to the

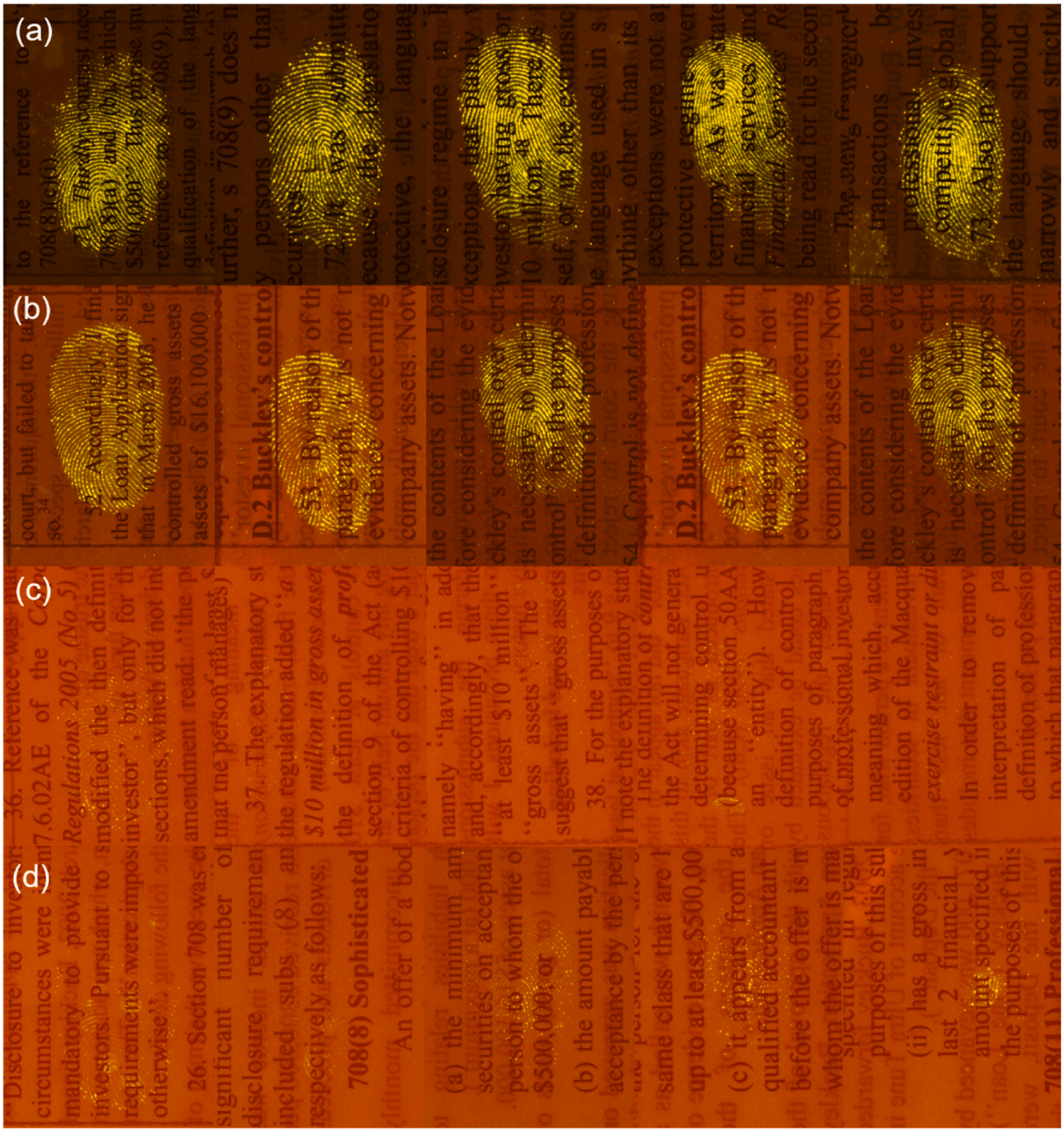


Fig. 10. Fingermarks from D3 developed on printed paper using the (a) petroleum ether (40–60 °C), (b) shellite, (c) kerosene and (d) white spirits formulations. Imaged using Rofin Polilight® PL500 (Rofin Australia Pty. Ltd., Australia) 505 nm light source with a Schott OG 550 nm (long pass) filter, photographic conditions were ISO 200, ½ sec shutter speed and f/11 aperture.

different chemical composition of the inks [16,29].

Throughout these comparisons, several trends were noted regarding the ink diffusion of pens. The pink permanent marker (P1) and red gel pen (P5) appeared to be particularly affected as they presented visual and slightly greater luminescence diffusion across all solvents. Bouzin et al [16]. observed the same trend in ink diffusion when comparing Solstice PF to HFE-7100 as carrier solvents for IND-Zn formulations. The diffusion of the pink/red pens is thought to be due to the high solubility of red colourants, particularly, the dye eosin, commonly used in red writing inks [16,30]. Additionally, two of the permanent markers (P1 and P6) exhibited greater diffusion than other pen types, and the extent of this diffusion was substantially greater for the white spirits and kerosene formulations. Xylene, a common solvent in permanent markers, is soluble in aromatic hydrocarbons, thus, the aromatic fraction of kerosene and white spirits is thought to be responsible for the greater extent of diffusion [29]. The felt tip liner (P8), a water-based ink, showed greater luminescence diffusion for shellite and petroleum ether formulations, however, the cause of this is currently unknown. Marginal differences were observed between the different time periods, including slight differences in the colour and brightness of luminescence diffusion over time, agreeing with literature observations [16]. Generally, greater diffusion was observed for white spirits and kerosene formulations, which are less volatile, causing the accumulation of excess solvent on the sample and, consequently, diffusion.

As the same proportion of polar co-solvents were used in each formulation, the carrier solvent was shown to impact ink diffusion, as observed in previous studies [16]. However, the effect of the carrier solvent may be more complicated, depending on the exact formulation used and possible interactions with the co-solvents. Nevertheless, the differences observed indicate that the chemistry of the carrier solvent may substantially impact evidence preservation, and thus their use must be carefully considered for casework.

Fig. 11. Fingermarks from D3 developed on recycled cardboard packaging using the (a) petroleum ether (40–60 °C), (b) shellite, (c) kerosene and (d) white spirits formulations. Imaged using Rofin Polilight® PL500 (Rofin Australia Pty. Ltd., Australia) 505 nm light source with a Schott OG 550 nm (long pass) filter, photographic conditions were ISO 200, ½ sec shutter speed and f/11 aperture.

Preliminary study of performance of ninhydrin with improvised solvents

As ninhydrin is still widely used, a small pilot study of investigating the performance of ninhydrin formulations based on improvised solvents for the detection of latent fingermarks was carried out. A simple ninhydrin-ethanol-petroleum ether formulation was selected [20] and adaptions were made to bring the high concentration of ninhydrin (15 g/L) more in line with general industry levels (5 g/L) to reduce costs [24,31].

The relative performance of the three improvised solvents was assessed in a head-to-head comparison by developing fingermarks from six donors (D3, D4, D6, D7, D10 and D11) on copy paper and lined notepaper. The fingermarks seen in Fig. 14 show limited differences between the solvents when substituted in the ninhydrin formulation, with high quality fingermarks obtained from D10, including good contrast and almost complete ridge development for all solvents. In comparison to the petroleum ether (40–60 °C) formulation, all improvised solvent formulations produced similar levels of ridge detail, however, a slight decrease in colour intensity was observed for kerosene and white spirits. The minor differences observed regarding the shade and intensity of Ruhemann's purple developed, however, this did not effect detectability of the developed marks. The performance of the ninhydrin formulations was compared across substrates as seen in Fig. 15.

Visual analysis of the fingermarks indicated that the blue hue of the note paper resulted in a slightly reduced contrast for some fingermarks. As a result, very fine/light impressions were somewhat difficult to distinguish on the lined paper, however, this effect was relatively minor.

Comparisons were also made on more complex printed substrates, assessing performance using split fingermarks from a single donor. This assessment found that all solvents were capable of producing fingermarks equivalent to grades of 3 and 4 at the 1st depletion (Fig. 16). The success of these improvised solvent formulations on a patterned substrate, typically classified as 'challenging' indicates that they may be

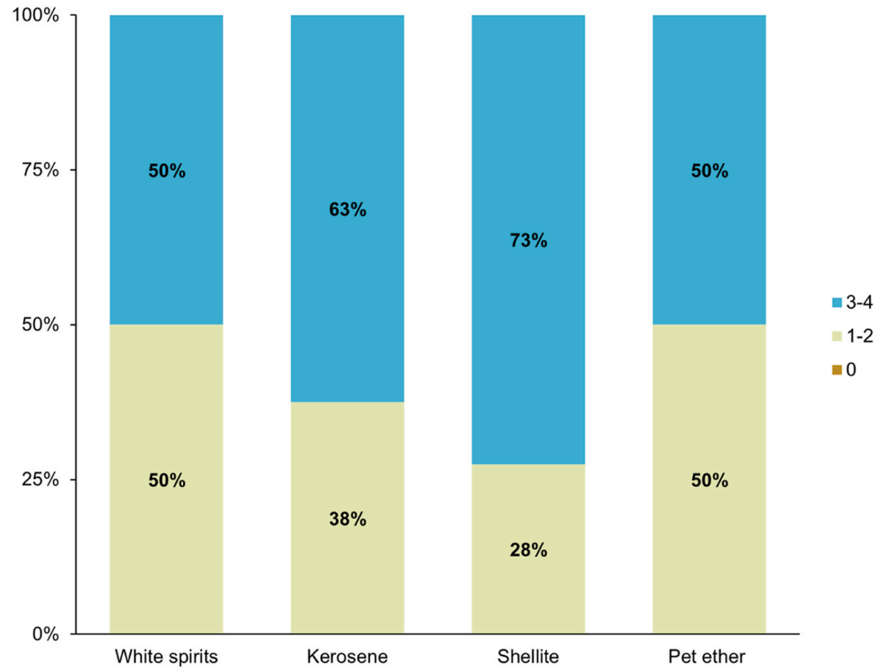


Fig. 12. Summary of results per formulation for all donors on printed copy paper, categorised into fingermarks that are undetectable (0), detectable but not suitable for comparison (1–2) and suitable for comparison (3–4). Pet ether = petroleum ether (40–60 °C).

Table 10
C1-C4 classifications of simple aromatics [26].

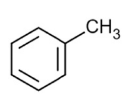
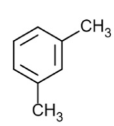
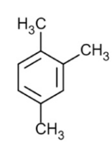
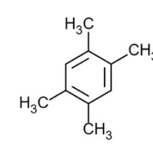
	C1	C2	C3	C4
Description	Monosubstituted benzene	Disubstituted benzene	Trisubstituted benzene	Tetrasubstituted benzene
Example	Toluene	Xylene	1,2,3-trimethyl benzene	1,2,4,5-tetramethyl benzene
				

Table 11
Summary of the chemical composition and physical properties of the carrier solvents in this study.

	Petroleum ether	Shellite	White spirits	Kerosene
BP*	40 – 60 °C	50 – 135 °C	149 – 194 °C	145 – 300 °C
***n*-alkanes**	C4-C6	C4-C7	C8-C12	C8-C15
Aromatics			C1-C4 <30 % aromatic*	C1-C4 ≤40 % aromatic*

* As reported in the manufacturer SDS.

useful for casework type substrates.

Ninhydrin has particular advantages over IND-Zn in a frugal context including its reduced cost and lack of need for specialised equipment. Whilst this work demonstrates that improvised solvents are able to achieve high-quality fingermarks for ninhydrin formulations, from here forward the focus of this study is on IND-Zn. Further studies on ninhydrin are required using a larger donor pool and wider range of substrates.

Frugal forensics assessment

The assessment tool developed by Bouzin et al. [2] based on Performance, Accessibility, Availability, Cost, Safety and Simplicity (PAACSS) was used to compare the improvised solvent formulations against the petroleum ether (40–60 °C) formulation, to determine whether the improvised solvents could be considered a sustainable alternative within a specific context (see Table 12). This study considered an international context of limited resource jurisdictions, using Australian costings. The safety considerations were judged with the assumption of effective controls (i.e. access to a fumehood/fume cupboard).

As described above, the shellite formulation slightly outperformed the petroleum ether formulation (awarded a +0.5 score) whilst the white spirits and kerosene formulations generally performed worse (awarded a −1 score). In relation to availability, all the improvised solvents can be locally sourced both in rural and metro regions in Australia and New Zealand, as well as remote locations such as Timor-Leste. The local supply reduces the effects of supply chain disruption in comparison to petroleum ether which must be sourced from a chemical supply company. The three alternative formulations were thus given a score of +2. All three improvised solvents offer a major benefit being significantly less expensive than petroleum ether (see Table 2), and the opportunity for their purchase in bulk may further reduce costs, leading to a score of +2 for cost.

Fig. 13. Comparison of the effect of carrier solvent on visible and luminescent ink diffusion on samples dried for 1 month. Samples photographed under white light and luminescent conditions. Luminscent conditions imaged using Rofin Polilight® PL500 (Rofin Australia Pty. Ltd., Australia) 505 nm light source with a Schott OG 550 nm (long pass) filter, photographic conditions were ISO 200, ½ sec shutter speed and f/11 aperture. Pet ether = petroleum ether (40–60 °C).

The major safety concern for petroleum ether is its extreme flammability compared to the improvised solvents (see Supplementary Information Table S2), however, use of a fumehood substantially minimises this risk. White spirits, shellite and kerosene have greater associated health risks, particularly due to the repeated exposure anticipated with the operational use of these solvents. In particular, unlike petroleum ether, shellite and kerosene are carcinogens, and may cause birth defects, with shellite being suspected of damaging fertility. As the primary route of exposure is inhalation, the use of fumehoods would be effective in greatly minimising these risks, therefore, kerosene and shellite formulations were given a score of −1, while the white spirit formulation was given a score of −0.5.

For the simplicity score, the key factor to consider is volatility. Due to the similar volatility of shellite and petroleum ether, their use in the IND-Zn formulation was analogous (shellite awarded a score of 0). Due to their lower volatility, the use of kerosene and white spirits was slightly less efficient than that of petroleum ether as samples had a greater drying time, however, this is not expected to majorly hinder workflow in an operational setting. Therefore, white spirits and kerosene were given a score of −0.5.

Based on the assessment tool, a total recommendation score of >1 was achieved for each improvised solvent, with the shellite formulation achieving the highest score of +3.5 (see Table 12). This result indicates that the use of improvised solvents offers a more sustainable formulation for limited resource jurisdictions. It is worth noting that this assessment tool is subjective, and results may depend on the assessor and their location. As such, it is essential that this evaluation is performed in the jurisdiction of the method's intended use, along with validation of the method under local conditions as required by the IFRG [18]. Additionally, this evaluation is intended for use as part of a larger framework where the attributes associated with negative scores can be identified and targeted, working towards a more sustainable approach.

Conclusions

A IFRG Phase 1 style study was carried out to investigate the use of improvised hydrocarbon solvents as sustainable alternatives for IND-Zn carrier solvents in limited resource jurisdictions such as Timor-Leste. It was found that shellite outperformed the other improvised solvents on multiple substrates and on weak fingermarks, however, all improvised solvents were able to provide useful fingermarks (grades 3 or 4). This indicates that, in terms of chemical composition, the ideal carrier solvent should be composed of short-chain alkanes with minimal aromatic components, affording a highly volatile formula. Additionally, longer chain n-alkanes and C1-C4 aromatics were found to increase visible and luminescent ink diffusion, which may be detrimental to evidence preservation in casework. The development of high-quality fingermarks on patterned copy paper suggests that improvised solvents may be applicable to evidence types typically encountered casework, however, a greater range of challenging substrates should be investigated. Lastly, the PAACSS evaluation tool was used to evaluate the applicability of the improvised solvent formulations for limited resource jurisdictions in an international context. All improvised solvent formulations achieved a total recommendation score >1, meaning they are all recommended as a sustainable alternative compared to the petroleum ether formulation.

The three selected solvents were also applied to a simple ninhydrin formulation to assess the suitability of such solvents to other amino acid sensitive reagents. Visual assessment of the fingermarks developed with the different carrier solvents revealed that kerosene, shellite and white spirits performed comparably to petroleum ether over three porous substrates that range in complexity. Minor variations in colour and intensity were observed between formulations although this did not significantly affect ridge detail. Further work should investigate the applicability of these solvents for ninhydrin formulations using objective assessment methods such as grading scales and statistics, as well as using

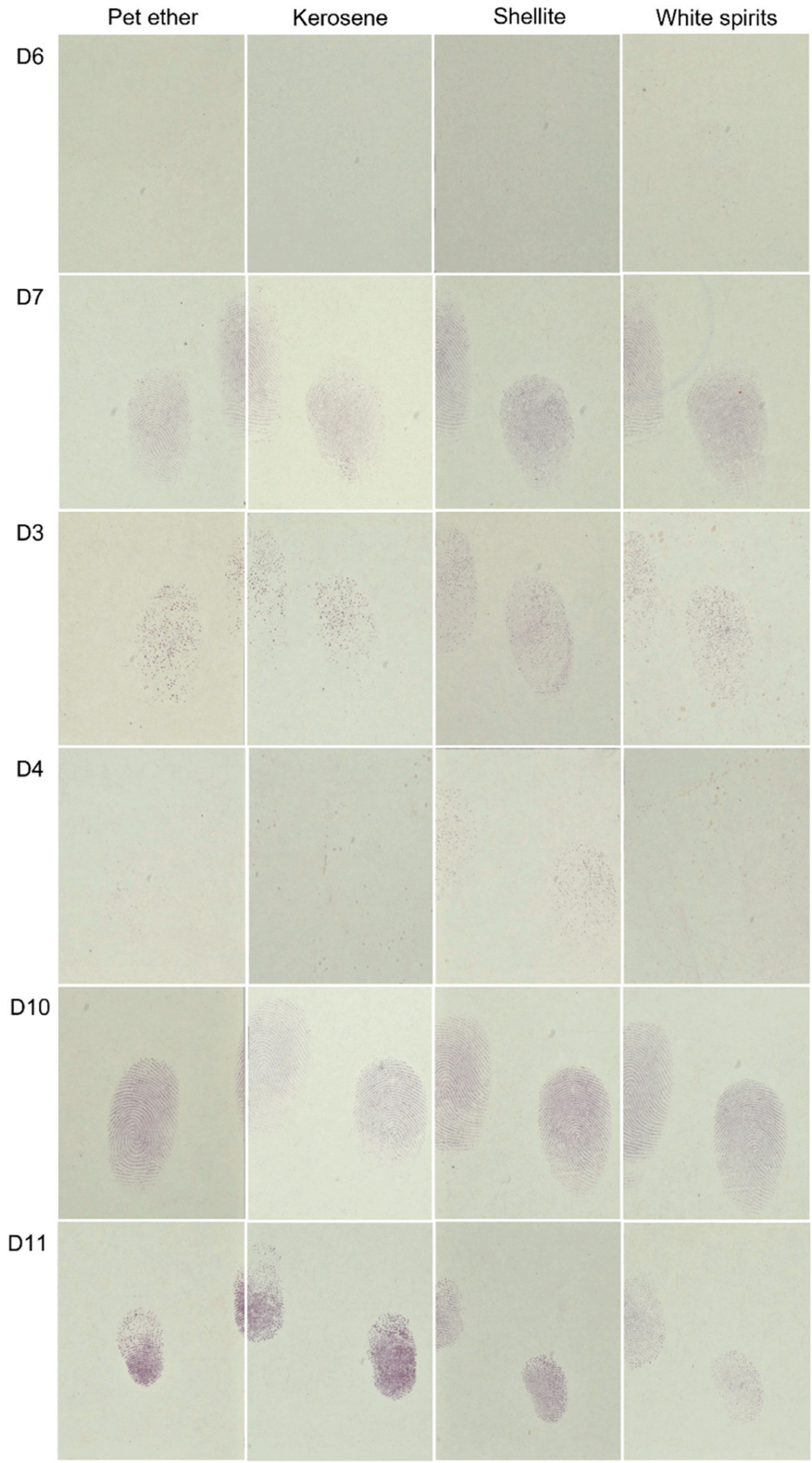


Fig. 14. Split fingermark comparison of ninhydrin formulations containing petroleum ether, kerosene, shellite and white spirits on copy paper for six donors. Pet ether = petroleum ether (40–60 °C).

a larger number of donors and substrates.

Future work should progress this modified IND-Zn technique to a Phase 2 study, optimising the reagent formula and testing this technique in sequence with other routine techniques. It should be noted however that subsequent treatments, such as physical developer or oil red O, are not usually applied in Timor-Leste and similar jurisdictions due to the added cost of additional treatments. There may also be other improvised solvents that could be investigated, for example in Timor-Leste alongside locally sourced kerosene and Zippo lighter fluid, automotive petrol fuel has been tested as a carrier solvent for IND-Zn (Fig. 17).

A thorough risk assessment must be conducted by each jurisdiction that intends to use this method as health and safety considerations are

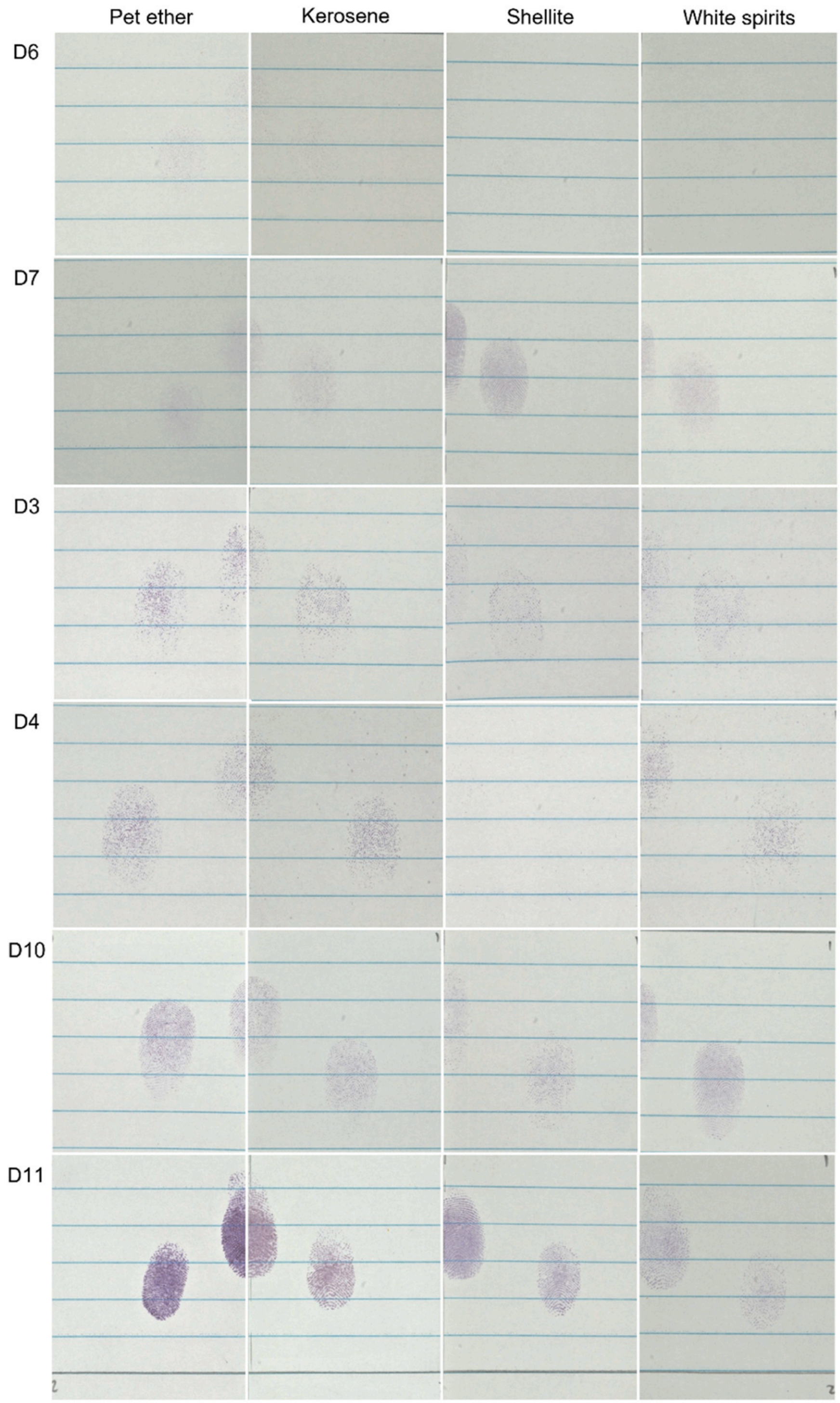


Fig. 15. Split fingermark comparison of ninhydrin formulations containing petroleum ether, kerosene, shellite and white spirits on notepaper for six donors. Pet ether = petroleum ether (40–60 °C).

concerns for these improvised solvents. Future work should also investigate the shelf-life of formulations containing improvised solvents as the non-polar nature of the solvent been shown to cause indanedione to precipitate over extended periods of time [16]. The ability to use inexpensive and more widely available improvised solvents as carrier solvents in amino acid sensitive reagent formulations, as demonstrated by this work, would benefit limited resource jurisdictions in not only the Global South, but those that may experience temporary austere conditions. In addition, the successful application of these solvents to IND-Zn formulations opens the door for their use in other techniques such as ninhydrin.

CRediT authorship contribution statement

Emma C Jones: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Jemmy T Bouzin:** Writing – review & editing, Methodology, Conceptualization. **Jordan FL Hooper:** Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Conceptualization. **Simon W Lewis:** Writing – review & editing,

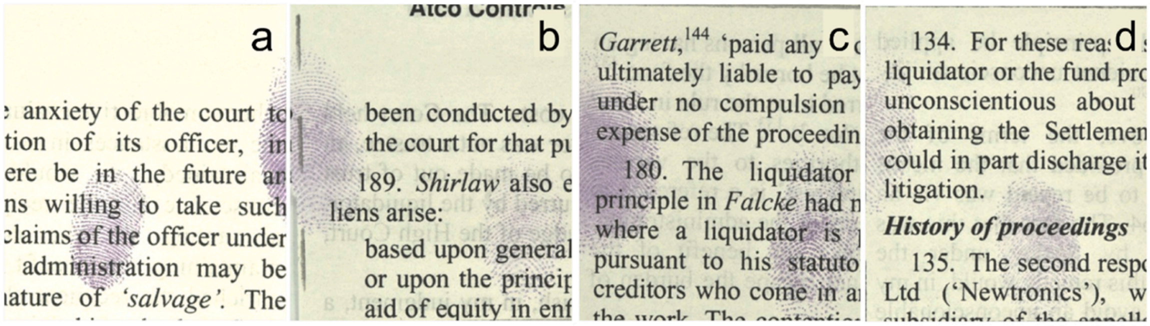


Fig. 16. Split fingermark comparison of ninhydrin formulations containing petroleum ether (left), kerosene (middle left), shellite (middle right) and kerosene (right) on printed paper for D11.

Table 12
Comparative scoring results for IND-Zn using improvised solvent formulations against nominal petroleum ether formulation.

Nominal method (petroleum ether)	Performance	Accessibility & Availability	Cost	Safety	Simplicity	Total Score	Recommendation
Shellite	+0.5	+2	+2	−1	0	+3.5	Strongly recommended
White spirits	−1	+2	+2	−0.5	−0.5	+2	Strongly recommended
Kerosene	−1	+2	+2	−1	−0.5	+1.5	Recommended

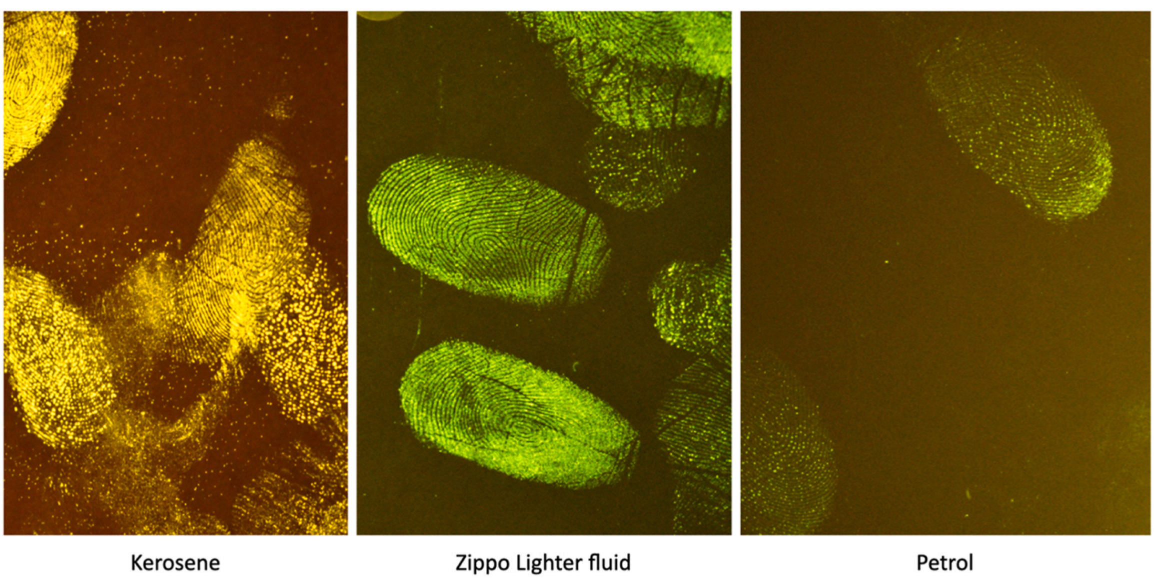


Fig. 17. Natural fingermarks on paper surfaces developed in Timor-Leste with IND-Zn using Timor-Leste sourced improvised solvents under operational conditions.

Visualization, Supervision, Project administration, Conceptualization. **Renee Wilson:** Writing – review & editing, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fsir.2024.100388.

References

[1] J.T. Bouzin, G. Sauzier, S.W. Lewis, Forensic science in Seychelles: an example of a micro-jurisdiction forensic delivery system, Forensic Sci. Int. Synerg. 3 (2021) 100139, https://doi.org/10.1016/j.fsisyn.2021.100139.
[2] J.T. Bouzin, T. Lópes, A.L. Heavey, J. Parrish, G. Sauzier, S.W. Lewis, Mind the gap: the challenges of sustainable forensic science service provision, Forensic Sci. Int. Synerg. 6 (2023) 100318.

[3] S.O. Idowu, R. Schmidpeter, L. Zu, The Future of the UN Sustainable Development Goals Business Perspectives for Global Development in 2030. 1st 2020. ed. CSR, Sustainability, Ethics & Governance, Springer International Publishing, 2020, https://doi.org/10.1007/978-3-030-21154-7.
[4] Democratic Republic of Timor-Leste. International Monetary Fund 2022.
[5] Forensics Review: Review of the provision of forensic science to the criminal justice system in England and Wales. (2019).
[6] L.M. Howes, R. Julian, R. Wilson, M.C. dos Santos, Forensic science capacity development: a case study of Timor-Leste, Forensic Sci. Int. Synerg. 9 (2024) 100553, https://doi.org/10.1016/j.fsisyn.2024.100553.
[7] A.A. Frick, P. Fritz, S.W. Lewis, CHAPTER 9: Chemical Methods for the Detection of Latent Fingermarks, in: J.A. Siegel (Ed.), Forensic Chemistry: Fundamentals and Applications, John Wiley & Sons, Incorporated, 2015, pp. 354–399, https://doi.org/10.1002/9781118897768.ch9.
[8] D.A. Crown, The development of latent fingerprints with ninhydrin, J. Crim. Law Criminol. Police Sci. 60 (2) (1969) 258–264, https://doi.org/10.2307/1142254.
[9] Fingermark Visualisation Manual. Defence Science and Technology Laboratory. (2022).
[10] M. Stoilovic, C. Lennard, Worshop Manual: Fingermark Detection & Enhancement. (Incorporating Light Theory and General Forensic Applications of Optical Enhancement Techniques), 6th ed., National Centre for Forensic Studies, Australia, 2012.
[11] N. Nicolasora, R. Downham, L. Hussey, A. Luscombe, K. Mayse, V. Sears, A validation study of the 1,2-indandione reagent for operational use in the UK: Part 1 — Formulation optimization, Forensic Sci. Int. 292 (2018) 242–253, https://doi.org/10.1016/j.forsciint.2018.04.046.
[12] I. Becker, M.-L. Heinrich, L. Schwarz, M. Bust, Process Instruction Indandione/Zinc for the visualization of latent fingermarks version 4, 1, Bundeskriminalamt, Wiesbaden, Germany, 2018.
[13] 3M Company Client Letter. 2022.
[14] J. Bouzin, Sustainable Latent Fingermark Detection Protocols for Remote-Location and Resource-Limited Jurisdictions, Curtin University, 2023.
[15] J.T. Bouzin, A.J. Horrocks, G. Sauzier, S.M. Bleay, S.W. Lewis, Comparison of three active 1,2-indanedione-zinc formulations for fingermark detection in the context of limited resources and supply chain risks in Seychelles, Forensic Chem. 30 (2022) 100439, https://doi.org/10.1016/j.forc.2022.100439.
[16] J.T. Bouzin, A.A. Frick, G. Sauzier, S.W. Lewis, Preliminary evaluation of Solstice® PF as a replacement carrier solvent for Australian fingermark detection, Forensic Sci. Int. 340 (2022) 111465, https://doi.org/10.1016/j.forsciint.2022.111465.
[17] Y.-B. Zhao, L.-X. Wang, W.-J. Li, W. You, K. Farrugia, Effect of carrier solvent in 1,2-indanedione formulation on the development of fingermarks on porous substrates, Forensic Sci. Int. 318 (2021) 110589, https://doi.org/10.1016/j.forsciint.2020.110589.
[18] International Fingerprint Research Group. Guidelines for the assessment of fingermark detection techniques. Journal of forensic identification. 2014;64(2): 174.
[19] S. Shahbazi, J.V. Goodpaster, G.D. Smith, T. Becker, Preparation, characterization, and application of a lipophilic coated exfoliated Egyptian blue for near-infrared luminescent latent fingermark detection, Forensic Chem. vol 18 (2019) 100208.
[20] M. Hilgert, in: Lewis S (Ed.), BKA Formul. Ninydrin (2023).
[21] J.A. D'Uva, N. Brent, R.E. Boseley, D. Ford, G. Sauzier, S.W. Lewis, Preliminary investigations into the use of single metal deposition II (SMD II) to visualise latent fingermarks on polyethylene 'zip-lock' bags in Western Australia, Forensic Chem. 18 (2020) 100229, https://doi.org/10.1016/j.forc.2020.100229.
[22] D. Hockey, A. Dove, T. Kent, Guidelines for the use and statistical analysis of the Home Office fingermark grading scheme for comparing fingermark development techniques, Forensic Sci. Int. 318 (2021) 110604, https://doi.org/10.1016/j.forsciint.2020.110604.
[23] X. Spindler, R. Shimmon, C. Roux, C. Lennard, The effect of zinc chloride, humidity and the substrate on the reaction of 1,2-indanedione–zinc with amino acids in latent fingermark secretions, Forensic Sci. Int. 212 (1) (2011) 150–157, https://doi.org/10.1016/j.forsciint.2011.06.005.
[24] M. Stoilovic, C. Lennard, Workshop Manual: Fingermark Detection & Enhancement, National Centre for Forensic Studies, 2012.
[25] A.A. Frick, F. Busetti, A. Cross, S.W. Lewis, Aqueous Nile blue: A simple, versatile and safe reagent for the detection of latent fingermarks, Chem. Commun. 50 (25) (2014) 3341–3343, https://doi.org/10.1039/c3cc49577a.
[26] ASTM International. Standard Test Method for Ignitable Liquid Residues in Extracts from Fire Debris Samples by Gas Chromatography-Mass Spectrometry. United States2022.
[27] J.T. Stimac, The search for safe, non-running solvents: a brief history, J. Forensic Identif. 50 (5) (2000) 455–461.
[28] Bleay S., Sears V., Downham R., et al. Fingerprint Source Book v2.0. 2nd ed. CAST; 2017.
[29] G. Sauzier, Ink Analysis, in: M.M. Houck (Ed.), Encyclopedia of Forensic Sciences, Third Edition (Third Edition), Elsevier, 2023, pp. 232–243, https://doi.org/10.1016/B978-0-12-823677-2.00036-2.
[30] Kunjappu J. Ink Chemistry Chem Br. 2003;39(3):22-25.
[31] F. Zampa, M. Hilgert, J. Malmborg, M. Svensson, L. Schwarz, A. Mattei, Evaluation of ninhydrin as a fingermark visualisation method – a comparison between different procedures as an outcome of the 2017 collaborative exercise of the ENFSI Fingerprint Working Group, Sci. Justice 60 (2) (2020) 191–200, https://doi.org/10.1016/j.scijus.2019.11.003.