

Detecting Narcotics in Solution Extracted From Dried Urine Stain on Cotton Fabric

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Abstract

The present study aims to present a new method to trace narcotics in the solution extracted from dried urine stain left on cotton fabric. Narcotic substance was traced in the 50 samples through two methods. In the first method, samples were directly examined through Thin Layer Chromatography (TLC). In the second method, a piece of cotton fabric was soaked in a beaker filled with urine sample. After full absorption, the piece of fabric was left to dry. Then it was soaked in distilled water and shaken so that the stain deposits would dissolve into water. Finally, the solution was extracted from the wet fabric by centrifugal spin and admixed with an equal amount of distilled water. A TLC test was run afterward. The TLC run on the main samples produced the following results: morphine, codeine and other opium alkaloids were detected in 38 samples; in 7 samples only codeine was found. Five samples produced no especial stains. The TLC performed on the solution tapped from the cotton piece of fabric produced similar results except for the lower density of stain colors. The results show that narcotics may be detected using dried urine stains on cotton fabric dissolved in distilled water.

Keywords: Narcotics, Urine stain, Crime scene, Thin Layer Chromatography, TLC

Introduction

Drug abuse is today one of the most abject ills plaguing the world, incurring an annual expenditure budget of USD 400 billion in the U.S. alone, covering expenses and costs such as treatment, job dismissal and crime¹. Drug abuse is one of the foremost factors spreading the seeds of crime, family violence and fatal or disabling incidences. These substances have significant physiological and psychological effects on individuals while committing a crime^{1,2}. In fact,

the majority of criminals constitute drug addicts and excessive drug abuse may lead to the death of the abuser^{3,4}. In the past two decades, drug facilitated crime has come to the fore and addressed extensively. This type of crime includes an extensive range of offences such as theft, rape, money extortion and even murder⁵.

Although collecting evidences and investigating the crime scene are the primary and most critical steps in a crime investigation process involving quick, systematic and precise steps in solving the case and

obtaining evidences and traces of drugs are critical instruments in solving the crime, establishing the presence of narcotics in such pieces of evidence faces challenges and limitations⁵. Therefore, numerous studies have addressed the problem of finding narcotics in biological samples from different parts of human body, including blood, plasma, hair, Cerebrospinal fluid, and urine⁶⁻¹⁰. Urine left in the crime scene is one of the most revealing instances of such evidence^{11, 12}. Some criminals tend to urinate over the body as a clue or psychiatry discharge or insulting the corpse or an emblematic signature. Furthermore, in severe cases of poisoning, patients discharge urine in the terminal stages of their lives. However, urine characteristics, including speed of drying, contamination, cadaveric transformation and putrefaction of the corpse impede urine sampling, especially from the bladder^{12, 13}. Common instances of urine found in crime scenes include dried urine stains in different parts of the scene, particularly the fabric evidence such as upholstery fabric. Extracting the urine from these stains for later experiments is conducted through sampling the stain and admixing distilled water, normal saline or other types of solutions to the exclusion of the fabric itself but fabric isn't used directly, yet. The present study aims to examine narcotics in the substance extracted from dried urine stain left on cotton fabric, compared with control samples.

Materials and Methods

The present study was conducted in 2009 at the reference laboratory of the Forensic Medicine Department of Province of Markazi, Iran. Of 150 received urine samples which had positive screening narcotics test (rapid morphine check using morphine test strip urine), 50 were selected randomly. In order to control the quality of the samples, screening tests

were performed again. Two methods were then utilized to test the presence of narcotics. In the first method, each sample was directly examined through Thin Layer Chromatography (TLC) once the narcotic substance was extracted from the urine. In the second method, TLC was performed on a solution extracted from cotton fabric. In this method, a 20*20cm piece of cotton cloth was chosen and soaked in 7 cc of the sample urine in a lab beaker. The solution was completely absorbed by the fabric with no sign of dripping. The soaked fabric was then placed in ambient temperature of 15-20 °C for 30 minutes to dry up. The dried fabric was then dipped into 7 cc of distilled water (same volume as urine sample) and shaken with a shaker for 30 minutes to dissolve the deposits absorbed by the cloth. Afterward, the solution was given 5 minutes of centrifugal spin at 1,000 rates per minute (rpm) to prepare a solution for extracting the narcotic substance. The TLC test was run later on. In both methods, the TLC results were confirmed by the GC/MSS instrument.

Narcotics Extraction from Urine

Narcotic substance was extracted through liquid-liquid extraction method. In this method, we used 10 cc HCL 10% to acidize 10 cc urine and then added 10-15 cc distilled water to the solution. The solution was then direct-heated for 20 minutes and then ammoniac was gradually added to basify the solution. The desired pH was maintained between 8.5 and 9. Next, we added 250cc of chloroform: isopropranolol compound (4:1, v/v) and shook the resultant solution for 20 minutes in a shaker. After forming the two-phase stage, we poured the solution into a decanter. We then separated the bottom phase containing narcotics, poured it into a beaker and boiled it in a bain-marie to be evaporated. The deposit left was used in TLC spotting.

TLC

1. TLC plates

A silica gel with fluorescent indicator UV245 plate with Ar Number of plates 805023 was used for TLC process. Every effort was made to ensure that the plates were free of any scratch, groove or stain with completely even and germ-free surfaces. A soft lead pencil was used to draw a non-scratching line 2 cm from the edge of the plate. On the opposite side a 1.5 cm wide piece of blank label was plastered on the back of the plate across the line for recording the sample details.

2. Spotting in TLC plate

We added 3 cc methanol to each study samples and also standard samples and agitated them to dissolve. We then started staining using a capillary tube from the line on the left of the plate. We would stop for a stain to dry before forming another stain. The maximum diameters of the stains were kept at 5 mm. Staining for each sample was performed 20 times along a standard stain. The distance between the stains was kept at a minimum of 2 cm. Since we used 2 chromatography tanks with different solvents, staining for each sample was performed on two different TLC plates. Once the stains were dried and there were no scratch, artifact or extra stains on the plates, the plates were immersed into the tanks. Otherwise, the foregoing steps had to be repeated.

3. Chromatography tanks

Two glass tanks measuring 22×9.5×21.5 cm with flat walls and even bottoms were used for chromatography. The brims of the tanks were identically curved with transparent walls so that one could see the solvent front cross over the TLC plate. The volume of the solvents in each tank was 100cc.

The solvent in the first tank included ethyl acetate: chloroform: dioxane: ammonia (10:25:60:5, v/v) and the solvent in the second tank included benzen: dioxane: methanol: ammonia (50:40:5:5, v/v). Once the solvents were prepared, we poured them into the respective tanks and agitated them for the solution to mix well. The solvent remained for 10 minutes sealed in the tanks to saturate and during this period the tank door was not open. The stain plates were then slipped into the tanks and remained there sealed. The plates were placed in the tanks in a way that they would remain straight, the upper surface of the plates would not contact with the glass walls and the solvent surface would lie beneath the stains to prevent dissolution of the stains in the solvent. Once the solvent reached the label on the top of the plate, the plates were removed from the tanks to dry and ready for spraying.

4. Emergence of stains and coloring the plates

UV and iodoplatinate indicator were used to detect and spot the stain on the TLC plates. To prepare the indicator, 0.25g hexachloroplatinate was solved in distilled water and admixed with 5g potassium iodide. Finally, 2cc dense HCL was added to the solution, reaching a total volume of 100cc.

5. Comparing emerged stains

The formed stains were compared with the standard narcotics stains (the narcotic contained in the opium which is used by the Reference Laboratory of Forensic Diagnosis and Laboratory of Iran and confirmed by the GC-MASS instrument) from four perspectives: R_f (Rate of Flow), the shape of the formed stain in that R_f, the size of the stain and its color.

6. Stain comparison results

The results prove narcotic positive if the stains are similar in the foregoing aspects with the standard stain and also if the results of the TLC plate stains used in the two tanks are similar as well.

Results

The direct TLC run on the main samples produced the following results: morphine, codeine and other alkaloids of the opium were found in 38 samples and in seven samples only codeine was found. No specific stain was found on the TLC plates for five samples. The TLC test on the solution extracted from the cotton fabric produced similar results, differing only in the density of stain colors.

Discussion

The results obtained in the present study showed that dissolving urine stain on a piece of cotton fabric in distilled water has no effect on the process of detecting narcotics in the samples, hence helping to spot narcotics in line with the primary sample. The only difference lies in the fact that in the TLC method color stains are thinner than those of the primary sample. Since the authors did not find any similar methods for extracting narcotics in the urine and because the results of the present study met adequate plausibility it seems that this method would be appropriate to investigate the abuse of narcotics in the crime scene victims along other biological samples.

Tsutsumi et al. recommend that the sample for urine verification and supplementary tests be obtained from the central area of the urine stain remained on the clothes in general and cotton or silk fabrics in particular¹⁴. Although we used a different method

and material (cotton cloth) and completely solved the stain in distilled water, the likely different interactions between the types of fabric and the substances in the urine, it is likely that the type of fabric may have a moderating effect on the results of the study. Hence, further research is encouraged varying the type of fabric used.

Although there was no false negative test in re-sampling the extracted solution from the fabric, adding distilled water might have created false negative results in the sampling test. TLC is a more precise method than strip sampling test, yielding a higher degree of precision in the study. Therefore, we recommend precise tests, such TLC, for using this method in extracting urine from fabric to find narcotics. On the other hand, since the amount of urine and the size of cotton fabric used were equal across samples, an equal amount of distilled water was utilized too. However, in real conditions the amount of distilled water needs to be particularly specified in order to prevent over-dilution of the samples. Hence, future researchers are encouraged to vary the size of fabric, the amount of urine and the size of stains.

Although laboratory studies have shown that morphine would be still detectable in urine stains even after 12 weeks¹⁵, utilizing different times for the dried state of the sample may contribute to the standardization of this method. In order to confirm the rigorous application of this method in crime scenes, it is also necessary to test the urine stains remaining on the pieces of cloths surrounding the corpse, including the clothing, mattress, blanket and the clothes of the living individuals found on the crime scene once this method is standardized.

Conclusion

The present study revealed that the urine stain

on the fabric may be used along distilled water to detect narcotics. For further research, it is strongly recommended to vary the amount of distilled water relative to the type of the fabric and the diameter of the urine stain so that a standard protocol will be designed to be used for different types of fabrics left on the crime scene.

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