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Characterization of Vermillion and Lipstick Using Thin-Layer Chromatography and Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy With Chemometrics

توصيف أحمر الشفاه والمسحوق الأحمر باستخدام كروماتوغرافيا الطبقة الرقيقة ومطيافية الأشعة تحت الحمراء بتحويل فورييه والانعكاس الكلي المخفف مع الكيمياء القياسية

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Abstract

The study employed Thin Layer Chromatography (TLC) and Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) to analyze local brands of vermillion and lipstick samples. In TLC, vermillion and lipsticks exhibited notable separation patterns among various brands. The ATR-FTIR spectrum analysis of both vermillion and lipstick samples revealed distinct spectral patterns. After analyzing the FTIR library's cosmetics spectrum, notable similarities were found among compounds like Butyl Stearate, Nonane, and Linoleic Acid. Principal Component Analysis (PCA) categorized by cosmetic type and form revealed significant variance within the datasets. PCA based on cosmetic type explained approximately 83.57% of the total variance, while PCA based on form elucidated about 90% of the variance. Support Vector Machine (SVM) analysis demonstrated high precision and recall for classifying cosmetic types and forms. SVM results showed a precision of 1.00 for both lipstick and vermillion, indicating accurate predictions. Additionally, Hierarchical Cluster Analysis (HCA) identified six clusters of data points with shared characteristics. Overall, the

Keywords: forensic sciences, trace evidence, cosmetics, vermilion, lipsticks, ATR-FTIR, HCA, PCA, SVM.

المستخلص

استخدمت الدراسة كروماتوغرافيا الطبقة الرقيقة (TLC) وتقنية مطياف الأشعة تحت الحمراء بتحويل فورييه والانعكاس الكلي المخفف (ATR-FTIR) لتحليل عينات من العلامات التجارية المحلية لأحمر الشفاه والمسحوق الأحمر. في تقنية TLC، أظهرت عينات أحمر الشفاه والمسحوق الأحمر أنماط فصل ملحوظة بين العلامات التجارية المختلفة. كشف تحليل طيف ATR-FTIR لعينات كل من أحمر الشفاه والمسحوق الأحمر عن أنماط طيفية مميزة. بعد تحليل مكتبة أطياف مستحضرات التجميل الخاصة بتقنية FTIR، وُجدت أوجه تشابه ملحوظة بين مركبات مثل ستيرات البوتيل والنونان وحمض اللينوليك. كشف تحليل المكونات الرئيسية (PCA) المصنف حسب نوع وشكل مستحضرات التجميل عن تباين كبير داخل مجموعات البيانات. أوضح تحليل PCA المستند إلى نوع مستحضرات التجميل ما يقرب من 83.57 ٪ من التباين الكلي، بينما أوضح تحليل PCA المستند إلى الشكل حوالي 90 ٪ من التباين. أظهر تحليل آلة المتجهات الداعمة (SVM) دقة واستدعاء عاليين لتصنيف أنواع وأشكال مستحضرات التجميل. أظهرت نتائج SVM دقة قدرها 1.00 لكل من أحمر الشفاه والمسحوق الأحمر، مما يشير إلى تنبؤات دقيقة. بالإضافة إلى ذلك، حدد تحليل التجميع الهرمي (HCA) ست مجموعات من نقاط البيانات

الكلمات المفتاحية: علوم الأدلة الجنائية، أدلة الآثار الضئيلة، مستحضرات التجميل، المسحوق الأحمر، أحمر الشفاه، تقنية مطياف الأشعة تحت الحمراء.

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study provides valuable insights into the composition and characteristics of vermillion and lipstick samples. The combination of TLC, ATR-FTIR spectrum analysis, PCA, SVM, and HCA offers a comprehensive approach for analyzing cosmetics

ذات الخصائص المشتركة. بشكل عام، تقدم الدراسة رؤى قيمة حول تكوين وخصائص عينات أحمر الشفاه والمسحوق الأحمر. يوفر الجمع بين تقنيات TLC وتحليل طيف ATR-FTIR وتحليل PCA و SVM و HCA نهجًا شاملاً لتحليل مستحضرات التجميل.

1. Introduction

The term "cosmetics" originates from Ancient Rome, and the practice of using makeup dates back centuries, with ancient Egyptians being among the earliest known users [1, 26]. In India, the use of cosmetics dates back to the Vedic and Puranic periods [2]. Cosmetic evidence can often be found as trace evidence at crime scenes, persisting on transferred objects, and serving as potential evidence. Vermilion and lipstick traces may be found in cases of sexual assault, murder, rape, and burglary. In cases of strangulation or suffocation, lipstick found on the victim's neck or face may indicate attempts to smother or cover the victim's mouth, providing crucial evidence. The color and brand of lipstick or vermilion can sometimes be linked to a suspect, particularly if the evidence is transferred onto clothing in cases of sexual assault, especially when the cosmetic is rare or distinctive. This information can aid investigators in narrowing down potential leads [3]. Identifying and confirming the presence of trace evidence is critical [4, 5], as it can help link the suspect, the crime scene, and the victim [6].

Common cosmetics recovered at crime scenes include vermilion and lipsticks. Vermilion, a red-orange powder, has been used for thousands of years, originally made by grinding cinnabar (Mercury Sulphide). It is commonly applied by married Hindu women on their foreheads. Traditional vermilion contains synthetic materials like rhodamine B and mercury sulphite, which give it a bright color [8,9]. The composition of vermilion varies depending on its origin and manufacturer [9]. Lipstick, contains 40-70% oils, 8-15% wax, and 0.5-8% coloring agents, with additional components like perfumes in smaller amounts [4]. Its color comes from organic dyes, and the composition varies by manufacturer [8]. There are no standard guidelines for manufacturing local vermilion or lipsticks, which can help differentiate between brands and sources [5].

Various analytical tools are used to identify vermilion and lipstick samples. Previous studies primarily used separation techniques thin layer chromatography (TLC), Gas chromatography (GC), and High-performance liquid chromatography (HPLC) [10], which are effective but destructive and require extensive sample preparation [4]. In contrast, spectroscopic techniques, Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR), Neutron Activation Analysis (NAA), and Raman spectroscopy are preferred due to their high discrimination power and non-destructive nature with minimal sample preparation [10].

ATR-FTIR is becoming an increasingly valuable tool across various forensic science fields, including the identification of body fluids [31], species differentiation [31], estimation of time since deposition [32], ink and toner analysis [33], paper differentiation [33], biochemical analysis of fingerprints [34], and narcotics identification [35]. Building on this trend, the present study aims to identify and differentiate local brands of vermilion and lipsticks using ATR-FTIR. Previous research has employed techniques like Thin Layer Chromatography (TLC) and various spectroscopic methods to distinguish between different brands [8]. While earlier studies primarily focused on analyzing

branded cosmetics for forensic applications [27-29], there remains a notable gap in examining local brands, as well as differentiating between various cosmetic types (e.g., stick, liquid, powder). To address this gap, this pilot study investigates the forensic differentiation of local brand vermilion and lipsticks from the Southern state of Karnataka. The study utilizes TLC for pigment identification and ATR-FTIR for spectral fingerprinting.

2.METHODOLOGY

2.1 Sample collection and preparation

The cosmetics samples (n= 37) were from local brands purchased within Bangalore city (brand details are provided in Supplementary Tables 1 and 2). All. The vermilion samples (n=24) were considered in its various forms—powder, stick, and liquid—while red shades of lipstick (n=13) were chosen from local brands sourced from different shops and flea markets. The samples were directly mounted on the diamond crystal of the ART FTIR instrument, requiring no further sample preparation. (brands detail in Supplementary Table 1, 2).

2.2 Thin layer chromatography (TLC)

For pigment separation in vermillion samples through Thin Layer Chromatography (TLC), non-polar solvent systems such as Toluene/Benzene (12:8), Toluene/Acetone (16:4), and a combination of Toluene, Acetone, and Benzene (16:2:2) were selected based on the study by Srivastava et al. (2012) [30]. These solvent systems were chosen for their ability to effectively separate non-polar pigments typically found in vermillion. Additionally, for lipsticks, moderately polar solvent systems like Chloroform: Methanol: Water (50:15:2) and Ethyl Acetate: Methanol: Ammonium Hydroxide (5:1:1) were employed to handle a wider range of pigment polarities. These systems were also used in a study by Bin Abdullaha et al. (2011) [15] for discriminating components of lipsticks using TLC.

Different solvent ratios were prepared in 20 mL beakers and covered with aluminum foil for 15 minutes to allow the solvent chambers to saturate. For pigment extraction, 0.1 g of vermillion and lipstick samples were weighed and dissolved in 2 mL of chloroform/ethyl acetate for lipsticks and the appropriate solvent system for vermillion. TLC plates (10x6 cm) were prepared by drawing a baseline 1.5 cm from the bottom. Sample extracts 1μl were spotted onto the plates using capillary tubes and allowed to dry. The plates were then placed in beakers containing solvents and allowed to run until 85% of the plate was covered. After the run, the plates were removed, dried, and the solvent front was marked. Vermilion samples were visualized under normal and UV light (short and long-range), while lipstick samples were visualized using iodine fumes. Finally, the Retention Factor (Rf) for each sample was calculated using the formula: Rf = Distance travelled by solute / distance travelled by solvent.

2.3 Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR)

The experiment was conducted on a Perkin Elmer FT-IR Spectrometer Spectrum 2 with a Diamond crystal. After each run, Carbon Tetrachloride was used to clean the sample area. The spectrum range was set from 4000-600 cm^{-1}, with 25 scans and a resolution of 4 cm^{-1}. A background scan was performed for each sample. Vermilion and Lipstick samples were placed directly on the crystal, and scanned with a pressure gauge set between 60-90 pa (Pascal). For liquid and stick samples, the force gauge was not used. Each sample was scanned in triplicates to ensure repeatability. ATR-FTIR library search was done to identify chemical compounds,

considering hits with scores of 0.8 or higher. The ATR-FTIR % transmittance spectra were saved as CSV files.

2.4 Chemometrics analysis

a. Data Analysis

Microsoft excel was used to enter ATR-FTIR spectral data (4000-600 cm^{-1}), which was then converted to .csv format for analysis in python (version 3.12.2) using libraries like Pandas, NumPy, Matplotlib, Seaborn, Scikit-learn, and SciPy, via Jupyter Notebook (version 7.1.0). Pandas was used for loading, cleaning and manipulating data, especially for the spectral data arrays. NumPy supported numerical operations on the data. Matplotlib and Seaborn were used for creating visualizations such as PCA plots, while Seaborn provided an attractive interface for statistical graphics. Scikit facilitated machine learning tasks of classification and PCA for dimensionality reduction. SciPy, built on NumPy, was used for peak detection and smoothing with functions such like find_peaks and savgol_filter.

b. Pre-Processing of ATR-FTIR Spectral Data

Before chemometrics analysis, ATR-FTIR spectral data was pre-processed through several steps: data cleaning, baseline correction, normalization, standardization, and peak smoothing. Data cleaning removed missing values and outliers. Baseline correction addressed baseline drift, while normalization (using Z score) adjusted features to have a mean of 0 and standard deviation of 1. This standardization prevents scale bias in statistical analyses and machine learning models. Peak smoothing was done with Savitzky-Golay filter. This filter is important in FTIR spectral data analysis as it reduces noise while preserving peak shapes. By smoothing the data through polynomial fitting, it helps to maintain key spectra features and ensures the overall quality of the spectra. It improves the clarity of weak peaks, making it easier to identify chemical components, thus enhancing the reliability of the analysis.

c. Principal Component Analysis (PCA)

Principal Component Analysis (PCA) is a statistical method used to reduce the complexity of large datasets by transforming them into a smaller set of uncorrelated variables called principal components. These components capture the maximum variance in the data, allowing for dimensionality reduction while retaining most of the original information. PCA helps in identifying patterns visualizing high dimensional data, and improving model performance by reducing noise.

d. Support Vector Machine (SVM)

Support Vector Machine (SVM) is a supervised machine learning algorithm used for classification and regression tasks. It works by finding the optimal hyperplane that best separates datapoints of different classes in a high-dimensional space. SVM aims to maximize the margin between the classes, ensuring the best possible separation. For the non-linear data, SVM uses kernel functions to transform the data into higher dimensions where a linear hyperplane can effectively separate classes.

e. Hierarchical cluster Analysis (HCA)

Hierarchical cluster Analysis (HCA) is a clustering method that organizes data into tree-like structure based on similarity. In SPSS version 29, HCA was performed using Ward's method and squared Euclidean distance on transformed Z scores. The algorithm iteratively merged or split clusters to form a hierarchical structure, visualized through dendrograms, revealing natural groupings and within the dataset.

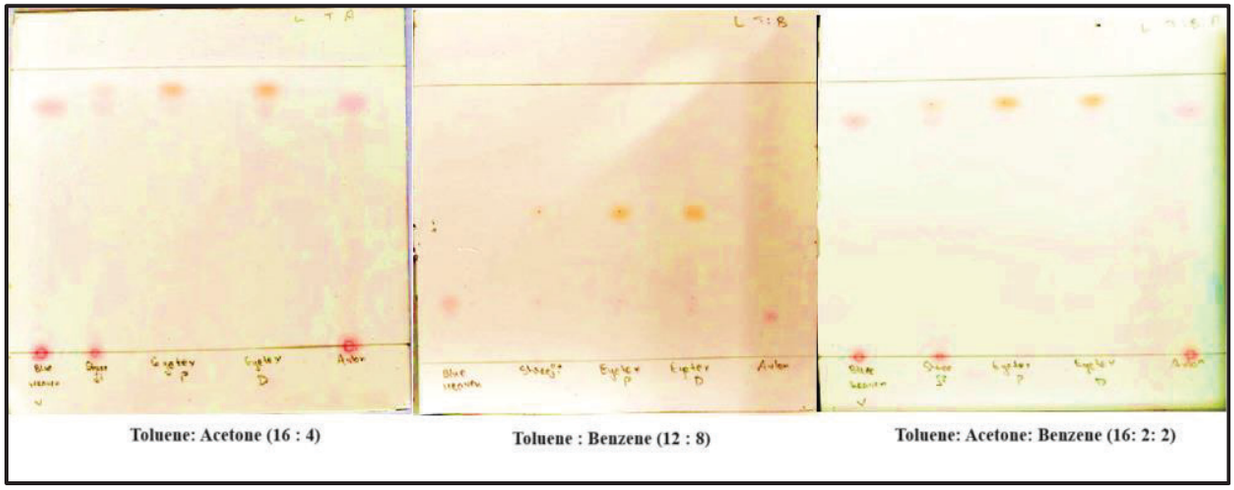

Figure 1- *Pigment separation for vermillion samples across three mobile phases (Toluene: Acetone (16:4), Toluene: Benzene (12:8), and Toluene: Acetone: Benzene (16:2:2).*

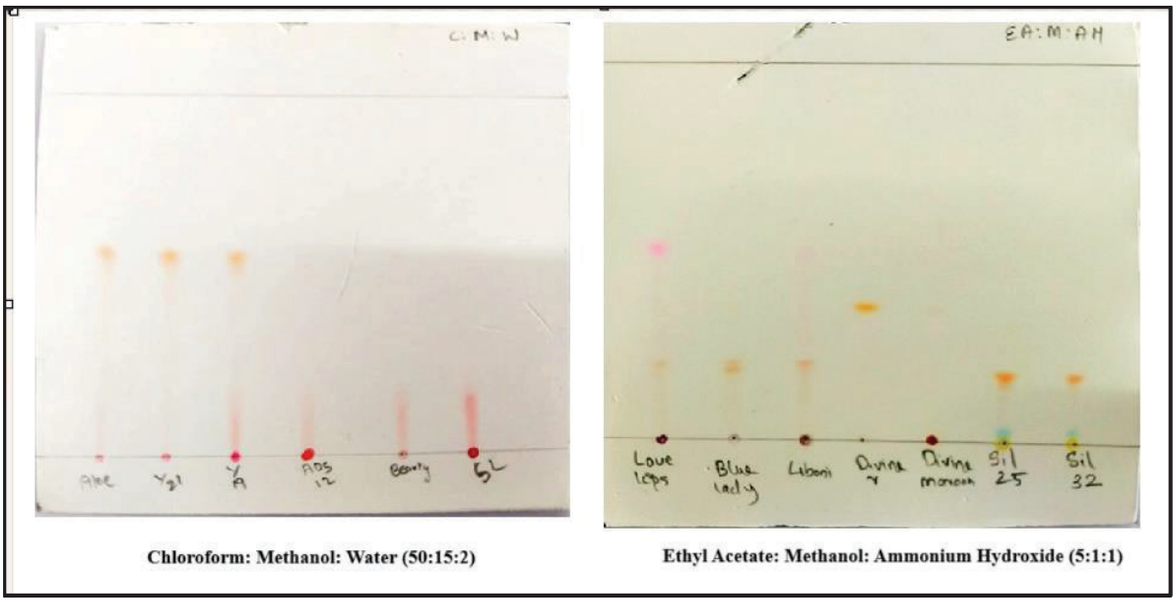

Figure 2- *Pigment separation for lipstick samples across three mobile phases (Chloroform: Methanol: Water (50:15:2) and Ethyl Acetate: Methanol: Ammonia Hydroxide (5:1:1).*

3.RESULTS AND DISCUSSION

3.1 Thin Layer Chromatography (TLC)

The Rf values for liquid vermillion varied across mobile phases. Mobile phase 1 (Toluene: Acetone 16:4) showed moderate separation (Rf values0.15 to 0.92), while mobile phase 2 (Toluene: Benzene 12:8) resulted in lower Rf values (0.15-0.58), indicating reduced mobility. Mobile phase 3 (Toluene: Acetone: Benzene 16:2:2) generally showed better separation, with Rf values above 0.8 for all the samples. For powder vermillion, mobile phase 1 provided the best separation, with Rf values up to 0.90, while mobile phase 2 showed mixed results, and mobile phase 3 produced weaker separation. For the stick vermillion samples, mobile phase 3 was the most effective overall, Figure 3 (supplementary table 3).

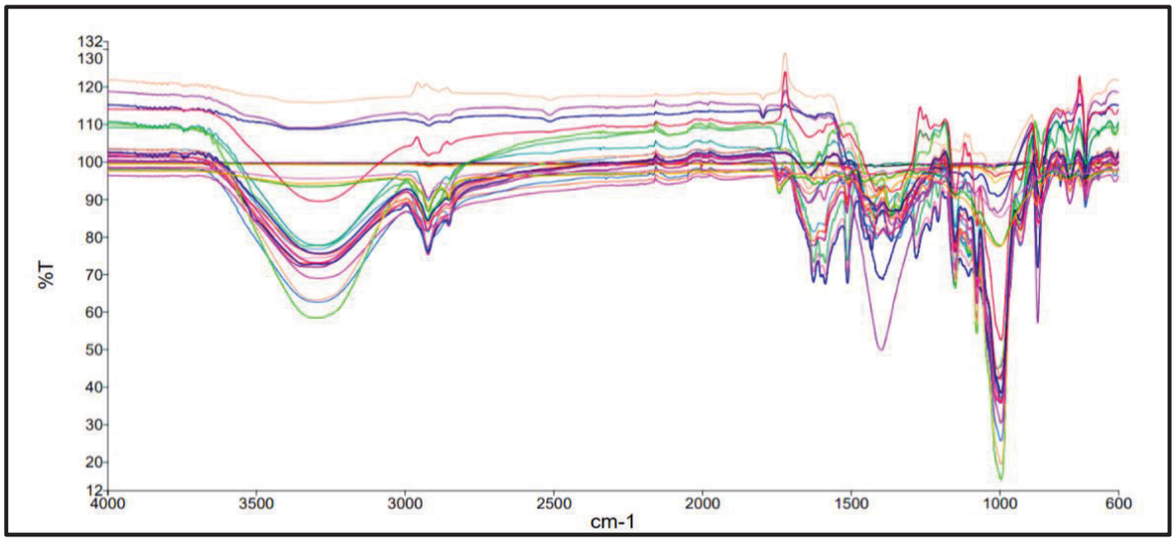

Figure 3- *Infrared spectra of Vermilion powder showing key peaks for OH-C-H and N-H stretching (4000-2800 cm-1), asymmetric methyl stretching (1395, 1380 cm-1), and C-O/C-C stretching (1027, 1006 cm-1).*

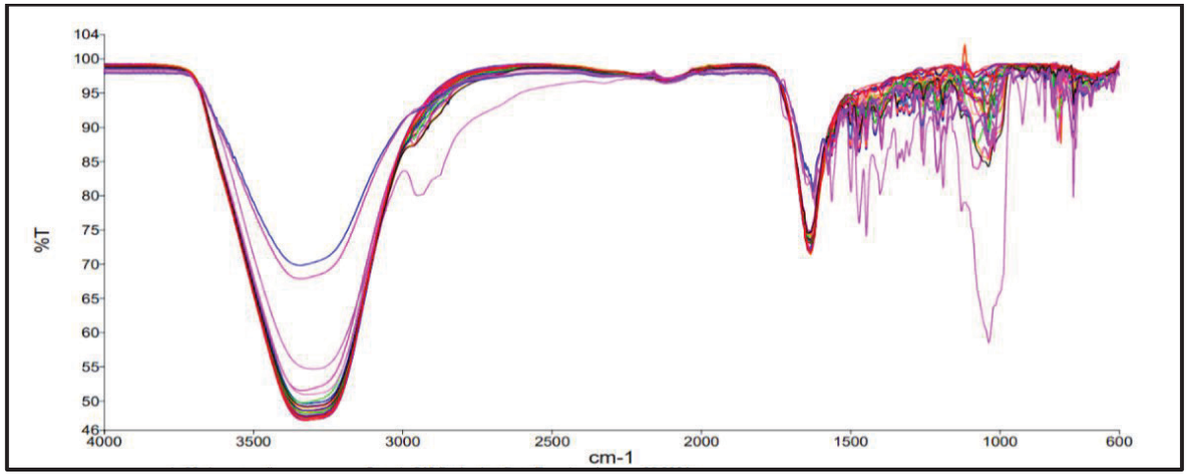

Figure 4- *Infrared spectra of Vermilion liquid with OH, C-H, and N-H stretching (4000-2800 cm-1), C=C stretching (1680-1600 cm-1), and asymmetric methyl stretching (1481, 1444 cm-1).*

The Rf values for separated pigments in lipstick samples varied across two mobile phases: Chloroform: Methanol: Water (50:15:2) and Ethyl Acetate: Methanol: Ammonia Hydroxide (5:1:1). In mobile phase 1 (Chloroform: Methanol: Water), most products showed low to moderate Rf values, with some exhibiting higher values (upto 0.86), indicating better separation, while others had lower values (around 0.15 to 0.16), suggesting poor pigment mobility. In mobile phase 2 (Ethyl Acetate: Methanol: Ammonia Hydroxide), the Rf values were more varied, with higher values (up to 0.73) for some products, indicating better separation. However, some samples showed very low Rf values (0.18), indicating reduced separation. Overall, mobile phase 2 generally provided better separation for most products, while mobile phase 1 showed more consistent, but lower, Rf values, Figure 3, (supplementary table 4).

In our study, effective separation was achieved using the selected mobile phase, aligning with previous research on pigment separation in

Table 1 - *Common and unique peaks identified in the vermillion samples of the present study.*

Range (cm-1)	Band Assignment	Reference
Vermilion powder sample		
4000-2800 cm^{-1}	OH-C-H, N-H stretching OH-C-H, N-H stretching	(Chopi et al., 2021)
1395, 1380 cm^{-1}	Asymmetric methyl stretching	(Chopi et al., 2021)
1275-1089 cm^{-1}	In-plane C-H bending	(Chopi et al., 2021)
1027, 1006 cm^{-1}	C-O or C-C stretching	Present study
871, 711 cm^{-1}	C-H deformation	Present study
Vermilion liquid sample		
4000-2800 cm^{-1}	OH, C-H, N-H stretching OH, C-H, N-H stretching	(Chopi et al., 2021)
1680-1600 cm^{-1}	C=C stretching	
1481,1444 cm^{-1}	Asymmetric methyl stretching	
1275-1089 cm^{-1}	In-plane C-H bending In-plane C-H bending	
Vermilion stick sample		
4000-2800 cm^{-1}	O-H, C-H, N-H stretching O-H, C-H, N-H stretching	(Chopi et al., 2021)
1560-1530 cm^{-1}	Asymmetric stretching Asymmetric stretching	(Chopi et al., 2021)
1481, 1444 cm^{-1}	Asymmetric methyl stretching	(Chopi et al., 2021)
1322-1258 cm^{-1}	Aromatic C-N stretching or C-O stretching	(Chopi et al., 2021)
753 cm^{-1}	-N-H wagging	Present study
1740 cm^{-1}	C=O stretching	Present study
1377, 1339, 1096, 848, 837cm^{-1}	C-O stretching	Present study
719 cm^{-1}	CH2 rocking mode	Present study

cosmetics. Ghosh and Sharma (2022) also used TLC to analyze vermilion samples and observed high interspecific variation in Rf values [18]. Bin Abdullah et al. (2011) used TLC with various mobile phases to separate pigments in lipsticks, categorizing them based on retention factor (Rf) values [15]. Joshi et al. (2013) similarly analyzed red-shade lipsticks but found no significant Rf variation between local and branded products [16]. Ezegbogu and Osadolor (2019) studied TLC retention factors in aged lipstick stains and found no significant differences in Rf values [17].

3.2 ATR-FTIR Spectrum analysis of the vermillion and lipsticks

The study presents an infrared spectroscopy analysis of different Vermilion samples, including powder, liquid, and stick forms, focusing on several specific absorption peaks. For the Vermilion powder sample, the key absorption bands observed were in the 4000-2800 cm^{-1} range, indicating OH-C-H and N-H stretching, peaks at 1395 and 1380 cm^{-1} for asymmetric methyl stretching, 1275-1089 cm^{-1} for in-plane C-H bending, 1027 and 1006 cm^{-1} for C-O or C-C stretching, and 871 and 711 cm^{-1} for C-H deformation, Figure 2. These findings align with Chophi et al. (2021) [9]. In the Vermilion liquid sample, the spectrum showed similar OH, C-H, and N-H stretching in the 4000-2800 cm^{-1} range, C=C stretching between 1680-1600 cm^{-1}, asymmetric methyl stretching at 1481 and 1444 cm^{-1}, and in-plane C-H bending at 1275-1089 cm^{-1}, Figure 4, corroborating Chophi et al. (2021) [9]. For the Vermilion stick sample, the spectra revealed a more complex set of absorption bands, including O-H, C-H, and N-H stretching in the 4000-2800 cm^{-1} range, asymmetric stretching at 1560-1530 cm^{-1}, and asymmetric methyl stretching at 1481 and 1444 cm^{-1}, Figure 5. Additional features included aromatic C-N or C-O stretching at 1322-1258 cm^{-1}, -N-H wagging at 753 cm-1, C=O stretching at 1740

Table 2 - *Common and unique peaks identified in the lipstick samples of the present study*

Range (cm-1)	Band Assignment	Reference
2850 cm^{-1}	Symmetric C H stretching vibrations	(Gladysz et al., 2017)
2916 cm^{-1}	asymmetric C H stretching vibrations	
2954 cm^{-1}	asymmetric CH3 stretching vibrations	
1730 cm^{-1}	C=O stretching vibrationsCH2 scissor deformation	
1465 cm^{-1}	C=O stretching	
1172 cm^{-1}	C H bending vibrations	
712 cm^{-1}	CH2 rocking mode	
2915 cm-1 and 2850 cm^{-1}	C-H stretches	(Wong et al., 2019)
805 cm^{-1}	Si-C stretch	
1060 cm^{-1}	Si-O-Si stretch	
1260 cm^{-1}	CH3 symmetric deformation of Si-CH3	
2850, 2915 cm^{-1}	Alkyl C-H stretches	
3000-3600 (Broad) cm^{-1}	O-H stretch	
2917.64 cm^{-1}	OH stretching or NH-- stretching	Kaur et al. 2020
2847.02 cm^{-1}	C-H stretching,	
1741.92 cm^{-1}	C-H stretching	
1462 cm^{-1}	CO stretching	
1378.11 cm^{-1}	OH- bending	
1241.08 cm^{-1}	CN- stretching	
1165.81 cm^{-1}	CO- stretching	
2955,2917 cm^{-1}	Asymmetric CH3 stretching C-H asymmetric, NH stretch	Present study
2849 cm^{-1}	C-H stretching	Present study
1739, 1009, 1462, 1377, 1172 cm^{-1}	C-O stretching CH or CO stretching OH bending C=O stretching, C-H bending	Present study
830 cm^{-1}	C-O stretching	Present study
729, 719, 669 cm^{-1}	CH2 rocking mode	Present study
2953 2921 2852 cm^{-1}	Asymmetric CH3 stretching C-H stretching C-H symmetric	Present study
1460 1377 cm^{-1}	C-H, C-O stretching C-O stretch, OH bending	Present study
722, 699 cm^{-1}	CH2 rocking mode	Present study

Table 3- *Library search results and hit score*

Compound	Hit Score	Function
Butyl Stearate	0.8	decrease viscosity and suspend colors for uniform application.
Nonane	0.94	functions as a perfuming agent, enhancing the fragrance of cosmetic products
1-Hexacosanol Synth	0.9	enhances its effectiveness as an emulsifier, thickener, and performance enhancer.
Linoleic Acid	0.76	functions as an emollient, thickening agent, skin restorative, antioxidant, and soothing agent
1-Bromoheptadecane	0.83	perfuming agent

cm^{-1}, and C-O stretching with CH2 rocking mode at several lower frequencies (1377, 1339, 1096, 848, 837, and 719 cm^{-1}) (Table 1). These results are consistent with the work of Chophi et al. (2021) [9], with some distinct differences observed for the stick sample.

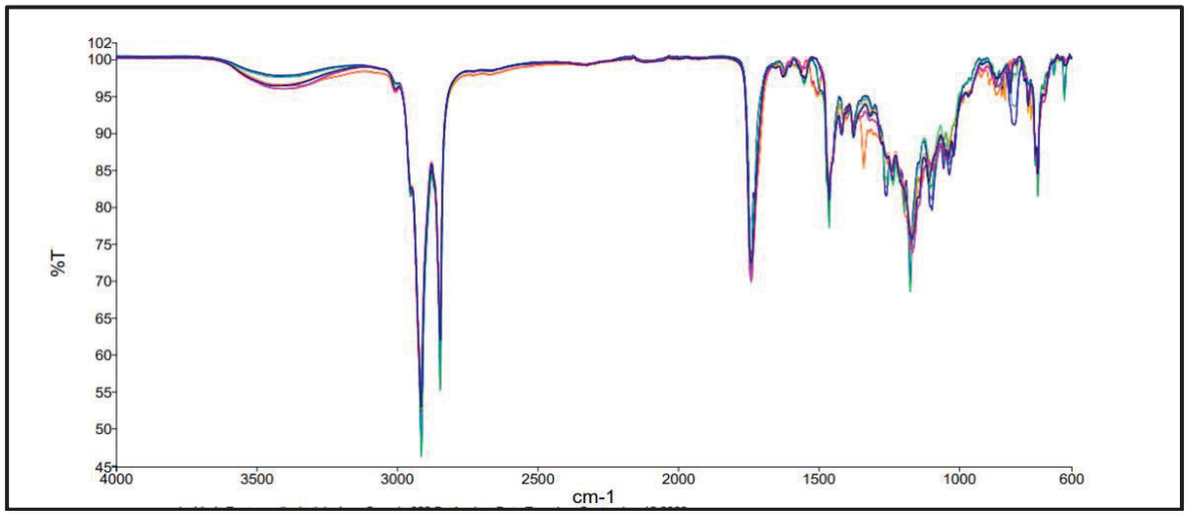

Figure 5- *Infrared spectra of Vermilion stick showing OH, C-H, and N-H stretching (4000-2800 cm-1), asymmetric stretching (1560-1530 cm-1), and C=O stretching (1740 cm-1).*

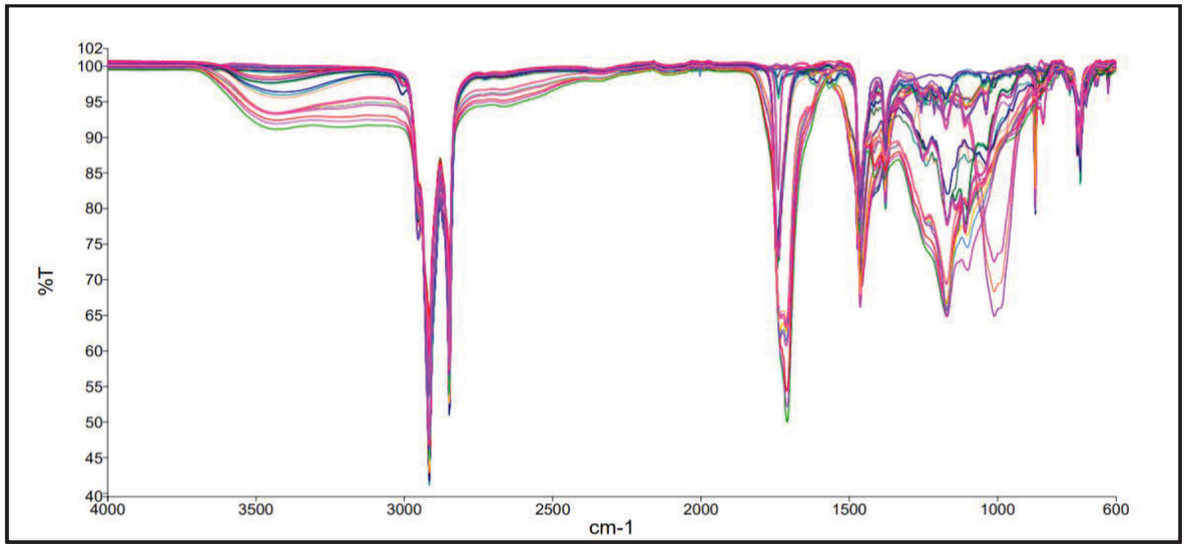

Figure 6- *Infrared spectra of lipstick samples showing key peaks at 2850 cm-1 and 2916 cm-1 for C-H stretching,2954 cm-1 for CH3 stretching, 1730 cm-1 for C=O stretching, and 712 cm-1 for CH2 rocking..*

The results from the current study on lipstick samples show several key infrared absorption peaks, Figure 6 that align with findings from previous literature, as well as some unique observations (Table 2). Common peaks identified across multiple studies include those at 2850 cm^{-1} and 2916 cm^{-1}, associated with symmetric and asymmetric C-H stretching vibrations, and at 2954 cm^{-1}, related to asymmetric CH3 stretching vibrations. Peaks at 1730 cm^{-1} and 1465 cm^{-1} correspond to C=O stretching and C-H bending vibrations, respectively. Additionally, 1172 cm^{-1} and 712 cm^{-1} are linked to C=O stretching and CH2 rocking mode. The current study further identified several distinct peaks, such as 1739 cm^{-1}, 1009 cm^{-1}, 1462 cm^{-1}, 1377 cm^{-1}, and 830 cm^{-1}, which correspond to C-O stretching, C-H or C-O stretching, OH bending, C=O stretching, and C-H bending. Furthermore, additional peaks at 729

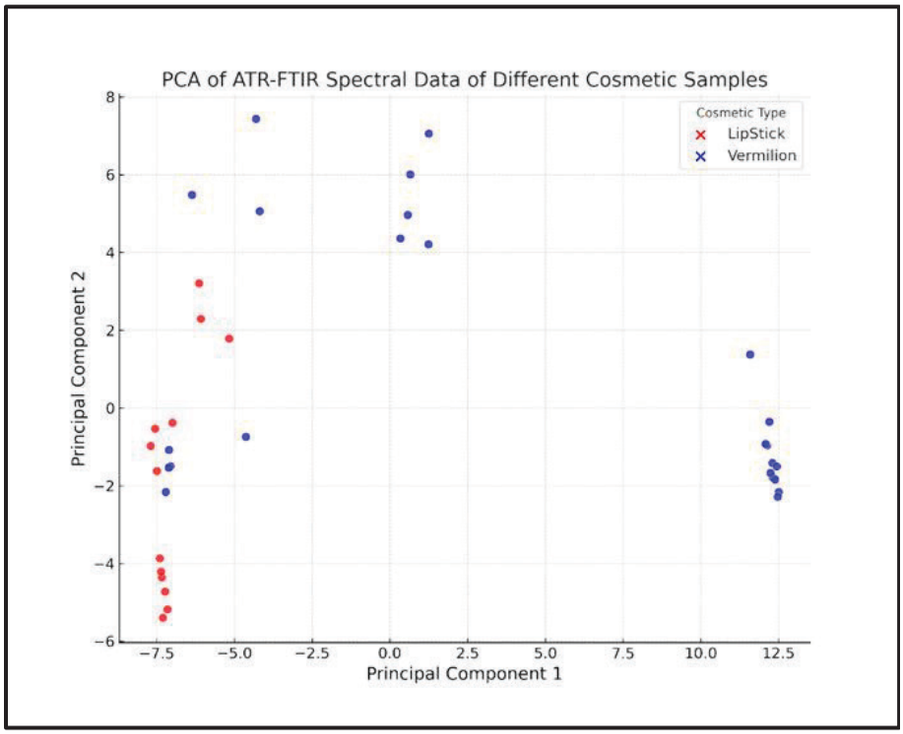

Figure 7-*PCA analysis of cosmetics (lipstick and vermillion), with PC1 explaining 71.25% and PC2 12.32%, together accounting for 83.57% of the variance.*

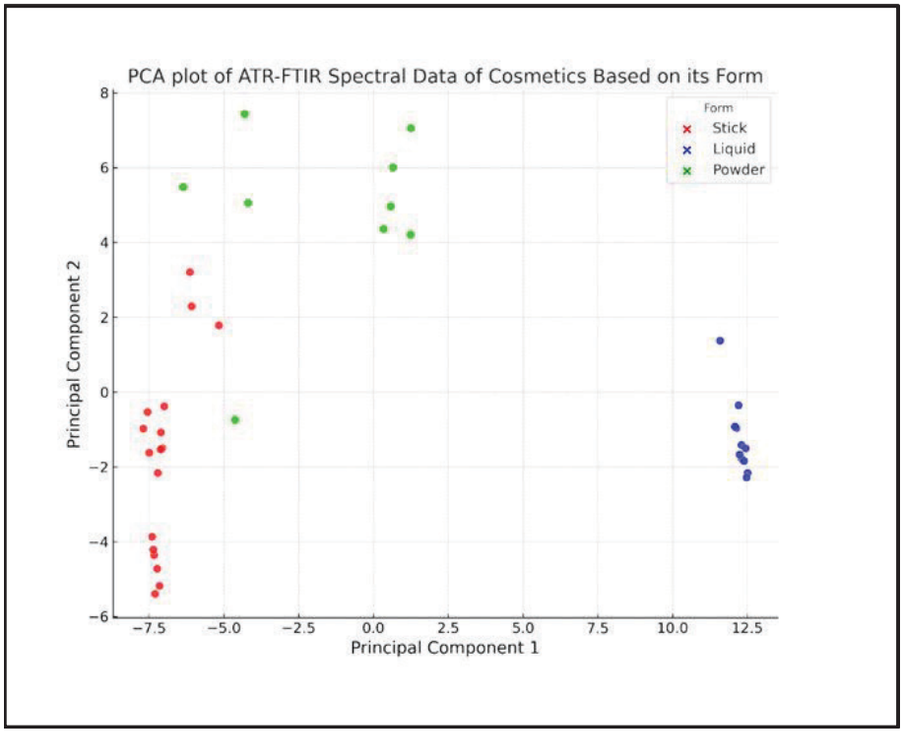

Figure 8- *PCA analysis based on form (stick, powder, liquid), with PC1 explaining 70% and PC2 20%, together accounting for 90% of the variance.*

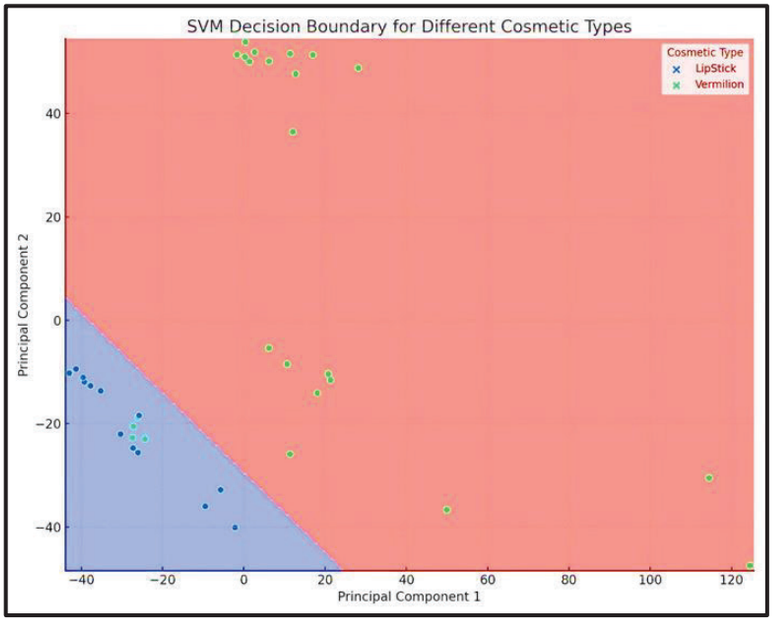

Figure 9- *SVM Decision Boundary for Different Cosmetics: Lipstick vs. Vermillion*

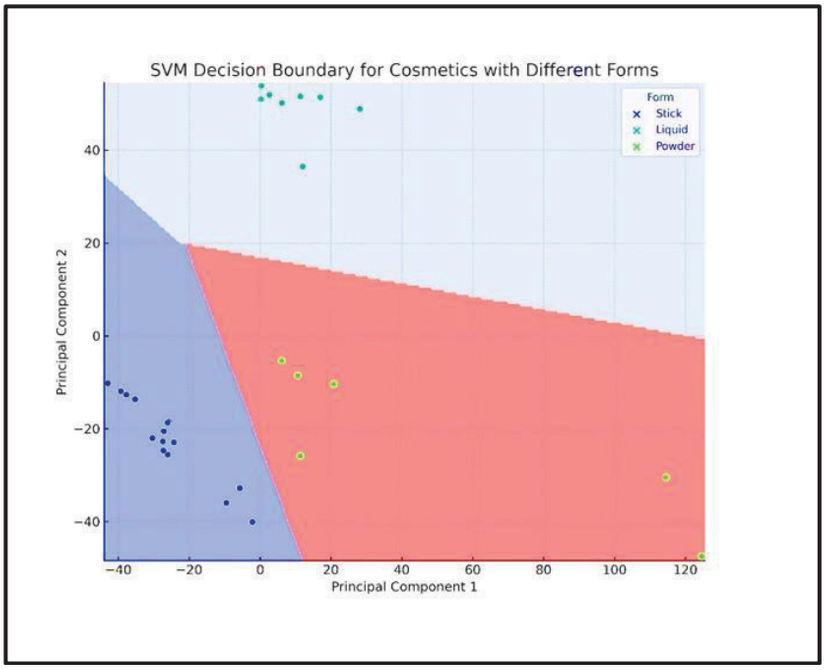

Figure 10- *SVM Decision Boundary for Different cosmetics Type: Stick vs. Liquid vs. powder*

cm^{-1} 719 cm^{-1}, and 669 cm^{-1} were observed for CH2 rocking mode, while 2953 cm^{-1}, 2921cm^{-1}, and 2852 cm^{-1} are linked to asymmetric CH3 stretching, C-H stretching, and C-H symmetric vibrations. These results contribute to a comprehensive understanding of the chemical composition of lipstick, highlighting both common and unique spectral features across different studies [4, 5, 13-14]. The peak ranges of lipsticks from 3000-700 cm^{-1} showed closer peak were similar to spectrum of other cosmetics i.e. eye

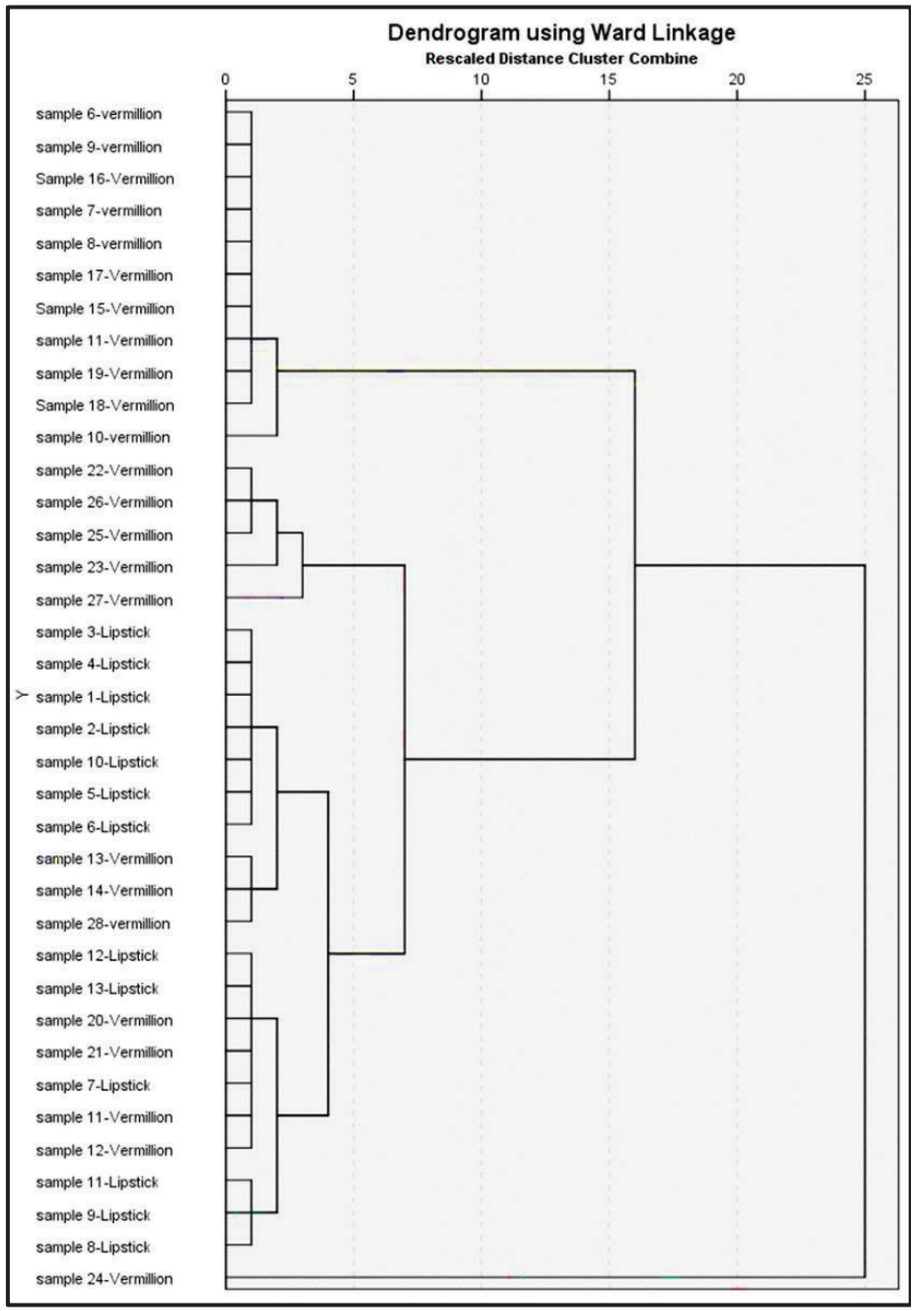

Figure 11- *PCA clustering of cosmetic samples based on type and form. Cluster 1 includes vermilion liquids, Cluster 2 consists of vermilion powders, Cluster 3 features lipstick sticks, Cluster 4 combines liquid and powder samples, Cluster 5 groups vermilion sticks and lipsticks, and Cluster 6 contains similar lipstick samples.*

pencil, depigmenting cream and eyeshadow [12] and foundation creams [6], indicating the common ATR spectrum for the cosmetics in this range.

After a comprehensive examination of the FTIR library's cosmetics spectrum, it became apparent that it shares significant similarities with five key compounds: Butyl Stearate, Nonane, 1-Hexacosanol Synth, Linoleic Acid, and 1-Bromoheptadecane.

This resemblance is striking, as indicated by their hit scores surpassing the notable threshold of 0.8. Such results suggest a substantial overlap in their spectral profiles, pointing towards potential correlations and shared traits among these compounds (Table 3).

3.3 Principal Component analysis (PCA)

Upon conducting Principal Component Analysis (PCA) categorized by cosmetic type, namely lipstick or vermillion, the distribution of variance explained by the principal components is as follows: Principal Component 1 (PC1) contributes to approximately 71.25% of the dataset's variance, while Principal Component 2 (PC2) accounts for about 12.32% of the variance. When considering both PC1 and PC2 collectively, they reveal approximately 83.57% of the total variance within the dataset (Figure 7). Based on the type of form i.e. stick, powder, and liquid. The PCA analysis variance explained by the principal components is as follows: Principal Component 1 (PC1) elucidates approximately 70% of the dataset's variance, while Principal Component 2 (PC2) accounts for roughly 20% of the variance. When considering both PC1 and PC2 collectively, they jointly explain about 90% of the total variance within the dataset (Figure 8)

ATR-FTIR and chemometrics have been used to discriminate and identify cosmetic samples. Gładysz et al. (2017) studied 37 lipstick brands in Poland, finding significant differences in the 3050–2775 cm-1 range and identifying six clusters, with 93% discriminatory power through correlation analysis [13]. Alblooshi et al. (2024) analyzed 20 MAC pink lipsticks, achieving 96% accuracy using PCA, noting variability in the 1460-720 cm^{-1} and 1652-463 cm^{-1} regions [19]. Chophi et al. (2020) used principal component analysis- linear discriminant analysis (PCA-LDA) to classify 38 red lipsticks, achieving 81.5% accuracy, with substrate-dependent peaks between 2000-600 cm^{-1} [4]. Wong et al. (2019) analyzed 40 wax and 20 liquid lipstick samples using UV-VIS (195-900 nm) and FTIR (4000-400 cm^{-1}) spectroscopy. Their PCA and LDA chemometric analysis achieved 93% accuracy for red lipsticks but only 73% for nude shades [21].

3.4 Support vector machine (SVM) analysis

In the SVM results, precision indicates the accuracy the model's positive predictions. It is calculated by dividing the number of true positive predictions by the total number of positive predictions (true positives plus false positives). In our study, a precision of 1.00 for both Lipstick and Vermilion means the model correctly predicted every sample as Lipstick or Vermilion. Recall, also known as Sensitivity, measures how well the model finds all the relevant cases within a category. It's the ratio of true positives to all the actual positives (true positives plus false negatives). Achieving a recall of 1.00 for Lipstick and Vermilion indicates that the model identified all samples of these types correctly in the test set. The F1-Score, which is the harmonic mean of precision and recall, gives us a balanced measure of the model's accuracy. It combines both precision and recall into a single metric. An F1-Score of 1.00 suggests that the model is exceptionally good at classifying LipStick and Vermilion, effectively balancing precision and recall. (Figure 9).

3.4.2 SVM analysis for cosmetic forms

In the cosmetic form context, the SVM model demonstrated remarkable performance metrics. With a precision of 1.00, the model accurately predicted every sample in the test dataset based on its form be it liquid, powder, or stick. Achieving a recall score of 1.00 indicates that the model successfully identified all Lipstick and Vermilion samples based on their forms within the test set. Furthermore, the

F1 Scores of 1.00 reflect the model's exceptional ability to classify these cosmetic forms i.e. liquid, stick, and powder, effectively balancing precision and recall. (Figure 10).

3.5 Hierarchical cluster Analysis (HCA)

The HCA analysis identified six clusters based on sample similarities. The first cluster, consisting of 10 vermilion liquid samples, includes Eyetex Divya (Sample 6), Eyetex Pallavi (Sample 9), Zovi (Sample 16), Milan Blue (Sample 7), Avlon International (Sample 8), Love Forever (Sample 17), Lakme (Sample 15), and another vermilion liquid (Sample 11). The second cluster contains three vermilion powder samples: Aashika (Sample 22), Vaisa (Sample 26), and Roli (Sample 25). The third cluster includes 7 lipstick samples in stick form: Yaris 21 (Sample 3), Yaris 19 (Sample 4), Aloe (Sample 1), Beauty Lips (Sample 2), Shills Soft Lips (Sample 10), Aroma (Sample 5), and ADS (Sample 7). The fourth cluster has 3 samples: Eyetex Kumkum (Sample 14) and vermilion liquid Glam 21 (Sample 18), along with Sridevi (Sample 28), where Glam 21 shows closer similarity to powder samples. The fifth cluster consists of 7 samples: Silvassa 32 (Sample 12), Silvassa 25 (Sample 13), Vermilion stick Liboni (Sample 20), Vermilion stick Blue Heaven (Sample 21), Lipstick Divine Red (Sample 7), Vermilion Blue Lady (Sample 11), and Vermilion stick Shingar (Sample 12). The sixth cluster includes 3 lipsticks: Blue Lady (Sample 11), Liboni Maroon (Sample 9), and Divine Maroon (Sample 8), which show greater similarity in composition. These clusters show high similarity depending on the form of vermilion or types of cosmetics (Figure 11). The current research identified six clusters among cosmetic stains, similar to previous studies. Ghazali and Ismail (2018) found six clusters in 12 red lipsticks with an 87% similarity index using ATR-FTIR analysis in the 1730-1701 cm^{-1} range [24]. Sharma et al. (2019) identified 8 clusters in 25 lipstick samples using ATR-FTIR, achieving 90% discriminatory power through hierarchical clustering [25].

The present study successfully separated pigments using compatible mobile phases and applied chemometric techniques like PCA and HCA. The SVM model accurately predicted cosmetic types and forms. A key strength is the inclusion of 23 vermilion and 13 lipstick brands available in Bangalore, covering powder, liquid, and stick forms for vermilion. Future research could enhance cosmetic stain analysis by combining multiple analytical techniques for more precise identification. Integrating ATR-FTIR with other spectroscopy methods could improve the detection of degradation in lipsticks, as seen in Gładysz et al. (2020) [20]. Expanding the study of aging and degradation in vermilion samples, as done by Chophi et al. (2020) [4], could also include more diverse substrates and environmental factors. Further improvements in dye separation, like those by Gładysz et al. (2019) [21], could involve optimizing capillary electrophoresis and pairing it with advanced chromatography or mass spectrometry for better precision. Additionally, combining MEKC with ATR-FTIR, as shown in Gładysz et al. (2020) [20], could offer more reliable results for identifying aged and contaminated stains. Lastly, expanding the use of Scanning Electron Microscopy/Energy Dispersive X-ray Spectroscopy (SEM-EDS), as demonstrated by Singh et al. (2023) [23], could help identify elemental variations in a broader range of cosmetic products, supporting forensic identification and authenticity verification. These combined approaches will enhance accuracy in forensic applications and consumer safety.

4. CONCLUSIONS

In conclusion, this study successfully demonstrates the separation and classification of vermilion and lipstick samples using TLC, FTIR, PCA, SVM, and HCA analyses. TLC results showed that mobile phase 3 was optimal for liquid and stick vermilion, while mobile phase 1 was best for powder vermilion. For lipsticks, mobile phase 2 provided superior separation, with mobile phase 1 showing consistent but lower Rf values. FTIR analysis revealed distinct absorption peaks for each cosmetic form, with similarities to compounds like Butyl Stearate and Linoleic Acid. PCA accounted for the majority of variance in both cosmetic types and forms, while SVM analysis achieved perfect classification accuracy. HCA further supported these findings, clustering samples based on their form and demonstrating the study's ability to differentiate liquid, powder, and stick cosmetics effectively. The forensic significance of these results lies in their ability to accurately trace the origin of cosmetic products and identify counterfeit or adulterated items in forensic investigations. This study offers a reliable approach for differentiating between various cosmetic forms, facilitating product verification and ensuring authenticity.

Conflict of interest

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Availability of data and material

The datasets used/or analyzed during the current study are available from the corresponding author on reasonable request.

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Supplementary Table 1- *Different brands of Vermilion samples used for analysis.*

Product (Vermilion)	Brand	Form	Manufacturer
Roli	Jyoti	Powder	Jaiprakash chemical.co
Manjal	Ashika	Powder	Ashika Incence Inc.
Milan	Blue heaven	Liquid	Blue heaven cosmetics, ltd
Vaisa	Ramji	Powder	n/a
Luxury	Ramji	Powder	n/a
Avlon	Avlon	Liquid	n/a
Pallavi	Eyetex	Liquid	Arvind lab
Divyaa	Eyetex	Liquid	Arvind lab
Pallavi	Eyetex	Stick	Arvind lab
Kumkum	Eyetex	powder	Arvind lab
Sindoor	Shingaar	Liquid	Shingar limited
Saubhagya premium Kumkum	Shingaar	Stick	Shingar limited
Kumkum	Shingaar	powder	Shingar limited
001 My red	color bar	Liquid	n/a
	Gopuram	Powder	Y.V.S and co
	Blue heaven	Stick	Blue heaven cosmetics, ltd
Love forever Sindoor	Swiss beauty	Liquid	Kascap glass pvt ltd
	Glam 21	Liquid	Love bird cosmetics
Kumkum	Sri devi	Powder	Sri devi camphor works
Jewel	Zovi colorberry	Liquid	Bevon Cosmetics M.L.N
Jewel	Lakme	Liquid	Aero care personal products LLp
	Shreeji	Liquid	n/a
Local		Powder	Maruthi enterprises
Liboni	Vermillion	Stick	n/a

Supplementary Table 2- *List of different brands of lipstick samples used for analysis.*

Product (Lipstick)	Brand	Manufacturer
Aloe Lipstick(18)	ADS	n/a
Lipstick 19	Yaris	n/a
Lipstick 21	Yaris	n/a
Beauty lips	ADS	n/a
Lipstick 12	ADS	n/a
Soft lips	Shills	P.R.C
Lipstick	Amona	A.B Cosmetics
	Liboni	n/a
Lipstick 7	Blue lady	n/a
Red	Divine	n/a
Maroon	Divine	n/a
Red Rouge 32	Silvassa	Aero Pharma
Lipstick 25	Silvassa	Aero Pharma

Supplementary Table 3- *Rf value for separated pigments of local brand of vermillion*

S.No.	Name of product	Cosmetic type	Mobile phase 1 Toluene: Acetone (16:4)	Mobile phase 2 Toluene: Benzene (12:8)	Mobile phase 3 Toluene: Acetone: Benzene (16:2:2)
1.	Lakme jewel liquid	Liquid Vermillion	-	-	-
2.	Shingar(liquid)	Liquid vermillion		P1 - 0.26	
3.	Colorbar	Liquid vermillion	P1 - 0.8	P1 - 0.2	P1 - 0.97
4.	Zovi jewel liquid	Liquid Vermillion	P1 - 0.81	P1 - 0.26	P1 - 0.87
5.	Love forever liquid	Liquid Vermillion	P1 - 0.81	P1 - 0.15	P1 - 0.87
6.	Glam 21 liquid	Liquid Vermillion	P1 - 0.8	P1 - 0.21 P2 - 0.26	P1 - 0.8
7.	Shreeji liquid	Liquid Vermillion	P1 - 0.84 P2 - 0.92	P1 - 0.23 P2 - 0.58	P1 - 0.15 P2 - 0.92
8.	Eyetex divya(liquid)	Liquid vermillion	P1 - 0.84 P2 - 0.86	P1 - 0.18 P2 - 0.15 P3 - 0.58	P1 - 0.92
9.	Milan blue heaven (liquid)	Liquid vermillion	P1 - 0.16	P1 - 0.23	P1 - 0.15
10.	Avlon international (liquid)	Liquid vermillion	P1 - 0.87	P1 - 0.15	P1 - 0.95
11.	Eyetex pallavi (liquid)	Liquid vermillion	P1 - 0.92	P1 - 0.23 P2 - 0.58	P1 - 0.92
12.	Eyetex kumkum powder	Powder Vermillion	P1 - 0.74 P2 - 0.81	P1 - 0.46	P1 - 0.71
13.	Shingar powder	Powder Vermillion	-	-	-
14.	Aashika(powder)	Powder Vermillion	P1 – 0.12 P2 – 0.18		P1 – 0.12 P2 – 0.16
15.	Gopuram (powder)	Powder Vermillion	P1 -0.85 P2 -0.86		P1 – 0.12
16.	Roli(powder)	Powder Vermillion	P1 - 0.06		P1 - 0.06
17.	Luxury (powder)	Powder Vermillion	P1 - 0.85 P2 - 0.90	P1 - 0.16	P1 - 0.83 P2 - 0.89
18.	Vaisa (powder)	Powder Vermillion	-	-	-
19.	Maruthi (powder)	Powder Vermillion	-	-	-

S.No.	Name of product	Cosmetic type	Mobile phase 1 Toluene: Acetone (16:4)	Mobile phase 2 Toluene: Benzene (12:8)	Mobile phase 3 Toluene: Acetone: Benzene (16:2:2)
20.	Sridevi (powder)	Powder vermillion	P1 - 0.89		P1 – 0.11
21.	Eyetex pallavi stick	Stick Vermillion	P1 - 0.78 P2 - 0.84	P1 - 0.15 P2 - 0.21 P3 - 0.50	P1 - 0.82 P2 - 0.89
22.	Shingar stick	Stick Vermillion	-	-	-
23.	Liboni stick	Stick Vermillion	P1 - 0.79	P1 - 0.26	P1 - 0.84
24.	Blue heaven stick	Stick Vermillion	-	-	-

Supplementary Table 4- *Rf value for separated pigments of local brand of lipsticks*

S.No.	Name of product	Cosmetic type	Mobile phase Chloroform: Methanol: water (50:15:2)	Mobile phase Ethyl acetate: methanol: Ammonia Hydroxide (5:1:1)
1.	ADS Lipstick	Lipstick	P1 – 0.16	P1 - 0.21 P2 - 0.24
2.	Aloe Lipstick	Lipstick	P1 - 0.58	P1 - 0.23 P2 - 0.30
3.	Amona lipstick	Lipstick	P1 - 0.16 P2 - 0.6 P3 - 0.66	P1 - 0.21 P2 - 0.23
4.	Beauty lips	Lipstick	P1 - 0.15	P1 - 0.23 P2 - 0.29
5.	Blue lady lipstick	Lipstick	P1 - 0.15 P2-0.66 P3 - 0.70 P4 - 0.78	P1 - 0.18 P2 - 0.23 P3 -0.29
6.	Divine lipstick (maroon)	Lipstick	P1 - 0.50	P1 - 0.36 P2 - 0.27 P3 - 0.30 P4 - 0.35 P5 - 0.73
7.	Divine lipstick (red)	Lipstick	P1 - 0.52 P2 - 0.86 P3 - 0.76	P1 - 0.36 P2 - 0.27 P3 - 0.35
8.	Liboni(maroon)	Lipstick	P1 - 0.16 P2 - 0.64 P3 - 0.83 P4 - 0.76 P5 - 0.81	P1 - 0.21 P2 - 0.52 P3 - 0.29 P4 - 0.32
9.	Shills soft lips(liquid)	Lipstick	P1 - 0.15	P1 - 0.23 P2 - 0.26
10.	Silvasa 25	Lipstick	P1 - 0.12 P2 - 0.15 P3 - 0.75	P1 - 0.18 P2 - 0.03 P3 - 0.29
11.	Silvasa 32	Lipstick	P1 - 0.15 P2 - 0.73	P1 - 0.18 P3 -0.29
12.	Yaris Lipstick (19)	Lipstick	P1 - 0.16 P2 - 0.56	P1 - 0.21 P2 - 0.24 P3 - 0.32
13.	Yaris Lipstick (21)	Lipstick	P1 - 0.56	P1 - 0.23 P2 - 0.32