Forensic Discrimination of Lipstick Stains Using Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy and Pattern Recognition Techniques

Mohd Afiq Mohd Azis[1], Naji Arafat Mahat[1,2,3,4*], Hasmerya Maarof[1,2], and Sarah Aina Azman[1]

[1]*Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia (UTM), 81310 Johor Bahru, Johor, Malaysia*

[2]*Investigative and Forensic Sciences Research Group, Universiti Teknologi Malaysia (UTM), 81310 Johor Bahru, Johor, Malaysia*

[3]*Center for Sustainable Nanomaterials, Ibnu Sina Institute for Scientific and Industrial Research, Universiti Teknologi Malaysia (UTM), 81310 Johor Bahru, Johor, Malaysia*

[4]*Center of Research for Fiqh Forensics and Judiciary, Universiti Sains Islam Malaysia, Bandar Baru Nilai, 71800 Nilai, Negeri Sembilan, Malaysia*

**** Corresponding author:***

tel: +60-194464040
email: naji.arafat@utm.my

Received: June 13, 2024
Accepted: July 31, 2024

DOI: 10.22146/ijc.97097

Abstract: *Lipstick stains discovered on objects (such as drinking cups) may be utilized as evidence and/or lead in forensic investigations by chemical analysis. Hence, this study analyzed two brands of lipstick stains' organic composition on paper cups over 0, 24, and 48 h of exposure using attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR), coupled with pattern recognition techniques of principal component analysis (PCA) and linear discriminant analysis (LDA). Spectral analysis revealed several functional group differences between the two brands of lipstick,* viz. *Wardah (W) and Silkygirl (S) stains, particularly in the fingerprint region of 1800–650 cm^{-1}. Application of PCA (variance of ≥ 70%) and LDA successfully provided the suitable means for categorically classifying (a) the different brands of lipstick, (b) the male and female volunteers, as well as (c) the different periods of exposure. The fact that the cross-validation correct classification rates for all the LDA models exceeded 90.0%, their suitability for forensic provenance application of lip stains appears statistically supported. Hence, such an approach to combine the non-destructive ATR-FTIR analysis with PCA and LDA is not only easy and cost-effective but also forensically relevant for enabling the data mining process for forensic intelligence.*

Keywords: *lipstick; ATR-FTIR; principal component analysis; linear discriminant analysis; forensic science*

■ INTRODUCTION

Lipstick is a type of cosmetic typically for women. In this modern age, various types of lipstick are made from trusted sources and are safe to style. However, in the time of Cleopatra, lipsticks made from carmine powder obtained from ants and beetles were popularly used as face decoration [1]. Nowadays, lipsticks are usually made from trusted oils, waxes, and colorants [2]. Lipsticks have been going through constant technological development and advancement to improve their qualities and application. Such improvements have dramatically changed the compositions of cosmetic products [3].

Lipstick traces left on surfaces like paper cups can provide sufficient chemical information that may link a suspect with the victim or crime scene [4]. Modern crime is often changing, modus operandi like using lipstick can prevent oneself from being accused of guilt and being sentenced, and wearing lipstick is also a way to avoid being arrested by the police [5]. Considering its transient nature and the possible alterations of the chemical compositions of lipstick stains over time [3], it is pertinent to be able to categorically classify the different types of lipstick stains on items like drinking cups exposed at varying durations. Despite being class characteristics, the information may help narrow the

search of suspects and convince the court of law to issue a remand before confirmatory identification can be made using DNA profiling and fingerprints.

A review of the literature reveals several studies focusing on harnessing chemical information from stains of lipsticks using chromatography techniques such as gas chromatography-mass spectrometer (GCMS) and high-performance liquid chromatography (HPLC) [3,6]. However, such techniques can be expensive, complicated to run, and destructive. Destruction of trace evidence by the analysis may deprive the right of the accused to have the exhibits re-analyzed by another forensic expert to refute the opinion provided by the prosecutor's expert witness. Hence, the use of non-destructive analytical techniques (e.g., attenuated total reflectance-Fourier transform infrared spectroscopy, ATR-FTIR) in forensic cases appears relevant [7].

Many studies [8] have shown the suitability of the chemical data to be integrated with pattern recognition techniques such as the unsupervised principal component analysis (PCA) and the supervised linear discriminant analysis (LDA). PCA is useful for revealing specific structures, while LDA is for predicting forensic provenance. Previous researchers have reported the high sensitivity of LDA for predicting forensic provenance and suggested their applications in developing forensic intelligence [8]. Hence, continuous evaluation of the techniques by analyzing varying potential exhibits like lipstick stains may further corroborate its practical value in forensic practical casework, a matter of forensic significance. The use of ATR-FTIR would maintain the integrity of the trace evidence, making it easy to operate and cheap, and the chemical data gathered can also be analyzed using PCA and LDA.

The approach may prove helpful for revealing specific patterns in the dataset that are difficult to examine with the naked eye [9], an integral aspect of forensic intelligence. Therefore, the predictive model developed in this study could be of applied value as the first step for developing suitable forensic intelligence before confirmatory identification can be made using individual characteristic means such as DNA profiling. Hence, this present research attempted to classify the different types of lipstick stains exposed to varying durations in the environment, using the spectroscopy data analyzed by PCA and LDA, which merits forensic consideration.

■ EXPERIMENTAL SECTION

Materials

Materials utilized included potassium bromide (KBr) powder (Specac, USA) and acetone (Sigma-Aldrich, Germany), as well as two different lipstick brands *viz.* Wardah Long Lasting Lipstick No. 12 Lustrous Red (PT Paragon Technology and Innovation, Indonesia) abbreviated as Wardah (W) and Silkygirl Powder Matte Lipcolor Lipstick No.4 Maroon) (The Alliance Cosmetics Group, Malaysia) abbreviated as Silkygirl (S).

Instrumentation

The instruments used were the ATR-FTIR spectrometer Frontier (Perkin Elmer, USA) and a data logger for recording temperature and humidity (Elitech, China). As for the statistical analysis, the IBM SPSS version 26 (IBM Corp., USA) and Minitab version 19.1 (Minitab LLC, USA) were utilized.

Procedure

Experimental design and sample deposition

The ethical approval (USIM/JKEP/2022-2025) for the present research was obtained from the Universiti Sains Islam Malaysia. This study involved analyzing the functional groups of partial lipstick stains from the lower lips of two volunteers (one male and one female) formed by two different lipstick brands, *viz.* W and S are of a similar color (red) on paper cups. Based on the statements made by merchants at Taman Universiti, Johor Bahru, Johor Malaysia, these two lipstick brands were among their best sellers. The inclusion criteria for recruiting the two volunteers were: (a) non-smoking healthy individuals, (b) within the age range of 20 to 25 years old, and (c) with no injury on their upper and lower lips. The opposite conditions were used as the exclusion criteria. This present research did not utilize the upper lip of the mouth, considering that under normal circumstances, any stains produced by it may be diluted by the liquid contained in the cup, diminishing its evidential value. The stains were exposed to the laboratory condition and

were analyzed using ATR-FTIR spectroscopy. Paper cups were utilized to mimic the crime scene situation whereby they were used for drinking by suspects and/or victims, leaving partial lipstick stains on the said objects. A data logger (Elitech, China) was placed in the laboratory to record the hourly temperature and relative humidity to which each stain on each paper cup was exposed.

To study the chemical decomposition of the lipstick stains, three different intervals (0, 24 and 48 h) were used, mimicking the real cases whereby the evidence found by the police will not be taken at 0 h; instead, it will exceed the period of the crime. Stains produced directly from the two lipsticks on paper cups (without the involvement of human lips) were used as the positive controls. On the other hand, small portions of fresh lipsticks were crushed with KBr into fine powders and served as the negative controls. Subsequently, the obtained spectral data were analyzed using the unsupervised PCA and the supervised LDA techniques to study the organization of the dataset and provide a prediction model.

For depositing a stain, each lipstick was freshly applied onto the cleaned lower lip of a volunteer prior to directly placing it at the outer part of a paper cup (within 2 s after the application on the lip), mimicking the natural movement of the lower lip during drinking. To produce the positive control stains, the lipsticks were directly applied on paper cups (length: about 2 cm). As for the negative control, a small portion of each lipstick was ground with KBr (sample:KBr of 100:1) into a powdery form using pastel and mortar. The powder from the mortar was then pressed into a 13 mm disc form using hydraulic pressure. The disc sample was then placed into a disc holder with a rectangular mount, directly placed into the ATR-FTIR, and analyzed.

Analysis of lipstick stains using ATR-FTIR

For studying the FTIR profiles of the lipstick stains from the two brands, triplicate scans on the wavenumber ranging from 4000–650 cm^{-1} were performed on the positive and negative controls as well as the left, right, and middle spots of lipstick stain samples placed at the outer portion of the paper cups. Analyzing the three spots of lip stain on the paper cup would provide the average of the spectrum. The data provided by previous researchers [5,7,10] were utilized to discuss the presence of functional groups and their wave numbers.

The portion of the cup that contained a lipstick stain was cut (5 × 3 cm) to enable the ATR-FTIR analysis. While cutting the paper cup was improvised in this present research, the condition and the techniques of the FTIR analysis were described by previous researchers [11], detailed below. Once the sample spectrum was recorded, the anvil was moved up and the sample was removed. The ATR crystal and anvil surface were cleaned using acetone and wipes before and after each analysis to remove the remaining smudge from the ATR crystal. The acquisition of IR spectra for the lipstick stains was performed using a ATR-FTIR spectrometer equipped with a zinc selenide (ZnSe) crystal ATR accessory and deuterated triglycine sulfate (DTGS) detector interfaced to a desktop equipped with the Spectrum 10 software. The background spectrum was run before the analysis of each sample was performed. The selected areas on the stains were scanned at 4 cm^{-1} (20 accumulations/sample) within the range of 4000 to 650 cm^{-1}. The spectral data were then utilized for the PCA and LDA assessments.

Pattern recognition technique

The entire dataset was examined to generically separate the lipstick stains according to gender (male and female), brands (W and S) and their exposure periods (0, 24 and 48 h), resulting in multiple layers of PCA and LDA for better provenance resolution. PCA and LDA were performed using the Minitab version 19 (Minitab Inc. Minitab Incorporated, Pennsylvania, USA) statistical software. For data preparation, a Microsoft Excel spreadsheet (Microsoft Corporation, Washington, USA) was used to preprocess the data through standardization before they were imported into the Minitab environment. A combination of PCA and LDA is very beneficial since it increases classification efficiency by simply recognizing the most important characteristics of the classification model [9].

Principal component analysis

Using PCA, suitable information was extracted from the spectral data (fingerprint region: 1500–650 cm^{-1}) of the lipstick stains (left, middle and right

sides), utilizing only selected few principal components (PCs) for explaining the organization of the dataset. The fingerprint region was chosen for the PCA and LDA because it showed higher variability in the functional groups than in the other part of the spectrum. Considering that this present research was aimed at differentiating/classifying the stains produced by the two lipstick brands at varying durations, using the most variable region of the spectrum may offer such advantages. Considering the inclusion of several PCs that carried more than 70% of variance has been proposed as suitable for representing the dataset in reduced dimension [8], the three PCs that accounted for 98.3% of the variation in the dataset observed were utilized for building up the PCA model. The score plot and loading plot describing the relationship between samples and variables were also obtained.

Linear discrimination analysis

Upon completion of PCA, the supervised method of LDA was performed for each dataset to classify the different groups of lipstick stains into one of several user-defined groups. By calculating the linear discriminant function, LDA creates a model that would correctly classify objects within a given dataset. By employing the cross-validation of the "leave-one-out" technique, the reliability of a classification model as a classifier for a given dataset can be developed by assessing the percentage of correct classification [8].

■ RESULTS AND DISCUSSION

Identification of Organic Composition of the Two Brands of Lipstick Stains on Paper Cups over Varying Periods of Exposure

Many forensically driven lipstick studies utilized destructive analytical instruments like GCMS and HPLC to study their chemical composition [12], which are not favorable to use if the amount of trace evidence is minute and transient [13]. Therefore, the use of non-destructive analytical instrumentation like the ATR-FTIR for chemical profiling, attempted here, merits forensic consideration. The whole study yielded a total of 337 ATR-FTIR spectra from 113 specimens (Table 1).

Since temperature and relative humidity may affect the chemical compositions of cosmetics such as lipstick, resulting in the loss of identity [14], such data in the laboratory were recorded and tabulated in Table 2. The temperature and relative humidity in the laboratory

Table 1. Details of specimens analyzed and the total number of ATR-FTIR spectra

No.	Specimens analyzed	Number of specimens	Number of spectra
1	Paper cup only (outer portion opposite its midline)	1	1
2	W lipstick only (crushed) as the negative control	1	3
3	S lipstick only (crushed) as the negative control	1	3
4	Lipstick stains of W on paper cups-without lips as the positive control	1	3
5	Lipstick stains of S on paper cups-without lips as the positive control	1	3
6	Lipstick stains of W on paper cups from lower lips (left, middle and right) at 0 h from both volunteers	18	54
7	Lipstick stains of S on paper cups from lower lips (left, middle and right) at 0 h from both volunteers	18	54
8	Lipstick stains of W on paper cups from lower lips (left, middle and right) at 24 h from both volunteers	18	54
9	Lipstick stains of S on paper cups from lower lips (left, middle and right) at 24 h from both volunteers	18	54
10	Lipstick stains of W on paper cups from lower lips (left, middle and right) at 48 h from both volunteers	18	54
11	Lipstick stains of S on paper cups from lower lips (left, middle and right) at 48 h from both volunteers	18	54
	TOTAL	113	337

Table 2. Temperature and relative humidity in the laboratory where the lipstick stain was exposed

Exposure periods	Temperature (°C)	Relative humidity (%)
0 h (measurement at one instance)	30.30	75.80
24 h (continuous)	30.01 ± 1.32	74.56 ± 3.19
	(28.60–33.00)	(66.40–77.70)
48 h (continuous)	29.30 ± 1.34	76.35 ± 2.99
	(27.00–33.00)	(66.40–79.60)

Temperature and relative humidity data were recorded *in situ* using a data logger and expressed as mean ± standard deviation (range). While the parameters were recorded continuously for the 24 and 48 h of exposure, the same was recorded once during the time of sampling for the lipstick stains at 0 h of exposure

during 48 h of observation were observed to be between 27.00–33.00 °C and 66.40–77.70%, respectively. These temperature and relative humidity data (Table 2) appear consistent with the data for indoor office rooms with various external shading devices in Malaysia reported by previous researchers [15].

A review of the literature reveals that majority of the previous forensic studies on lipstick stains (a) involved only one gender [5,16] and/or did not even mention the gender of volunteer [4], (b) analyzed directly the chemical compositions of lipstick alone [5], (c) and/or the stains on substrates [4], and (d) did not compare the spectral data among the different groups of lipsticks and/or stains [4]. Moreover, (e) the specific study focusing on variations of the chemical composition of lipsticks and their corresponding lip stains over different periods of exposure in the environments, (f) especially that of tropical countries like Malaysia, remains unreported. Hence, this present research utilized lip stains deposited by both the male and female volunteers on paper cups, as well as attempted to compare the spectral data between the two brands over varying periods of exposure in indoor conditions before performing the PCA and LDA, which appears to fit into the gap of knowledge. Because review of the literature does not reveal any specific data concerning the specific brands and models of lipstick analyzed here, a suitable discussion for comparing the spectral data with the literature could not be attempted.

The comparison of the classes of chemical groups identified at the observed peaks of crushed specimens of lipstick is provided in Table 3. Results revealed that both lipstick brands had similar three peaks within the region of 2950–2850 cm^{-1} of the spectra, representing the O–H (alcohol), N–H (amine), and C–H (alkane) stretching. These functional groups are associated with the typical components of surfactants as well as pH adjusters (e.g. triethanolamine, diethanolamine, and ethanolamine) for cosmetics products like lipsticks [17].

While peaks for C=O stretching (esters or ketones), C–H stretching (methylene group) and C–O stretching (secondary alcohol) were observed at 1737, 1463, and 1110 cm^{-1} for the crushed W, the same remained absent for the crushed S specimens. On the contrary, peaks for C–N stretching (aliphatic amines group) (1260 cm^{-1}), S=O stretching (mica compound) (1063 cm^{-1}), C=C bending (trisubstituted alkene) (843 cm^{-1}) and C=C bending (disubstituted alkene) (802 cm^{-1}) observed for the crushed S were absent in the crushed W specimens. Hence, utilizing the ATR-FTIR spectra data appears to facilitate discrimination of these two brands of lipsticks (Table 3).

Organic compositions of the different lipstick brands and their stains

The comparisons of classes of chemical groups in the data are presented in Table 3. The plain paper cup contained N–H stretching (amine group) (2916 cm^{-1}), C–H stretching (alkane group) (2849 cm^{-1}), C–H stretching (methylene group) (1464 cm^{-1}) and CH_2 rocking mode (alkane group) (720 cm^{-1}). While all the functional groups observed for the plain paper cup also prevailed for the stains produced by W on the same object, three additional peaks were observed for the latter. These additional peaks were observed at 1738, 1168 and 807 cm^{-1}, representing C=O stretching (esters or ketones), C–O stretching (aromatic compound) and C=C bending

Table 3. Classes of functional groups identified at the observed peak of a plain paper cup, Wardah (W) (crushed and stains), and Silkygirl (S) (crushed and stains)

Presence of possible chemical groups	Specimens analyzed and the wavenumbers (cm^{-1})				
	Plain paper cup	Wardah (W)		Silkygirl (S)	
		Crushed	Stains	Crushed	Stains
O–H stretching (alcohol)	**Absence**	2954	**Absence**	2959	**Absence**
N-H stretching (amine group)	2916	2918	2916	2918	2917
C–H stretching (alkane group)	2849	2849	2849	2849	2849
C=O stretching (esters or ketones)	**Absence**	**1737** *	**1738** *	**Absence**	**Absence**
C–H stretching (methylene group)	1464	**1463** *	1464	**Absence**	1463
C–N stretching (aliphatic amines group)	**Absence**	**Absence**	**Absence**	**1260** ▲	**Absence**
C–O stretching (aromatic compound)	**Absence**	1171	**1168** *	1132	**Absence**
S=O stretching (mica compound)	**Absence**	**Absence**	**Absence**	**1063** ▲	**1034** ▲
C–O stretching (secondary alcohol)	**Absence**	**1110** *	**Absence**	**Absence**	**Absence**
C=C bending (trisubstituted alkene)	**Absence**	**Absence**	**Absence**	843	**842** ▲
C=C bending (disubstituted alkene)	**Absence**	**Absence**	807	802	798
CH_2 rocking mode (alkane group)	720	**Absence**	720	**Absence**	717

Identification of chemical functional groups was made using the data by [5,7,10]

* denotes the specific peaks for W; ▲ denotes the specific peaks for S

(disubstituted alkene), respectively (Table 3).

In comparison to the peaks observed for the paper cup, three additional peaks (1034, 842, and 798 cm^{-1}) were observed for the S stains that corresponded with the presence of S=O stretching (mica compound), C=C bending (trisubstituted alkene) and C=C bending (disubstituted alkene), correspondingly. Interestingly, analysis of the ATR-FTIR spectra further revealed peaks specific for the individual lipstick stains of W and S that may enable forensic classification. While three specific peaks can be assigned for W stains, two specific peaks can be identified for S stains. The three peaks for W were C=O stretching (esters or ketones) (1738 cm^{-1}), C–O stretching (aromatic compound) (1168 cm^{-1}) S=O stretching (mica compound) (1089 cm^{-1}). As for S, the two peaks were S=O stretching (mica compound) (1034 cm^{-1}), and C=C bending (trisubstituted alkene) (842 cm^{-1}). Hence, the application of ATR-FTIR for differentiating the two lipstick stains on the paper cup proves to be empirically supported (Table 3).

Variations of organic composition for the different lipstick stains over periods of exposure and gender

Fig. 1 represents the ATR-FTIR spectra for the W stain (alone) and its lip stains on paper cups (at 0, 24, and 48 h) produced by the male and female volunteers, respectively. In addition, comparisons of chemical groups (identified at observed peaks) for the W and S lip stains on such paper cups are provided in Table 4. Results revealed that the W and lip stains from male and female volunteers exposed to the laboratory condition at 0, 24, and 48 h shared the same chemical groups with similar peaks. The chemical groups were N–H stretching (amine), C–H stretching (alkane), C=O stretching (esters or ketones), C–H stretching (methylene), C–O stretching (aromatic), and CH_2 rocking mode (alkane). Notwithstanding, while C=C bending (disubstituted alkene) was observed at 807 cm^{-1} in the W stain (alone), the same was not detected in any of the lip stains produced by W from both the male and female volunteers exposed at 0, 24, and 48 h (Fig. 1 and Table 4).

A similar pattern prevailed for the S and lip stains from the male and female volunteers exposed to the same laboratory condition for 0, 24, and 48 h. Interestingly, the discriminating presence of the N–H bending (amide group) in the lip stains produced by the female volunteer was observed at 0 (753 cm^{-1}), 24 (757 cm^{-1}), and 48 (753 cm^{-1}) h of exposures. As for the

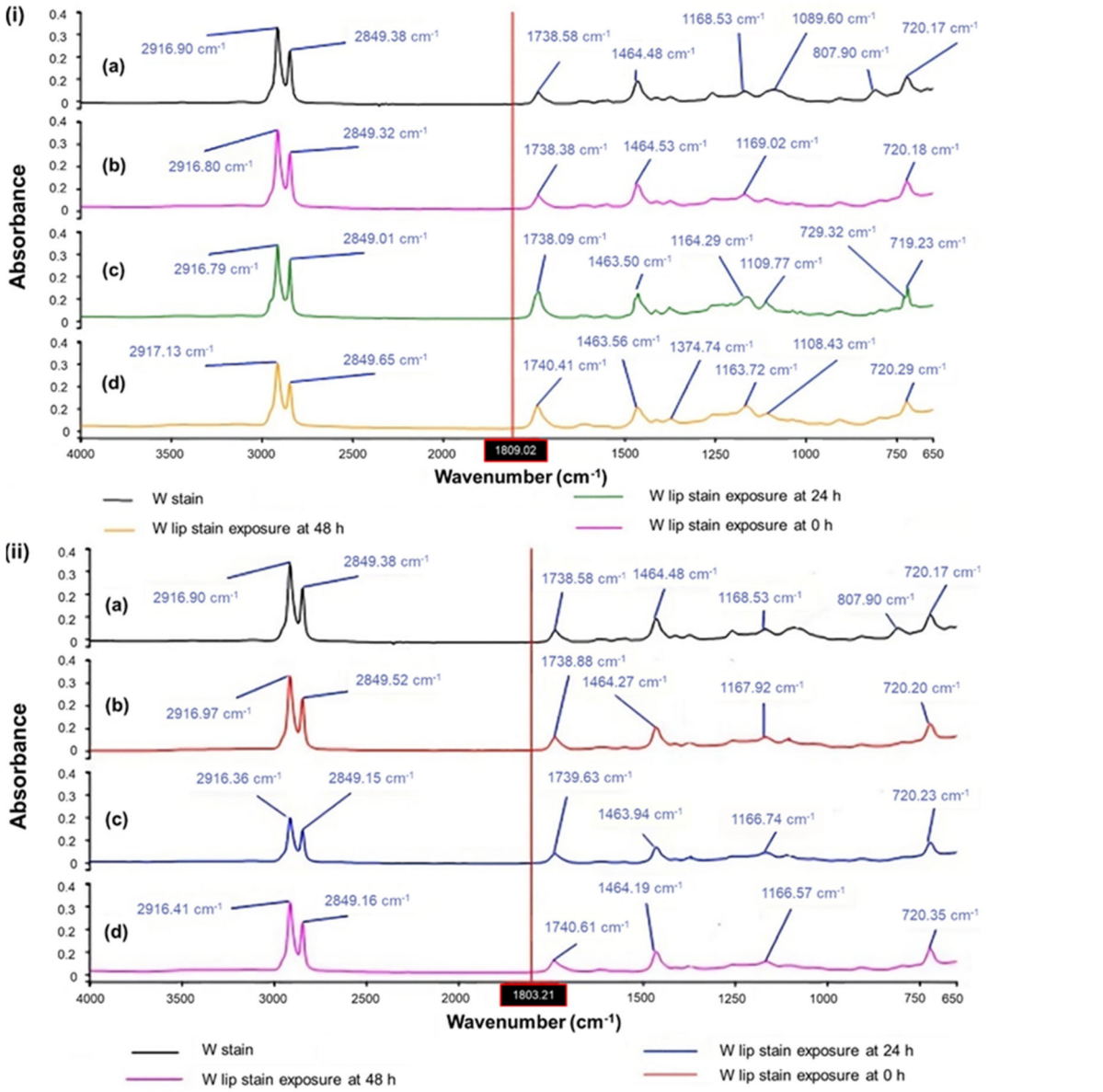

Fig 1. ATR-FTIR spectra of the (a) W stain (alone) as well as its lip stains produced by (i) male and (ii) female volunteers on the paper cup exposed to the laboratory condition (temperature: 27.00–33.00 °C; relative humidity: 66.40–79.60%) at (b) 0, (c) 24 and (d) 48 h of exposures

male volunteer, the N–H bending (amide group) was observed at 24 (755 cm^{-1}) and 48 (753 cm^{-1}) h only (Table 4). Such variations in chemical groups observed between the lipstick stains and those of lip stains may be attributable to the possible differences in skin pH, physiological factors, and activities, as well as hormonal fluctuations, following the application of lipsticks on the lips.

Because empirical studies focusing on such aspects remain unreported in the body of literature, specific attempts to address these knowledge gaps appear scientifically and forensically relevant. Although small variations can be seen in the lip stains between the male and female volunteers (especially for the S) and at certain durations of exposure, the data may not sufficiently support its discriminatory ability, considering the small

Table 4. Classes of functional groups were identified at the observed peak of W and S stain (alone), and lip stains were produced by male and female volunteers exposed to paper cups at 0, 24, and 48 h

Presence of possible chemical group	Specimens analyzed and the wavenumbers (cm^{-1})							
	W stain (alone)	Lip stain exposure durations (h)			S stain (alone)	Lip stain exposure durations (h)		
		0	24	48		0	24	48
N–H stretching (amine group)	2916	2916 (2916)	2916 (2916)	2917 (2916)	2917	2916 (2916)	2916 (2915)	2917 (2917)
C–H stretching (alkane group)	2849	2849 (2849)	2849 (2849)	2849 (2849)	2849	2848 (2848)	2849 (2846)	2849 (2849)
C=O stretching (esters or ketones)	1738	1738 (1738)	1738 (1739)	1740 (1740)	**Absence**	**Absence (Absence)**	**Absence (Absence)**	**Absence (Absence)**
C–H stretching (methylene group)	1464	1464 (1464)	1463 (1463)	1463 (1464)	1463	1464 (1462)	1464 (1466)	1464 (1465)
C–N stretching (aliphatic amines)	**Absence**	**Absence (Absence)**	**Absence (Absence)**	**Absence (Absence)**	1256	1259 (1256)	1254 (1254)	1256 (1256)
C–O stretching (aromatic compound)	1168	1169 (1167)	1164 (1166)	1163 (1166)	1034	1048 (1036)	1038 (1037)	1037 (1040)
C=C bending (trisubstituted alkene)	**Absence**	**Absence (Absence)**	**Absence (Absence)**	**Absence (Absence)**	842	842 (841)	841 (845)	841 (842)
C=C bending (disubstituted alkene)	807	**Absence (Absence)**	**Absence (Absence)**	**Absence (Absence)**	798	801 (800)	799 (807)	800 (801)
N–H bending (amide group)	**Absence**	**Absence (Absence)**	**Absence (Absence)**	**Absence (Absence)**	**Absence**	**Absence (753) ▲**	**755 * (757) ***	**753 * (753) ***
CH_2 rocking mode (alkane group)	720	720 (720)	719 (720)	720 (720)	717	718 (697)	717 (700)	697 (717)

Identification of chemical functional groups was made using the data by [5,7,10]. While values without parentheses represent the male volunteer, the ones in parentheses () represent the female volunteer

* denotes specific peaks for male; ▲denotes specific peaks for female

sample size of volunteers. In this context, studies covering the different types of specimens reported improved forensic provenance discriminatory ability with the use of PCA and LDA [8,16,18]. As such, these pattern recognition techniques were used to classify the different lipstick stains.

Overall Approach of PCA and LDA

Since ATR-FTIR analysis of lipsticks and their respective lip stains would produce a substantial number of variables, the underlying patterns within the dataset may be challenging to observe. This has led many researchers to opt for the use of pattern recognition techniques such as PCA and LDA in the forensic provenance analysis of lipsticks and their corresponding lip stains [4,7]. In this regard, many researchers have suggested the integration of spectral data from the fingerprint region (1800–650 cm^{-1}) into the PCA and LDA environments [8,18]. This is because such a fingerprint region consists of the largest number of variations that can be assigned to specific groups [16,19-20].

Being the unsupervised method, PCA would prove useful for reducing large datasets into more manageable PCs to understand the organization of the overall dataset [20]. In this context, researchers have suggested the inclusion of two to three PCs that are accountable for at least 70% of variations as representative of the dataset in a much-reduced dimension [8]. Considering that the variance for the two PCs analyzed for all the PCA in this research exceeded that of 90%, the utilization of two PCs for constructing the 2-dimensional score plots appears statically supported. As the supervised method, LDA has been shown to discriminate the different types of products/items successfully, and as such, its utilization for forensic intelligence has been suggested [18]. Hence,

the discriminant models were constructed using LDA to classify the datasets into the different lipstick brands, genders, brands, and periods of exposure in this present research using the iterative approach. For accepting the models, LDA cross-validation correct classification rates of ≥ 90.0% were considered [8].

Organization of the Dataset and Discrimination of the Two Lipstick Brands

Fig. 2(a) represents the two-dimensional (2D) PCA score plot for the two lipstick brands using the first two PCs. The fact that the first two PCs were responsible for 95.5% of the variations in the dataset (PC1 = 52.0%; PC2 = 43.5%), their utilization for constructing the 2D PCA score plots appears appropriate. Results revealed that the PCA model was able to exclusively separate the S lipstick from that of the W. While most of the data for W can be correctly classified into its larger group, several data ramified into a sub-group, necessitating the use of LDA for discriminating these two lipstick brands.

The 2D LDA plot for the two lipstick brands using a discriminant function is presented in Fig. 2(b). As can be seen, the application of the LDA markedly improved the groupings of the W and S lipsticks compared to PCA, offering the LDA cross-validation correct classifications rate of 99.0%. Such value of correct classification rate fell well within the acceptable rate of 90% prescribed by previous researchers [8]. In addition, the results were also consistent with those reported by earlier researchers [7,16] that discriminated against the different brands of lipstick with similar color, ranging between 96 to 100% correct classification rates. This is attributable to the explanatory nature of PCA, which deals with the organization of the data, unlike the classificatory nature of LDA which would permit suitable prediction models for forensic provenance [8].

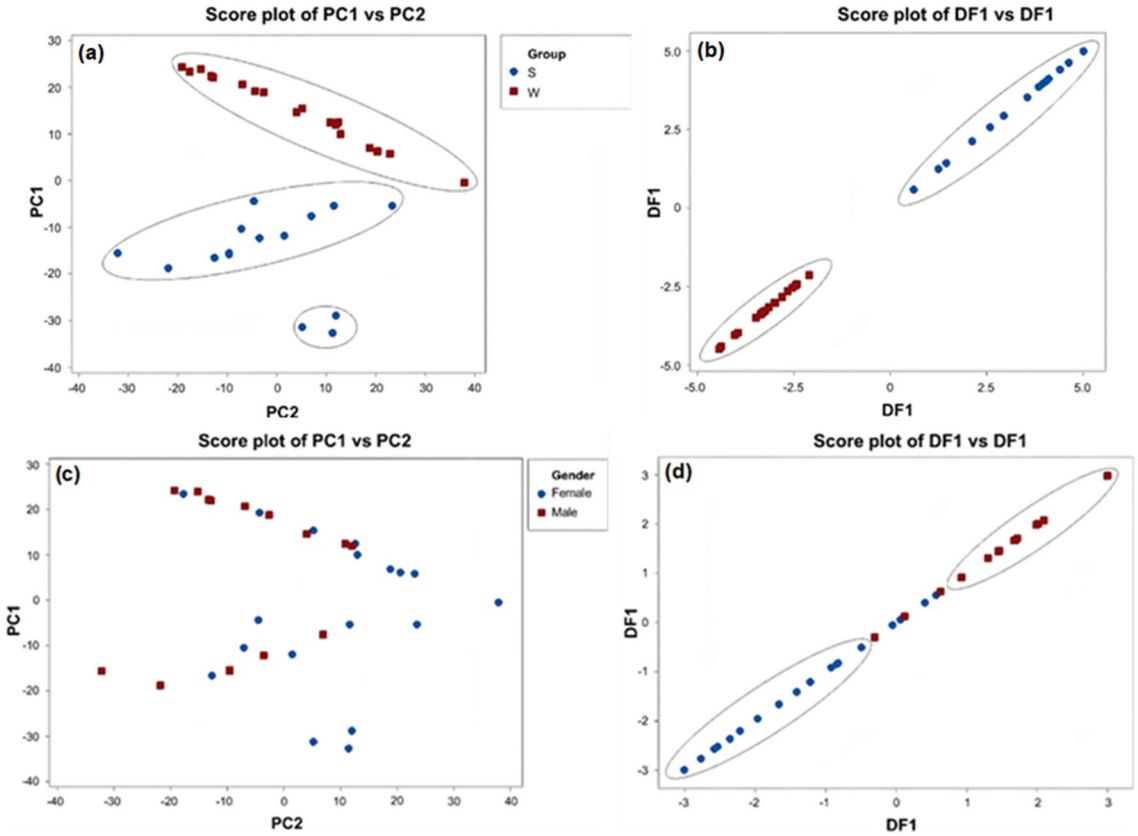

Fig 2. The 2D PCA and LDA score plots for two lipstick brands using the (a) first two PCs (variance: 95.5%) and (b) two discriminant functions (correct classification: 99.0%), as well as for the gender of volunteers using the (c) first two PCs (variance: 95.4%) and (d) two discriminant functions (correct classification: 91.0%)

Organization of the Dataset and Discrimination of Gender

Identification of suspects and victims is pivotal in forensic investigations [13], and in most cases, secondary identification through class characteristic evidence (e.g. male or female) is needed to narrow down the search before performing confirmatory identification using DNA profiling and fingerprints. Therefore, developing an empirical approach for statistically discriminating lip prints recovered from crime scenes into male or female is paramount, and such an aspect was evaluated in this present research. Despite its importance in forensic investigation, a specific study focusing on discriminating genders from lip stains has not been reported in the literature. Fig. 2(c) and 2(d) depict the 2D PCA score plot for the gender of volunteers using the first two PCs and the 2D LDA plot for the gender of volunteers using two discriminant functions, respectively.

It was observed that the first two PCs corresponded with 95.4% variance in the dataset, with PC1 and PC2 representing 51.3 and 44.1%, respectively. Despite the large variance, the 2D PCA model did not appear to provide clear grouping between the genders, with many parts of the data intermingling. One suitable explanation for this phenomenon is that the lip prints on the paper cups were made almost instantaneously after the lipstick was applied to the lips. Such a short interval between the application of lipstick and its lip stain deposition on the paper cup may not render sufficient time for the biochemical changes on the lips to take place. Hence, this situation may result in high similarities in the chemical composition of the lip prints between male and female volunteers, justifying the use of the more sensitive supervised method of LDA for discriminating the gender. The 2D LDA plot for the gender of volunteers revealed two general groups i.e., male and female (cross-validation correct classifications rate of 91.0%), with several misclassified female data. Although the 2D LDA model is acceptable with cross-validation correct classification rate ≥ 90% [8], one possible way to improve the LDA model for discriminating gender is by increasing the sample size, an avenue for further studies.

Organization of the Dataset and Discrimination of Exposure Periods Based on Brands and Gender

One of the key elements in forensic investigations is to prove or disprove an alibi claimed by a suspect, placing him or her at the crime scene when the crime was committed [13]. To do that, suitable means must be developed to provide statistical support for classifying whether an object can be considered an exhibit or simply an artifact to the crime scene. Because the varying pH levels of the skin, constituents of sweat glands and moisture, as well as the assemblage of skin microbiomes, could affect the interaction of lipstick components and its degradation patterns, future research emphasizing these issues should be considered. Moreover, a literature review does not reveal any specific studies emphasizing variations in ATR-FTIR spectra of lip stains over varying periods of exposure analyzed using pattern recognition techniques such as PCA and LDA. Considering the sensitivity and accuracy of PCA and LDA for organizing and discriminating complex chemical data among various forensically driven studies [8,18], it is worthwhile to explore if the approach can be used to detect variability in the chemical composition over a short period of exposure (e.g., 0, 24 and 48 h). Should the approach for dating relatively short periods of exposure be feasible, advocating its usefulness for longer periods of exposure is logically supported, and verification of such a proposition may form an interesting future study.

The male volunteer's PCA and LDA score plots are represented in Fig. 3(a) and 3(b), respectively. The same is true for the female volunteers (Fig. 3(c) and 3(d)). The result of the 2D PCA score plot for W lip stains exposed at varying durations for male volunteers (Fig. 3(a)) revealed a total variance of 96.6%, which fell well within the suggested values of ≥ 70% by Shadan et al. [8]. It can be seen from the score plot that the PCA model for male volunteers successfully organized the dataset into the appropriate three groups, *viz.* 0, 24, and 48 h of exposure. Expectedly, the application of LDA resulted in the same grouping as demonstrated by the PCA model with a cross-validation correct classification rate of 99.0%. As for the female volunteer, the 2D PCA score plot for

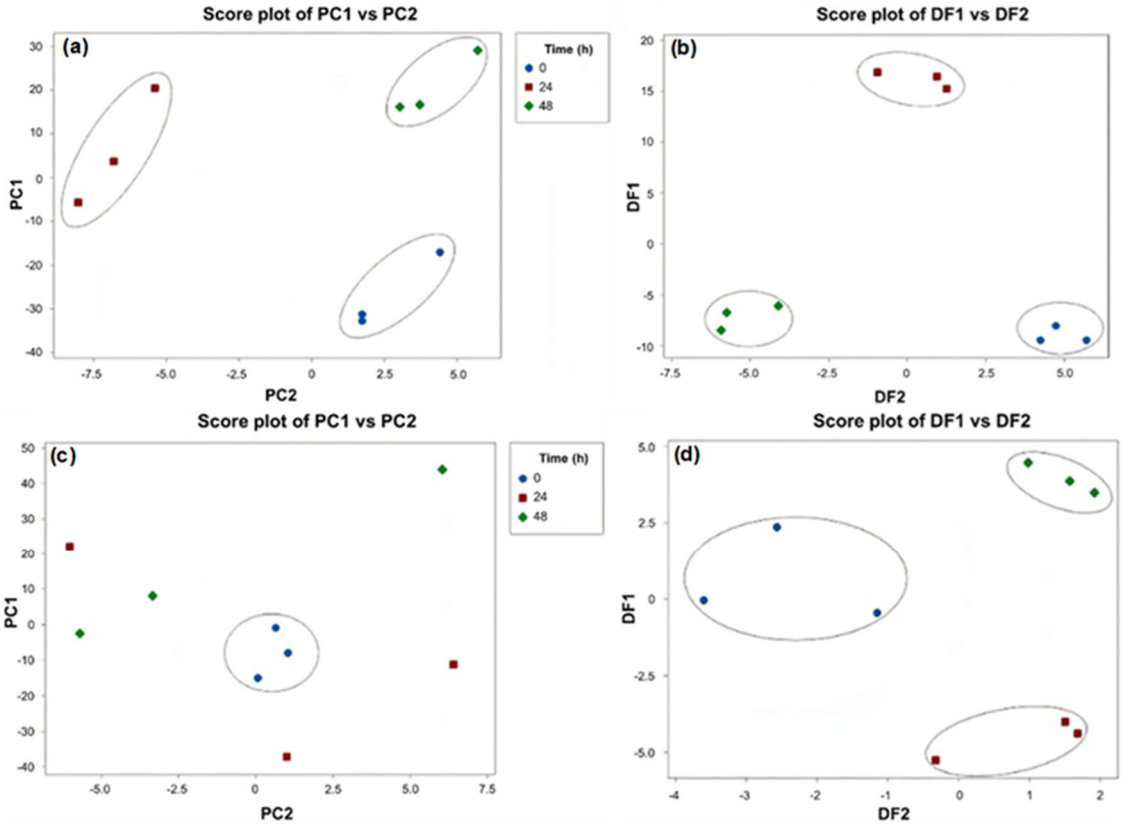

Fig 3. The 2D PCA and LDA score plots for W lip stains exposed at varying durations for the male volunteer using the (a) first two PCs (variance: 96.6%) and (b) first two discriminant functions (correct classification: 99.0%), as well as for the female volunteer using the (c) first two PCs (variance: 97.6%) and (d) first two discriminant functions (correct classification: 99.0%)

W lip stains exposed at varying durations was found to demonstrate 97.6% of the total variance. Despite such a high total variance for the two first PCs, the PCA model only managed to correctly organize the data for 0–h exposure within the same group; the same was not the case for the remaining two periods of exposure (24 and 48 h). However, the subsequent analysis using the 2D LDA successfully discriminated the three periods of exposure for the lip stains produced by W in the female volunteer. This finding corroborated sufficiently with the fact that the incorporation of LDA has resulted in better forensic provenance classification, as reported by previous researchers on other materials [8].

While correspondingly Fig. 4(a) and 4(b) depict the PCA and LDA score plots for male volunteers, the same for female volunteers are presented in Fig. 4(c) and 4(d). The 2D PCA score plot for S lip stains exposed at varying durations for the male volunteer had 92.8% of the total variance from the first two PCs. Similar to the observation recorded for the lip stains produced by the W lipstick, at varying periods of exposure by the female volunteer, the relatively high total variance did not translate into a proper organization of the dataset by the PCA model. Only with the use of the LDA model can categorical discrimination of the S lip stains by the male volunteer at varying periods of exposure be successfully made (Fig. 4(b)). As for the female volunteer, the 2D score plot of PCA for S lip stains successfully organized the dataset into the three respective periods of exposure with a total variance of 95.3% for the first two PCs (Fig. 4(c)). The subsequent utilization of LDA revealed the same groupings as that of PCA with a cross-validation correct classification rate of 95.3% (Fig. 4(d)). Considering the successful discrimination of lip stains produced by the two brands, exposed to varying periods of exposure in the laboratory, it can be construed that this

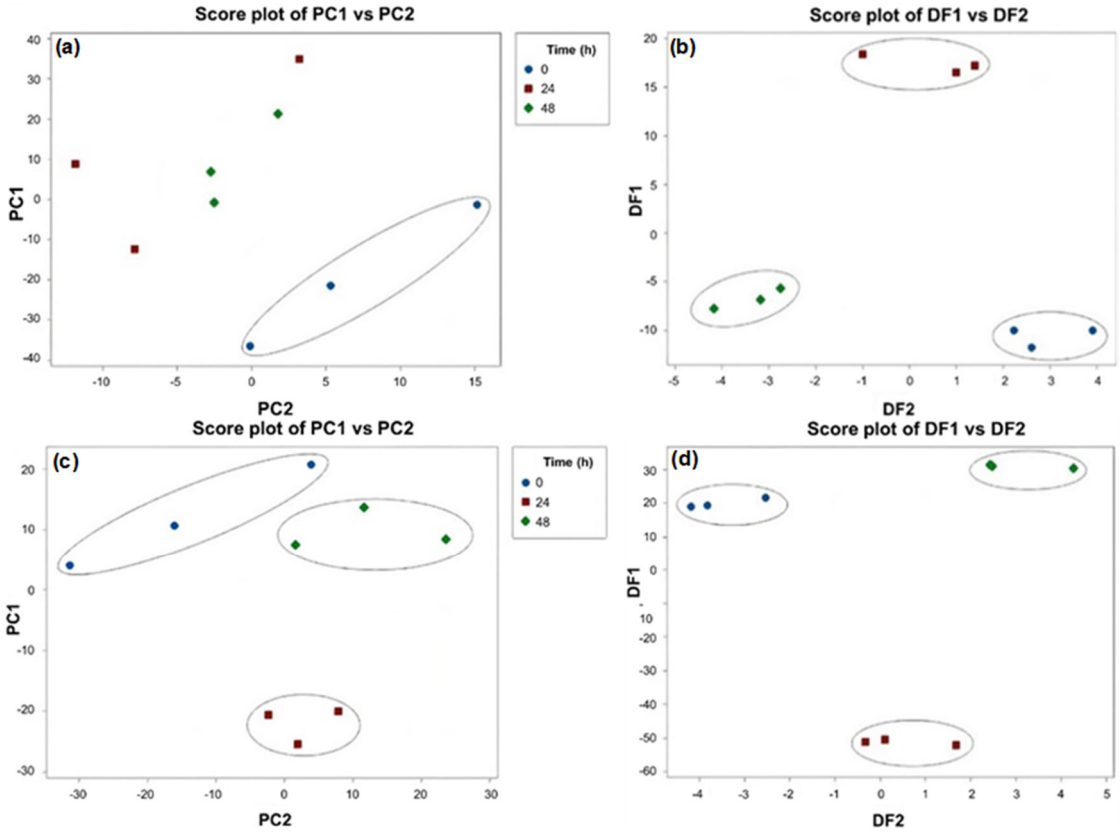

Fig 4. The two-dimensional PCA and LDA score plots for S lip stains exposed at varying durations for the male volunteer using the (a) first two PCs (variance: 92.8%) and (b) first two discriminant functions (correct classification: 98.5%), as well as for the female volunteer using the (c) first two PCs (variance: 95.3%) and (d) first two discriminant functions (correct classification: 98.4%)

piece of approach that combined the ATR-FTIR spectra with that of PCA and LDA may be a feasible strategy for dating the forensic evidence to prove or disprove one's alibi. A larger sample size and volunteers prove necessary to further elucidate this approach's real practical value, including varying lipsticks.

Limitation

This present research utilized only two brands of lipstick, analyzed in triplicates. Therefore, incorporating (a) other varying brands of lipstick, (b) a higher number of replicates for each brand, (c) a larger number of volunteers, (d) with differing age groups, (e) dietary patterns, and (f) medical conditions may prove forensically relevant for developing more robust forensic intelligence, especially for exploring the effect of gender. These aspects shall be considered for future research with a similar context.

■ CONCLUSION

This present research successfully identified the functional groups of the two brands of lipstick stains on paper cups exposed to 0, 24, and 48 h in laboratory conditions. Results revealed that W lip stains contained stretching signals of N–H amine, C–H alkane, C=O esters or ketones, and C–O aromatic groups. As for the S, the organic composition included N–H amine, C–H alkane, C–H methylene, CH_2 alkane, S=O mica, C=C trisubstituted alkene and disubstituted alkene. Furthermore, the differentiation of the lip stains based on the periods of exposure was possible following careful examination of the ATR-FTIR spectra. Secondly, the application of PCA and LDA successfully provided a suitable means for categorically classifying (a) the different brands of lipstick, (b) the male and female volunteers, as well as (c) the different periods of

exposure based on brands and gender. Because the cross-validation correct classification rates for all the LDA models exceeded 90.0%, their suitability for forensic provenance application of lip stains appears statistically supported. Hence, such an approach to combine the non-destructive ATR-FTIR analysis with PCA and LDA is not only easy and cost-effective but also forensically relevant for enabling the data mining process for forensic intelligence.

■ ACKNOWLEDGMENTS

This research was supported by the Ministry of Higher Education Malaysia Fundamental Research Grant Scheme (FRGS/1/2023/STG01/UTM/02/1) and the Universiti Teknologi Malaysia Fundamental Research Grant (Q.J130000.3854.22H68).

■ CONFLICT OF INTEREST

The authors declared no conflict or competing interest.

■ AUTHOR CONTRIBUTIONS

Mohd Afiq Mohd Azis: Formal analysis, Investigation, Data curation, Writing – original draft, Visualization. Naji Arafat Mahat: Supervision, Conceptualization, Methodology, Writing – review & editing, Project administration, Funding acquisition, Resources. Hasmerya Maarof: Co-supervision, Methodology, Writing – review & editing. Sarah Aina Azman: Writing – review & editing. All authors read and agreed to the final version of this manuscript.

■ REFERENCES

[1] Gładysz, M., Król, M., and Kościelniak, P., 2021, Current analytical methodologies used for examination of lipsticks and its traces for forensic purposes, *Microchem. J.*, 164, 106002.

[2] Wong, J.X.W., Sauzier, G., and Lewis, S.W., 2019, Forensic discrimination of lipsticks using visible and attenuated total reflectance infrared spectroscopy, *Forensic Sci. Int.*, 298, 88–96.

[3] Gładysz, M., Król, M., Chudecka, A., and Kościelniak, P., 2020, Application of spectroscopic and separation techniques to the examination of the chemical composition stability of lipsticks exposed to various factors and storage conditions, *Forensic Sci. Int.*, 309, 110230.

[4] Gładysz, M., Król, M., and Kościelniak, P., 2017, Differentiation of red lipsticks using the attenuated total reflection technique supported by two chemometric methods, *Forensic Sci. Int.*, 280, 130–138.

[5] Chophi, R., Sharma, S., and Singh, R., 2020, Forensic analysis of red lipsticks using ATR-FTIR spectroscopy and chemometrics, *Forensic Chem.*, 17, 100209.

[6] Ezegbogu, M.O., and Osadolor, H.B., 2019, Comparative forensic analysis of lipsticks using thin layer chromatography and gas chromatography, *Int. J. Chem. Mol. Eng.*, 13 (5), 231–235.

[7] Kaur, K., Yadav, P.K., Bumbrah, G.S., and Sharma, R.M., 2020, Forensic classification of lipsticks using attenuated total reflectance – Fourier transform infrared (ATR-FTIR) spectroscopy, *Vib. Spectrosc.*, 110, 103146.

[8] Shadan, A.F., Mahat, N.A., Wan Ibrahim, W.A., Ariffin, Z., and Ismail, D., 2018, Provenance establishment of stingless bee honey using multi-element analysis in combination with chemometrics techniques, *J. Forensic Sci.*, 63 (1), 80–85.

[9] Gautam, R., Vanga, S., Ariese, F., and Umapathy, S., 2015, Review of multidimensional data processing approaches for Raman and infrared spectroscopy, *EPJ Tech. Instrum.*, 2 (1), 8.

[10] Sharma, S., Chophi, R., Kaur, H., and Singh, R., 2020, Differentiation of cosmetic foundation creams using attenuated total reflection Fourier-transform infrared spectroscopy: A rapid and nondestructive approach in trace evidence analysis, *J. Forensic Sci.*, 65 (3), 751–761.

[11] Hussin, F.N.N.M., Attan, N., and Wahab, R.A., 2020, Extraction and characterization of nanocellulose from raw oil palm leaves (*Elaeis guineensis*), *Arabian J. Sci. Eng.*, 45 (1), 175–186.

[12] Gładysz, M., Król, M., Własiuk, P., Piwowar, M., Zadora, G., and Kościelniak, P., 2018, Development

and evaluation of semi-destructive, ultrasound assisted extraction method followed by gas chromatography coupled to mass spectrometry enabling discrimination of red lipstick samples, *J. Chromatogr. A*, 1577, 92–100.

[13] Saferstein, R., 2017, *Criminalistics: An Introduction to Forensic Science*, 12th Ed., Pearson, New Jersey, US.

[14] NPRA, 2018, *Guideline on Good Distribution Practice*, National Pharmaceutical Regulatory Division Ministry of Health Malaysia, 3rd Ed., 1–36. http://www.npra.moh.gov.my.

[15] Arifin, N.A., and Denan, Z., 2015, An analysis of indoor air temperature and relative humidity in office room with various external shading devices in Malaysia, *Procedia – Soc. Behav. Sci.*, 179, 290–296.

[16] Sharma, V., Bharti, A., and Kumar, R., 2019, On the spectroscopic investigation of lipstick stains: Forensic trace evidence, *Spectrochim. Acta, Part A*, 215, 48–57.

[17] Fiume, M.M., Heldreth, B.A., Bergfeld, W.F., Belsito, D.V., Hill, R.A., Klaassen, C.D., Liebler, D.C., Marks, J.G., Shank, R.C., Slaga, T.J., Snyder, P.W., and Andersen, F.A., 2015, Safety assessment of ethanolamine and ethanolamine salts as used in cosmetics, *Int. J. Toxicol.*, 34 (Suppl. 2), 84S–98S.

[18] de Bruin-Hoegée, M., van der Schans, M.J., Langenberg, J.P., and van Asten, A.C., 2024, Biomarker profiling in plants to distinguish between exposure to chlorine gas and bleach using LC-HRMS/MS and chemometrics, *Forensic Sci. Int.*, 358, 112022.

[19] Arora, T., Verma, R., Kumar, R., Chauhan, R., Kumar, B., and Sharma, V., 2021, Chemometrics based ATR-FTIR spectroscopy method for rapid and non-destructive discrimination between eyeliner and mascara traces, *Microchem. J.*, 164, 106080.

[20] He, X., and Wang, J., 2020, Rapid and nondestructive forensic identification of tire particles by attenuated total reflectance – Fourier transform infrared spectroscopy and chemometrics, *Anal. Lett.*, 53 (5), 714–734.