Malaysian Journal of Chemistry, 2024, Vol. 26(5), 498-504
(Special Issue: *4th IKMPB Online Symposium 2024 - Development of Chemistry to Accomplish a Sustainable World*)

Forensic Analysis of Eyeshadows using ATR-FTIR Spectroscopy and Chemometrics

Nurul Aliah Fadhlina Azhar and Hamizah Md Rasid*
School of Chemistry and Environment, Faculty of Applied Sciences, Universiti Teknologi MARA,
40450 Shah Alam, Selangor, Malaysia
*Corresponding author (e-mail: hamizah7708@uitm.edu.my)

Eye cosmetics, like eyeshadows, are commonly used and easily transferred, making them potential crucial trace evidence at crime scenes. This study focuses on resolving criminal cases by connecting victims, suspects, and crime scenes through the analysis of eyeshadow. Conventional methods like flame atomic absorption spectroscopy and x-ray fluorescence are destructive, hindering forensic examination. Instead, this research employs attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy coupled with chemometric methods like principal component analysis (PCA) and principal component analysis-linear discriminant analysis (PCA-LDA). ATR-FTIR spectroscopy reveals compositional variations among eyeshadows, with unique peaks representing functional groups. PCA aids in pattern recognition, and PCA-LDA achieves 100% calibration accuracy and 45% validation accuracy for brand differentiation. However, a preliminary substrate study suggests challenges in analyzing eyeshadows on materials like cotton cloth and glass, leading to difficulties linking stains to their source. The study aims to establish a non-destructive, rapid, and reproducible method for the forensic analysis of eye cosmetics using ATR-FTIR spectroscopy, contributing to the understanding and application of this technique in forensic science.

Keywords: ATR-FTIR; chemometrics methods; eyeshadow analysis

Received: January 2024; Accepted: July 2024

Cosmetic products are used on the human body for purposes like cleansing, beautification, enhancing attractiveness, or altering appearance. Eye cosmetics, like eyeshadow, are commonly used in everyday life and can easily transfer onto objects like clothing, handkerchiefs, and tissue paper when contact occurs during criminal activities, potentially serving as physical or trace evidence. Since they are not visible to the naked eye, criminals do not typically remove or damage them. In forensic science, identifying the source of trace evidence is vital, as it can establish links between suspects, victims, and crime scenes, serving as significant and credible evidence in court [1]. Analyzing circumstantial evidence can also provide insight into the perpetrator, their methods, the sequence of events, and the nature of the crimes. While the color of eye-cosmetic evidence helps distinguish between questioned and recovered samples in forensic examinations, subjective interpretation may be needed when similar shades are present. To address this, instrumental analysis provides objective results. Recent technological advancements and analytical procedures have increased the value of cosmetic evidence in cases involving rape, assaults on women, murder, anonymous letters, theft, and burglary, where it was once considered unimportant.

Several studies have used different analytical methods to examine cosmetics, focusing on identifying toxic or trace amounts of heavy metals used in their production. These methods include flame atomic absorption spectroscopy (FAAS), x-ray fluorescence (XRF), and inductively coupled plasma-mass spectrometry (ICP-MS). While these techniques offer stability and accuracy, they also come with limitations. In a study by Marguí et al. [2], despite being rapid and cost-effective, the XRF method required larger sample amounts, making it unsuitable where eye-cosmetic samples are usually limited and considered trace evidence. Another method, ICP-MS, is time-consuming, expensive, destructive, and complex to operate as noted by Liu et al. [3]. AAS, mentioned by Liu et al. [3], has low sensitivity and is only suitable for detecting single elements in cosmetics analysis. Lastly, FAAS is not suitable for the current study because it is destructive, involves sample preparation, and works best with large liquid samples [4]. Because many existing methods are either destructive or semi-destructive, they cannot be used effectively in forensic examinations, as they might destroy trace evidence.

Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy offers several advantages, including minimal sample preparation, easy cleanup, and the ability to analyze samples in their natural state without the need for heating, pressing into pellets, or grinding. Since such evidence is often found in small amounts, the analysis method

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†Paper presented at the 4th IKMPB Online Symposium 2024 – Development of Chemistry to Accomplish a Sustainable World

must be non-destructive, sensitive, reliable, quick, eco-friendly, and have a high degree of confidence. It should also require little to no sample preparation. ATR-FTIR spectroscopy possesses all these qualities, making it highly beneficial for the forensic community [5]. However, analyzing and classifying eye cosmetic samples without chemometric techniques can also be challenging [6]. Given these limitations and the scarce research on differentiating and analyzing various brands of eye cosmetics, an alternative approach is needed. Nowadays, many researchers combine ATR-FTIR spectroscopy with chemometric methods such as principal component analysis (PCA) and linear discriminant analysis (LDA) for forensic analysis [7]. Chemometric methods are employed to manage large ATR-FTIR spectral datasets and aid in objectively reducing and interpreting the data. Thus, techniques like PCA help reduce key variables, while LDA is used for prediction and classification [8]. Wong et al. [9] emphasized that chemometrics enhance objectivity, reliability, and reproducibility, and reduce human bias in data assessment, leading to more transparent decision-making.

This study proposes using ATR-FTIR spectroscopy along with chemometric methods like PCA and PCA-LDA to distinguish between different eyeshadow brands and analyze eyeshadow stains on various surfaces such as cotton cloth and glass.

EXPERIMENTAL

Sample Collection and Preparation

Common brands of eyeshadow were purchased from local markets in Kuala Lumpur and Selangor. A total of 10 pink eyeshadows in a similar hue from 10 different brands were collected. Table 1 displays the brands and hues of the eyeshadow samples used in this study. Each eyeshadow sample, approximately 1.5 mg in weight, was placed onto the crystal with good surface contact ensured using a tightened anvil. Triplicate eyeshadow samples were used for the blank procedure. In the substrate study, each brand of eyeshadow was applied onto selected surfaces like cotton cloth and glass. A blank substrate served as a background, and eyeshadow residue on the same surface was considered the sample. Subtraction was performed across the entire mid-infrared band, from 4000-650 cm^{-1}. After each new sample, a fresh background measurement was taken to avoid cross-contamination. Spectroscopic-grade acetone was used to clean the crystal surface between each sample analysis during the experiment.

Instrumentation

Perkin Elmer Spectrum One FTIR spectrometer was used with germanium ATR crystal to analyze the samples. Spectrum v5.0.1 software was employed to record spectra in the mid-infrared band from 4000-650 cm^{-1}. The ATR-FTIR spectrometer settings included a scan time of 16 scans and a resolution of 4 cm^{-1}. To remove background effects, two fresh air measurements were taken before each new sample analysis. After each sample analysis, the ATR crystal was cleaned using spectroscopic-grade acetone.

Data Analysis

Chemometric methods, specifically PCA and LDA, were utilized to distinguish between samples. The analyses were performed using Unscrambler X software (Version 10.5.1 (64 bit), CAMO AS, Norway). Before chemometric analysis, baseline correction and smoothing techniques were applied to enhance data quality. All ATR-FTIR spectra recorded between 4000 and 650 cm^{-1} were utilized.

Table 1. Description of eyeshadow samples that were analyzed

Sample code	Brand	Series
E1	SILKYGIRL	Full Bloom
E2	JILL LEEN	Show Color Mini
E3	PINKFLASH	PinkDessert
E4	GOGO TALES	Little Daisy
E5	ROM&ND	Better Than Eyes
E6	I’M MEME	Color Pattern
E7	INNISFREE	Airy
E8	ETUDE	Play Color Eyes
E9	HOLIKA HOLIKA	My Fave Mood
E10	PERIPERA	All Take Mood Technique

PCA was used to simplify complex data from ATR-FTIR spectroscopy, aiding in identifying differences and similarities among samples. It reduces data dimensionality, thereby facilitating interpretation. PCA was performed on the dataset with IR spectral data specified as X-variables. The number of Principal Components (PCs) retained was determined based on the variance explained by each component. Scores, loadings, and variance explained were visualized using score plots, loading plots, and a scree plot to assess data structure and variability. Subsequently, LDA was executed to further differentiate between eyeshadow brands. The dataset was structured with samples as rows and IR spectral data as columns, including a categorical Y-variable representing eyeshadow brands. LDA was applied specifying the range for X-variables and Y-variables. A Prediction Matrix and Confusion Matrix were employed to validate model performance and ensure reliability.

RESULTS AND DISCUSSION

The discussion is organized into four sections; ATR-FTIR spectroscopy, PCA, PCA-LDA, and substrate analysis.

ATR-FTIR Spectroscopy

The initial findings revealed that each eyeshadow produced distinct spectral peaks, albeit with variations in peak shape, intensity, and wavenumbers especially in the fingerprint region. Additional peaks that emerged were observed to characterize each sample. This study confirmed that ATR-FTIR spectroscopy can effectively provide unique spectra due to the different components used in eyeshadow production. Despite some similar peaks resulting from common macromolecules, each sample exhibited distinct spectral patterns, particularly in the fingerprint region. To discern compositional differences among eyeshadow brands, ATR-FTIR was employed to obtain their spectra, which were divided into two regions: one from 4000 cm^{-1} to 1800 cm^{-1} and the other from 1800 cm^{-1} to 650 cm^{-1}. Notably, the region between 2800 cm^{-1} and 1800 cm^{-1} had fewer pronounced peaks in all samples.

Figure 1 shows a typical eyeshadow spectrum. In the range of 4000 cm^{-1} to 2000 cm^{-1}, there was a distinct sharp peak at 3675 cm^{-1} indicating the presence of free O-H groups. Additionally, three distinct peaks at 2959 cm^{-1}, 2919 cm^{-1}, and 2851 cm^{-1} were identified in this range, indicating the presence of aliphatic compounds in eyeshadow samples. The peak at 2959 cm^{-1} is also associated with talc and mica [8]. Below the fingerprint region (1800 cm^{-1} – 650 cm^{-1}), several peaks were observed. Peaks at 1739 cm^{-1}, 1538 cm^{-1}, and 729 cm^{-1} are attributed to amide groups, representing amide I (C=O stretching), amide II (N-H / NH_2 bending/ N-C=O stretching), and amide III (N-H out of plane bending), respectively. The peak at 1472 cm^{-1} resulted from methylene scissoring vibrations, while the peak at 1260 cm^{-1} originated from the C-O stretching of an aromatic ester. Peaks at 975 cm^{-1}, 800 cm^{-1}, and 666 cm^{-1} were attributed to the silica group by the silica group (Si-H band vibrations) [1].

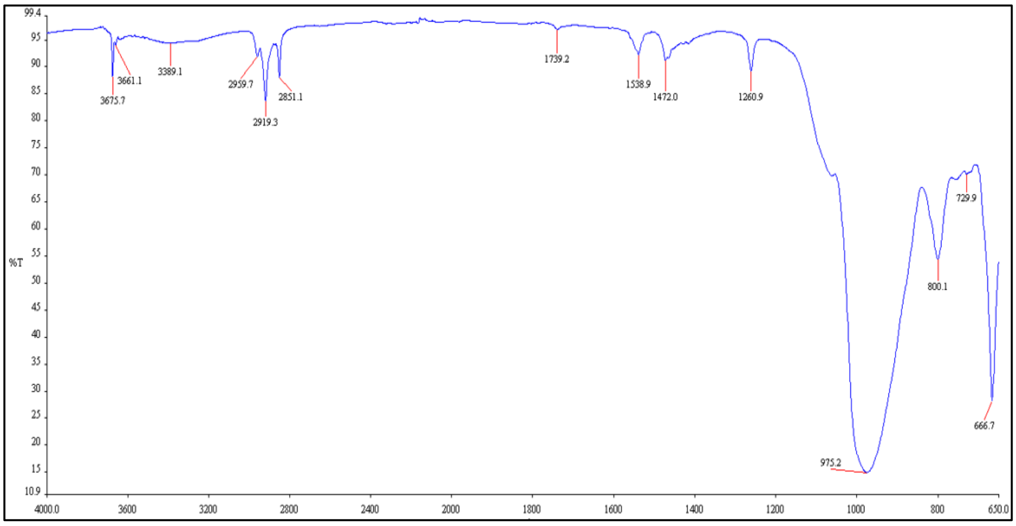

Figure 1. Typical spectrum of eyeshadow represented by sample E4

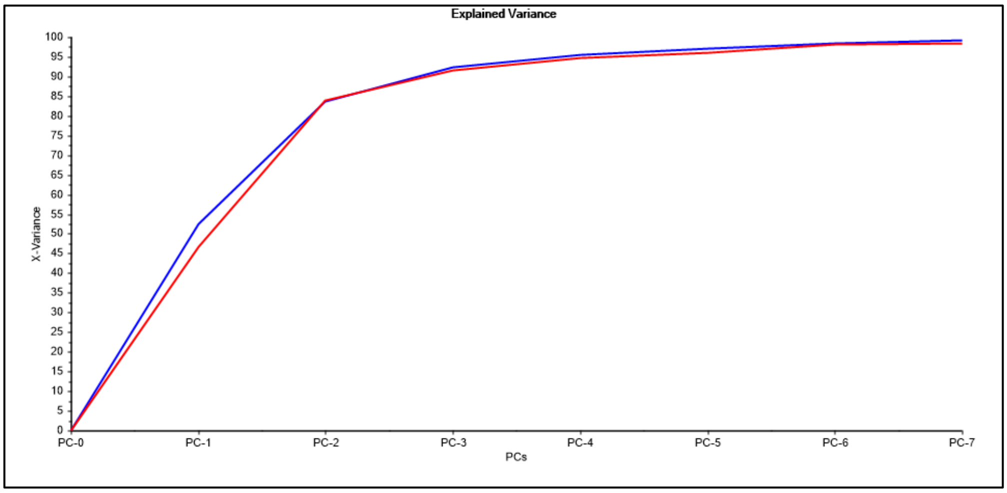

Figure 2. Scree plot.

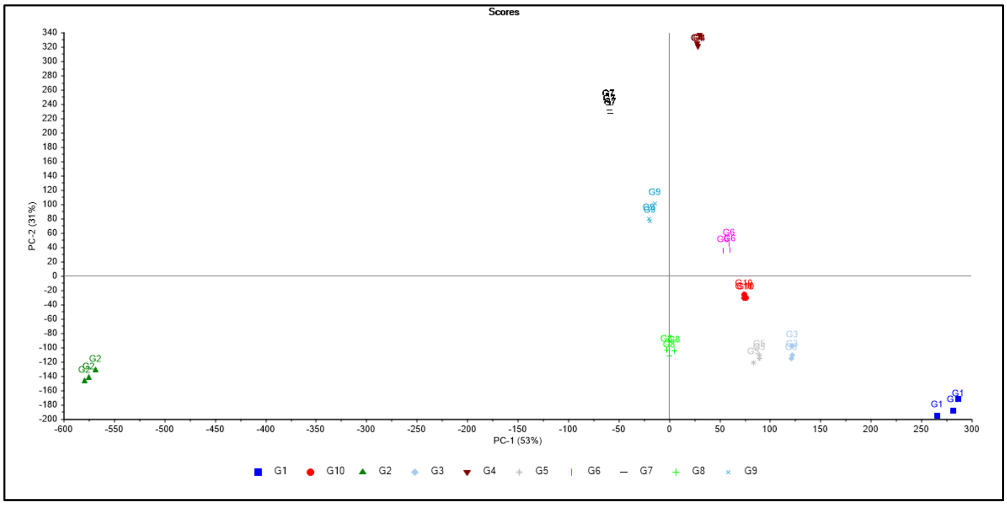

Figure 3. 2D PCA score plot of eyeshadow samples.

PCA

To determine if eyeshadow brands could be distinguished from each other, a chemometric approach was used to analyze the pattern or clustering of eyeshadow samples. PCA was performed on all ATR-FTIR spectra recorded between 4000 cm^{-1} and 650 cm^{-1}. Figure 2 displays the number of principal components (PC) needed to explain the dataset's variance. In this study, PCA produced three significant principal components (PC1: 53%, PC2: 31%, and PC3: 9%), accounting for a total of 93% of the dataset's variance. Various combinations of these components were tested to visualize the data optimally. A plot of PC1 and PC2, which accounted for 84% of the variation, proved most effective for sample differentiation. Figure 3 represents a 2D scatter plot of PC1 and PC2. It clearly shows that samples from the same brands are grouped, indicating similar compositions. Additionally, samples with similar chemical compositions are located near each other; for example, samples from G3 and G5 are close. However, since PCA is not a classification method, it does not determine whether a sample has been accurately classified into its correct class.

The combined loading plot of PC1 and PC2 can be divided into three regions: R-1 (4000 cm^{-1} - 1363 cm^{-1}), R-II (1363 cm^{-1} - 1011 cm^{-1}), and R-III (1011 cm^{-1} - 725 cm^{-1}), as shown in Figure 4. The loading plot shows the relationships between the original variables and the PCs. In R-I, PC1 and PC2 had a positive correlation. However, there was a negative correlation observed for a PC1 peak at 3651 cm^{-1}, possibly due to the presence of free hydroxyl groups in the samples, while PC2 remained positively correlated. The peaks between 2859 cm^{-1} and 2771 cm^{-1} in this region may be attributed to aliphatic compounds. Moving to R-II, PC1 showed a positive correlation while PC2 might have had a negative correlation. Peaks in this region could result from various factors like amide I's C=O stretching, amide

II's N-H/NH_2 bending and N-C=O stretching, methylene stretching, and the aromatic esters' C-O stretching. In R-III, PC1 exhibited a negative correlation, while PC2 showed a positive correlation. This region is mainly composed of silica group peaks and N-H amide III bending in the samples.

PCA-LDA

A combination of PCA and LDA was used to classify and assess the differentiation between eyeshadow brands more accurately. Combining PCA and LDA, as recommended by Sharma et al. [8], helps identify key features contributing to dataset variance and enhances classification and discrimination. While PCA clustered different brands, as shown in Figure 3, further analysis was needed to determine accurate classification. Therefore, a linear discriminant model was constructed using the first three PCA principal components to report classification accuracy. For calibration, three independent samples per brand were replicated, resulting in 30 samples for model development. With wavenumbers ranging from 4000 cm^{-1} to 650 cm^{-1}, the training/calibration set for the LDA calibration model included 30 samples. Given that each sample class was regarded as a separate class, 10 sample classes were created by the LDA model. The prediction of the validation set was then made using the calibration model. To assess the model's accuracy, the predicted class and the actual class were compared. Figure 5 illustrates the LDA discrimination plot. The LDA model achieved 100% classification accuracy, correctly distinguishing all eyeshadow samples by brand, as detailed in Table 2. No misclassifications occurred, even when comparing multiple samples, demonstrating the powerful discriminating ability of the developed LDA model. This study highlights how incorporating chemometric methods like PCA and LDA with ATR-FTIR spectroscopy yields effective classification and discrimination of eyeshadow samples.

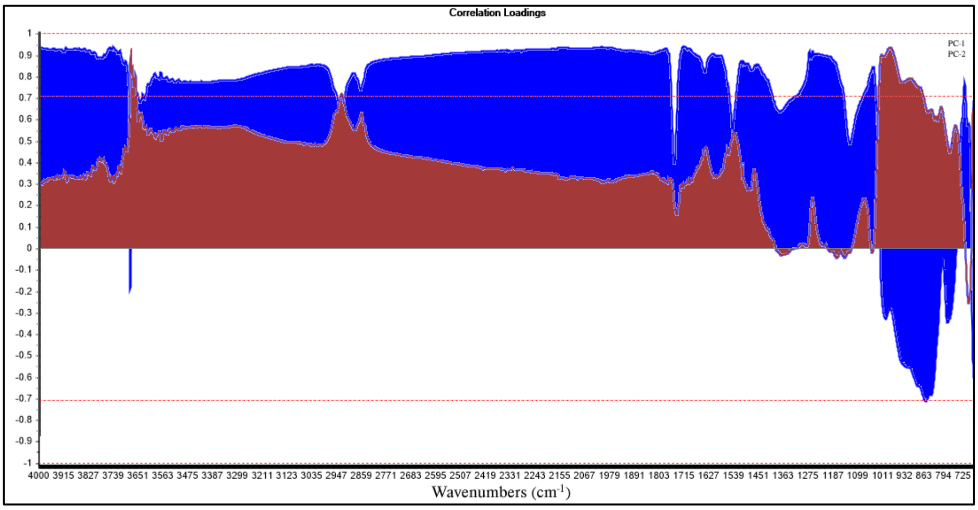

Figure 4. Loading plot of PC1 and PC2.

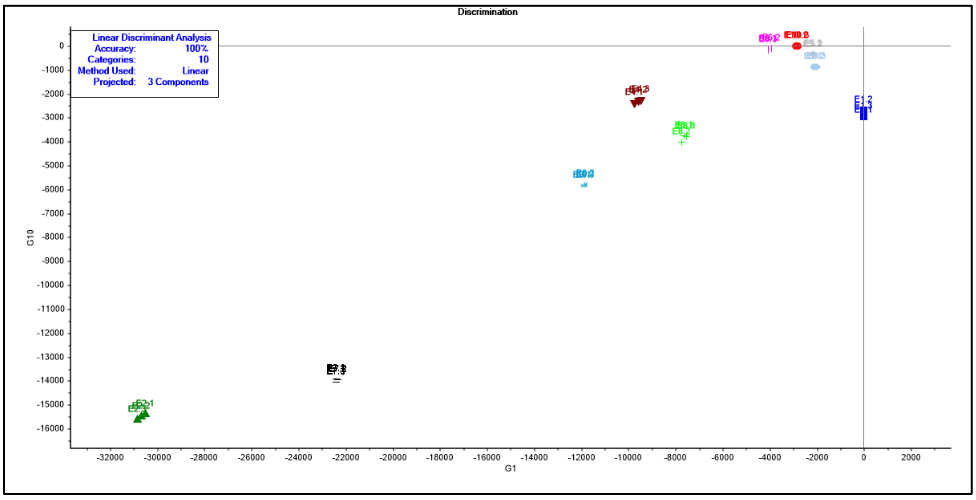

Figure 5. LDA discrimination plot of eyeshadow samples.

Table 2. Results of PCA-LDA for the classification of eyeshadow samples.

Eyeshadow	Group	Correct	Incorrect (classified as)	% correct classification
E1.1, E1.2, E1.3	G1	3	0	100
E2.1, E2.2, E2.3	G2	3	0	100
E3.1, E3.2, E3.3	G3	3	0	100
E4.1, E4.2, E4.3	G4	3	0	100
E5.1, E5.2, E5.3	G5	3	0	100
E6.1, E6.2, E6.3	G6	3	0	100
E7.1, E7.2, E7.3	G7	3	0	100
E8.1, E8.2, E8.3	G8	3	0	100
E9.1, E9.2, E9.3	G9	3	0	100
E10.1, E10.2, E10.3	G10	3	0	100
Total		30	0	100

Table 3. Results of PCA-LDA for classification of eyeshadows on substrates.

Eyeshadow	Group	Correct	Incorrect (classified as)	% correct classification
E1 (c)	G1	0	1 (E1 into G6)	0
E2 (c)	G2	0	1 (E2 into G4)	0
E3 (c)	G3	0	1 (E3 into G4)	0
E4 (c)	G4	1	0	100
E5 (c)	G5	0	1 (E5 into G4)	0
E6 (c)	G6	1	0	100
E7 (c)	G7	1	0	100
E8 (c)	G8	1	0	100
E9 (c)	G9	1	0	100
E10 (c)	G10	0	1 (E10 into G6)	0
E1 (g)	G1	0	1 (E1 into G4)	0
E2 (g)	G2	0	1 (E2 into G4)	0
E3 (g)	G3	0	1 (E3 into G4)	0
E4 (g)	G4	1	0	100
E5 (g)	G5	0	1 (E5 into G4)	0
E6 (g)	G6	1	0	100
E7 (g)	G7	1	0	100
E8 (g)	G8	0	1 (E8 into G4)	0
E9 (g)	G9	1	0	100
E10 (g)	G10	0	1 (E10 into G4)	0
Total		9	11	45

Note: (c) and (g) indicate cotton cloth and glass substrate

Substrate Analysis of Eyeshadow Traces

A PCA-LDA combination was used for classification to better understand and differentiate eyeshadow traces found on various surfaces during real criminal investigations. In this study, the effects of cotton cloth and glass substrates on eyeshadow analysis were explored. Samples from 10 eyeshadow brands were applied onto chosen substrates, including cotton cloth and glass, following the same experimental parameters used for brand analysis.

By measuring the substrate's background, the substrate-induced peaks were removed, revealing that major peaks remained consistent across substrates with slight variations in peak strength. However, a few small peaks disappeared due to substrate effects. PCA was conducted on the 20 individual data points to obtain PC1-3, which were then used as input for the LDA model. This model was cross-validated using the same 20 stained samples to ensure its accuracy and robustness on the chosen substrates, as detailed in Table 3. The cross-validation accuracy was 45%, meaning nearly half of the stained samples were correctly classified on cotton cloth and glass. However, stains from E1, E2, E3, E5, and E10 on cotton cloth could not be assigned to their respective groups and were misclassified as G6 and G4. On the other hand, stains from E1, E2, E3, E5, E8, and E10 on glass substrates could not be assigned to their respective groups and were misclassified as G4. This indicates that the substrate presence has altered the eyeshadow samples' spectra, making classification and discrimination challenging. Chophi et al. [10] mentioned that eyeshadow stains do not adhere well to plastic and glass substrates whereas cotton has absorbent properties, and the eyeshadow may be absorbed into the fibers of the fabric, which explains the misclassification of these samples.

CONCLUSION

In summary, ATR-FTIR spectroscopy with chemometrics effectively differentiated eyeshadow brands in this study. ATR-FTIR enabled fast, repeatable, and non-destructive analysis. Chemometric tools like PCA and PCA-LDA improved pattern recognition with statistical confidence, overcoming the limitations of visual comparisons. The PCA-LDA calibration achieved 100% accuracy for eyeshadow samples. A preliminary stain analysis on cotton cloth and glass was conducted to mimic real crime investigations, cross-validating the PCA-LDA model with 45% accuracy. Approximately half of the eyeshadow stains were correctly classified, while others were misclassified, likely due to substrate effects. Recommendations include increasing sample replicates for substrate analysis and conducting further investigations on the effect of different substrates, aging, and environmental factors on sample analysis

ACKNOWLEDGEMENTS

The authors would like to express their gratitude to the Universiti Teknologi MARA, Malaysia for funding this work. This work was funded by Special Research Grant no: 600-RMC/GPK 5/3(139/2020).

REFERENCES

1. Chophi, R., Sharma, S., Sharma, S. and Singh, R. (2019) Trends in the forensic analysis of cosmetic evidence. *Forensic Chemistry*, **14**, 100165.
2. Marguí, E., Dalipi, R., Borgese, L., Depero, L. and Queralt, I. (2019) Possibilities and drawbacks of total reflection X-ray fluorescence spectrometry as a fast, simple, and cost-effective technique for multielement analyses of cosmetics. *Analytica Chimica Acta*, **1075**, 27–37.
3. Liu, Y., Chu, Y., Hu, Z., Zhang, S., Ma, S., Khan, M. S., Chen, F., Zhang, D., Guo, L. and Lau, C. (2020) High-sensitivity determination of trace lead and cadmium in cosmetics using laser-induced breakdown spectroscopy with ultrasound-assisted extraction. *Microchemical Journal*, **158**, 105322.
4. Ahmed, A. Y., Asada, A. and Hamza, I. (2021) Cobalt and lead concentrations in cosmetic products sold at local markets in Saudi Arabia. *Toxicology Reports*, **8**, 1693–1698.
5. 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. *Microchemical Journal*, **164**, 106080.
6. Yadav, P. K. and Sharma, R. M. (2022) Forensic Analysis of cosmetic-exposition of significance, limitations, and challenges. *Indian Journal of Criminology*, **49(2)**, 17–31.
7. Alkhuder, K. (2022) Attenuated total reflection – Fourier transform infrared spectroscopy: a universal analytical technique with promising applications in forensic analyses. *International Journal of Legal Medicine*, **136**, 1717–1736.
8. Sharma, S., Chophi, R., Kaur, H. and Singh, R. (2019) Differentiation of Cosmetic Foundation Creams Using Attenuated Total Reflection Fourier-Transform Infrared Spectroscopy: A Rapid and Non-Destructive Approach in Trace Evidence Analysis. *Journal of Forensic Sciences*, **65(3)**, 751–761.
9. Wong, J. X., Sauzier, G. and Lewis, S. W. (2019) Forensic discrimination of lipsticks using visible and attenuated total reflectance infrared spectroscopy. *Forensic Science International*, **298**, 88–96.
10. Chophi, R., Sharma, S., Jossan, J. K. and Singh, R. (2021) Rapid and non-destructive analysis of eye cosmetics using ATR-FTIR spectroscopy and chemometrics. *Forensic Science International*, **329**, 111062.