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Identification of Illegal Coal Tar Dyes Constituents in Mucous Cosmetics by HPLC Method

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ABSTRACT

Illegal coal tar dyes are reported to possess strong toxicity and carcinogenecity when added in mucous cosmetics. According to CNS, current methods in analyzing organic coloring materials (dyes) are mainly filter paper chromatography and thin-layer chromatography. In this study, we adopted a refined HPLC procedure reaching optimal conditions for the separation and identification of the coloring constituents allegedly added in mucous cosmetics. Ultimately, the analytical efficiency and precision are greatly improved. The optimal conditions for the HPLC method were found with a column of Cosmosil 5C18-AR-II; with the first mobile phase using 0.07 M ammonium acetate solution in 0.01 M tetrabutylammonium bromide (CH_3COONH_4) : CH_3CN : CH_3OH = 55:35:10 for analyzing 16 hydrophilic coal tar dyes, while the second mobile phase uses CH_3OH : H_2O = 95:5 for analyzing 6 hydrophobic dyes and a photodiode array detector within UV-VIS wavelength. By applying this HPLC method, a total of 22 banned coal tar dye constituents can be satisfactorily separated. It is also found that the detection limit can be improved to 0.05 μg/mL.

Key words: **high performance liquid chromatography (HPLC), illegal coal tar dyes, mucous cosmetics.**

INTRODUCTION

According to the Law for the Sanitary Control of Cosmetics and Related Regulations announced by Department of Health, ROC, on May 27, 1991, the dyes applied on the manufacture of cosmetics should be approved by central sanitary authorities. Unapproved dyes for cosmetics are not allowed to use. However, the types of dyes disallowed in cosmetics vary from country to country. In one incident, cosmetics exported from Taiwan were found to contain unapproved dyes by the another government. Therefore, it is necessary to develop a rapid and precise analytical method for identification of coloring constituents in cosmetics.

According to Food and Drugs Administration of USA, coloring dyes are divided into three categories. The first category is food, drugs, and cosmetics dyes (F.D.&C dyes) allowed to be used

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in food, drugs, and cosmetics. The second category is drugs and cosmetics dyes (D&C dyes) allowed to be used in drugs and cosmetics, while used as additives in food is prohibited. The third category is drugs and cosmetics dyes for external use only (Ext.D&C dyes). They are prohibited from being used in lip-contacting mucous cosmetics. In Republic of China, the coloring dyes are categorized into two groups according to The Law for the Sanitary Control of Cosmetics and Related Regulations[1]. The first group of 65 dyes can be used in cosmetics. The second group including 25 dyes is not allowed to be used in mucous cosmetics. In this study, an HPLC method was developed to separate and identify 21 dyes in the second group as well as Amaranth (Red No. 2), which was excluded in use for cosmetics due to safety consideration. The structures of these compounds are presented in Figure 1. The official method for the detection of organic coloring materials is based on Chinese National Standard (CNS) No. 9189S2077[2,3]. The coal tar dyes constituents in cosmetics are extracted by organic solvents. Solvent selection is based on the partition coefficient between dyes and solvents. The extracts are then analyzed by Thin Layer Chromatography (TLC) and Paper Chromatography (PC). These methods, however, are complicated. The spots on TLC or PC readily diffuse with significant tailing effects. It is difficult to identify the compounds when their R_f values are similar.

The purpose of this study was to establish an analytical method to simplify the extraction and identification procedures. An HPLC method coupled with a photodiode array detector was carried out in this study. Twenty-two coloring dyes were well separated and the UV-VIS spectra of which were also obtained. Both separation and identification of dyes were improved and the limit of detection was also enhanced using this method.

Cosmetics especially lipstick is getting popular because the living standard is getting better in Taiwan. Lipstick is classified into the second category in mucous cosmetics. In addition to wax lipids, coloring dyes are the most important ingredients of lipstick. The newly developed method was also used to analyze coloring dyes in commercial lipstick in order to check for illegal coloring dyes.

MATERIALS AND METHODS

I. *Materials*

(I) *The Source of Test Samples*

Twenty lipstick samples were purchased from cosmetics vendors in Nankang area of Taipei City or obtained from local health authorities.

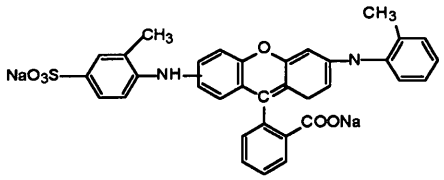
(II) *Reagents*

1. LC grade methanol, acetonitrile, and chloroform were obtained from Lab-scan Co. (Ireland)
2. Acetic acid (reagent grade) was the product of Wako Inc. (Japan)
3. Ammonium water solution (reagent grade) was purchased from Nacalai Tesque Inc. (Japan)
4. Ammonium acetate (reagent grade) was obtained from Merck Co. (Germany)
5. Tetrabutylammonium bromide (reagent grade) was the product of Fluka Co. (Switzerland)
6. Ethanol (95%) was obtained from Bureau of Taiwan Tobacco and Wine Monopoly.

(III) *Standards of Coloring Dyes*

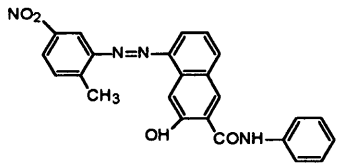
1. Violamine R, CI 45190
2. Medicinal Scarlet, CI 26105
3. Brilliant Fast Scarlet, CI 12315
4. Permanent Red F5R, CI 15865
5. Orange SS, CI 12100
6. Fast Light Yellow 3G, CI 18820
7. Alizarine Violet NR, CI 60730
8. Naphthol Green B, CI 10020
9. Ponceau 3R, CI 16155
10. Ponceau 2R, CI 16150
11. Ponceau SX, CI 14700
12. Oil Red X, CI 12140
13. Fast Red A, CI 15620
14. Orange I, CI 14600
15. Hansa Yellow, CI 11680
16. Polar Yellow 5G, CI 18950

(1): R26



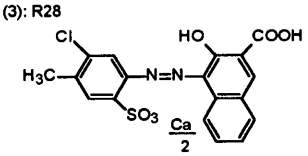
Cosmetics Red NO.26 Violamine R CI 45190

(2): R27



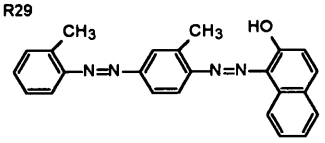
Cosmetics Red NO.27 Brilliant Fast Scarlet CI 12315

(3): R28



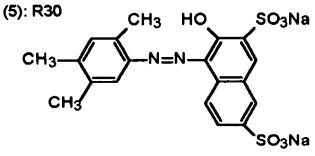
Cosmetics Red NO.28 Permanent Red F5R CI 15865

(4): R29



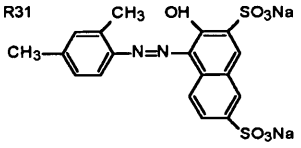
Cosmetics Red NO.29 Medicinal Scarlet CI 26105

(5): R30



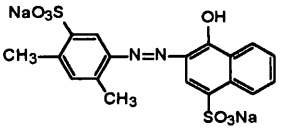
Cosmetics Red NO.30 Ponceau 3R CI 16155

(6): R31



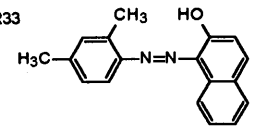
Cosmetics Red NO.31 Ponceau 2R CI 16150

(7): R32



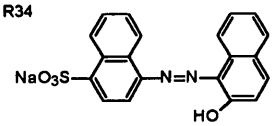
Cosmetics Red NO.32 Ponceau SX CI 14700

(8): R33



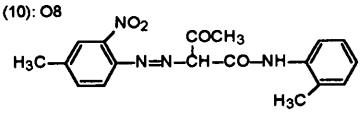
Cosmetics Red NO.33 Oil Red XO CI 12140

(9): R34



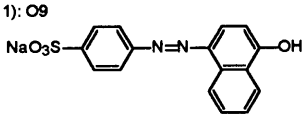
Cosmetics Red NO.34 Fast Red A CI 15620

(10): O8



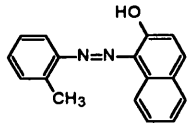
Cosmetics Orange NO.8 Hansa Orange CI 11725

(11): O9



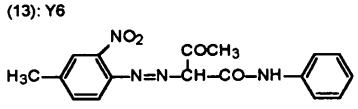
Cosmetics Orange NO.9 Orange I CI 14600

(12): O10



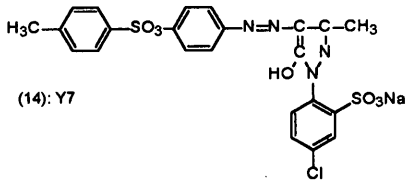
Cosmetics Orange NO.10 Orange SS CI 12100

(13): Y6



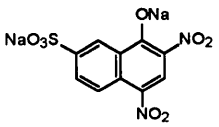
Cosmetics Yellow NO.6 Hansa Yellow CI 11680

(14): Y7



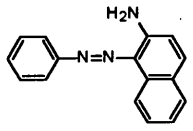
Cosmetics Yellow NO.7 Polar Yellow 5G CI 18950

(15): Y8



Cosmetics Yellow NO.8 Naphthol Yellow S CI 10316

(16): Y9



Cosmetics Yellow NO.9 Oil Yellow AB CI 11380

Figure 1. Structures of coal tar dyes prohibited in mucous cosmetics.

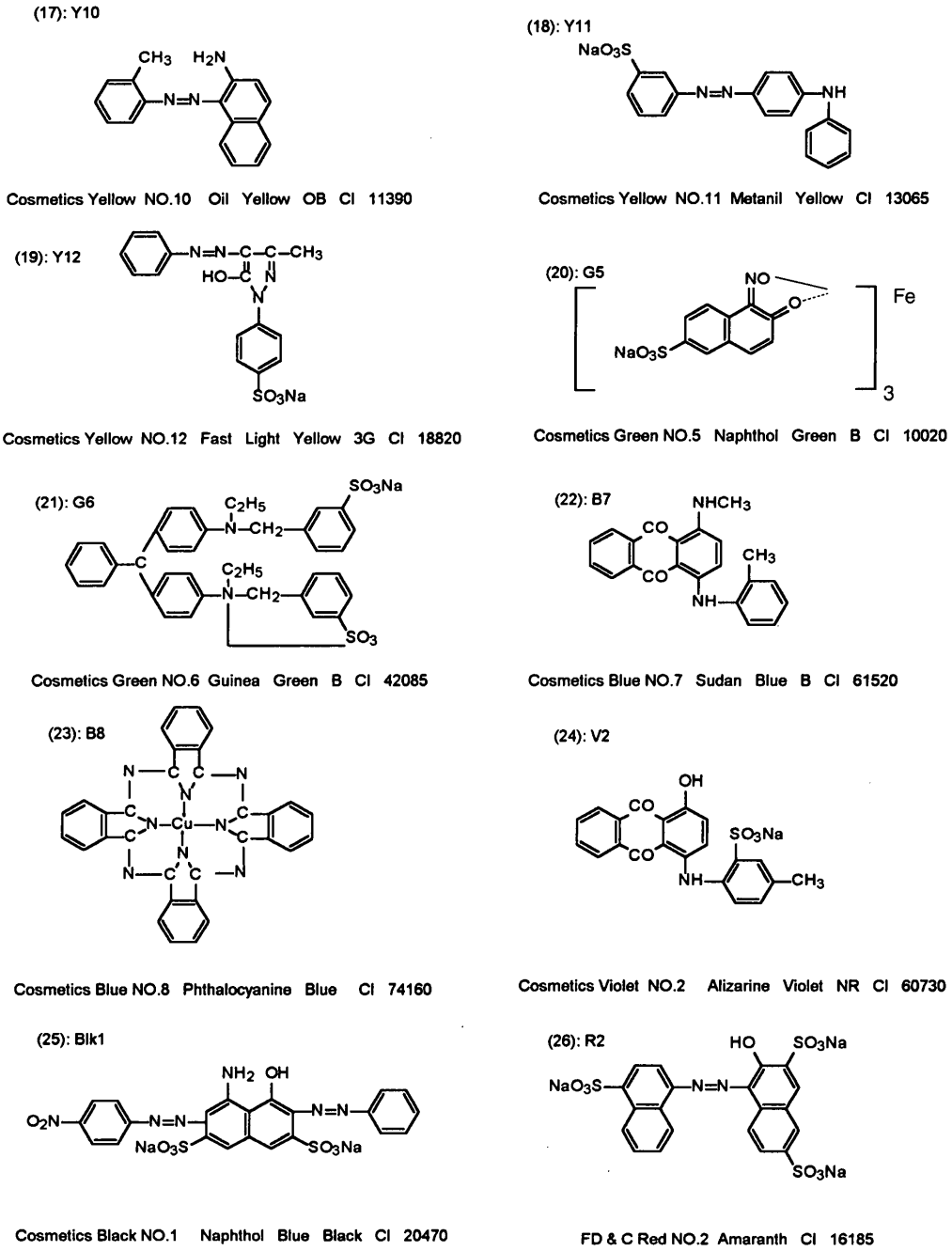


Figure 1. (continued)

17. Naphthol Yellow S, CI 10316
18. Oil Yellow AB, CI 11380
19. Metanil Yellow, CI 13065
20. Guinea Green B, CI 42085
21. Naphthol Blue Black, CI 20470
22. Amaranth, CI 16185

Standards #1 and #2 were purchased from Sigma Co. (USA). Standards #3~7 were purchased from Kuei Ssu Chemical Co., Ltd (Japan). Standard #8 was obtained from Wako Co: (Japan). Standards #9~22 were purchased from Tokyo Kasei Kogyo Co., Ltd (Japan).

II. *Method*

(I) *Instruments*

1. Waters HPLC system (USA) including

(1) Model 510 HPLC solvent delivery pump.
(2) Model 990 photodiode array UV-VIS detector.
(3) Model 5200 integrator.

2. Milli-Q water purification system (USA).
3. Singen/Htw D-7700 sonicator (Germany).

(II) *Preparation of Standard Solution*

1. Preparation of 16 water soluble standard solutions

Sixteen water soluble coloring dyes including Standards #1, 4, 6,7, 8, 9, 10, 11, 13, 14, 16, 17, 19, 20, 21, and 22 were individually dissolved in distilled water to make 100 ppm and 1 ppm standard solutions.

2. Preparation of 6 lipid soluble standard solutions

Six lipid soluble coloring dyes including Standards #2, 3, 5, 12, 15, and 18 were separately dissolved in acetonitrile: chloroform (4/1) solution, which was then sonicated to make 100 ppm and 1 ppm standard solutions.

(III) *Preparation of Sample Solutions*

1. Preparation of water soluble sample solutions (solution A)

The lipstick sample (100~200 mg) was accurately weighed out and dissolved in 5~10 mL of alcohol, which was then water-bathed at 60~70°C with stirring. Acetic acid solution accompanied with a fat-free cotton thread was added into the above solution, which was kept at the same temperature until the dyeing process was finished. The cotton thread was washed with water and then immersed in 10% ammonium water upon heating in order to dissolve out the coloring dyes from cotton thread. The finial solution was concentrated and filtered prior to HPLC analysis.

2. Preparation of lipid soluble sample solutions (solution B)

The lipstick sample (100~200 mg) was weighed into a beaker and 5 mL of acetonitrile: chloroform (4/1) solution was added. The sample was crashed with a glass bar, strongly stirred, and sonicated in order to fully extract the coloring dyes from sample. After centrifugation, the supernatant was collected. The residue was re-extracted with 5 mL of above extraction solvent, which was then centrifuged to obtain the supernatant. The above extraction procedure was repeated until no more color appeared on extraction solvent. The combined supernatants were then concentrated and filtered prior to HPLC analysis.

(IV) *HPLC Conditions*

1. Column: Cosmosil 5C18-AR-II (4.6 mm × 250 mm).
2. Mobile phase: a mobile phase of 0.07 M ammonium acetate solution with 0.01 M tetrabutylammonium bromide: acetonitrile: methanol (55/35/10) and another mobile phase of methanol: water (95/5) were performed for HPLC analysis of sample solutions A and B, respectively. A photodiode array detector with a scan range of 220~650 nm was used. The VIS at 520 nm and UV at 254 nm were used to detect the compounds of interest in sample solution A, while VIS at 470 nm was used to monitor the coloring dyes in sample solution B.
3. Flow rate: 1.2 mL/min.
4. Injection volume: 20 μL.

RESULTS AND DISCUSSION

The Law for the Sanitary Control of Cosmetics and Related Regulations ban 25 organic coloring dyes and Amaranth from being used in mucous cosmetics due to their potential mutagenecity and carcinogenecity[4-6]. Our goal is to develop an HPLC method to separate and identify 22 unauthorized coal tar dyes, which are commercially available. These 22 dyes can be divided into water and lipid soluble according to their solubility characteristics. Water soluble dyes possess excellent water solubility because their chemical structures contain SO_3Na, which shows an acidic

characteristic and readily dissociates in water. Lipid soluble coloring dyes are readily soluble in benzene, chloroform, or toluene. However, These non-polar solvents could generate a huge solvent peak so as to interfere with the analysis of compounds of interest when they are introduced to HPLC. Non-polar solvents could also damage the packing materials of C_{18} column. To eliminate this unfavorable effect resulting from the use of non-polar solvents, a solvent mixture of acetonitrile: chloroform (4/1) accompanied with a sonication process for improving solubility was performed in this study. An optimum ratio of acetonitrile: chloroform (4/1) was thus selected because the solubility of dyes decreases with increasing ratio, while solvent peak becomes larger when the ratio of which was less than 4.

I. *Pretreatment of Test Samples*

Water-soluble coloring dyes in lipstick are less extractable because they are easily trapped by waxes including bee wax, Carnauba wax, Candelilla wax, and paraffin, which generally exist in lipstick samples. This difficulty was overcome using ethanol as a solvent accompanied with gentle heating to dissolve the waxes. The acidic water-soluble dyes were freely released from lipstick samples and then re-extracted using a cotton-dyeing process[7]. Lipid soluble coloring dyes, however, was partitioned into a solvent system containing acetonitrile: chloroform (4/1).

II. *Analytical Conditions for HPLC*

An HPLC with gradient elution was routinely used to analyze coal tar dyes[8-13]. With a gradient elution, one can achieve a better separation on HPLC chromatogram when multiple components are monitored at one injection. This method, however, was inconvenient to operate. Baseline shifting and poor reproducibility were also observed while using gradient elution. Therefore, an isocratic elution was used instead in this study.

(I) *Analysis of Water Soluble Dyes*

1. Column selection

A non-polar C_{18} column was selected for the analysis of water soluble dyes in cosmetics. This column was selected because it was capable of retaining the ion-pair complex, which was formed via a pair ion binding reaction between dyes and ion-pair reagent added in mobile phase. Two C_{18} columns, Inertsil ODS-2 and Cosmosil AR-II columns, were tested. The first column resulted in broader peaks while the second column yielded sufficient resolution to discern peak shapes.

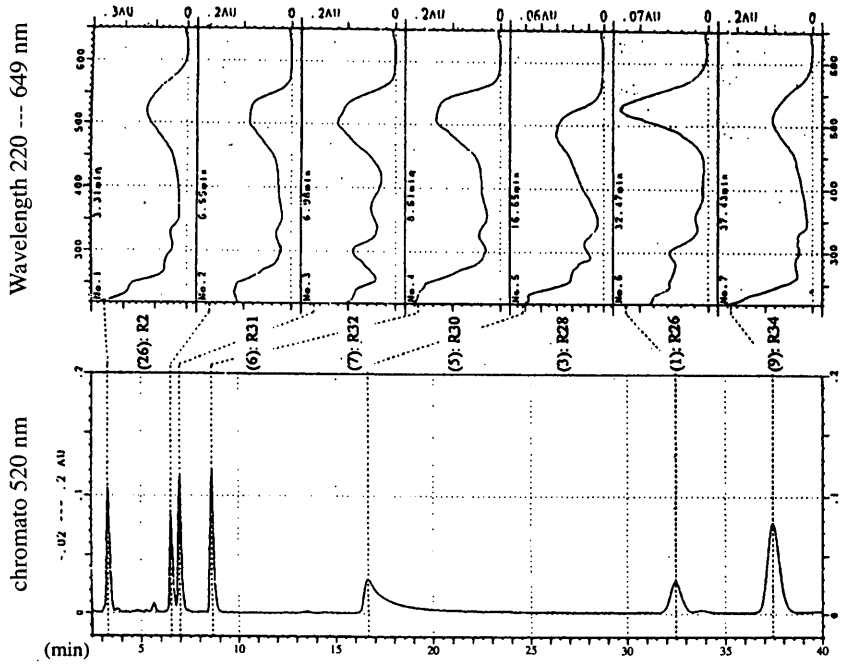
2. Effect of ion-pair reagent

Ion-pair reagents have been reported being used to analyze coal tar dyes[14-17]. Water soluble dyes can react with ion-pair reagents in mobile phase such as water/methanol system to yield a pair ion complex, which is less polar than water soluble dyes so as to be more retained by non-polar column. Several chemicals are routinely used as ion-pair reagents such as tetrabutylammonium hydroxide (TBAH), tetrabutylammonium bromide (TBA-Br), tetrahexylammonium bromide (THA-Br), cetyltrimethylammonium bromide (cetrimide), and tetrabutylammonium phosphate (TBAP). Cetrimide was excluded in this study because it was difficult to elute out of column so as to reduce the lifetime of column. It was also unfavorable to use THAB because it was required to adjust pH values.

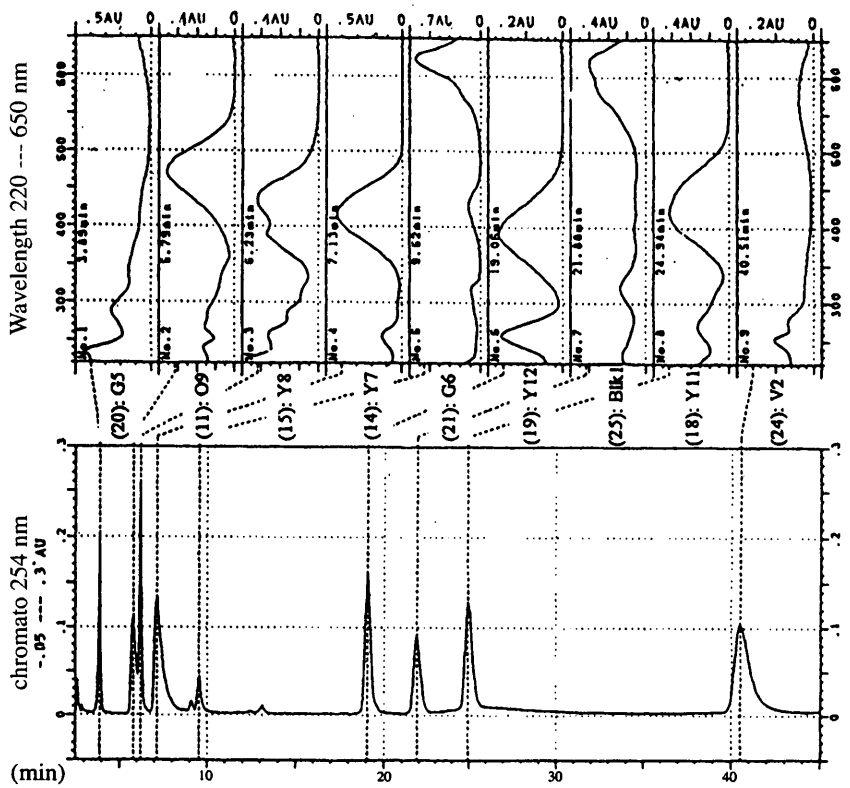
TBA-Br was selected as ion-pair reagent in this study. Results showed that capacity factors of dyes rose with increasing TBA-Br concentration. The optimum concentration of TBA-Br was found to be 0.01 M. When the concentration of TBA-Br was higher than 0.015 M, Peaks #16 and 17, as well as peaks #19 and 21 began to overlap. In addition, it took up to 60 min to finish the analysis when a concentration of 0.02 M TBA-Br was used.

3. Effect of organic phase in mobile phase

A satisfactory resolution was not achieved with the mobile phase merely consisting of water/methanol or water/acetonitrile. A tri-phase



(a) red



(b) non-red

Figure 2. Chromatogram of sixteen hydrophilic coal tar dyes prohibited in mucous cosmetics. Operating conditions, mobile phase: 0.07M ammonium acetate (containing 0.01M TBA-Br)-ACN-MeOH=55: 35: 10; detection wavelength: (a) red:520 nm; (b) non-red: 254 nm.

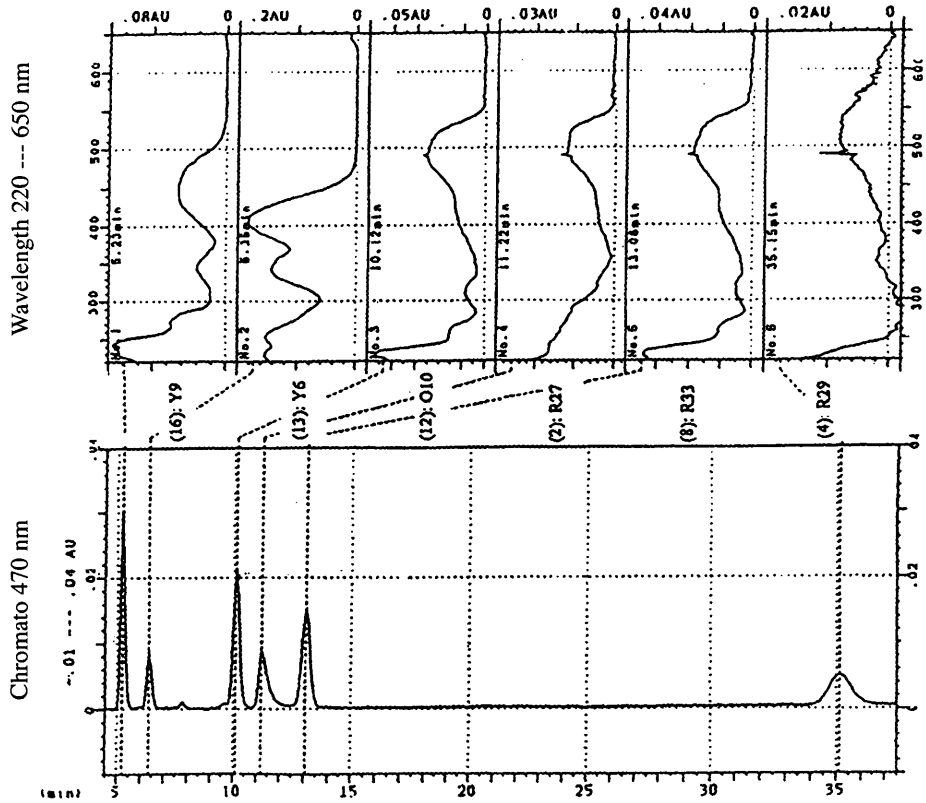


Figure 3. Chromatogram of six hydrophobic coal tar dyes prohibited in mucous cosmetics. Operating conditions, mobile phase: Methanol: H_2O=95 : 5, detection wavelength: 470nm.

Table 1. The limits of detection in hydrophilic coal tar dyes

Color series	Wavelength (nm)	No	Coal tar dyes	S/N ≧ 5 (μg/mL)
red series	520	26	R2	0.1
		6	R31	0.1
		7	R32	0.1
		5	R30	0.075
		3	R28	1
		1	R26	0.2
		9	R34	0.2
non-red series	254	20	G5	0.075
		11	O9	0.25
		15	Y8	0.0625
		14	Y7	1
		21	G6	1
		19	Y12	0.1
		25	Blk1	0.5
		18	Y11	0.25
		24	V2	0.25

Table 2. The limits of detection in hydrophobic coal tar dyes

Color series	Wavelength (nm)	No	Coal tar dyes	S/N ≧ 5 (μg/mL)
Yellow series	470	16	Y9	0.05
		13	Y6	0.05
Orange series	470	12	O10	0.085
Red series	470	2	R27	0.1
		8	R33	0.075
		4	R29	0.15

system (water/methanol/acetonitrile) was therefore used in this study. The resolution among acid coloring dyes with less capacity factor was reduced with increasing methanol ratio. A 10% methanol was thus fixed while modifying the ratio of acetonitrile and water. According to our study, a mobile phase containing 35% acetonitrile was capable of yielding a sufficient resolution that was on the basis of a capacity factor less than 17 (retention time < 40 min) as shown in Figure 2.

4. Effect of ammonium acetate

The capacity factors of Standards #7 and 13 were significantly increased by adding TBA-Br into mobile phase, the retention times of which were about 70 and 80 min, respectively. The capacity factor ratio of above standards was decreased when ammonium acetate was introduced into mobile phase. Ammonium acetate also improved the peak shape of these two compounds. The capacity factors of all analytes were decreased with increasing ammonium acetate concentration. A concentration of 0.07 M ammonium acetate was selected in this study because a satisfactory separation of water soluble coloring dyes as well as an analytical time of less than 40 min was capable of being accomplished.

(II) *Analysis of Lipid Soluble Dyes*

Six sharp peaks of lipid soluble coloring dyes were observed when they were individually analyzed by HPLC with a mobile phase of 100% methanol. Standard #2 was retained the most in column. Its retention time was less than 15 min. However, when a mixture of 6 standards were injected, a co-eluted peak containing Standards #3 and 5 appeared. A mobile phase containing 5% water in methanol was capable of totally separating 6 lipid soluble dyes as shown in Figure 3.

(III) *Wavelength Selection*

A wavelength at 520 nm was found to be most favorable for the detection of red color, a major color widely applied on lipsticks, because the wavelength range located at visible wavelength is less interfered by the other non-color components in lipsticks. A wavelength at 254 nm, which belonged to UV absorbance wavelength normally absorbed by coal tar dyes, was used to detect the color other than red such as green, orange, yellow, black, and purple in color. A wavelength at 470 nm, which usually performed to detect yellow color, was the optimum wavelength to monitor 6 lipid soluble dyes according to our study.

III. *Analytical Results*

This HPLC method was also used to analyze 20 commercial lipstick samples. No illegal coal tar dyes constituents were found in 20 test samples indicating the Law for the Sanitary Control of Cosmetics and Related Regulations was obeyed by the manufacturers.

In terms of limit of detection (LOD), Standards #15 and 18 exhibited excellent sensitivity, the LOD of which was down to 0.05 μg/mL (s/n ≥5). The least sensitive dyes were Standards #16 and 20. The results of this investigation are presented in Tables 1 and 2. The method developed in this study was more sensitive than TLC, which was routinely used to analyze the coal tar

dyes constituents in lipsticks. A ng detection level was achieved using this method.

ACKNKOWLEDGMENT

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以高效液相層析法鑑定黏膜用化妝品中之非法定煤焦色素

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摘 要

非法添加於黏膜化妝品的煤焦色素具有強毒性或致癌性，依據我國國家標準(CNS)，化妝品中有機性著色劑之檢驗方法係採用濾紙層析法與薄層層析法。本研究係採用高效液相層析法，尋找最佳條件以分離並鑑別摻加於黏膜化妝品中非法定煤焦色素，以提高檢驗效率及精確度。高效液相層析法條件：層析管爲Cosmosil 5C18-AR-II。移動相：(a)分析16種水溶性煤焦色素使用0.07 M醋酸銨內含0.01 M溴化四丁基銨：乙腈：甲醇 = 55：35：10。(b)分析6種脂溶性煤焦色素使用甲醇：水 = 95：5。偵測器爲具有可見光波長之光二極體陣列檢測器(photodiode array detector)。以本法分析22種非法定之煤焦色素均可得到良好之分離效果，最低偵測濃度0.05 μg/mL。

關鍵詞：高效液相層析法，非法定之煤焦色素，黏膜化妝品。