



A PRELIMINARY STUDY ON FORENSIC EXAMINATION OF VERMILION STAINS BY USING THIN LAYER CHROMATOGRAPHY

Forensic Science

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ABSTRACT

Cosmetic commodities are often turned up as trace manifest at the crime scenes such as lipstick, blood, soil, glass, sindoor, paints etc.

BACKGROUND: Sindoor is trace evidence and readily swapped from one article to another. It may serve to associate the victim to the suspect or crime scene and suspect to the victim or crime scene.

RESULT: In the suggested course, 15 sindoor samples of the varied brands were solicited. 10 solvent systems were worked on each sample of sindoor in which the four solvent systems [methanol: acetone (8:2), Acetonitrile: Acetone:methanol (2:4:4), Acetonitrile: methanol (2.5:7.5) and benzene: methanol: acetic acid (8;1:1)] were established for the evaluation of sindoor specimens by employing Thin Layer Chromatography.

CONCLUSION: sindoor tints that are encountered at the crime scene can be singled out or scrutinized by testing it with its substantive origin by thin layer chromatography with newly improved solvent system.

KEYWORDS

sindoor stains, solvent systems, TLC, hRf value, ANOVA

INTRODUCTION

The cosmetic daubs are the trace pieces of evidence that may be raised at the site of the atrocity. They are employed as corroborative evidence in the court of law, which may facilitate to tie the suspect to the victim or to the crime scene. Customarily stumbled cosmetic products are lipstick, sindoor, nail enamels etc. Locard principle of exchange asseverates that trace evidence are easily relinquish from one place to another (such as lipstick, sindoor etc.).

Vermillion (sindoor) daubs may be encountered as trace evidence at the crime scene in assorted types of disputes (molestation, sexual assault etc). Sindoor is still an illustrious pooja item, which is offered to Hindu gods as well as a goddess [1]. In Nepal, patrons are looked upon as a god and the visitors are usually accosted in the house putting a crimson speck in the forehead. The vermilion is a fundamental element in any rituals [2]. Sindoor manufactured on profit-oriented basis embrace synthetic/organic pigments and chemicals. Voguish sindoor mainly uses vermilion (an orange-red tint), which is prepared from the grounded cinnabar (mercury sulphide). Natural or traditional sindoor was formed with turmeric and alum or lime, or from other herbal ingredients [3].

Varied chromatographic and instrumental approaches are accessible for the analysis of cosmetic products. In the preceding researches, Sharma et al. (2006) examined 36 samples of liquid vermilion by using TLC and Chandel R.S. et al. (2013) analyzed 15 samples of sindoor by using TLC [4-5]. In the suggested research endeavor, an undertaking has been carried out to identify the origin of sindoor traces by matching the smear with the substantive source of sindoor, by investigating with Thin Layer Chromatography using a newly developed solvent system which accorded better results as contrasted to the solvent systems that were being used hitherto.

HYPOTHESIS

H₁- There is no significant difference in the hRf value of different brands of sindoor's obtained by different solvent systems.

H₂- There is a significant difference in the hRf values of different brands of sindoor's obtained by different solvent system.

MATERIALS AND METHODOLOGY

Materials

In the present study fifteen samples of different brands of sindoor were collected for the study that is as follows: (1) Milap (2) Maakumkum (3) Pooja-G kumkum powder (ahuja product Delhi) (4) Namonavkarsindoor (5) Kanavkumkum powder sample (6) Kumkum silky gold sindoor (7) Sapanasuhagkumkum powder (8) Sagan singarsindoor (9) Om sapna (10) S sapnakumkum (11) Sapanasindoor (12) S singarkumkum powder (13) S saganasindoor (14) Suhag khushisindoor (15) Suhag shringar.

Method

Preparation of Sindoor Samples

Sindoor specimens were prepared by smearing minuscule portion of each sample on sections of cotton cloth. The stained cotton pieces were marked progressively and to ward off cross-contamination, were separated cautiously.

Extraction

The cotton sections were later settled in three test tubes and 1ml of water, acetone, and methanol was added to each tube subsequently and the test tubes were shaken for 5mins. The extract of each tube was later turned over to another clean Petri-dish for further analysis.



Fig1. Preparation of Sindoor Samples

Solvent Used for TLC

The following solvent systems (mobile phase) were investigated on each sample of sindoor to identify out the most apt solvent system that could be implemented comprehensively for Thin Layer Chromatographic estrangement of all taints of sindoor (table1).

Table1. Various Solvent Systems Tried for TLC Analysis

Solvent System	Solvent Systems	Composition (v/v/v)	Development Time
1.	Chloroform: acetone: methanol	60:20:20	1 hour
2.	Chloroform: acetone	80:20	1 hour
3.	Benzene: toluene	60:40	1 hour
4.	Toluene: acetone	90:10	1 hour
5.	Benzene: toluene: methanol	70:20:10	1 hour
6.	Benzene: toluene: acetone	45:45:10	1 hour
7.	Methanol: acetone	80:20	1 hour
8.	Acetonitrile: acetone: methanol	20:40:40	50 min
9.	Acetonitrile: methanol	25:75	1 hour 20 min
10.	Benzene: methanol: acetic acid	80:10:10	1 hour

Statistical Analysis

Analysis of variance (ANOVA) is a collection of statistical models and their associated procedures (such as 'variation' among and between groups) used to analyze the differences among group means.

RESULT AND DISCUSSION

In the suggested research, the solvent system chloroform, acetone and methanol (6:2:2) separates the components of eight brands of sindoor samples (Sapna suhag kumkum powder, Sagan singar sindoor, Om sapna, S sapna kumkum, Sapna sindur, S singar kumkum powder, S

sagan sindur, Suhag khushi sindoor) at the different hRf value and a number of spots were visible.

The solvent system methanol and acetone (8:2), acetonitrile, acetone and methanol (2:4:4), acetonitrile and methanol (25:75) and benzene, methanol and acetic acid (8:1:1) separates the components of all the brands of sindoor samples by giving various spots at different hRf values (table2). All the other solvent system used in the research does not show any remarkable results.

Table2. A Comparative Study of All the Four Solvent Systems on the Basis of Spot Color and hRf Values

Sindoor Brand	Methanol: Acetone (80:20)		Acetonitrile: Acetone: Methanol (20:40:40)		Acetonitrile: Methanol (25:75)		Benzene: Methanol: Acetic Acid(80:10:10)	
	Colour of Spots	hRf Values	Colour of Spots	hRf Values	Colour of Spots	hRf Values	Colour of Spots	hRf Values
1	Pink	58	Light red	96	Pink Pink	88 96	Pink Orange Orange	23 40 97
2	Pink Pink	11 35	Dark pink Yellow Light pink	17 25 95	Pink Yellow Light pink	12 16 80	Pink Yellow	18 23
3	Light pink	57	Orange	96	Pink Pink Red	12 76 93	Orange	95
4	Orange Pink	37 53	Red	12	Red	94	Orange Orange	21 41
5	Yellow Pink Red	10 35 57	Purple Yellow Pink	19 29 95	Light pink Yellow Pink Red	13 17 72 94	Blue Pink Violet Yellow	8 10 13 18
6	Pink	9	Light purple	19	Pink Yellow	11 14	Blue Pink Yellow	7 14 17
7	Orange	83	orange	95	Orange	95	Orange Pink	8 11
8	Pink Orange	55 79	orange	83	Orange	96	Orange Pink Light orange	9 12 27
9	Pink Orange	66 87	Pink Orange	63 98	Pink Orange	81 97	Orange Pink Orange	22 29 46
10	Pink Pink Orange	74 86 90	Pink Pink Orange	18 64 95	Pink Orange	81 98	Orange	58
11	Pink Orange	79 88	Pink Orange	65 95	Pink Orange	82 95	Pink	54
12	Orange	75	Orange	95	Orange	96	Pink Orange	17 40
13	Orange	74	Orange	96	Orange	92	Orange Orange	37 96
14	Pink Orange	58 77	Pink Orange	64 96	Pink Orange	74 95	Pink Orange Orange	10 15 93
15	Pink Orange	89 95	Pink Orange	70 97	Pink Orange	73 97	Pink Orange Orange	10 14 32

On the assumption of analysis of variance proceeding, the null hypothesis is rejected and the alternate hypothesis is accepted as the calculated F value (8.81) is greater than critical F value (2.769). The alternate rationale hints that there is a substantial disparity in the hRf values of distinctive brands of sindoor's samples procured by four established solvent systems. Therefore, the proceeds and statistical evaluation so obtained, proposes that the four solvent systems developed, separates out chemical components of sindoor samples which significantly aids in the identification on the premise of hRf values (table3).

Table3. Statistical Interpretation of the Result

Source of Variation	Sum of Squares (SS)	Degree of Freedom (df)	Mean Squares (MS)	F ratio
Between Treatments	SS _{between} = 17656.8	3	MSB = 5885.6	F = 8.81
Within Treatments	SS _{within} = 37399.7	56	MSW = 667.85	F (3,56) = 2.769

Total	SS _{total} = 50471.53			

Cosmetic products lipsticks, sindoor, nail enamel etc. are frequently encountered as trace evidence at a crime location. Sindoor can be valued as corroborative evidence that serves to prove the *corpus delicti*. Very limited effort has been undertaken on the study of sindoor stains by TLC. Singh, R. and Jasuja, O.P. (2005) analyzed liquid lipsticks by using Thin Layer Chromatography. They worked fifteen samples of liquid lipsticks (five brands) that were studied by using TLC. They tested twenty-one solvent systems for TLC in which two solvent systems toluene: isopropyl alcohol (9:1) and toluene: acetone (9:1), dye contents of liquid lipsticks were isolated. In the toluene: isopropyl alcohol (9:1) solvent system, the segregation was not as distinctive as in the toluene: acetone (9:1) solvent system. They noted that a few spots were conspicuous in daylight examination and none of the additional spots could be seen when the plate was exposed to long as well as short wavelength UV light. But when exposed to iodine fuming, a number of spots were appeared [6].

Chandel R. S. et al. in the year 2013, examined 15 samples of sindoor of diverse brands and found that the solvent system butanol: propanol: water (60:30:10) was strongest for the dissolution of assorted components of sindoor by using TLC [5].

CONCLUSION

In the present research, it is confirmed that Thin Layer Chromatography is one of the best preliminary technique for the comparative investigation of suspected sindoor samples based on the R_f values and the number of spots. Selected sindoor samples showed that all samples can be characterized from each other by employing the accompanying solvent systems-

1. **Methanol:** Acetone
2. **Acetonitrile:** Acetone: Methanol
3. **Acetonitrile:** Methanol
4. **Benzene:** Methanol: Acetic acid

The other solvent system separates only a few brands of sindoor. The TLC plates, when subsequently visualized with the strong daylight gives an excellent partition of constituents of all the samples under analogous experimental conditions. Each sindoor sample revealed a peculiar combination of spots having different R_f values under comparable conditions. The proceeding of Analysis of Variance (ANOVA) obtained, advocates that the chemical components of sindoor samples separates out by the four solvents developed which further boosts in the recognition of the assumption of R_f values.

In the suggested research endeavor, it is determined that if the traces of sindoor, when present on the victim or culprit, can be trialed with the tint of the substantive origin of sindoor by Thin Layer Chromatography using a newly developed solvent. These offer better results as compared to the solvent systems that were being used hitherto. In prospective considerations, the sindoor stains can be investigated by other chromatographic procedures with further samples of sindoor stains.

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