Forensic Discrimination of Lipsticks by Thin Layer Chromatography and Gas Chromatography-Mass Spectrometry

Ahmad Fahmi Lim bin Abdullah[a], Yathindra Marimuthu[a], Chang Kah Haw[a], Nurul Fatihah binti Mohamad Said[b], Noor Zuhartini Md Muslim[a], Nik Fakhuruddin Nik Hassan[a], Mohamad Hadzri Yaacob[a] and Yew Chong Hooi[c]

[a] *Forensic Science Programme, School of Health Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan*
[b] *Faculty of Applied Science, Universiti Teknologi MARA, 40450 Shah Alam, Selangor*
[c] *Royal Malaysia Police Forensic Laboratory, 43200 Cheras, Selangor*

ABSTRACT: Cosmetic evidence such as lipstick recovered from a crime scene can prove useful to link a suspect with the victim or crime scene and therefore need to be carefully analysed during crime investigation. 53 lipstick samples of different brands of similar colour were selected for this study. Colouring agent was analysed by thin layer chromatography (TLC) and organic components were subjected to gas chromatography-mass spectrometry (GC-MS). Using three different solvent systems [methylene chloride; chloroform: methanol: water (50: 15: 2), ethyl acetate: methanol: ammonium hydroxide (5: 1: 1)] by TLC, lipstick samples of colours indistinguishable on visual observation could be grouped into eight subgroups. Subsequent GC-MS analysis enabled identification of all the samples by comparing chromatograms acquired by scan mode. Lipstick sample from the scene of crime and from suspected sources can be analysed using both these techniques and comparison of pattern of band separation and chromatogram can aid in discriminating lipstick traces in a simple and quick manner.

Keywords: lipstick, forensic analysis, solvent system, GC-MS, TLC, forensic chemistry

Introduction

Cosmetic evidence recovered from a crime scene can link a suspect to a victim or to a crime scene and therefore needs careful and thorough attention in crime investigation. Traces of lipstick are one such evidence. Lipstick smears could be found left on drinking cups, glasses, cigarette butts, tissue papers or handkerchief [1]. In certain cases, trace amount of lipstick was transferred to the clothing of perpetrator who attacked a female [2-4]. Thus, forensic analysis of lipsticks was often found to be crucial in the investigation of criminal cases. The identification and determination of components in a lipstick sample have to be conducted with rapid methods [2-7].

Lipsticks contain wax, oil and colouring agents as three main ingredients [1, 3, 6]. Wax enables the adjustment of the staying powder properties to heat and hardened texture on application. Meanwhile, oil provides shiny and glide quality. During manufacturing process, the number of dyes or colouring agents used is limited and combination of dyes give rise to variation in shade. Colouring agents can be either synthetic or natural dyes which can further be categorized into oil-soluble or water soluble dyes. Therefore, lipstick of same colour may contain varied colouring agents [6].

Various methods of forensic lipstick analysis were reported [2, 3, 5, 7-20]. Forensic analysis of lipstick by thin layer chromatography (TLC) was conducted in previous studies for the separation of these colouring agents in a mixture with development of bands that varied in colour and number[2, 3, 5, 6]. Small amount of lipstick (approximately 10 μg) could lead to good comparisons in TLC [6]. Oil soluble and water soluble dyes were separated in different solvent systems respectively [7]. Several authors have described various TLC solvent system for separation in respective studies [2, 3, 6, 7]. In addition, TLC gives rise to different retardation factor (R_f) value for different components present in a lipstick sample. The samples of lipstick were compared and grouped into different groups in which they were distinguishable on comparison.

Gas chromatography (GC) is a technique for the separation of complex mixtures especially with organic compounds based on the differences in partitioning behaviours between mobile gas phase and stationary phase in the column. Combination of GC with mass spectrometry technique allows the identification of chemical structures of organic compounds as in lipstick. The presence of such compounds differing in chemical structures produced different chromatographic patterns on analysis. According to Russell and Welch (1984), difference in the composition of the lipstick can be seen not only between manufacturers but also samples from a single manufacturer [6].

Usually, lipstick smears collected from a scene of crime contained only trace amount of sample [3, 8]. However, study conducted by Russell and Welch (1984) showed that small quantity of extracted lipstick smear could be compared with those samples from direct extracts of lipstick effectively by TLC and GC-MS [6]. In this study, TLC and GC-MS techniques were carried out on trace lipstick smears from different brands and also different series from the same brand for comparison and discrimination.

Experimental

i) Reagents, materials and apparatus

The samples, 53 lipsticks were obtained from shops and consisted of commonly used lip cosmetics in Malaysia, representing 15 different manufacturers. Commonly available solvents and other chemical substances, unless otherwise stated, were of analytical grade.

Silica gel aluminium plates, (F 254, 20 cm × 20 cm), were obtained from Merck Darmstadt Germany. A model of gas chromatography-mass spectrometer (Varian CP-3800 GC equipped with Saturn 2200 MS detector) with a VF -1MS capillary column (30 mm × 0.25 mm × 0.25 μm) was used.

ii) Sample preparation

Fifty three lipstick samples of different brands of very similar shade were selected from among the 70 samples in our collection and labeled according to different manufacturers. All the lipsticks were red with very similar shades.

Lipstick was smeared on filter paper (110 mm in diameter; Whatman, Schleicher & Schuell) and a small portion of filter paper (4 mm x 4 mm) was placed into a labeled tube. Petroleum ether (1.0 mL) was added to the tube and placed in desiccators for removal of waxes and oil. This step was repeated for twice. Petroleum ether extract was combined and kept for GC-MS analysis. The filter paper containing colouring agent was decanted followed by the addition of methanol-ammonium hydroxide (95:5 v/v). The solution containing extracted dyes was transferred to a small glass vial and gentle heating was applied to aid evaporation. Methanol (2 drops) was added before the spotting of sample on TLC plate.

Three solvent systems were prepared and labelled as solvent system A, B and C respectively. All solvent used were of analytical grade.

Solvent system A- Methylene Chloride
Solvent system B- Chloroform: Methanol: Water (50: 15: 2)
Solvent system C- Ethyl acetate: Methanol: Ammonium hydroxide (5: 1: 1)

iii) TLC separation

Silica gel aluminium plates (20 cm x 20 cm) were heated at 100^{o}C for 1 h and cooled at room temperature. Sample was spotted and the plate was developed in a TLC chamber with respective solvent system. Examination of developed TLC plate was performed under white and ultraviolet lights. R_f value of different visible bands was recorded.

iv) GC-MS analysis

Combined petroleum ether (2.0 mL) was carefully concentrated to about 500 μL. 100 μL of this extract was transferred into GC vial. 500 μL of n-hexane was also added to the vial.

The GC-MS analysis was carried out on a Varian CP-3800 GC equipped with Saturn 2200 MS detector, a VF -1MS capillary column (30 mm × 0.25 mm × 0.25 μm). Helium was used as carrier gas with a flow rate of 0.8 mL/min. The injector was set at 250 ^{o}C and the transfer line was set at 280 ^{o}C. The oven temperature was programmed as follows: an initial temperature of 65 ^{o}C was held for 5 min, followed by an increase of 5 ^{o}C/min to 300 ^{o}C which was held for 10 min. The samples (1 μL) were introduced in

splitless mode. Mass spectra were acquired in scan mode from m/z 50-500.

Results and Discussion

Most of the lipstick samples could be separated from others through TLC technique using solvent system B and C. The number of coloured spots observed varied from one to four in orange, pink and yellow shades in addition to several spots which fluorescence under UV light (**Table 1**). This suggests that manufacturers of lipsticks applied different or a combination of colouring agents to give the desired colour that appears indistinguishable to normal visual examination in white light especially when present at trace amount.

During TLC using solvent system A, spot was not observed indicating that lipstick samples used has no or little oil-soluble colouring agent that might be separated by dichloromethane. Both the solvent system B and C produced good separation for the samples analysed permitting sample grouping based on their differing R_f and colour of spots. Solvent system C gave sharper bands as compared to solvent system B. However, it is worth mentioning that some bands produced by solvent system C were not well separated using a 15 cm x 15 cm plate. Solvent system C which gave sharper bands allows better discrimination especially in separating K1, L1, M1 and N1 which appeared to be indistinguishable using solvent system B.

By TLC, every lipstick sample could be discriminated from the rest or could be assigned to different groups with solvent system B and C (**Table 2**). From a total of 53 samples examined, 16 of them showed different pattern of separation in both the solvent systems. The samples (H1 and H2; E6, E8 and E9; H5, H6 and H7) which originated from the same manufacturer were grouped into a single group based on R_f calculated. Groupings (a total of 11 groups) of other samples either from a single manufacturer or different manufacturers were shown in **Table 2**.

The method limit of detection was determined by smearing lipstick on filter papers. The amount of lipstick per unit area of filter paper was determined before going through the extraction process and TLC determination. The minimum amount of lipstick present on filter paper and produced a positive result using the experimental parameters is 154 µg, on a surface of 2 mm by 2 mm.

Table 2: Grouping of samples based on R_f and colour of spots

Group	Sample
1	A5, G2, I1, I2, K1, L1, M1, O1, P1
2	B1, C1, D6, N1, Q1
3	A2, A3, E3
4	D1, D2, F3
5	D5, G1, J1
6	E6, E8, E9
7	H5, H6, H7
8	A1, C2
9	B2, D3
10	E1, H3
11	H1, H2
Ungrouped	A4, B3, C3, C4, C5, C6, D4, D7, E2, E4, E5, E7, F1, F2, H4, Q2

In each lipstick, differences in organic components were found to produce different GC-MS chromatogram patterns attributable to variation in chemical structures. With GC-MS operating at scan mode, comparison of the 53 chromatograms shows all of them have different peaks patterns (**Fig. 1**). Nevertheless, staged chromatograms of H1, H2 and H4 as well as G1 and G2 show some similarities, respectively. It is worth nothing that H4 is different from H1 and H2 by TLC. Similar is the difference between G1 and G2. A careful comparison of overlaid chromatograms of H1 and H2 shows that they are not identical, when adjacent peak height ratios were considered (**Fig. 2**). It is pertinent to mention that in this study no attempt was made to identify the peak producing compounds since visual comparison of the chromatogram patterns is sufficient to discriminate the samples.

Table 1: Analysis result of different kinds of lipstick samples with solvent system B and C

Sample	Brand	Solvent system B		Solvent system C	
		No. of spot	R_f	No. of spot	R_f
A1	Follow Me	1	0.29 (lo)	1	*0.48 (lo)
A2	Follow Me	2	*0.66, 0.32 (lo)	2	*0.46, 0.39 (lo)
A3	Follow Me	2	*0.66, 0.33 (o)	2	*0.46, 0.38 (lo)
A4	Follow Me	2	*0.78, 0.32 (lo)	2	0.45 (lp), 0.38 (lo)
A5	Follow Me	1	0.20 (lo)	1	0.38 (lo)
B1	Safi	1	0.32 (lo)	2	*0.43, 0.36 (lo)
B2	Safi	2	*0.25(lo), **0.18 (lp)	3	**0.35 (p), 0.34 (lo), *0.26 (lo)
B3	Safi	3	**0.80 (lo), **0.75 (lo), *0.25 (lp)	4	*0.42 (lp), *0.38 (lo), *0.28 (lo), *0.21 (lo)
C1	Safi	1	0.25 (lo)	2	*0.38, 0.30 (lo)
C2	Safi	1	0.31 (lo)	1	*0.35 (lo)
C3	Safi	2	0.43 (lo), *0.17 (lp)	3	*0.55(p), 0.48(lp), 0.37 (lo)
C4	Safi	2	*0.79 (lp), 0.43 (lo)	1	*0.37 (lo)
C5	Safi	2	0.27 (lp), 0.06 (lo)	3	*0.37, 0.31(lo), 0.09 (y)
C6	Safi	1	0.20 (lo)	2	*0.31(lo), 0.11 (y)
D1	Safi	1	0.29 (lo)	2	*0.42 (lp), *0.37 (lo)
D2	Safi	1	0.28 (lo)	2	*0.42 (lp), *0.35 (lo)
D3	Safi	2	0.36 (lo), *0.10 (lp)	3	*0.43 (p), 0.38 (lp), 0.35 (lo)
D4	Safi	1	0.28 (lo)	2	0.33 (lo), 0.33 (lp)
D5	Safi	2	0.24(lo), *0.19 (lp)	2	**0.33 (lo), 0.33 (lp)
D6	Safi	1	0.37 (lo)	2	*0.38 (lp), 0.33 (lo)
D7	Safi	1	*0.24(lo)	3	*0.38 (p), *0.30(lo), 0.29 (lp)
E1	Irise Colour	1	0.37 (lo)	2	*0.41(lp), 0.35 (lo)
E2	Irise Colour	4	**0.65 (lp), *0.28 (lo), *0.17 (lo), *0.04 (lp)	3	*0.37 (lp), *0.30 (lo), *0.17 (lo)
E3	Irise Colour	2	**0.65 (lp), 0.26 (lo)	2	*0.37 (lo), 0.28 (lo)
E4	Irise Colour	2	*0.20 (lo), *0.09(lp)	2	**0.33 (p), *0.24 (lo)
E5	Irise Colour	3	**0.67 (lp), *0.20 (lo), *0.07 (lp)	3	*0.51 (p), *0.50 (lo),*0.41 (lo)
E6	Irise Colour	4	**0.70 (lo), *0.28 (lo), * 0.19 (lo), *0.07 (lo)	5	**0.44 (p), *0.42 (lp), *0.38 (lo), *0.29 (lo), **0.26 (lo), 0.24 (y)
E7	Irise Colour	2	*0.85 (lp), *0.39 (lp)	4	*0.43, *0.37 (lp), *0.26 (lo), **0.18 (lo)
E8	Irise Colour	4	**0.85 (lp), *0.39 (lo), *0.22 (lo), *0.08(lp)	6	**0.47 (lp), *0.44 (lp), *0.38 (lo), *0.35 (lo), **0.26 (lo), **0.16 (y)
E9	Irise Colour	4	**0.72 (o), *0.28 (lo), *0.20 (lo), *0.06 (lp)	6	*0.54 (lp), *0.51 (lp), **0.44 (p), **0.37 (o), **0.28 (lo), **0.19 (y)
F1	Tracia Teen	2	**0.75 (lp), *0.67 (lo)	2	*0.46(lp), *0.42 (lo)
F2	Tracia Teen	1	*0.25 (lo)	2	*0.49 (lo), *0.43 (p)
F3	Tracia Teen	1	0.23 (lo)	3	*0.50(lp), *0.37 (lo)
G 1	Fanbo	2	0.31 (lo), *0.07 (p)	2	**0.32 (p), 0.26 (o)
G 2	Fanbo	1	0.31 (o)	1	0.26 (o)
H 1	Avon	2	0.31 (o), *0.17 (lp)	2	0.25 (o), **0.23 (lp)
H 2	Avon	2	0.32 (o), *0.19 (lp)	2	0.25(o), **0.22(lp)
H 3	Avon	1	0.30 (o)	2	**0.40 (p), 0.25 (o)
H 4	Avon	3	0.27 (p), *0.16 (lp), 0.08 (lp)	3	0.31(p), **0.24 (o), 0.23 (lp)
H 5	Avon	1	0.26 (o)	1	0.23 (o)
H 6	Avon	1	0.27 (lo)	1	0.25 (lo)
H 7	Avon	1	0.26 (o)	1	0.26 (lo)
I 1	L'oréal Paris	1	0.31 (o)	1	0.26 (o)
I 2	L'oréal Paris	1	0.31 (lo)	1	0.27 (o)
J 1	Silky Girl	2	0.33 (o), *0.09 (p)	2	**0.32 (p), 0.27 (o)
K 1	Dior	1	0.30 (o)	1	0.26 (o)
L 1	Estēe Lauder	1	0.31 (o)	1	0.27 (o)
M 1	Beauté de Kosé	1	0.31 (o)	1	0.31 (o)
N 1	Shiseido	1	0.31(o)	2	*0.35 (lp), 0.30 (o)
O 1	Lancôme	1	0.30 (o)	1	0.30 (o)
P 1	Maybelline	1	0.28 (o)	1	0.30 (o)
Q 1	Nutrimetics	1	0.29 (o)	2	*0.35 (lp), 0.30 (o)
Q 2	Nutrimetics	3	**0.72 (Y), 0.31 (o), *0.25 (p)	4	0.29 (o), **0.28 (o), **0.23 (p), **0.14 (y)

lo-light orange, lp-light pink, o-orange, p-pink, y-yellow, *- weak fluorescence, **- strong fluorescence

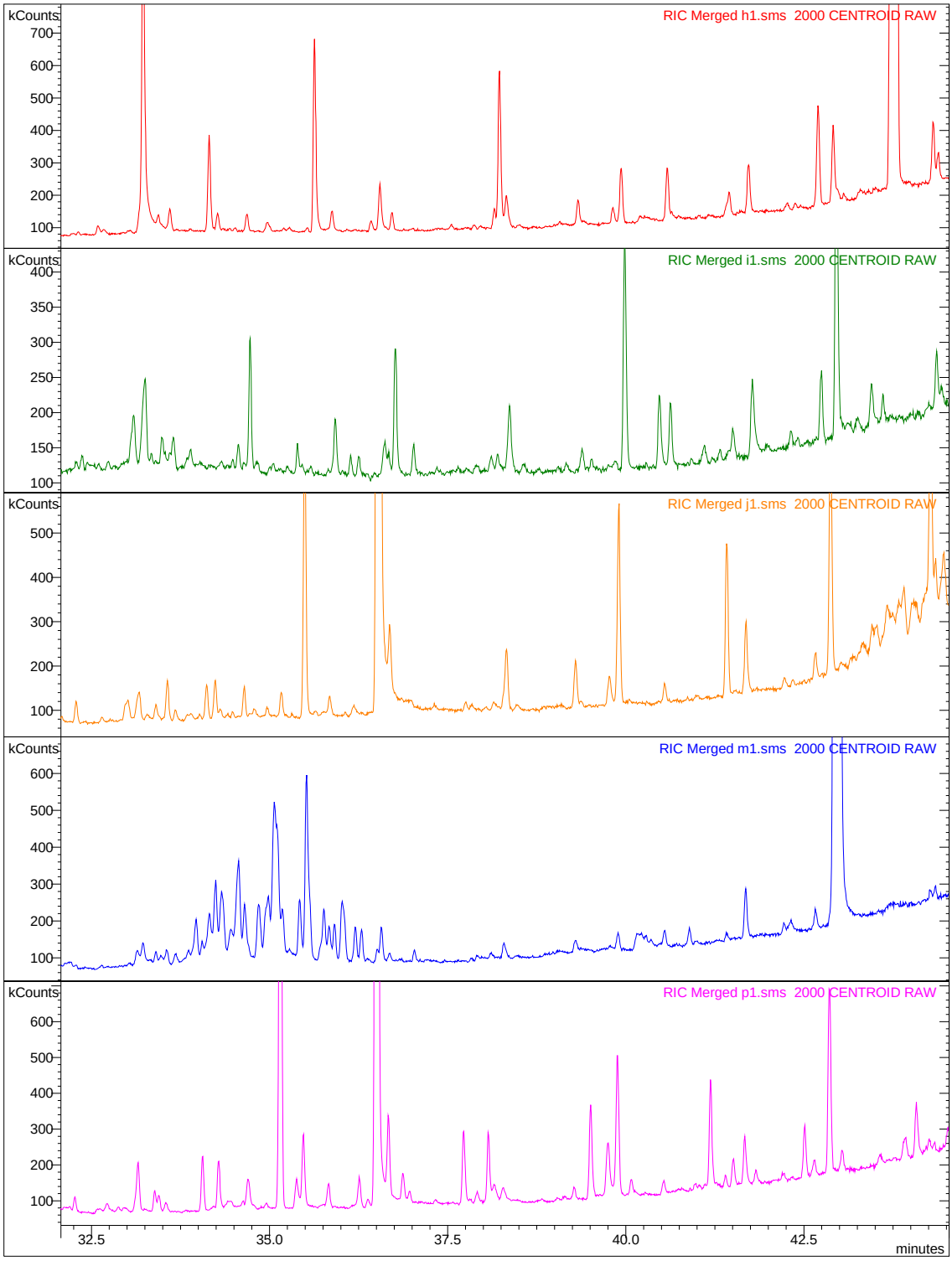

Fig. 1: Staged chromatograms show different lipsticks (H1, I1, J1, M1 and P1) give distinguishable chromatogram patterns

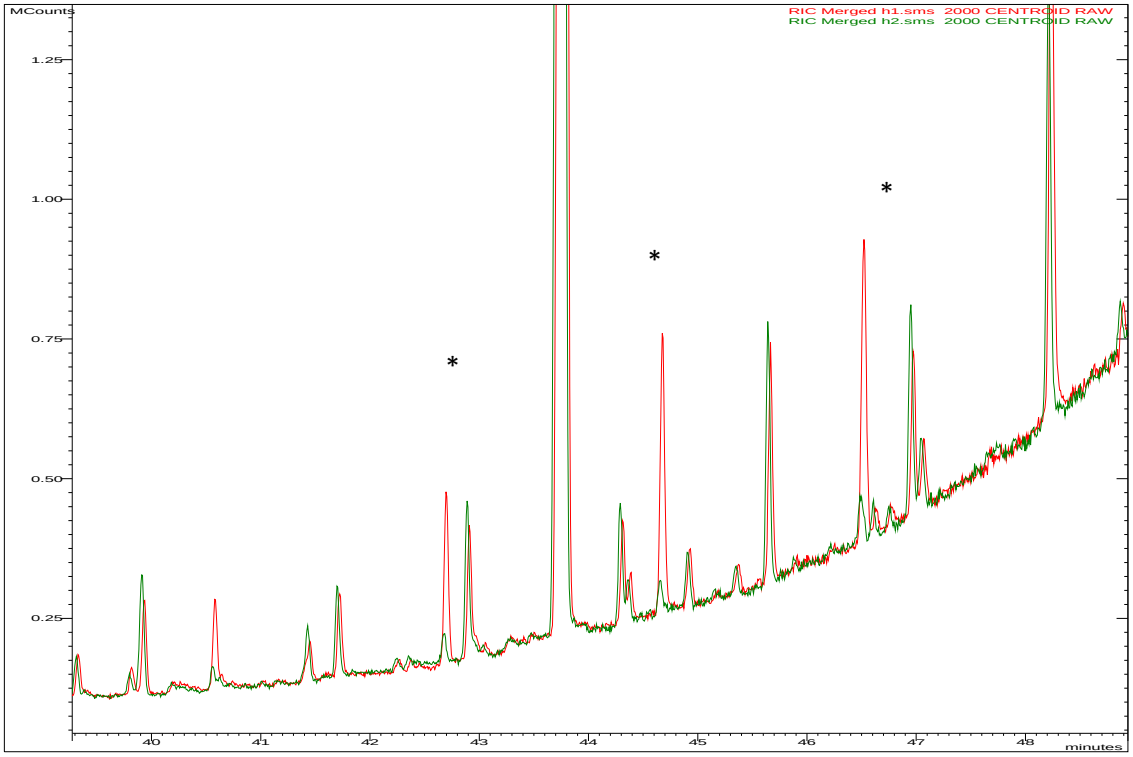

Fig. 2- Overlay of chromatograms of two very similar samples, H1 (red) and H2 (green) shows different peak height ratio to adjacent peaks in regions marked *

Conclusion

TLC analysis of lipstick traces is shown to be a good screening technique for discriminating samples during initial investigation while GC-MS can serve as a confirmatory technique since it enables discrimination based on the chemical structures of the sample. Our study showed that all lipstick samples that remained indistinguishable during visual analysis could be discriminated from each other by a combination of both TLC and GC-MS techniques. With lipstick sample from the scene of crime and also samples from suspected sources, both these techniques can be carried out together for comparison purpose. Generation of pattern of TLC separation and chromatogram in a suspected sample that differed from the pattern produced by the crime scene sample may lead to exclusion in a simple and quick manner.

Acknowledgements

The authors thank Universiti Sains Malaysia for funding this work (Grant no 304/PPSK/6139036).

References

1. Weisz, A., S.R. Milstein, and A.L. Scher. (2007). Colouring Agents in Cosmetic Products (Excluding Hair Dyes): regulatory Aspects and Analytical methods, in Analysis of Cosmetic Products, A. Salvador and A. Chisvert, Editors. Elsevier: London.
2. Andrasko, J. (1981). Forensic Analysis of Lipsticks, Forensic Science International. 17: p. 235-251.
3. Barker, A.M.L. and P.D.B. Clarke. (1972). Examination of Small Quantities of Lipsticks, Journal of Forensic Science Society. 12: p. 449-451.
4. Kaegy, R.L. (1983). Examinations of Cosmetic Smudges Including Transesterification and Gas Chromatographic/Mass Spectrometric Analysis, Journal of Forensic Sciences. 28(3): p. 623-631.
5. Jiang, H. and Y. Yan. (2007). Analysis of Lipsticks by TLC, Detergent and Cosmetics. 30(23-25).
6. Russell, L.W. and A.E. Welch. (1984). Analysis of Lipsticks, Forensic Science International. 25: p. 105-116.
7. Sjoberg, A.M. and C. Olkkonen. (1985). Determination of Synthetic Organic Colours in Lipsticks bt Thin-layer and High-performance

Liquid Chromatography, Journal of Chromatography. 318: p. 149-154.
8. Choudhry, M.Y. (1991). Comparison of Minute Smears of Lipsticks by Microscopectrophotometry and Scanning Electron Microscopy/Energy-Dispersive Spectroscopy, Journal of Forensic Sciences. 36(2): p. 366-375.
9. Ehara, Y. and Y. Marumo. (1998). Identification of Lipstick Smears by Fluorescence Observation and Purge-and-Trap Gas Chromatography, Forensic Science International. 96: p. 1-10.
10. Engebretson, A. and D.M. Besemann. (2007). Forensic Lipstick Analysis using Chemical Fingerprinting via Gas Chromatography (Abstracts of Papers), in 233rd ACS National Meeting, March 25-29, 2007. Chicago, IL: SciFinder Scholar.
11. Gagliardi, L., et al. (1998). Identification of Xanthene Dyes in Lipsticks by Reversed-phase High-performance Liquid Chromatography, Journal of Chromatography. 448: p. 296-300.
12. Gagliardi, L., et al. (1987). Identification of Cosmetic Dyes by Ion-pair Reversed-phase High-Performance Liquid Chromatography, Journal of Chromatography. 394: p. 345-352.
13. Griffin, R.M.E., et al. (1994). An Improved High-Performance Liquid Chromatography System for the Analysis of Basic Dyes in Forensic Casework, Journal of Chromatography A. 674: p. 271-280.
14. Lawrence, J., et al. (1982). Gas chromatographic analysis of chlorophenylmercapturic acid lindane metabolites, Journal of Chromatography. 236(2): p. 403-419.
15. Misra, G. and V.K. Mittal. (2004). Neutron Activation Analysis of Lipsticks using g-Ray Spectrometry, Journal of Applied Apectroscopy. 71(2): 270-274.
16. Reuland, D. and W.A. Trinler. (1980). A Comparison of Lipstick smears by High Performance Liquid Chromatography, Journal of Forensic Science Society. 20: p. 111-120.
17. Reuland, D. and W.A. Trinler. (1984). A Comparison of Lipstick Smears by High Performance Liquid Chromatography. Part II. The Effects of Wear-time and Subject on the Chromatograms, Journal of Forensic Science Society. 24: p. 509-518.
18. Rodger, C., et al. (1998). The in-situ Analysis of Lipsticks by Surface Enhanced Resonance Raman Scattering, The Analyst. 123: p. 1823-1826.
19. Verdu, F. and A. Castello. (2006). Development of latent lip prints on multicoloured surfaces, a problem resolved using fluorescent dyes, Indian Internet Journal of Forensic Medicine and Toxicology. 4(2).
20. Verdu, F., A. Castello, and M. Alvarez-Segui. (2005). Luminous Lip-prints as Criminal Evidence, Forensic Science International. 155: p. 185-187.

Additional information and reprint requests:
Ahmad Fahmi Lim bin Abdullah, PhD
(E-mail: fahmilim@kb.usm.my)
Forensic Science Programme
School of Health Sciences
Universiti Sains Malaysia
16150Kubang Kerian, Kelantan, Malaysia