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Comparative Forensic Analysis of Lipsticks Using Thin Layer Chromatography and Gas Chromatography

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***Abstract*—**Lipsticks constitute a significant source of transfer evidence, and can, therefore, provide corroborative or inclusionary evidence in criminal investigation. This study aimed to determine the uniqueness and persistence of different lipstick smears using Thin Layer Chromatography (TLC), and Gas Chromatography with a Flame Ionisation Detector (GC-FID). In this study, we analysed lipstick smears retrieved from tea cups exposed to the environment for up to four weeks. The n-alkane content of each sample was determined using GC-FID, while TLC was used to determine the number of bands, and retention factor of each band per smear. This study shows that TLC gives more consistent results over a 4-week period than GC-FID. It also proposes a maximum exposure time of two weeks for the analysis of lipsticks left in the open using GC-FID. Finally, we conclude that neither TLC nor GC-FID can distinguish lipstick evidence recovered from hypothetical crime scenes.

***Keywords*—**Forensic science, chromatography, identification, lipstick.

I. Introduction

LIPSTICKS are primarily used as beauty products and are intended to add colour and texture to the lips. They often come in a wide range of colours and finishes such as matte, satin and lustre which serve as sunscreens and make the lips more succulent. A large proportion of research bordering on lipstick use aimed to determine the ingredients present in different lipsticks and their potential to cause disease [1]-[4]. Nonetheless, since Edmond Locard first used cosmetics to aid a homicide investigation in 1912, forensic scientists began to probe the usefulness of lipsticks in solving crimes and establishing direct or indirect human identification using lipstick evidence [5].

Lipstick smears constitute a significant type of transfer evidence commonly found at crime scenes, and due to their ease of transfer and prevalent use, they can be recovered from an array of forensic evidence such as clothing or bedding in rape cases, as smears on glasses, cups or cigarette butts or even used tissue paper [6]. The accurate analysis of lipstick smears can, therefore, provide corroborative or inclusionary evidence to aid criminal investigation. The main research approaches adopted in the forensic analysis of lipstick smears either aim to retrieve DNA from lipsticks deposited on surfaces or determine the unique composition of different lipstick brands.

Even though it is possible to obtain full DNA profiles following secondary transfer of lipstick smears [7], conflicting opinions exist on the efficiency of DNA recovery from lipsticks. Reference [8] recorded an 18% success rate in the recovery and profiling of DNA retrieved from lipstick smears, while highlighting DNA degradation, stochastic amplification and PCR inhibition as the major challenges encountered, others have successfully retrieved and profiled DNA from a range of lip products. Even though the artifacts encountered in the later experiment was overcome by additional DNA purification procedures, it is important to note that the success recorded (80%) is largely attributable to the other lip products examined such as lip glosses, balms, pencils and glazes rather than lipsticks [9].

Similarly, conflicting outcomes were also obtained from the chemical analysis of lipstick smears using different chromatographic and spectroscopic methods. For example, while [10] were unable to match different lipstick samples by comparing their red dye pigments using TLC, others have successfully identified different lipstick specimens using TLC [11]. Even though [12] had successfully distinguished 117 lipstick samples by combining TLC, X-ray analysis and High-Performance Liquid Chromatography (HPLC), [13] obtained inconsistent results when worn and non-worn lipstick samples were compared using HPLC.

To date, several analytical approaches such as fourier transform infrared spectroscopy [14], attenuated total reflectance infrared spectroscopy [15], purge-and-trap gas chromatography [16], gas chromatography-mass spectrometry [17], microspectrophotometry [18] and Raman spectroscopy [19] have yielded positive results in the analysis of lipstick smears. These successes have prompted recent proposals for the creation of lipstick databases in order to permit the forensic profiling of unknown lipstick samples [14]. While this approach may seem to be pleasant for the forensic community, the cost of developing method-specific databases and the unpredictability of branded lipstick formulations make this proposal seem like a bridge too far.

Of all the analytical techniques available in literature, Raman spectroscopy proposes very bright prospects as it has a discriminating power of 95%, requires minimal sample preparation and is non-destructive, thus making it an indispensable tool in forensics [19]. In spite of its rich prospects, scientists have had to consider alternative means of analysing lipstick smears as Raman is expensive and thus unavailable in most forensic laboratories. Moreover, Raman Spectroscopic analyses usually require specialised training which most centres may not readily afford.

The present study will, therefore, examine the n-alkane

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content of lipstick smears using GC-FID as it is hoped that this will open new roads to the development of easily accessible and cost-effective methods for the forensic analyses of lipstick stains. Furthermore, since TLC is relatively cheap and readily available in most centres, this study will also explore the likelihood of individualizing lipstick smears using TLC while running a side-by-side comparison with GC-FID. This research is also designed to reappraise the factors (sample size, age, homogeneity, purity and background material) believed to affect the discriminatory power of different chromatographic procedures on lipstick smears [12].

II. Materials and Methods

A. Research Description

This research was designed as a cross-sectional study with purposive approach. Eight lipstick samples (local and foreign) were purchased from a local supermarket in Benin City, Nigeria, and grouped based on their colour, consistency, and manufacturer/brand names. Eight research participants were randomly selected from the University of Benin community. Each volunteer drank from three sets of tea cups after applying the lipstick samples provided.

The original lipstick samples were analysed first, after which the first set of tea cups were swabbed immediately, and analysed accordingly. The second and third sets of cups were left in the open laboratory and labelled appropriately. The lipstick smears on these cups were swabbed after two weeks, and four weeks respectively, then analysed accordingly.

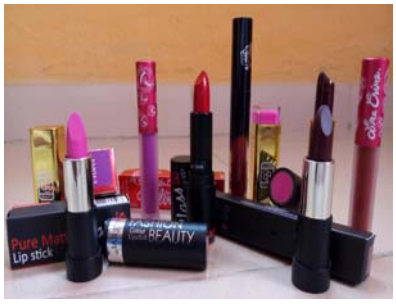

Fig. 1 Lipstick samples analysed in this study

TABLE I
Description of the Lipstick Samples Analysed in this Study

Code Name	Brand	Colour	Consistency
A	Beyond Beauty	Brown	Solid
B	Beyond Beauty	Pink	Solid
C	Fashion Beauty	Brown	Solid
D	Fashion Beauty	Pink	Solid
E	Lime Crime Velvetine	Maroon	Liquid
F	Lime Crime Velvetine	Pink	Liquid
G	Lock fit	Brown	Liquid
H	Pure Matte	Red	Solid

B. Sample Analysis

1. TLC

TLC was performed on 10 x 20 cm standard quality silica gel plates. The lipstick-stained swab sticks were each placed in pre-labelled conical flasks. 5mL of the extracting solution (dichloromethane) was transferred to each flask and mixed vigorously for about 10 minutes to extract the lipstick from the swab sticks. With the aid of a ruler and pencil, a straight line was drawn 1.5 cm from one end of the TLC plate after which the TLC plates were labeled appropriately. Each plate bore four labels, corresponding to the labels on the conical flasks.

A capillary tube was used to place a dot of the lipstick extract just above the penciled line. 20 mL of the mobile phase (acetone, ethanol, ammonium hydroxide, and water in the ratio 5:5:2:1) was transferred into the chromatographic jar. The TLC plate was then carefully inserted into the jar, sample end down. The jar was secured properly, and sample elution was monitored for about 10 minutes. The TLC plates were observed under direct sunlight and iodine vapour.

The *retention factor* for the different bands obtained from each sample was calculated thus:

$$Retention\ factor = \frac{Distance\ Covered\ by\ each\ lipstick\ component}{Distance\ Covered\ by\ the\ Solvent\ front} \quad (1)$$

2. Gas Chromatography (GC-FID)

The stoppered jar to be used for extraction was washed clean and dried with hexane after which the absolute weight of the empty jar was noted. The swabbed lipstick smear was pre-soaked in a stoppered jar containing 20 mL of dichloromethane and agitated vigorously for 10 minutes. The supernatant - organic layer was siphoned into a clean dry beaker. 20 mL of dichloromethane was again added to the stoppered jar and the process of agitation and siphoning repeated. The beaker was kept in a fume cupboard until all the solvent evaporated. The extract was analysed as described below.

3. Column Chromatography (Clean Up)

A clean 20mL column was clamped in a retort stand. The column was then packed with glass wool and depressed to the 2mL mark with a glass rod. Activated silica gel was packed in the column up to the 12 mL mark after which 2 0mL of hexane was run through the column. 2 mL of hexane was then added to the extract and agitated vigorously. The reconstituted extract was poured into the packed column and allowed to sink completely in the column. Elution was achieved by pouring 40mL of hexane through the column. The eluent was collected into a beaker and placed in a fume cupboard until evaporation was complete. The extract was again reconstituted by adding 1 mL of hexane and transferred to GC vials for onward analysis.

Sample analysis was executed using a GC-FID (7820A GC System Agilent) according to the manufacturer's instructions. The J&W column incorporated into the instrument has a length of 30m, diameter of 320 µm, film of 0.25 µm and an injection temperature of 350 ^{0}C. The FID detector has a split less inlet system with a heater temperature of 350 ^{0}C, hydrogen flow of 40 mL/min, air flow of 400 mL/min, makeup flow of nitrogen at 25 mL/min and an equilibration time of 1 minute at 60 ^{0}C. The instrument run time was set to 40 minutes at 330 ^{0}C and a pressure of 12 psi.

C. Data Acquisition and Analysis

For the TLC analysis, the retention factor of each spot per smear (see Fig. 2) was calculated using (1), and the mean of the retention factors for each lipstick per treatment was subjected to further statistical analysis. For the GC-FID data, the concentration of n-alkanes in each lipstick smear per treatment was averaged, and the means were subjected to further statistical analysis. Statistical analysis was done using the Statistical Package for Social Sciences (SPSS) version 21.0 (IBM Inc., USA). The analysis of variance (ANOVA) was used to compare means and results were expressed in mean ± standard error of the mean. A p-value of less than 0.05 ($p < 0.05$) was considered significant.

III. Results

The retention factor of each spot per smear as obtained from the TLC separation was calculated as the ratio of the distance moved by the dye to that moved by the solvent front and the mean value of the retention factors was calculated. The ANOVA analysis was used to determine the degree of variation among the mean retention factors of each smear (see Table 2). The within run comparison of means indicate the degree of variation of the dye content of each lipstick sample over time. The different treatments include the original lipstick sample, direct smear, and the smears obtained after exposure to the environment for two weeks and four weeks. Conversely, the between run comparison of means was used to compare the degree of variation of the mean retention times among the different lipstick samples.

The average concentration of n-alkanes in each lipstick sample per treatment as obtained from the GC-FID analysis was calculated and subjected to further ANOVA analysis (see Table III). The within run comparison of means was used to determine the n-alkane content of each lipstick sample over time while the between run comparison of means was used to compare the difference in the n-alkane content among the different lipstick samples.

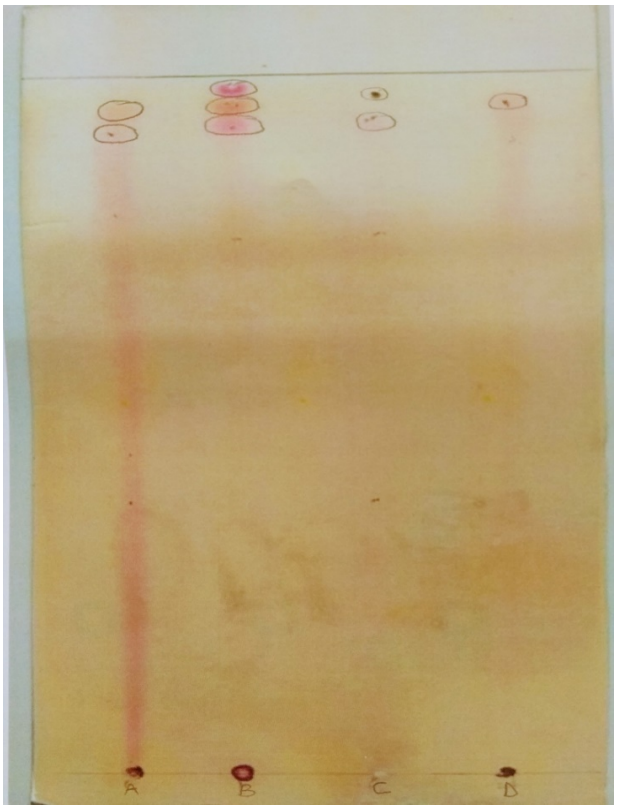

Fig. 2 Thin layer chromatogram (visualized with Iodine fumes) showing the elution of the dyes in some of the lipstick smears analysed in this study after an exposure time of two weeks. Eluted dyes are highlighted in the chromatogram

TABLE II
ANOVA Table Comparing the Means of the Retention Factors of the Dyes in Each Lipstick Sample Per Treatment as Obtained from the Thin Layer Chromatograms. Within Run Mean Comparisons are Shown in the Extreme Right Column, While Between Run Comparisons are Shown in the Bottom Row

Lipstick Sample	Original Sample	1st Smear	2nd Smear (after 2 weeks)	3rd Smear (after 4 weeks)	P-Value
A	0.890[a]	0.795[a]	0.787[a]	0.753[a]	0.88
B	0.540[a]	0.980[a]	0.980[a]	0.955[a]	0.38
C	0.880[a]	0.985[a]	0.970[a]	0.950[a]	0.06
D	0.903[a]	0.908[a]	0.750[a]	0.700[a]	0.43
E	0.774[a]	0.640[a]	0.685[a]	0.710[a]	0.92
F	0.933[a]	0.900[a]	0.943[a]	0.920[a]	0.89
G	0.913[a]	0.937[a]	0.820[a]	0.700[a]	0.38
H	0.920[a]	0.935[a]	0.930[a]	0.930[a]	0.99
P-Value	0.52	0.29	0.63	0.61	

Means in the same columns and rows with similar superscripts are not significantly different (P>0.05)

IV. Discussion

In spite of the rich prospects lipsticks might offer as forensic tools due to their frequent appearance at crime scenes and relative ease of recovery; analysts have had to contend with the choice of analytical technique while putting variables such as repeatability, reproducibility, turn-around-time, and cost into perspective. Furthermore, the heterogeneity of lipstick constituents has made it very difficult for researchers to develop a one-size-fits-all analytical technique. In this study, the individualization and persistence (up to a period of four weeks) of lipstick smears recovered from a hypothetical crime scene were assessed using TLC and GC-FID.

A within-run comparison of the mean *retention factors (rf)* obtained from the analysis of the lipstick samples using TLC showed that there was no significant difference in the number, and retention factors of bands obtained from each sample after each treatment, i.e. original sample, direct smear, smear analysed after a two-week exposure time, and smear analysed after a four-week exposure time. This finding implies that lipstick smears can be detected using TLC up to four weeks after exposure without appreciable changes in the number of dyes detected per time. In the same vein, the between-run analysis of the lipstick smears examined in this study showed no significant difference in the mean retention factors obtained after each stage of the analysis. This outcome, however, indicates that TLC cannot be used to individualize lipstick smears retrieved from hypothetical crime scenes irrespective of the duration of exposure.

These findings are in agreement with those observed by [20] who concluded that analysis of the red pigments present in foreign and local lipsticks contain no unique feature upon which conclusive individualizations can be made. In another study, [21] were only able to group 30% of the lipstick analysed in their work but were unable to differentiate one lipstick sample from another using TLC. On the other hand, [14] suggested that TLC is ideal for the forensic match of cosmetic lip products although their work was limited on the analysis of aged lip gloss samples only which have different compositions when compared with lipsticks. Overall, these outcomes show that lipstick do not contain unique dyes with which they can be differentiated for forensic purposes.

In another study, [12] opined that lipsticks could be identified for forensic purposes if several separation techniques were combined. It was also concluded that the probability of finding two indistinguishable lipsticks after employing a combination of colour comparison, X-ray analysis, TLC, and High-Performance Liquid Chromatography (HPLC) is less than 1/7000. This study, which did not consider the discriminatory power of TLC technique when used alone also suggested that interference from the material bearing the lip stain, as well as the age of the lipstick sample may introduce a significant bias in the authenticity of conclusions reached using TLC. The present study, however, contradicts this assumption as it has shown that age (at least, up to a period of four weeks) does not have any significant effect on the quality of results obtained from lipstick identification using TLC. It also submits that background materials do not affect the results obtained from chromatographic separations of lipsticks using TLC since there was no significant difference in the mean retention factors obtained from the analyses of the original lipstick samples and those lifted off tea cups using sterile swab sticks.

A between-run comparison of the mean concentration of the n-alkanes in the lipstick smears using GC-FID showed a highly significant difference ($P<0.01$) after a two-week exposure period. This finding implies that the n-alkane content of lipsticks will vary appreciably if left in the open for up to two weeks. However, this variation cannot be used as a basis for distinguishing lipsticks using GC-FID since there was no significant difference in the n-alkane concentration of the lipstick smears obtained from the analysis of the original, first and second treatments respectively. The declining concentration of n-alkanes in lipsticks after exposure to the environment is attributable to their volatility.

TABLE III
ANOVA TABLE COMPARING THE MEAN CONCENTRATION OF THE N-ALKANES IN EACH LIPSTICK SAMPLE PER TREATMENT AS OBTAINED USING THE GC-FID. WITHIN RUN MEAN COMPARISONS ARE SHOWN IN THE EXTREME RIGHT COLUMN, WHILE BETWEEN RUN COMPARISONS ARE SHOWN IN THE BOTTOM ROW

Lipstick Sample	Original Sample	1st Smear	2nd Smear (after 2 weeks)	P-Value
A	0.131^{ab}	0.096^{a}	0.185^{b}	***0.03**
B	0.212^{a}	0.168^{a}	0.104^{a}	0.42
C	0.121^{a}	0.105^{a}	0.093^{a}	0.44
D	0.139^{a}	0.179^{a}	0.099^{a}	0.58
E	0.190^{a}	0.137^{a}	0.089^{a}	0.46
F	0.168^{a}	0.168^{a}	0.068^{b}	***0.02**
G	0.118^{a}	0.099^{a}	0.158^{a}	0.14
H	0.145^{b}	0.070^{a}	0.085^{a}	****0.00**
P-Value	0.56	0.63	****0.01**	

Means in the same rows with similar superscripts are not significantly different ($P>0.05$)
*Significant at $P<0.05$
**Highly Significant at $P<0.01$

In the same vein, a within-run comparison revealed a significant difference between the chromatographic peaks obtained for the original sample, first, and second smears for samples A, F, and H. While samples A and H are solid in consistency, F is liquid. The disparity observed in these samples may be as a result of transfer inconsistency as liquid lipsticks are more likely to be retained on the lips of the wearer than solid lipsticks. The within-run inconsistencies observed in this study imply that GC-FID analysis targeting the n-alkane content of lipsticks is a poor marker for the forensic identification of lipsticks. Moreover, markedly diminished peaks were obtained using GC-FID after an exposure time of four weeks. This implies that GC-FID cannot be used to differentiate and/or detect lipstick smears if left in the open for more than two weeks.

Considering the analytical and technical suitability of both methods in the forensic identification of lipstick smears, TLC seems more useful for the detection of lipsticks if exposed for up to four weeks, and perhaps for even longer periods. Furthermore, the measurement of the n-alkane content of

different lipstick brands using GC-FID is a poor diagnostic tool for the forensic identification of lipsticks, especially if they have been left in the open for over two weeks. To sum up, this study shows that neither TLC nor GC-FID guarantees the reliable individualization of lipstick smears for forensic purposes. Even though GC-FID is significantly costlier than TLC for lipstick analysis, it offers no appreciable advantage over TLC in the forensic analysis of lipstick smears.

V. Conclusion

Judging by the data obtained from this research, we, therefore, conclude that TLC gives more consistent results than GC-FID when identical samples are analysed under similar conditions. Despite its significantly higher cost, GC-FID offers no appreciable advantage over TLC in the forensic analysis of lipstick stains. We also show that neither the dye content nor n-alkane composition of lipsticks are reliable markers for the forensic identification of lipsticks.

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