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Forensic examination of lipsticks as trace evidence under different environmental conditions

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Abstract

Trace evidence such as fingerprints, biological fluids, fibres, glass, hairs, soils, and cosmetics have been considered most of the important materials on the crime scene, which can be potentially transferred or exchanged between two surfaces when one makes contact to another. Cosmetic products are one of the valuable trace pieces of evidence on crime scene that are generally used for beautification, moisturize, reinforcement of the skin, nutritiveness, and improve the feeling or sensory aspects. In this study, different lip cosmetic products including wax lipstick, liquid lipstick, gloss and lip balm were investigated as forensic evidence. For this, 102 of lip cosmetic samples (20 of lipstick, 14 of lip gloss, 22 of lip balm and 46 of liquid lipstick) were analyzed by using Fourier-transform Infrared spectroscopy (FTIR). The differentiation between lip cosmetic products is detected. The effect of environmental conditions is studied. For this, lip cosmetic products are kept in various water medium such as simulated sea water, tap water, and distilled water in order to track the chemical stability by FTIR. Moreover, they are kept in different time of intervals from 1 day to 1 month to investigate the change over time. Staining of lip products to various substrates including fabric, glass and paper towel have been also explored. The effect of substrate type, lip cosmetic products, time and water sources have been systematically explored by FTIR and video spectral comparator (VSC). All the samples have been examined under different light sources such as visible light, UV-A (365 nm), infrared (695 nm) and spot (fluorescence) to observe the distinctive features of lip cosmetic samples on different substrates. The results have demonstrated that lip products including wax, liquids lipstick, gloss and lip balm have distinguishing character which is observed by FTIR and VSC8000. Moreover, the chemical stability of lip cosmetic products under different water sources could be characteristic tools for differentiating of lip cosmetic products. The outcomes show that cosmetic trace evidence found in water source could be critical evidence in crime scene investigation.

1. Introduction

Violence against women has become a big problem to be solved for many countries, which has been recognized as a global pandemic by United Nations Secretary [1]. In crime scene, it could be countered various evidence that helps to solve the cases. Detection, quantification, and characterization of the evidence play a critical role for solving the crime [2].

Forensic scientists follow up various evidence, namely classified as physical, chemical, and biological evidence. Cosmetics, one of the encountered pieces of evidence in the event of crime against woman, could be detected in different surfaces such as clothes, cigarette butts, drinking cups, mirror, facial cleanser cotton, and many others [3]. Cosmetics cover various commercial products including eyeliner, lipstick, vermilion, hand cream, nail polish, mascara, eye creams, and so on. Even cosmetic products give a valuable information as forensic evidence in crime scene investigation, some limitations including their abundance in different brands, containing wide variety of chemicals could make it non-utilizable evidence at crime scene. Therefore, characterization and differentiation of cosmetic products using destructive and non-destructive methods have gained immense attention in forensic science [2,4,5].

A variety of method have been extensively used to discriminate the cosmetic products. Cosmetic products can be classified into makeup products, hair care, skin care, intraoral product, nail care and perfumes [6]. In crime scene, numerous cosmetic products can be countered as trace evidence such as lipstick, eyeliner, vermilion, nail polish, kajal, toothpaste and so on. The identification of cosmetics has been part of forensic investigations since 1912, when Edmond Locard found that the pink powder under a murder suspect's fingernail was chemically identical to a makeup powder found in the victim's room [7]. The first studies that involved examining lip cosmetic stains as evidence and comparing their chemical composition in forensic analyzes appeared in the 1950s [8]. Lip cosmetics are one of the most used cosmetic products for beautification, moisturize, reinforcement of the skin, nutritiveness, and improve the feeling or sensory aspects which dates to Mesopotamian civilizations. In terms of forensic science, lipstick can be transferred to many objects including cigarette butts, fabrics, tissue papers, food and beverage products, pillows, and many other surfaces in the criminal events such as child abuse, sex assault, kidnapping, murder, rape etc. [8-11]. Moreover, evidence could be found in different places such as in soil, in lake or sea water. The stains on the evidence therefore can be structural change.

Recently, lip cosmetic products can be classified in different category including lipstick, lip balm, lip gloss, lip liner, liquid lipstick, lip tint and lip chalk according to the chemical compositions, physical structure, physical appearance, applying type, color, gloss and mattness etc. Up to now, various materials such as colored stones, ocher, lead, snake blood, insects, bromine mannite and iodine mixtures, vegetable oils and so on have been used to form lip cosmetic products [12]. With the increasing industrialization and globalization, various chemicals were used to fabricate different lip cosmetic products in high amounts. Although many manufacturers use different chemical for the fabrication of lip cosmetic products, there are 3 chemicals that almost all manufacturer uses including wax, oil and pigment. Wax is responsible for the consistency. hardness, and stability of the lipstick. It softens and smoothes the lips. The types of wax used can be classified as herbal (carnauba, candelilla), animal (beeswax), mineral (ozokerite, paraffin), and synthetic (polyethylene) [9,13].

While waxes maintain structural integrity, they cause the gloss of the lipstick to decrease and the product to become sticky. Oils are used to ensure the diminishing shine and reduce the stickiness of the lipstick. Oils also ensure that the pigments mix homogeneously and make the lips softer. Natural oils such as castor oil, jojoba oil, and lanolin are frequently used in these products [8,13]. Colorants that determine the color tones of lipsticks are synthetic (tartrazine, erythrosine, eosin, phloxine), natural (carmine), organic and inorganic dyes, especially iron (II) and (III) oxides, or titanium (IV) oxide, and mineral pigments (lead white, iron yellow) which adjusts the brightness, opacity, and concealment of the color [8]. Some manufacturers could add other chemicals such as filters. emulsifiers. colorants with different concentration depending on manufacturer [8,14,15].

Lip cosmetics can be found also different forms and types including solid or liquid. Also, they can be named as gloss, balm or lipstick. While wax lipstick consist of wax, liquid lipstick generally contains solvent (usually alcohol), which make them liquid. Wax is also added in some product as a thickening agent. In liquid lipstick forms, solvent is added which rapidly evaporate and leave a thin layer of paint on the skin. Isododecane is one of the most used solvents for liquid lipstick. Moreover, low-density siloxane polymers such as polydimethylsiloxane, trimethylsiloxysilicate, and cyclopentasiloxane have been extensively used to increase the permanence of products, provide creamy form, soften and increase film-forming properties [16,17].

Although lip balms are similar to lipstick in terms of structure, their wax and oil content are much higher than lipsticks. Since their primary purpose is not to provide color, they contain very little pigment [18]. To increase antioxidant properties synthetic antioxidants such as butyl hydroxytoluene (BHT), ascorbic acid, kojic acid, mercury, and hydroquinone etc. can be used. For the high sun protection factor (SPF), chemical sunscreens can be used such as octyl methoxycinnamate, octocrylene, octyl salicylate, benzophenone-3, etc. [13,19]. Aromas are generally used to suppress the bad taste of chemicals [13].

The purpose of lip glosses is to make the lips look brighter. Lip gloss formulations contain more oils and significantly lower amounts of waxes and colorants compared to lipsticks [20]. Like most lip products, lip glosses contain tocopherol (vitamin E) to protect the lips. They typically contain highly tacky film formers such as polybutene. These film formers provide a more transparent and fluid texture and have a high refractive index for shine. Although it is not possible to make a definitive identification of cosmetic stains at the crime scene due to reasons such as the lack of clarity of the complex ingredients of the products, the existence of secret formulas, and global mass production, this evidence helps establish a connection between the suspect, the victim, and the crime scene [21].

The discrimination of chemical composition of lip cosmetic products is critical parameter to solve criminal cases in forensic science. For this, various methods such as atomic absorption spectrometry (AAS), inductively coupled plasma mass spectrometry (ICP-MS), thin layer chromatography (TLC), gas chromatography- mass spectrometry (GC-MS), high performance liquid chromatography (HPLC), Fourier-transform infrared spectroscopy (FTIR) have been performed to differentiate the cosmetic evidence [22-24].

Gładysz et al. [25] have used attenuated total reflectance - Fourier transform infrared spectroscopy (ATR-FTIR) spectroscopy in combination with chemometric techniques to distinguish 38 lipstick samples. Chopi et al. [26] performed the distinguishing of 25 of lip cosmetic samples via cluster analysis and principal component analysis (PCA). However, these studies focused on restricted colors (usually red and tan). This highlights the lack of lipsticks in other shades and even other lip cosmetics in similar studies. Kaur et al. [3] stated that ATR-FTIR is an excellent technique for classifying lipstick samples of different colors and brands in their study with 47 brown, 57 pink and 55 red lipstick samples using ATR-FTIR spectroscopy and chemometric methods. Jasuja and Singh have investigated the liquid lipstick by using TLC method to discriminate various liquid lipstick samples [16]. Wong et al. have investigated

the use of spectroscopic methods to examine the 40 of lipstick and liquid lipstick staines. They have used ultraviolet-visible spectroscopy (UV-Vis) and ATR-FTIR to distinguish between stains, each with a different color (red and nude shades). They also succeeded in distinguishing 40 lipsticks with red and nude shades using PCA and linear discriminant analysis (LDA) [17]. Gardner et al. [27] have used Raman spectroscopy to discriminate 34 lip glosses and 17 lip balms. it was reported that fluorescence was more common especially in products containing dyestuffs. However, it was stated that most of the dye-free samples did not contain fluorescence. Salahioglu et al. [28] examined lipstick stains on fabric, cigarette butts and tissue paper by Raman spectroscopy. It was reported that the aim of the study was to keep the stains for a long time and to show their time-dependent changes, but they did not find any significant changes over a two-year period. However, in some lipstick spectra, it was found that the intensity of the C=C band at 1655 cm⁻¹ and the -CH band at 3011 cm⁻ ¹ decreased and disappeared over time. It was aimed to determine the effect of environmental conditions on the samples as time passed, since the sample expired due to the waiting period in the forensic examination process and many cases were sent to forensic science laboratories. Kaur et al. [29] have studied lip gloss and used the thin layer chromatography (TLC) method for branded products. With this method, they aimed to develop a new solvent system for the separation of various components of lip glosses. However, it was stated that the results were not reproducible in the nonbranded lip gloss sample. Sharma et al. [20] examined the time-dependent changes of the samples in TLC and FTIR study with 25 lip gloss samples. It was concluded that new and old lip gloss (with 1 month aged) stains can be analyzed by applying TLC and FTIR techniques. It was determined that the components could be distinguished for a period of 1 month, but the color intensity decreased as time passed. In a possible case, this will help the suspect to be associated with the crime scene in a temporal sense. Ka Khei et al. [30] have studied the effect of substrate type for discrimination of lipstick samples by using ATR-FTIR. They used various substrates such as wet tissue, nitrile gloves, white paper, and polyester with 10 brands of lipsticks.

It was demonstrated that among them, FTIR is nondestructive method spectroscopic method which is widely used for the analysis of various forensic evidence including soil, cosmetic products, hair, polymer etc. [31]. This technique allows rapid, robust, non-destructive, and reproducible results with minimal sample size and does not require any pre-treatment, solvent, standards for analyzing of samples. In a crime scene, lipstick-stained objects as physical evidence could be encountered in different environmental conditions such as buried, in forest, in different water such as lake, sea or dam. Therefore, the determination of structural change in these environmental conditions is gaining importance in the forensic field. Until now, most of studies have examined fresh lip cosmetic samples and only few studies investigated the effect of environmental conditions on the structural change of lip cosmetics as evidence [26]. Gładysz et al. [32] conducted a stability experiment with various lipstick products in their study. For this, they studied the effect of time, surface and lights to examine the structural change against the environmental conditions. It was found some structural change with time, but no consistent changes were observed. They also studied the effect of surface by glass, fabric, paper to examine the surface type, but they could not conclude about the effect of the surface on stability studies. It was also mentioned that fabric surface is found to be challenging substrate to work the lipstick on the surface.

The present study aims to differentiate the lip cosmetic products and also examines the effect of time, substrate type and environmental conditions. The structural analysis of samples is achieved by FTIR. The images of samples in different substrates are captured using Video spectral comparator (VSC) 8000. Also, various light sources including visible light, UV light (365 nm), infrared (695 nm) and spot (fluorescence) by VSC 8000 have been used to differentiate the lip cosmetic products groups. Lips samples are stained to various substrates such as document paper, fabric (cotton), and paper towel in order to examine the permanence of lip cosmetic stain marks under different environmental conditions. It is also aimed to investigate the stability of lip cosmetic stains on the different substrates against to the environmental conditions. For this, lip cosmetic staines in different substrates are dipped in various water sources such as tap water, simulated sea water, distilled water. The stained substrates are also kept at different time of intervals (from 1 day to 1 month) in order to examine the change over time. Even all lip cosmetic groups including lip gloss, lip balm, wax lipstick and liquid lipstick looks similar when viewed with naked eye, they can be discriminated by VSC under different light sources and FTIR which make them preferable method because of their non-destructive character. The outcomes show that FTIR is important technique for the discrimination of lip cosmetic samples in crime scene. The use of VSC8000 also is a valuable step to investigate the different type of evidence which can be utilized under different light of source including UV light, infrared, and spot (fluorescence).

2. Materials and method

2.1. Sample collection and preparation

This study groups all the lip cosmetic samples in 4 categories under lipstick, liquid lipstick, lip balm and lip gloss. For the study, 46 of wax lipstick, 22 of lip balm, 20 of liquid lipstick, and 14 of lip gloss have been used. All the samples are collected from various district bazaars and grocery chains in Turkey. The samples are named with different codes to avoid confusion at the classification stage. In this nomenclature, the first letter in the codes indicates the type of sample (lipstick, liquid lipstick, balm, gloss); the other two letters represent the brand of the sample. The numbers next to the codes represent different products of that brand. Letters representing the type of sample; the letter "**R**" for lipstick, the letter "**L**" for liquid lipstick, the letter "**N**" for lip balm and the letter "**P**" for gloss. While choosing the

letters representing the brand, if the brand name consists of two or more words, the initials of the first two words, if it consists of a single word, the first two letters of the word are taken as basis. The details including sample code, sample type and brand are given in Table 1. Samples coded LMA-06 for liquid lipstick, RBE-02 for lipstick, NGA-02 for lip balm and PBC-01 for lip gloss were used and were never changed during the experiment. Since the color appears more intense, care was taken to choose red color, but pink color was preferred due to the higher color intensity in lip balm.

Table	1.	Nomencla	ture o	of samr	oles
Iable	1.	Nomencia	luiel	л зашр	JIES.

Sample	Туре	Brand	Sample	Туре	Brand
LAV-01	Liquid lipstick	Avon	NBE-02	Lip balm	Beaulis
LBA-01	Liquid lipstick	The Balm	NBE-03	Lip balm	Beaulis
LBE-01	Liquid lipstick	Beaulis	NBL-01	Lip balm	Blistex
LBE-02	Liquid lipstick	Beaulis	NBO-01	Lip balm	Bote
LBE-03	Liquid lipstick	Beaulis	NFL-01	Lip balm	Flormar
LBE-04	Liquid lipstick	Beaulis	NGA-01	Lip balm	Gabrini
LCR-01	Liquid lipstick	Crystal	NGA-02	Lip balm	Gabrini
LEL-01	Liquid lipstick	Estel'la	NGR-01	Lip balm	Golden Rose
LEL-02	Liquid lipstick	Estel'la	NHH-01	Lip balm	Himalaya Herbals
LEL-03	Liquid lipstick	Estel'la	NKI-01	Lip balm	Kiva
LEN-01	Liquid lipstick	Enigma	NMA-01	Lip balm	MonAmour
LEN-02	Liquid lipstick	Enigma	NMN-01	Lip balm	Maybelline New York
LFA-01	Liquid lipstick	Farmasi	NNI-01	Lip balm	Nivea
LFL-01	Liquid lipstick	Flormar	NPB-01	Lip balm	PrettyBeauty
LFL-02	Liquid lipstick	Flormar	NPB-02	Lip balm	PrettyBeauty
LFL-03	Liquid lipstick	Flormar	NUS-01	Lip balm	Ushas
LGO-01	Liquid lipstick	Gossamer	NVE-01	Lip balm	Vesslina
LGR-01	Liquid lipstick	Golden Rose	PAA-01	Lipgloss	Markasız
LGR-02	Liquid lipstick	Golden Rose	PBC-01	Lipgloss	BriConti
LGR-03	Liquid lipstick	Golden Rose	PBO-01	Lipgloss	Bonica
LGR-04	Liquid lipstick	Golden Rose	PBO-02	Lipgloss	Bonica
LGR-05	Liquid lipstick	Golden Rose	PBO-03	Lipgloss	Bonica
LLL-01	Liquid lipstick	Light of Life	PBO-04	Lipgloss	Bonica
LLP-01	Liquid lipstick	Loreal Paris	PBO-05	Lingloss	Bonica
LMA-01	Liquid lipstick	MonAmour	PEN-01	Lingloss	Enigma
LMA-02	Liquid lipstick	MonAmour	PES-01	Lingloss	Essence
LMA-03	Liquid lipstick	MonAmour	PGR-01	Lingloss	Golden Rose
LMA-04	Liquid lipstick	MonAmour	PIR-01	Lingloss	Irshi
LMA-05	Liquid lipstick	MonAmour	PLP-01	Lipgloss	Lancome Paris
LMA-06	Liquid lipstick	MonAmour	PMA-01	Lipgloss	MonAmour
LMN-01	Liquid lipstick	Maybelline New York	PTI-01	Lipgloss	Tikatti
LMT-01	Liquid lipstick	Makeup Time	RAP-01	Lipstick	Afrodit Paris
LMT-02	Liquid lipstick	MakeupTime	RAV-01	Lipstick	Avon
LNW-01	Liquid lipstick	NewWell	RAV-02	Lipstick	Avon
LNW-02	Liquid lipstick	NewWell	RAV-03	Lipstick	Avon
LNW-03	Liquid lipstick	NewWell	RAV-04	Lipstick	Avon
LNW-04	Liquid lipstick	NewWell	RBE-01	Lipstick	Beaulis
LNW-05	Liquid lipstick	NewWell	RBE-02	Lipstick	Beaulis
LNW-06	Liquid lipstick	NewWell	RBE-03	Lipstick	Beaulis
LNW-07	Liquid lipstick	NewWell	RBE-04	Lipstick	Beaulis
LPA-01	Liquid lipstick	Pastel	RBE-05	Lipstick	Beaulis
LPA-02	Liquid lipstick	Pastel	RBE-06	Lipstick	Beaulis
LPA-03	Liquid lipstick	Pastel	RBE-07	Lipstick	Beaulis
LRS-01	Liquid lipstick	Rosie Style	RBE-08	Linstick	Beaulis
LUS-01	Liquid lipstick	Ushas	RBE-09	Lipstick	Beaulis
LUS-02	Liquid lipstick	Ushas	RCH-01	Linstick	Chanlanya
NAN-01	Lin balm	Antipodes	RES-01	Linstick	Estel'la
NBB-01	Lip balm	BeeBeauty	RGR-01	Lipstick	Golden Rose
NBB-02	Lip balm	BeeBeauty	RMI-01	Lipstick	Miniso
NBB-03	Lip balm	BeeBeauty	RMN-01	Lipstick	Maybelline New York
NBE-01	Lip balm	Beaulis	RPA-01	Lipstick	Pastel

2.2. Instrumentation

The cosmetic samples are analyzed by the Agilent Cary 630 FTIR Spectrometer and the MicroLab FTIR software of the same company accredited with this device. Spectra were obtained between 4000 and 650 cm⁻¹ with a spectral resolution of 4 cm⁻¹. The data were converted to the appropriate file format using the OMNIC program. Orange Data Mining for PCA analysis were used. Samples were taken from three different point and compared in order to obtain good reproducibility, which is presented in Figure 1. The reproducibility test was carried out for all lip cosmetic samples including lip stick, liquid lipstick, lip palm and lip gloss.

Video Spectral Comparator 8000 (VSC-8000) was used to visualize the samples and show their variations under different wavelengths of light. The lip cosmetic samples are stained on A4 paper and images are taken. All the images are taken by VSC8000 under visible light, UV light, infrared (695 nm) and fluorescence (645 nm).

2.3. Sample analysis

For the structural analysis of samples, all lip products are stained on the glass microscope slides and allowed to dry for 20 min. After drying, the samples were scraped from the glass with metal spatula and collected in sample container.

The collected samples then analyzed by FTIR. Before analysis, the crystal was cleaned with alcohol, a paper towel, and a lint-free cloth prior to each analysis in order to avoid any foreign impurities. In order to get reliable and reproducible results, samples were taken from 3 different areas of lip cosmetic products.

2.4. The effect of substrate

In order to examine the effect of substrate type, different materials such as fabric (100% cotton), glass and paper towel have been used. Lip cosmetic products are gently stained to substrates at once then kept for 20 min for drying.

2.5. The effect of water source

Various water sources have been used to examine the environmental conditions. For this, distilled water, tap water and simulated sea water (3.5% NaCI) are used. For the stability experiments, stained substrates are dipped in various water source and kept at room conditions. Moreover, all the samples are kept in water sources at different time of intervals (1 hour, 1 day, 1 week, 1month) to investigate the change over time.

2.6. Data analysis

The PCA method, one of the chemometric methods, was preferred so that the analyzes made in the data analysis could be calculated statistically and the difference from each other could be displayed. Orange Data Mining program was used during the processing of this method. At this stage, PC-1 and PC-2 values were considered.

3. Results and discussion

3.1. Visual comparison of lip cosmetic products

All samples are stained to white copy paper in order to observe under different light of sources. For this, the images under visible light, UV light, IR and fluorescence light by VSC 8000 are captured which is given in Figure 1.



Figure 1. Liquid lipstick samples on VSC 8000 -1.

VSC 8000 have been used to differentiate the samples under various light sources. When the samples are examined with naked eye, it was observed that it was quite difficult to discriminate them in the same color group. However, they can be differentiated under IR and spot (fluorescence). For instance, LCR-01 and LMA-06 have similar images under visible light, but it can be differentiated in spot (fluorescence) which can be explained by the chemical ingredients. Manufacturers use various chemicals to produce lip samples. Moreover, use of UV light for differentiation of samples also provides efficient results. While all lipstick and liquid lipstick samples appear darker in UV light, most lip balm and lip gloss samples appear paler. Although all the samples examined under fluorescent light show glows that cannot be included in a grouping, it has been seen that the lip gloss samples have different glows from the others. It is thought that the cause of the glows here is due to components with different fluorescence properties. Components with different fluorescence properties emit light at different wavelengths and intensities. It can cause different glitters under the same light.

The sparkle appearance of lip gloss samples is effective in showing its glittery structure. Lip gloss samples examined under fluorescence light are thought to be discriminated from others with this feature. The images of all other samples under different light sources were shown in Figure 1-9.



Figure 2. Liquid lipstick samples on VSC 8000 -2.



Figure 3. Liquid lipstick samples on VSC 8000 -3.



Figure 4. Liquid lipstick samples on VSC 8000 -4.



Figure 5. Lipstick samples on VSC 8000 -1.



Figure 6. Lipstick samples on VSC 8000 -2.

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	Visible Light	UV – 365 nm	IR – 695 nm	Flouresance (spot) - 645 nm
NAN-01			6999-09	C 1
NBB-01			THE REAL	
NBB-02				
NBB-03				
NBE-01				
NBE-02			stantik.	-
NBE-03				
NBL-01	Cont.			
NBO-01	~	C		
NFL-01				
NGA-01	Section of the sectio	Sec. 1995		
NGA-02		-		
NGR-01				

Figure 7. Lip balm samples on VSC 8000 -1.

	Visible Light	UV – 365 nm	IR – 695 nm	Flouresance (spot) - 645 nm
NHH-01	ATTE .		STR.	
NK1-01			Service !!	
NMA-01		-	25330 BODA	
NMN-01			-	
NNI-01	Ser A			
NPB-01			distant.	
NPB-02			Constanting of	
NUS-01	622	6		
NVE-01	(1))			

Figure 8. Lip balm samples on VSC 8000 -2.



Figure 9. Lip gloss samples on VSC 8000.

3.2. Characterization of lip cosmetic samples

ATR-FTIR was used to identify the compositional variations among the samples. In order to get a reproducible result. FTIR are acquired from 3 different part of lip cosmetics. The results are given in Figure 2. The results have shown that all spectra for L (liquid lipstick), R (lipstick), N (lip balm) and P (lip gloss) samples were exactly superimposed with each other, which confirms the homogeneity test studies for all lip cosmetic samples.

3.3. Stability test

Lip cosmetics are physical evidence which could be transferred to paper, clothes or another person. The presence of lipstick-stained evidence could play an important role in order to solve the case [33]. The evidence could be everywhere such as inside a trash box, in the forest, in the lake or sea water etc. for this, we investigate the effect of different parameter in order to

comprehend the structural chance towards to time, substrate type and water source.

The study on the effect of substrate is vital in order to simulate crime scene. To simulate the substrate type, lip cosmetic products are stained to glass, fabric and paper towel. For these surfaces, a laboratory slide as a glass, a fabric cut from a 100% cotton t-shirt, and Z-fold paper towels were used. Then, lip cosmetic-stained substrates are dipped in different water source including tap water, distilled water, and simulated sea water. Moreover, to examine the time, stained substrates is kept in water sources at different time of intervals. Images of lip gloss stained on the glass surface in different water sources are shown in Figure 3. As a representative example, lip glossstained glass in water were shown. It can be seen that lip gloss stains undergo change in different water sources. All lip products stain in water sources have spread to surface. Lip gloss stains become lost in distilled water at the end of 1 month. On the tap water, the lip gloss stains get loose when the test is maintained from 1 hour to 1 month.

Nevertheless, lip gloss stains are observed at the end of 1 month. Similarly, lip gloss stains can be seen in the simulated sea water on the whole stability test process. The stability images of lip cosmetic-stained objects in different water source were shown in supplementary document (Figure 10-20).



Figure 10. Stability test of liquid lipstick on glass.

	To	1 Hour	1 Day	1 Week	1 Month
Tap Water	Ö	8		8	3
Distilled Water	Ö			3	B
Simulated Water	8	-	2	-	*

Figure 11. Stability test of lipstick on glass.



Figure 12. Stability test of lip balm on glass.



Figure 13. Stability test of liquid lipstick on fabric.



Figure 14. Stability test of lipstick on fabric.



Figure 15. Stability test of lip balm on fabric.



Figure 16. Stability test of lip gloss on fabric.

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Figure 17. Stability test of liquid lipstick on paper towel.

	To	1 Hour	1 Day	1 Week	1 Month
Tap Water					
Distilled Water					
Simulated Water					

Figure 18. Stability test of lipstick on paper towel.



Figure 19. Stability test of lip balm on paper towel.



Figure 20. Stability test of lip gloss on paper towel.

In order to examine the chemical stability of samples on different surfaces, a series of experiment were carried out. For this, all lip cosmetic products are stained to different surfaces. Besides, all samples are kept under different water source at different time of intervals. The structural of lip product samples analyzed by FTIR (Figure 4). The band between 3100-3700 cm⁻¹ indicated the presence of hydroxyl groups due to intermolecular hydrogen bonding. The peaks occurring between 2800-3050 cm-1 were attributed to the presence of -CH bonds [6,25]. The peaks in the ranges 1730-1740 cm⁻¹ confirms the presence of C=O stretching vibration. Peaks between 1370-1560 cm-1 are attributed to aromatic compounds, peaks between 1440-1465 cm⁻¹ are attributed to -CH₂ shear deformation. The peaks between 850-1270 cm⁻¹ attribute Si-O stretching of silicates, and peaks between $630\text{-}790~\text{cm}^{\text{-}1}$ are attributed to Si-O and Si-O-Al stretching vibration [25,34,35]. The peak in 1040 cm-1 is attributed to the Si-O-Si stretching vibration of siloxanes [17,36]. It could be seen that FTIR results give a distinctive result among the samples. For instance, there is no clear peak on the sample L in spectral range 1730-1780 cm⁻¹ while sharp peak of carbonyl groups is observed in the sample R, N and P. Besides, there is hydroxyl peaks between 3300-3600 cm⁻¹ in the sample of L and R while no visible peaks are seen in the sample of N and P.

In order to investigate the effect of time on the chemical stability, all lip product samples are kept under water (tap water, distilled water, simulated sea water) from 1 hour to 1 month. The results showed that there is no significant change of samples under the water from 1 hour to 1 month. It could be said that it is hard to determine the applied time of lip cosmetic products on glass. Furthermore, lip cosmetic products on different surfaces including fabric and paper towel are characterized by FTIR. The FTIR results of other lip products including liquid lipstick, lip balm and lip gloss on fabric and paper towel are given in Figure 21 and Figure 22 and Figure 23 respectively. Similarly, there is no significant change in the FTIR to discriminate the time of applying, the substrate and the water.

3.4. Principle Component Analysis (PCA)

PCA analysis was applied to all T_0 samples and classification was observed (Figure 5-6). In the PCA analysis of the samples, it is generally seen that the liquid lipsticks are separated from the other samples. Especially LMT-01 and LMA-01 coded samples are clearly separated from other samples.

Lipstick, lip balm and lip gloss samples are grouped together to show their similarities.



Wavenumber (cm⁻¹)

Figure 21. The FTIR spectra of liquid lipstick in water sources at different time of intervals.



Figure 22. The FTIR spectra of lip balm in water sources at different time of intervals.



Figure 23. The FTIR spectra of lip gloss in water sources at different time of intervals.

4. Conclusion

In this study, four different lip cosmetic products including wax lipstick, liquid lipstick, lip balm and lip gloss have been examined as forensic evidence. FTIR is carried out to identify the characterization and differentiate samples. Moreover, VSC 8000 have been used for imaging of lip cosmetic-stained substrates under different light of sources to observe the structural change against the environmental conditions. The effect of lip cosmetic product types, time, type of substrate, and environmental conditions are systematically investigated. The stability studies have demonstrated that the stain on the different substrates shows differentiating results which is captured by VSC 8000. By taking account that stained (with lip cosmetic products) evidence could be encountered in different environment, the effect of chemical and physical change of stained objects in purified water, tap water and simulated sea water are investigated. Accordingly, there is no significant structural change in water source and as the times changes, however, they can be discriminated visually using optical microscopy or Video spectral comparator.

Author contributions

Seyda Turkay: Investigation, analysis, writing-original draft, visualization, methodology **Soner Kizil:** Investigation, analysis, writing-original draft conceptualization, methodology, visualization, writing-reviewing and editing, supervision.

Conflicts of interest

The authors declare no conflicts of interest.

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