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Presumptive drug identification by ninhydrin fingerprint analysis

Erin Lange, Felicity Carlysle-Davies *

University of Strathclyde, Royal College Building, 204 George St, Glasgow G1 1XW, UK

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ABSTRACT

A known method of smuggling drugs into prisons is by infusing papers with these illicit substances, and sending them to prisoners through the mail. During the preparation of these drug-infused samples, there is potential for direct contact between the hands and paper, leading to the deposition of fingerprints. These fingerprints would not be visible to the naked eye, but can easily be rendered visible using the ninhydrin method for latent fingerprint detection. This reaction is well known to produce a visible purple coloured fingerprint on the surface of the material in a well-documented, consistent manner. This research, however, demonstrates variations of this reaction in the presence of illicit drugs on the surface of the paper being analyzed. The fingerprints have been demonstrated to vary in shade and intensity of colour in the purple/blue/grey region following the ninhydrin process when different drugs have been infused in the paper material. This phenomenon has the potential to be used as a presumptive indicator of any drugs that may be present in infused papers.

1. Introduction

Since 2017, a surge in cases of drug smuggling into prisons has become apparent [1], and a common smuggling method is by infusing paper items, such as letters and greeting cards, with the illicit substance [1]. Opioids [1], methamphetamine [1], and New Psychoactive Substances (NPS) [1,2] have all been identified in smuggling attempts into prisons in this way. Whilst the presence of illicit drugs may be the focus of investigation for samples of this type where drugs are suspected, other types of analysis can be of interest. Due to the nature of direct handling of paper samples during the drug infusion process, it may be of interest to additionally perform latent fingermark analysis on seized papers.

Ninhydrin is a well-known chemical that has the potential to detect latent fingerprints and render them visible to the naked eye. When a fingerprint is deposited on a surface, amino acids primarily from eccrine glands on the fingertips are left in the shape of the ridges of the fingerprint. Secretions from sebaceous glands are also likely to be present on the fingertips and deposited onto a surface if the hand recently touched the face or scalp [3]. Ninhydrin is able to react with these amino acids to form a molecule commonly known as Ruhemann's purple [4,5]. As the name suggests, a purple colour will become visible over the area where amino acids were deposited, allowing fingerprints to be detected visually. However, the intensity and shade of the colour can vary depending on which amino acids have been deposited to form the fingerprint [4]. This detection technique works well on porous surfaces [5], which makes it an ideal way to detect the presence of fingerprints on paper materials.

In addition to its uses to detect latent fingerprints, ninhydrin also has a history as a stain for TLC plates [6,7]. Ninhydrin can be used to visualize primary and secondary amines [8] which makes it an effective staining agent for these compounds. Many illicit substances fall into the amine classification, including methamphetamine [9] and cocaine [10], which indicates that they may be able to be visualized through the use of ninhydrin. While amino acids are known to be rendered visible by ninhydrin via the formation of Ruhemann's purple [4,5], there may be additional detection capabilities of this technique in the case of drug-infused papers due to the presence of these illicit substances.

This research combines the areas of latent fingerprint detection and drug analysis, and there is potential for development of testing methods that cover both these interests. If a drug is able to be presumptively detected alongside latent fingerprint development, this would reduce analysis times as only one method would need to be performed on a sample instead of multiple tests. In addition, it is possible that the presence of illicit substances could be flagged on a seized sample during a fingerprint analysis, even if no drugs had previously been suspected. This would be beneficial in order to efficiently identify any samples on which further testing should be done.

2. Materials and methods

Ethical approval for donation of fingerprints on paper samples was granted by the University of Strathclyde.

* Corresponding author.

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Ninhydrin, methamphetamine, MDMA, amphetamine sulfate, barbital and cocaine were obtained from Sigma. Synthetic cannabinoids AKB-48 and THJ-018 were obtained from Fine Chemicals, Scientific Supplies Ltd. Morphine was sourced from W + R Patrick LTD and diamorphine was purchased from Johnson Matthey Mcfarlane Smith. Methanol was obtained from VWR, and the Novec-71DE engineering fluid was from Apollo Scientific. Fisher Scientific was the source for acetic acid, ethyl acetate, and absolute ethanol. Samples of A4 paper, magazine paper, and greeting cards were obtained from commercial retailers.

2.1. Infusion of drug into paper

Standard drug solutions of methamphetamine, MDMA, barbital, amphetamine sulfate, morphine, diamorphine, and cocaine were prepared at 50 mg/mL in methanol. Synthetic cannabinoids AKB-48, and THJ-018 were prepared at 50 mg/mL in acetonitrile. 50 μL of each drug was infused onto 3 cm by 3 cm squares of A4 printer paper, matte magazine paper, and greeting card, for a total of 9 samples of each drug. Using a pipette, the 50 μL aliquot of the drug was deposited onto the centre of each paper and was left to dry in a petri dish. This process was done in triplicate for each drug on each paper type. Blank samples of each paper type were also prepared by infusing pure methanol and pure acetonitrile, as well as blank samples that had not been infused at all.

2.2. Ninhydrin fingerprint analysis on infused papers

Ninhydrin working solution was prepared by mixing 5 g ninhydrin with 4.5 mL absolute ethanol and 2 mL ethyl acetate to form a slurry, and then 5 mL of acetic acid was added. 5.2 mL of this stock solution was then mixed with 95 mL of Novec-71DE engineering fluid to make the ninhydrin working solution, and a portion was poured into a 250 mL beaker.

One to two fingerprints were deposited on the surface of each infused paper by a single donor. Two fingerprint donors were recruited to this study, and fingerprints were deposited from all ten fingers. A mix of primed fingerprints was used, where the finger touched the forehead prior to depositing the fingerprint, along with non-primed fingerprints. Each prepared sample was fully soaked in the ninhydrin working solution. This solution was changed for fresh solution each time a sample with a different infused drug was soaked. The soaked samples were then placed in a humidity chamber at 70 °C for 15 min. The papers were removed from the humidity chamber and observations were recorded.

3. Results and discussion

The focus of this study was to investigate the use of the ninhydrin process for latent fingerprint detection, specifically on drug-infused papers. From the results, it became evident that it is possible to obtain visible fingerprints on paper surfaces using this method, in the same manner as for un-infused papers. However, it was consistently observed that papers infused with amine-type drugs resulted in varying fingerprint colours over the infused- and un-infused portions of the paper. This gave a clear indication of where the drug was present on the paper. The image in Fig. 1 is an example of the phenomenon observed, where two different colours are apparent on the same fingerprint based on where the drug is present on the paper.

During sample preparation, a consistent volume of 50 μL of drug solution was chosen from method optimization during preliminary testing. This volume was deposited near the centre of each paper using a micro-pipette, which spread as it soaked into the paper without covering the entire surface. As in the example in Fig. 1, this allowed both fingerprint colours to develop on the same fingerprint, and could be compared with the knowledge that the drug was the only variable present in any given sample. Within one single fingerprint, the combination of eccrine sweat and any sebaceous secretion present would be consistent throughout, meaning that the different colours develop even with the same fingerprint residue make-up. In order to compare the fingerprint colours of drug-infused samples to un-infused blank samples, a set of blank papers and solvent-blank papers were tested in addition to the chosen drugs.

The blank samples all exhibited a purple or pink/purple fingerprint, which aligns with the expected results for the ninhydrin reaction in normal circumstances, as given in Table 1. The blank samples and solvent-blank samples did not exhibit any variations in colour throughout the full fingerprint or between the three paper types, which is expected for this well-known method. The results in Table 2 list the fingerprint colours that were observed for all tested drugs. Following the ninhydrin reaction, the amphetamine class drugs and the morphine

Table 1
Colour of fingerprints on blank paper samples.

Sample	Colour of Fingerprint		
	A4	Magazine	Card
Blank	Purple/pink	Purple	Purple
MeOH blank	Purple/pink	Purple	Purple
Acetonitrile blank	Purple/pink	Purple	Purple

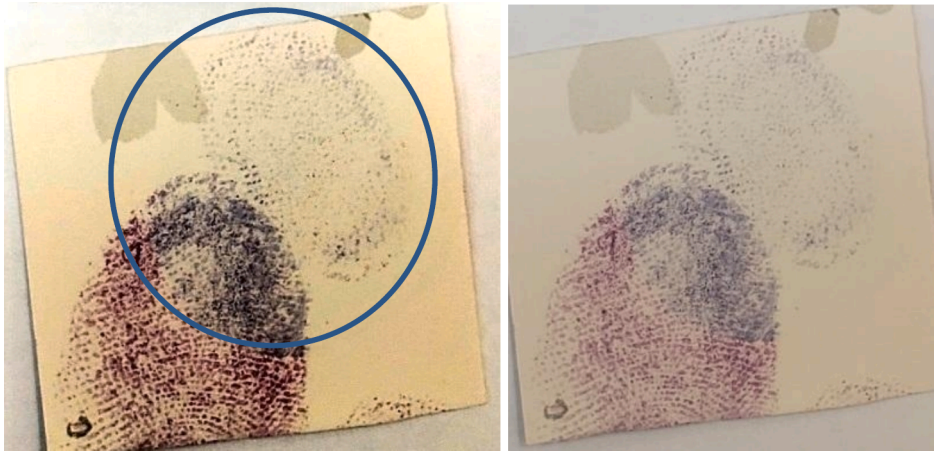

Fig. 1. Greeting card sample containing infused cocaine exhibiting a dark purple/blue colour over the infused area of the paper (circled), and a lighter purple over the un-infused portion of the sample. [Left image: brightness adjusted. Right image: original image for reference]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Description of fingerprint colours over the drug infused area of A4 paper, magazine paper, and greeting card samples.

Drug Class	Infused substance	Colour of Fingerprint		
		A4	Magazine	Card
Amphetamine	Methamphetamine	Dark/ bright purple	Dark/bright purple	Dark purple/ blue
	MDMA	Dark purple	Dark purple, some staining of infused area	Dark purple
	Amphetamine Sulfate	Infused area fully stained dark purple	Infused area fully stained dark purple	Infused area fully stained dark purple
Barbiturates	Barbital	No distinction from blank	No distinction from blank	No distinction from blank
Cannabinoid	AKB-48	No distinction from blank	No distinction from blank	No distinction from blank
	THJ-018	No distinction from blank	No distinction from blank	No distinction from blank
Opioid	Morphine	No distinction from blank	Dark purple	Dark purple
	Diamorphine	Grey/ purple	Grey/blue	Light grey/ purple grey
Cocaine	Cocaine	Purple	Grey/blue	Grey/blue/ purple

appeared a dark purple/blue colour, while the diamorphine and cocaine appeared to be light grey/blue coloured, each distinct from the blank samples. As in Fig. 1, the colours that differed from the blanks were consistently observed only on the portion of the fingerprint that was directly over the area infused with the drug. In the case of amphetamine sulfate, the entire area of the paper that was infused with drug was stained dark purple. This was the only drug where this occurrence was observed clearly to the naked eye. Some staining of the drug-infused area was also visible on the MDMA samples, however this was not immediately or as clearly visible as in the amphetamine sulfate samples. Examples of the results for the blank samples, and for each drug can be found in Appendix A.

From the data in Table 2, it was evident that the barbiturate and synthetic cannabinoid drugs did not result in any colour variations from the blank samples. However, the amphetamine and opioid class drugs, as well as cocaine, consistently resulted in varying fingerprint colours. All observed colours remained consistently in the purple to blue range, with the most common colour variation being a dark purple (Fig. 2).

While this study primarily considered the fingerprint colour variations by drug class, the class of organic compounds each drug fits into also provides insight on why the colours differ. Ninhydrin is useful as a method of detection for primary and secondary amines [11], and for amino acids by its use as a TLC visualization agent [7]. The drugs used in this study are a mix of primary amines (amphetamine sulfate), secondary amines (methamphetamine, MDMA), and tertiary amines (cocaine, morphine, diamorphine) Drugs with other functionalities were also used for the purpose of gaining an understanding how the visual results of this ninhydrin reaction differ on a wider range of compounds. These include the ketone and amide functional groups with THJ-018 and AKB-48, respectively, and barbital also includes the amide functionality. It was observed in the results that neither the synthetic cannabinoid drugs nor barbital reacted with the ninhydrin, since there were no fingerprint colour variations observed. It is known that amides do not produce a reaction with ninhydrin [12], which aligns with what was observed for

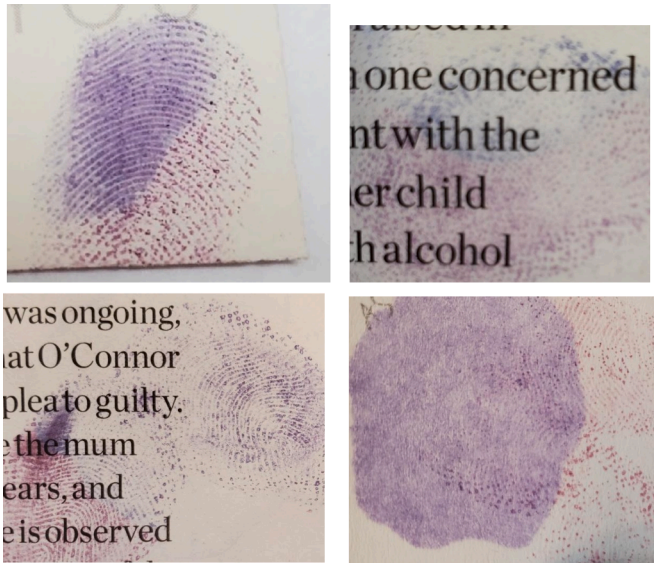

Fig. 2. Examples of fingerprints with differences in colour over the infused and uninfused portion of the paper. Infused drugs shown are methamphetamine on card (top left), cocaine on magazine (top right), morphine on magazine (bottom left), and amphetamine sulfate on A4 (bottom right).

the barbital and AKB-48 samples. In considering the observed results for the amine drugs, the amphetamine sulfate was unique from the other tested drugs since the entire area became stained purple instead of just the fingerprint itself. This drug was the only primary amine tested, which can explain these observed results. The reaction between primary amino functional groups and ninhydrin has been well studied [13,14] and it can be suggested that the observed results are due to the differences of how ninhydrin reacts with primary, secondary, and tertiary amino compounds. In the case of amphetamine sulfate, Ruhemann's purple colour was observed over the entire area of the paper that contained the infused primary amine, regardless if a fingerprint was present on that area of the paper. From this, it is evident that the ninhydrin was reacting primarily with the drug itself to produce the colour. Since this was not observed for the secondary and tertiary amine drugs, the ninhydrin reaction could not have been occuring in the same way over the entire drug-infused area. In this case of the secondary and tertiary amines, the amino acids present from the fingerprint appear to be an essential component in the reaction in order to produce a colour on the papers. Rather than the ninhydrin primarily reacting with the drug itself, as in the case of amphetamine sulfate, it is suggested that the ninhydrin is reacting mainly with the deposited amino acids, and the presence of the secondary and tertiary amine drugs are affecting the visual outcome of the reaction.

Colour variations through ninhydrin reactions have been previously reported through performing the reaction with the presence of different compounds. In a 2009 study [6], ninhydrin was investigated as a TLC plate stain for the visualization of azides. This study found that using their developed method, which converts each azide to its corresponding amine prior to ninhydrin staining, a range of colours will appear on the TLC plate. The apparent colours were mainly in the blue/purple/pink range, and differed for each compound. These results align with this study in demonstrating that ninhydrin may produce varying colours depending on the amine that is present for the reaction. A 2014 study also demonstrated varying colours by using ninhydrin as a TLC plate visualization agent [7]. This paper focused on rendering amino acids visible on TLC plates for identification using ninhydrin in the presence of 2-furoic acid. With this acid present in the reaction, the amino acids could be distinguished based on colour, mainly in the violet/pink/red/brown colour range. These results indicate that it is possible to use varying colours produced by a ninhydrin reaction to indicate if specific

compounds may be present, which supports the findings of this study. It is apparent that varying colours by a ninhydrin stain can be expected if the right conditions are present, which aligns with the results observed in this study, with the presence of certain illicit substances.

It is important to consider that some variation in results may occur due to variations in the fingerprints themselves. From preliminary testing, it was determined that it would not be possible to obtain a high number of good quality fingerprints using the same finger for each deposition on a set of samples. This was due to the residue on the tip of the finger being depleted after each deposited fingerprint, as would be expected. As a result, all 10 fingers of each donor were used for fingerprint deposition. This was done in both natural conditions (no prior priming of the fingertip), and primed conditions with prior touching of the hair or forehead. During initial testing of the ninhydrin process for fingerprint visibility, there was no observable difference in results based on these varying fingerprint deposition conditions. Preliminary testing was done over several days, with no variations in results observed between each day's set of samples. As a result, this variance in sample deposition was deemed acceptable for the purposes of this study, considering that it would allow for a greater amount of high quality fingerprints to be deposited in a short time span.

Overall, there was no distinction between the results for the three paper types of any tested drug. This aids in demonstrating the consistency of the fingerprint colour variations over drug-infused areas of the papers, even when varying sample types. The consistent results here indicate that the sample surface does not have a major impact on the reaction, which means that testing using this method could be used on a wide variety of paper samples. While more research would need to be done using different types of paper to further confirm this, with fewer limitations on sample types this method can be used on, the value of this method increases.

An important factor to consider with these results is the amount of drug that was infused into each paper sample, when comparing the conditions presented in this study to real life samples. Seized drug-infused paper samples have been reported to range from <0.05 mg/cm^2 to 1.17 mg/cm^2, and in some cases up to 2.38 mg/cm^2 [15]. This study used 50 μL of a 50 mg/mL drug solution infused into each 3 cm $\times$ 3 cm paper sample. This is equivalent to a final concentration of 0.28 mg/cm^2, which falls on the lower end of the range identified in previously seized samples. Further research should be done to investigate the effect that drug concentration has on the results. This should particularly focus on determining the lower concentration limit where drug identification is presumptively possible by observing the fingerprint colours, in addition to identifying if increased concentrations affect the shade or vibrancy of the altered colours.

Applications of these colour differences could be used as an initial detection method for drug presence, similar to presumptive testing. If these alternative colours are observed during a routine analysis for the presence of fingerprints, this could indicate the presence of drugs infused in the material and could lead to further investigation. Presumptively, this could indicate which type of drug is present depending on the specific colour, and where on the sample the infusion is located. However, since it was evident that not all drugs resulted in a colour different from the blank samples, such as the synthetic cannabinoids, this could not be used as a confirmatory method to determine drug presence or absence, but only as an indicator. Simultaneous detection of drugs and fingerprints also has the benefit of efficiency, as only one test would need to be performed rather than two. This would be particularly useful as an initial indicator of if illicit drugs are present on a paper sample in cases where drugs are not suspected to be present, so may not be tested for. A limitation presented in this research though is that evidently not all drugs produce a colour changing reaction that differs from blank samples. As a presumptive test, this method could likely not be used on synthetic cannabinoids or barbiturate class drugs as indicated from these results.

Further research should be done to determine the limits of drug concentration in terms of observing a change in fingerprint colour, as well as investigating a wider range of drugs to gain a wider understanding of this phenomenon. In addition, this study specifically tested fingerprints that had been deposited after drug infusion was complete and the papers were dry. However, it may also be beneficial to test this method on samples that have had fingerprints deposited before the drug infusion process, to determine if the quality of fingerprints are affected by the wet solvent.

Additional research should also be performed to investigate the potential of using ninhydrin as a reagent for presumptive drug-detection. During latent fingerprint analysis, ninhydrin reacts with the fingerprint residue left of the paper surfaces and becomes a bright purple colour, and this research demonstrated that when certain drugs are present, the colour varies. Further investigation into this reaction could provide additional methods for presumptive drug detection and investigation. Even without the presence of fingerprints, if the substances that react with ninhydrin could be added to a paper sample via a spray or by soaking the sample in a solution, then it is possible that the same phenomenon observed in this study would take place. The aim of a method such as this would be a result of the area(s) of paper infused with a drug appearing as a colour distinctly different from a blank, similar to the results observed here for the amphetamine sulfate samples.

4. Conclusions

The research in this study demonstrated that using the ninhydrin method to detect latent fingerprints on drug-infused paper material resulted in variations in the observed fingerprint colours. The colours that appeared over drug-infused areas of the paper were consistently different from both blank samples and un-infused areas of the sample on three types of paper. This phenomenon was particularly evident with the amphetamine and opioid classes of drugs, as well as with cocaine. However, no colour variations were observed for the barbiturate and synthetic cannabinoid drug classes. These results have been determined to be due to the class of organic compound of each tested drug, with the amine drugs producing varying colours due to their reactivity with ninhydrin. Overall, this research presented a process that has the potential to be useful in the forensic analysis of drug-infused papers by way of presumptive testing, and simultaneous fingerprint and drug detection, but further work may still be required for full development and understanding of its potential.

CRediT authorship contribution statement

Erin Lange: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Felicity Carlysle-Davies:** Writing – review & editing, Supervision.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.forc.2024.100597.

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