DISCRIMINATION OF VARIOUS COSMETIC PRODUCTS USING ATR-FTIR SPECTROSCOPY AND CHEMOMETRICS

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UNIVERSITI SAINS MALAYSIA

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DISCRIMINATION OF VARIOUS COSMETIC PRODUCTS USING ATR-FTIR SPECTROSCOPY AND CHEMOMETRICS

by

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Thesis submitted in fulfilment of the requirements for the degree of Bachelor of Science in Forensic Science

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CERTIFICATE

This is to certify that the dissertation entitled Discrimination of Various Cosmetic Products using ATR-FTIR Spectroscopy and Chemometrics is the bona fide record of research work done by Ms Nur Syuhada binti Ismail during the period from October 2024 to February 2025 under my supervision. I have read this dissertation and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation to be submitted in partial fulfilment for the degree of Bachelor of Science in Forensic Science.

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DECLARATION

I hereby declare that this dissertation is the result of my own investigations, except where otherwise stated and duly acknowledged. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at Universiti Sains Malaysia or other institutions. I grant Universiti Sains Malaysia the right to use the dissertation for teaching, research and promotional purposes.

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(NUR SYUHADA BINTI ISMAIL)

Date: 9/2/2025

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LIST OF ABBREVIATIONS

ATR-FTIR	Attenuated Total Reflection-Fourier Transform Infrared
ANN	Artificial Neural Network
BHT	Butylated Hydroxy Toluene
CA	Cluster Analysis
DNA	Deoxyribonucleic Acid
DPT	Desktop Publishing Tool
EU	European Union
FDA	Food and Drug Administration
LDA	Linear Discriminant Analysis
IR	Infrared
PCA	Principal Component Analysis
PCs	Principal Components
PLS	Partial Least Squares
PEGs	Polyethylene Glycols
RSD	Relative Standard Deviation
R&D	Research and Development
SPF	Sun Protected Factor
SIMCA	Soft Independent Modelling of Class Analogy
TLC	Thin Layer Chromatography
UV	Ultraviolet
UV-Vis	Ultraviolet–Visible
UVB	Ultraviolet B
2D	Two-Dimensional
3D	Three-Dimensional

DISKRIMINASI PELBAGAI PRODUK KOSMETIK MENGGUNAKAN SPEKTROSKOPI ATR-FTIR DAN KEMOMETRIK

ABSTRAK

Dalam dunia moden hari ini, kosmetik telah menjadi sebahagian penting dalam kehidupan seharian kita. Produk kosmetik boleh menjadi bukti yang berharga di tempat kejadian jenayah kerana kesan kosmetik tergolong dalam kategori bukti pemindahan. Sentuhan antara pengguna kosmetik (sama ada mangsa atau pelaku) dengan objek atau individu lain sering menyebabkan pemindahan sisa kosmetik dan membantu menghubungkan individu dengan lokasi. Walau bagaimanapun, kajian forensik mengenai analisis dan pembezaan pelbagai jenis produk kosmetik masih terhad. Dalam kajian ini, teknik spektroskopi ATR-FTIR yang pantas dan tidak merosakkan sampel telah digunakan untuk membezakan 16 sampel produk kosmetik. Analisis Komponen Utama-Analisis Diskriminan Linear (PCA-LDA) digunakan bagi melengkapkan perbandingan spektrum visual serta memberikan tafsiran hasil yang lebih objektif. Model ramalan PCA-LDA mencapai ketepatan 100%, di mana semua sampel, tanpa mengira jenis kosmetik, diklasifikasikan dengan betul mengikut jenama masing-masing. Ujian buta yang dilakukan ke atas tiga sampel tidak diketahui berjaya dihubungkan kepada sumber asalnya. Dalam kajian ketahanan, produk kosmetik terpilih dibiarkan pada suhu bilik, dan sampel yang telah berusia sehingga 12 jam masih menunjukkan ciri spektrum yang serupa dengan sampel segar, membolehkan proses pengecaman dilakukan. Kajian ini membuktikan bahawa kaedah yang digunakan adalah pantas, menjimatkan kos, tahan lasak, serta tidak merosakkan sampel, menjadikannya sesuai untuk pembezaan pelbagai produk kosmetik dalam siasatan forensik.

DISCRIMINATION OF VARIOUS COSMETIC PRODUCTS USING ATR-FTIR SPECTROSCOPY AND CHEMOMETRICS

ABSTRACT

In today's world, cosmetics have become an essential part in our everyday lives. Cosmetic products can serve as valuable evidence at crime scenes, as cosmetic smudges fall under the category of transfer evidence. The contact between a cosmetic user (whether victim or perpetrator) and objects or other individuals often results in the transfer of cosmetic residues, which can help link individuals to specific locations, events, or interactions. Furthermore, there has been limited forensic studies focus on analysis for discrimination of different types of cosmetic products. In this study, Attenuated Total Reflectance-Fourier Transform-Infrared (ATR-FTIR) spectroscopy, a rapid and non-destructive has been utilised to differentiate a total of 16 cosmetic products of four different types. Principal Component Analysis-Linear Discriminant Analysis (PCA-LDA) was employed to complement visual spectral comparisons and generate objective interpretation of the results. 100 % accuracy of predictive PCA-LDA model was established with all samples regardless of cosmetic types were correctly classified to their respective brands. The blind test was conducted with three unknown samples, which were correctly linked with their respective source. In persistence study, selected cosmetic products were exposed at room temperature and aged samples for up to 12 hours still showed similar spectral features similarly to their fresh samples that allowed identification. The current work demonstrated a quick, cost-effective, robust, and non-destructive method for differentiation of various cosmetic products in forensic investigation.

CHAPTER 1

INTRODUCTION

1.1 Cosmetic Product

In today's world, cosmetics have become an essential part in our everyday lives. Cosmetics were once only used for special events or particular beauty rituals, but today people of all ages and genders use them extensively to improve their appearance, express who they are, and keep themselves clean. The range of cosmetic items has increased to meet diverse needs and preferences from makeup and skincare to haircare and fragrance. The factors that contributing to these changes are due to the media trends, changing societal standards, and the increasing significance of self-care in preserving mental well-being. Therefore, nowadays, cosmetic products are used almost in all age groups including infants, children, and adults in the form of facial creams, moisturisers, body lotions, lip balms, lip sticks, deodorant and perfume.

By definition, a cosmetic product is any substance or mixture intended to be placed in contact with the external parts of the human body, with the exclusive or with main objective of cleaning, changing the look, protecting and maintaining good condition, or eliminating body odours (Faria-Silva *et al.*, 2022). According to the European Union (EU), cosmetics range from daily hygiene products, such as soap, shampoo, deodorant, toothpaste, to luxury beauty products, such as fragrances and makeup. On the other hand, the Food and Drug Administration (FDA) defines cosmetics as products meant to be used by humans on their bodies for the purpose of cleansing, beautifying, appealing enhancement, or adjusting appearance. Cosmetic products are designed and manufactured following strict criteria and guidelines established by the FDA, EU, or authorities of the various countries to ensure their safety for human use and ensure they lead to the desired effect. Therefore, cosmetic products include a variety of materials and products used in daily routine of an individual, such as body lotions, facial and bodily cleansers, hair products, makeup, and fragrance product.

1.2 Forensically Important Cosmetic Products

According to Locard's Exchange Principle, "every contact leaves a trace." Cosmetic products, therefore, can serve as valuable evidence at crime scenes, as cosmetic smudges fall under the category of transfer evidence. The contact between a cosmetic user (whether victim or perpetrator) and objects or other individuals often results in the transfer of cosmetic residues, which can help link individuals to specific locations, events, or interactions. Currently, cosmetic evidence encountered at crime scenes is not only exclusively associated with crimes against women, such as violent physical and sexual assaults, but also with a female perpetrator of a crime (AlSaeed *et al*., 2022).

Moreover, cosmetic product is useful as corroborative evidence where more information can be provided regarding the criminal, the tools they used, and the sequence of events which making it useful for explaining crimes in court. For example, the colour of cosmetic evidence enables quick differentiation between questioned and recovered samples in forensic investigations. However, when the colours of the cosmetic product appear similar, there is a risk of biased interpretation which necessitating instrumental analysis to obtain the qualitative data. Cosmetic evidence is often encountered in cases like rape, sexual assault, homicide, theft, and home invasions cases where this type of evidence was once considered insignificant. However, with the recent advances in technology and analytical techniques have in this modern society leads to increase its evidentiary value but the search continues for a suitable, non-destructive method of cosmetic evidence analysis that can deliver definitive identification beyond scientific doubt.

1.3 Problem Statement

Cosmetic products traces or smudges are commonly found at the crime scene or on the victim which often resulting to many past studies of forensic analysis corelated with cosmetic products. However, only a few forensic studies focus on analysis for distinguishing between different types of cosmetic products such as between face moisturiser, antiperspirant like deodorant and body lotions. According to Chophi *et al*. (2019), the complexity of cosmetic products is due to their diverse compositions and textures, which has spurred a growing focus on developing new and improved analytical methods for forensic analysis on cosmetic products. Hence, the advances in analytical techniques have helped address some limitations in examining cosmetic smudges and understanding their implications in forensic scenarios.

Moreover, most forensic analysis of cosmetic products has focused on makeup products like lipstick, lip gloss, nail polish, face powder, and eye makeup, due to their widespread daily use especially among women. Mostly, past research investigated the discrimination between different brands but same types of cosmetic product. For instance, Mohamad Asri *et al*. (2021) focused on discrimination of different brands of facial creams, Mohamed Ghazali & Ismail (2018) examined the discrimination of different brands of lipstick and Chophi *et al*. (2021) studied on the analysis of eye-cosmetics such as eyeliners and eyeshadow.

Furthermore, the evidentiary potential of cosmetic traces is often underestimated in the realm of forensic analysis. Current forensic methods may overlook cosmetics as valuable corroborative evidence, focusing instead on more conventional traces like DNA. In cases such as sexual assault, investigators primarily seek DNA evidence. However, when DNA is unavailable, unrecovered, or analysis proves inconclusive, cosmetic traces left at the crime scene can become essential evidence. These traces can provide critical links between individuals and crime scenes, offering insight that may otherwise be missed in the absence of DNA.

1.4 Significance of the Study

The use of cosmetic products for skin decoration or protection dates back thousands of years, and such products remain highly popular in modern society (Wong *et al*., 2019). Nowadays, the widespread availability of diverse brands has contributed to the growing use of cosmetic products among both men and women. As a result, cosmetics have become a routine part of daily life for many individuals. This increasing prevalence also enhances the evidential value of cosmetic traces as trace evidence in forensic investigations.

On the other hand, owing to its tendency to persist over prolonged periods, cosmetic evidence recovered from crime scenes can assist in linking a suspect to a certain crime or victim, or exonerating a suspect (AlSaeed *et al*., 2022). Determining the origin of cosmetic traces helps linking the suspect to a crime and supports suspect exclusion which effectively narrowing the focus of ongoing investigations. This is especially valuable in cases such as homicides, sexual assaults, and crimes where women may be involved as perpetrators.

Furthermore, ATR-FTIR (Attenuated Total Reflectance - Fourier Transform Infrared) spectroscopy has various advantages such as being rapid, easy to analyse, and non-destructive, with little to no sample preparation needed to obtain the spectra (Nimi *et al*., 2023). At the same time, the discrimination power of ATR-FTIR spectroscopy in analysing forensic evidence improved by combining with chemometrics techniques. According to Sharma *et al*. (2019a) the discriminating power of visual analysis is found to be 98.0%, while principal component analysis (PCA) and linear discriminant analysis (LDA) in chemometrics analysis further increased the discriminating power to 99.3% and 100%, respectively. Therefore, ATR-FTIR and chemometrics method would be able to confirm if the cosmetic traces originated from the same brand of cosmetic product in the suspect's possession and at the crime scene based on the spectrum comparison and discrimination analysis.

1.5 Objectives

The objectives of this study were as follows:

1.5.1 General Objective

The general objective of this study was to study the significance and evidential value of various cosmetic products (facial foundation, body lotion, face moisturiser, antiperspirant) as trace evidence in forensic investigation using ATR-FTIR spectroscopy and chemometrics.

1.5.2 Specific Objectives

1. To characterise various types of cosmetic products.
2. To discriminate various types of cosmetic products.
3. To establish the potential of residual traces from cosmetic products as trace evidence and their persistence for forensic intelligence purposes.

CHAPTER 2

LITERATURE REVIEW

2.1 Trace Evidence and Its Evidential Value

Generally speaking, "trace evidence" refers to any kind of evidence that appears in tiny quantity enough which can be exchanged between two surfaces either between places, objects or people without being notice. The most common examples of trace evidence include hair, glass, fibres, paints, gunshot residue and ignitable liquids. Historically, trace evidence has been the discipline of forensic science that deals with the minute transfer of materials based on the theory that when two objects come in contact, each one would leave with something from the other (Florida, 2022). This is also sometimes referred to as Locard's Exchange Principle "every contact leaves a trace".

Although the capacity for trace materials to indicate a common source is not individualising without a physical fit, it is important to recognize that trace evidence can offer more than a possible/potential "source attribution". Trace materials can be used as exclusionary or associative evidence, and they can provide valuable information at the onset of an investigation to corroborate case activities and eyewitness accounts. The transfer and persistence of trace materials can provide useful information to reconstruct how a criminal event took place, expose relevant links, and reveal factors that can lead to the recovery of additional evidence (Trejos *et al*., 2020).

Besides that, trace evidence can act as associative evidence in the absence of biological evidence like DNA, because quite often, there is little to no probative biological evidence can be found in a casework which make the trace evidence remains a critical to link in solving the case. For example, finding unusual trace evidence can help detectives focus their search and steer them toward a more focused and productive path of investigation in the absence of a suspect. Thus, trace evidence can helps provide links between the victim and suspect, the victim and scene and the suspect and scene thereby supporting or refuting aspects of a criminal investigation.

Furthermore, trace evidence has exclusionary value in excluding potential suspects or scenarios. For example, trace evidence can act as exclusionary evidence if cosmetic smear found on suspect doesn't match with cosmetic product at the crime scene so, it can be used to eliminate them from suspicion. On the other hand, it is also having corroborative value which can help to support or verify statements made by witnesses, suspects, or victims. For instance, if a suspect claim that they didn't meet with the victim but there is a presence of cosmetic smear on their clothes which might possibly match with the cosmetic product a victim used then, the cosmetic traces (trace evidence) could challenge the testimonies given by the suspect. Therefore, the strength of trace evidence lies in its ability to corroborate or challenge testimonies and exclude or include suspects with a high degree of precision.

2.2 Challenges and Issues in Trace Evidence Analysis

The investigation process of a case from the crime scene to the courtroom is intricate and time-consuming. It requires the presentation of probative evidence to fulfil the burden of proof and establish the truth behind an allegation. In the last 15 years, forensic science has been facing a number of significant challenges, and it has been recently considered to be at a crossroads (Roux *et al.*, 2015). In this context, trace evidence recovered from crime scenes plays an important role in bridging gaps in the investigative process by linking suspects, victims, and crime scenes through the analysis of microscopic materials. However, despite its significance, there are several challenges and issues in trace evidence analysis that can affect its reliability and application in criminal investigations.

Trace evidence is often found in very small quantities since it is not readily visible and can be transferred when contact is made with an individual or an environment. For example, light material of trace evidence like hair and fibre tends to be lost over time due to less persistent in retaining at a place. In this context, the process of 3R’s of evidence which are recognition, recording and recovery become challenging (Robertson & Roux, 2010). Hence, one of the most significant challenges of trace evidence analysis is the tricky recognition step leads to small sample size making it difficult to analyse and identify.

On the other hand, trace evidence found at crime scene often mixed with or contaminated with other materials or items present the location. For instance, trace evidence such as smears of cosmetic product can be sticky and adhesive making them prone to picking up particles like dirt or dust. The large numbers of adhering particles, as well as their variety, provide an extremely rich source of potential information, but they pose a correspondingly complex analytical problem (Stoney & Stoney, 2015). As the contaminating materials may interfere with the analysis and increase the complexity of the samples, this can lead to inaccurate results and produce false positive.

Furthermore, forensic expert is responsible for presenting the findings of analysis in court, but the interpreting trace evidence analysis is quite challenging for forensic scientists to deliver the context of the results. This is because quantifying the likelihood of a match between trace evidence and a source is complex so, it can be challenging to articulate the strength of evidence in court. For example, terms like "match," "consistent," and "most likely" are often used to present results in a clear and understandable manner for the court. Therefore, evaluating the significance of trace evidence requires a thorough understanding and in-depth knowledge regarding the relevancy of its presence at a specific crime scene with all the related circumstances. The interpreting trace evidence also needs a database to compare materials and a proper method to analyse them using suitable references. Hence, it requires significant training and research and development (R&D) capacity to generate the required knowledge base, develop and maintain databases to better assess the relevance of the transferred material (Roux *et al*., 2015).

2.3 Forensic Analysis of Cosmetic Products

Over the years, there are quite a lot of publications have explored the potential of cosmetic products as trace evidence in forensic investigation. However, most past studies have primarily focus on cosmetic products such as foundation or facial creams, lipsticks and eye makeup. Hence, due to the gap in this specific area of knowledge, other cosmetic products traces could often be overlooked when encountered in homicides or sexual assaults crime scene investigations. Several analytical techniques have been studied in combination for the forensic analysis of cosmetics products.

Firstly, forensic analysis of cosmetic product of aged lip-gloss stains by using Thin Layer Chromatography (TLC) and FTIR analysis was performed by Sharma *et al*. (2016). This study was conducted because of very little work on the analysis of lip gloss using TLC and FTIR analysis are available at that time. In this study, twenty-five lip-gloss samples were analysed using twenty different solvent systems. In the result of TLC analysis, they found that the Rf values almost close to each other but the difference in the intensity of the spots with the aged lip gloss stains was highly observed. Then, for the samples under strong sunlight for two to three days at temperature 35-38°c showed a change in colour spots only, but for separation of components through TLC showed similar Rf values and the number of spots as that of fresh samples. Thus, the researcher concluded that the stains of lip gloss is not much affected by the passage of time (at least up to one month) and the components present in the lip gloss still could be separated up to one month period successfully, but there was a decrease in the intensity of the colour.

There are a few publications performed forensic analysis of lipsticks. In 2019, Wong *et al*. (2019) analysed 22 red-shaded and 18 nude-shaded lipsticks using visible absorbance and diffuse reflectance spectroscopy, as well as attenuated total reflectance Fourier transform infrared spectroscopy. In another studies, Chophi *et al*. (2020b) performed analysis on 38 different red shade lipsticks of 20 different manufacturers using attenuated total reflectance-Fourier transform-infrared (ATR-FTIR) spectroscopy. Meanwhile, Alblooshi *et al.* (2024) analysed 20 different pink shade lipsticks of the same manufacturer using Vacuum FT-IR, and Raman spectroscopy.

In the study, Wong *et al*. (2019) found that ATR-FTIR spectroscopy afforded the most accurate results due to its increased chemical specificity but since the discrimination of the lipstick is not only based on different chemical components, but variations in their relative abundance. Hence, reinforcing the need for a multivariate statistical approach such as PCA scores plot has been used in the study. Additionally, when using visible spectroscopy, they found that absorbance mode gave better discrimination of red-shaded lipsticks whereas diffuse reflectance spectroscopy was more discriminatory of nude-shaded lipsticks. As previously mentioned, multivariate statistical approach needs to reinforce in this analysis of lipstick due to the respective reason and it has been proved in the study performed by Chophi *et al*. (2020b) in which the discriminating power of PCA for the samples in the study was found to be 100%. In order to investigate whether different series of same manufacturer got classified into their respective group, PCA-LDA was performed, and the obtained accuracy of the classification was 81.48% since some of the samples were misclassified. Moreover, the combination between vacuum FT-IR and Raman spectroscopy shows a solid reliable result on lipsticks differentiation of up to 95.8% of all samples in the study conducted by Alblooshi *et al.* (2024) and the use of PCA as a chemometric tool in the analysis of infrared spectroscopy data for lipstick samples has provided promising results in characterising and clustering different types of lipstick based on their spectral signatures in the fingerprint region.

Furthermore, multivariate statistical approach like chemometrics analysis also works on other types of cosmetic product such as facial foundation or foundation cream. In study conducted by Sharma *et al*. (2019a), chemometric methods such as PCA and LDA were applied to make the interpretation of results more objective since a few samples showed similar spectra due to similar chemical constituents and could not be discriminated from each other. At first, the discriminating power of visual analysis was found to be 98.0% but in combination with chemometric tools it increased up to 100% where PCA provided discrimination power of 99.3% and LDA increased the discrimination between samples of foundation creams to 100% with 0% rate of false classification. Therefore, PCA-LDA can be considered as one of the best discriminant models for the classification and separation of foundation creams of different brands.

The analysis of cosmetic foundations was also conducted using near-infrared spectroscopy (Skobeeva *et al*., 2022). This study reported that selected techniques proved to be promising for database construction and as a preliminary method of analysis, with 93% of the spectra being correctly classified. Notably, they discovered that darker foundation shades were less likely to be correctly classified (90% classified correctly) compared to lighter ones (96.7% classified correctly).

In another study, Arora *et al*. (2021) performed the analysis of eye cosmetic to discriminate between eyeliner and mascara traces. The ATR-FTIR spectroscopy was successfully applied for the spectral characterisation, discrimination and classification of 102 samples (62 eyeliners from 37 brands and 40 mascaras from 24 brands) into their respective classes of eyeliners and mascaras. Cross-validation was also conducted resulting in the accuracy upwards of 91.7%. The sensitivity of 0.9 and specificity of 1.0, proving the model to be highly accurate. Hence, the model was extremely helpful to carry out the forensic analysis of such products being found in the cases involving crime against the women as well as used as writing instruments for suicide note, threatening and anonymous letters. Although these products are found in trace quantity but still visible due to their dark shades.

On the other hand, the persistence of moisturiser products on human skin was also investigated in relation to sexual assault investigation. In the study performed by Raynor *et al.* (2021), they discovered that petrolatum-based moisturiser persisted on human skin for 13.5 ± 0.7 hours while the glycerol-based moisturiser persisted for 19.8 ± 1 hours. Petrolatum had the shortest skin surface lifetime (12 hours) while acetyl alcohol had the longest (23.5 hours) may be related to the increased chemical interaction with the skin surface. Hence, exercising and bathing activities correlated with a decrease in moisturiser persistence while skin being covered was associated with an increase in moisturiser persistence, meaning the moisturiser lifetimes reported here may underestimate those in covered intimate areas. The observed persistence lifetimes give optimism that moisturisers can be sampled and used as forensic evidence in sexual assault investigations.

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

Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy is an effective analytical technique that analyses a sample's molecular composition. ATR-FTIR spectroscopy is a specific configuration of FTIR spectroscopy that employs the ATR sampling method, enabling convenient analysis of solid, liquid, and semi-solid samples without extensive sample preparation. It combines the ATR sampling approach with the concepts of infrared spectroscopy to obtain valuable information about the functional groups and the chemical bonds that are present in a sample. This spectroscopy method could discriminate the source of origin of similar objects from different brands. Thus, in forensic science investigation, many trace evidence has been studied by using this technique such as cosmetic product traces such as eyeliner and mascara traces (Arora *et al*., 2021), foundation creams (Sharma *et al*., 2019a), lipsticks (Chophi *et al*., 2020b; Kaur *et al*., 2020) and suncreen stains (Angrish *et al.*, 2020).

ATR-FTIR is the standard technique for FTIR measurement because of ATR crystal is used as a sample support where the ATR crystal usually made of IR transparent materials such as zinc selenide (ZnSe), germanium (Ge) or diamond. Those reflectance crystal provides good contact with the specimen surface. According (Alkhuder, 2022), ATR-FTIR is a universal and highly accurate analytical method that can be used for a quick in situ screening and identification of a wide range of materials and stains in criminal investigations. This technique also has several advantages over other techniques used in forensic analyses such as it does not require any specific preparations to be carried out on the sample prior to testing. Besides that, only a small amount of the test sample is needed to perform the measurement, and no necessary reagents are required for the ATR FTIR–based analysis. Additionally, ATR-FTIR is a non-destructive technique that preserves the tested sample without causing any alterations. This means that it allows the sample to be used for further forensic analyses after undergoing ATR-FTIR spectroscopy.

ATR-FTIR spectroscopy measures how much infrared light is absorbed by a sample when it comes into contact with a high-refractive-index crystal, ATR crystal. **Figure 2.1** shows the diagrammatic representation of ATR-FTIR spectroscopy. In ATR sampling, the infrared (IR) light travels through a crystal, is totally internally reflected at least once at the crystal-sample interface; and the reflected light travels to the FTIR detector. During the internal reflection, a part of the infrared light passes through the ATR crystal covered on the top by the sample, where it can absorb and this is called the evanescent wave (Ausili *et al.*, 2015). The absorbed radiation will reach the IR detector and IR spectrum is generated. Then, the important information on the molecular composition, structure, and chemical characteristics of the sample can be gleaned by examining the resulting infrared spectrum.

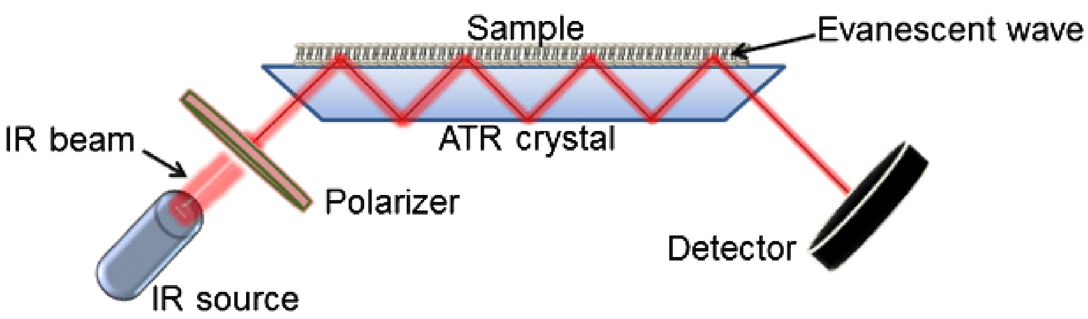

Figure 2.1: Diagrammatic representation of ATR-FTIR spectroscopy (Ausili *et al.*, 2015)

The electromagnetic spectrum of infrared region is in between of 12500 to 500 cm^{-1} . The infrared (IR) spectrum is part of the electromagnetic spectrum and is typically divided into three regions: near-infrared, near-IR (region 12500 to 400 cm^{-1}), mid-infrared, mid-IR (region 4000 to 400 cm^{-1}) and far-infrared, far-IR (region 400 to 10 cm^{-1}). The most commonly analysed in FTIR spectroscopy is mid-IR (region 4000 to 400 cm^{-1}) where during the analysis, the molecules in a sample will absord the mid-IR in the mid-IR region, enabling the fundamental vibrations of specific functional groups to be detected. **Table 2.1** shows the bond streching frequencies and their corresponding ranges.

Table 2.1: Frequency range of function groups

Bond	**Type of Compound**	**Frequency Range, (cm^{-1})**
C–H	Alkanes	2850 – 5970
		1340 – 1470
C–H	Alkenes	3010 – 3095
		675 – 995
C–H	Alkynes	3300
C–H	Aromatic Rings	3010 - 3100
		690 - 900
O–H	Monomeric Alcohols, Phenols	3590 – 3650
		3200 – 3600
		3500– 3650
		2500 – 2700
N–H	Amines, Amides	3300 - 3500
C=C	Alkenes	1610 - 1680
C=C	Aromatic Rings	1500 - 1600
C≡C	Alkenes	2100 - 2260
C–N	Amines, Amides	1180 – 1360
C≡N	Nitriles	2210 – 2280
C–O	Alcohols, Ethers, Carboxylic Acids, Esters	1050 – 1500
C=O	Aldehydes, Ketones, Carboxylic Acids, Esters	1690 - 1760
NO_2	Nitro Compounds	1500 - 1570
		1300 - 1370

2.5 Chemometrics Analysis

Chemometrics is a chemical discipline that uses mathematics, statistics, and formal logic to design or select experimental procedures to provide the most relevant information by analysing data and gaining knowledge about chemical systems. The main application of chemometric methods is the design of experiments that optimises the method and evaluates chemical analysis data with the minimum number of trials. Chemometric techniques (multivariate analysis and other statistical methods) are recognised as powerful tools in forensic science to interpret and optimise analytical procedures (Soria, 2022). The analytical method produces masses of dataset even for a single sample and or a large number of samples, the amount of output data will increase tremendously which makes the task of expert much tedious, time-consuming and also, the manual examination can provide false positive results. Therefore, the advanced chemometric methods have emerged to analyse the large and complex dataset (Kumar & Sharma, 2018).

Chemometric techniques are particularly used in analytical or physical chemistry and metabolomics, and instrumentation and methods are advanced by the improvement of chemometric methods. The use of chemometrics is typically applied to spectral datasets generated by spectroscopic techniques, some of which include Raman, Fourier Transform Infrared Spectroscopy (FTIR), ultraviolet–visible (UV-Vis), and fluorescence spectroscopy (Webster, 2020). Thus, chemometric techniques offers a lot of advantages such as provide accurate and significant results in a short time in forensic analysis involving the use of those spectroscopic techniques.

There are a few techniques use in chemometric analysis which are cluster analysis (CA), principal component analysis (PCA), linear discriminant analysis

(LDA), soft independent modelling of class analogy (SIMCA), artificial neural network (ANN), and partial least squares (PLS) (Kumar & Sharma, 2018). However, the spectroscopic data from the analysis of the cosmetic products in this study were interpreted using a combination technique of PCA and LDA. These two methods were selected due to their ability to rapidly and effectively analyse large dataset while providing accurate group or class predictions.

2.5.1 Principal Component Analysis (PCA)

In the multivariate analysis, principal component analysis (PCA) is probably the oldest and best-known technique, introduced by Pearson in 1901 and developed by Hotelling in 1933. PCA is used to reduce the dimensionality of a large number of interconnected variables to a few principal components (PCs) with minimal information loss since it is an unsupervised classification method. PCs, also known as component scores, are used to define the spatial relations between the variables, and the variance explained by each PC is expressed in terms of its eigenvalue. PCs having eigenvalues greater than one are selected only (Sharma & Sharma, 2022).

Principal component analysis (PCA) offers several advantages in forensic analysis including provide better data insight for dealing with duplication in the data set, lessening the complexity and enhancing computational efficiency. PCA is a technique for reducing the dimensionality of such datasets, increasing interpretability but at the same time minimising information loss (Jolliffe & Cadima, 2016). This makes data analysis more efficient and manageable. Additionally, PCA help in enhancing discrimination of data from the sample analysis. PCA improves the ability to distinguish between different groups or classes, such as differentiating substances, trace evidence, or suspect profiles.

Furthermore, PCA also can visualised the data by enabling the graphical representation of multidimensional data, making it easier to identify patterns, clusters, and outliers in forensic evidence. According to Mendlein *et al*. (2013), there are a couple of ways to visualise the data or result of PCA. First one is the conventional method which is score plot, it is where the score of one PC is plotted against another score for specific sample. In this plot, samples that the cluster are closely correlated, the samples plotted distinct apart are distinct. Another visualisation method known as loading plots. It involves plotting the factor loadings which represent the cosine of the angle between the PC and each variable. A positive cosine indicates a positive correlation between variables, while a negative cosine reflects a negative correlation. When the cosine is close to zero, the variables are interpreted as uncorrelated. The last method uses the Kaiser criterion to determine the PC's total. Eigenvalues greater than one are considered significant.

Generally, principal component analysis is a bilinear modelling method used to interpret the data obtained by ATR FTIR spectroscopy by identifying the differences and similarities between samples. It is known as the dimension reduction or data compression tool as it helps to interpret complex data with large number of variables to an easy understandable form and it is highly useful tool to demonstrate the significant relationships between selected samples and helps to summarise and visualise the original data.

2.5.2 Linear Discriminant Analysis (LDA)

Linear Discriminant Analysis (LDA) is another form of dimensionality reduction technique first developed by Ronald Fisher in 1936 (Saheed, 2023). It is a technique that builds a mathematical function to maximize the separation between known classes of samples. It condenses the dimensions of the complex dataset by reducing a huge number of original variables to few new composite dimensions (called as canonical functions), without or with a minimum loss of information from original (Kumar & Sharma, 2018).

Linear discriminant analysis mainly works to provide more class separability and to draw a decision among the specified classes without changing the shape and location of the original data sets. The use of LDA was accomplished by the exclusion of the lesser variables by using PCA. Therefore, the use of PCA which is a variable reduction tool requires careful evaluation of the principal components to be used before applying LDA (Sharma *et al.*, 2019a). This is because the use of too many PCs can introduce to excessive noise potentially resulting in inaccurate analysis.

In short, PCA and LDA are compliment with each other due to although PCA is a method for uncovering relationships in large multidimensional data sets, it is not sufficient for developing a classification rule that can accurately predict the class-membership of an unknown sample, and thus, LDA is also performed (Sharma & Sharma, 2022). On the other hand, PCA also in need to reduce the number of variables in a dataset where the resultant PCs are used in LDA for class prediction. Therefore, combining PCA-LDA techniques for classification and discrimination is preferable, practical, and effective in improving classification performance.

CHAPTER 3

METHODOLOGY

3.1 Material and Methods

The analytical grade isopropanol was purchased from Merck (Germany) and used to clean ATR crystal. A Bruker Alpha II FTIR spectroscopy (Bruker Corporation, Billerica, MA), equipped with a diamond crystal attenuated total reflectance (ATR) accessory was utilised for ATR-FTIR spectroscopy in this study.

3.2 Sample Collection

Four different brands classified under 4 types of cosmetic products which were facial foundation, body lotion, face moisturiser and antiperspirant were purchased from the supermarkets in Kota Bharu district, Kelantan, Malaysia. A total of 16 samples of cosmetic products were studied with one duplicate sample were obtained for each similar brand and type. The details of various tested samples are tabulated in **Table 3.1** and their images are shown in **Figures 3.1 to 3.4.**

Table 3.1: The details of various tested cosmetic product samples

Sample code	Type	Brand	Manufacturer	Country of origin
S1A	Facial foundation	Wardah colour fit matte foundation	PT Paragon Technology and Innovation	Indonesia
S2A		Maybelline New York fit me	Yichang Tianmei International Cosmetic Limited Company	China
S3A		Silky girl skin perfect liquid foundation	Alliance Cosmetic Group	Malaysia
S4A		Catrice true skin hydrating foundation	Cosnova Beauty	Italy
S5A	Body lotion	Nivea extra bright C&A vitamin lotion	Beiersdorf Global AG	Thailand
S6A		Vaseline healthy bright superfood freshlock cranberry vitamin C	Unilever	United states
S7A		Enchanteur insta white perfumed lotion	Wipro Unza	Malaysia
S8A		Citra pearly grow UV	PT Unilever	Indonesia
S9A	Face moisturiser	Aiken prebiotic hydra plus	Wipro Unza Sdn. Bhd.	Malaysia
S10A		Simple hydrating light moisturiser	Unilever	Hungary
S11A		Beauty of Joseon Dynasty cream	Cosmecca Korea Co., Ltd.	Korea
S12A		The originate hyalucera moisturiser	Shanghai Ayara Cosmetics Co., Ltd.	China

Table 3.1: Continued

Sample code	Type	Brand	Manufacturer	Country of origin
S13A	Antiperspirant spray	Rexona free spirit	Thai Daizo Aerosol Co., Ltd.	Thailand
S14A		Enchanteur perfume deo mist	Wipro Unza	Indonesia
S15A S15B		Safi hijabista body care antibacterial deo	Wipro Unza Sdn. Bhd.	Malaysia
S16A		Silkygirl french sunflower	Alliance Cosmetics Group	Malaysia

a) b) c) d)

Figure 3.1: Facial foundation samples a) Wardah colour fit matte foundation b) Maybelline New York fit me c) Silky girl skin perfect liquid foundation d) Catrice true skin hydrating foundation

a) b) c) d)

Figure 3.2: Body lotion samples a) Nivea extra bright C&A vitamin lotion b) Vaseline healthy bright superfood freshlock cranberry vitamin c c) Enchanteur insta white perfumed lotion d) Citra pearly grow UV

a) b) c) d)

Figure 3.3: Face moisturiser samples a) Aiken prebiotic hydra plus b) Simple hydrating light moisturiser c) Beauty of Joseon dynasty cream d) The originate hyalucera moisturiser

a) b) c) d)

Figure 3.4: Antiperspirant samples a) Rexona free spirit b) Enchanteur perfume deo mist c) Safi hijabista body care antibacterial deo d) Silkygirl french sunflower