Identification of Oil-Soluble Coal Tar Dyes in Cosmetics Using Reversed-Phase TLC/ Scanning Densitometry

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A simple and rapid analytical method of oil-soluble coal tar dyes in cosmetics was established using reversed-phase TLC/scanning densitometry. Eleven kinds of oil-soluble coal tar dyes were able to be separated completely on reversed-phase TLC plates by the complementary use of 2 solvent systems. The solvent systems were A; *n*-hexane/2-butanone solution (5 : 1, v/v), solvent system B; acetonitrile/methanol solution (5 : 1, v/v). Then we measured visible absorption spectra of spots developed on the reversed-phase TLC plates by scanning densitometer to identify these coal tar dyes. The proposed method was successfully applied to the identification of oil-soluble coal tar dyes in commercial cosmetics.

Key words —— oil-soluble coal tar dye, reversed-phase TLC, scanning densitometry, cosmetic

INTRODUCTION

Coal tar dyes are added to many cosmetics and these cosmetics are used daily by many people. Therefore, coal tar dyes must not only provide a pleasing aesthetic effect, but also they are safe for the human body.

Regarding the use of coal tar dyes in cosmetics, there are 83 kinds of coal tar dyes available for use in drugs and other products under the standards and testing methods approved by the Ministry of Health and Welfare ordinance. Eleven kinds of oil-soluble coal tar dyes are permitted.[1,2]

Currently, coal tar dyes in cosmetics are generally analyzed using TLC and HPLC.[3–7] Moreover, the method using TLC is widely applied because is simple and can separate the components comparatively well after developing for short time. In particular, reversed-phase TLC, which shows good reproducibility for Rf value, is utilized for routine test of coal tar dyes in foods.[8–12] However, the information obtained from TLC only demonstrates color tone and Rf value. It is very dangerous to identify the coal tar dyes by these factors. Therefore, in the past, spots of on the TLC plate are scraped off, re-extracted, then identified by measuring visible absorption spectrum, but these operations are complicated and time-consuming. Moreover, it is not possible to disregard damage to the sample.

The authors previously reported an analytical method for water-soluble coal tar dyes in cosmetics using reversed-phase TLC/scanning densitometry.[13]

In this study, using a simple and rapid analysis method, we analyzed 11 kinds of oil-soluble coal tar dyes permitted in cosmetics using reversed-phase TLC, then tried to identify these coal tar dyes by directly measuring the visible absorption spectra of spots of coal tar dyes developed on the TLC plate using scanning densitometer. We report our results in this paper.

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MATERIALS AND METHODS

Reagents and Reference Standards ——

Reference Standards for Oil-Soluble Coal Tar Dyes: Yellow No. 404 (Y404) and Yellow No. 405 (Y405) were obtained from the National Institute of Health Sciences (Tokyo, Japan), Yellow No. 204 (Y204), Violet No. 201 (V201) from Sigma (Missouri, U.S.A.), Orange No. 403 (O403), Red No. 215 (R215), Red No. 225 (R225), Blue No. 403 (B403), and Green No. 202 (G202) from Tatumikasei Corporation (Yokohama, Japan), Red No. 501 (R501) and Red No. 505 (R505) from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). All the other reagents were of analytical grade from Wako Pure Chemical Industries Ltd. (Osaka, Japan).

Preparation of Standard Solutions: Standard solutions for coal tar dyes were prepared in chloroform to give concentrations of 10 μg/ml, respectively.

Sample Preparation ——

Commercial Products for Samples: Commercial products for samples were two kinds of nail enamels, a hair stick, and a pomade.

Preparations of Sample Solutions: To about 0.2 g of nail enamel add 2 ml of *n*-hexane, shake for 5 min, let stand, and obtain fractionated *n*-hexane of the upper layer as the sample solution.

To about 1 g of hair stick add 10 ml each of *n*-hexane and acetonitrile, shake for 15 min, let stand, and obtain fractionated *n*-hexane of the upper layer as the sample solution.

Pomade was prepared by the third method of preparation of test solution described in Methods of Analysis in Health Science, (Kanehara, Japan, 2000, pp. 665–670).[14)]

Operating Conditions ——

TLC Conditions: The TLC plate was an RP-18F_{254}S (Art. 15389, Lot No. 45398571, E. Merck, Darmstadt, Germany), and the solvent systems were A; *n*-hexane/2-butanone solution (5 : 1, v/v) and B; acetonitrile/methanol solution (5 : 1, v/v).

Scanning Densitometric Conditions: The dual wavelength flying spot scanning densitometer used in the study was a CS-9000 from Shimadzu (Kyoto, Japan). The wavelength scanning range was from 370 to 700 nm, slit size was 0.4 × 0.4 mm and the visible absorption spectrum was measured by reflectance spectrophotometry.

RESULTS AND DISCUSSION

Examination of Sample Preparation

Since the dye component of nail enamel was extracted by hexane as mentioned in the Materials and Methods Section, the extract was used as the sample solution. Since the dye component was extracted in the upper hexane layer when hair stick was treated with a mixture of hexane and acetonitrile (1 : 1), the extract was used as the sample solution. Since extraction from pomade was impossible by these methods, it was prepared by the third method of preparation of test solution described in Methods of Analysis in Health Science, (Kanehara, Japan, 2000, pp. 665–670).

Examination of Developing Solvents

We examined developing solvents for separating 11 kinds of standard coal tar dyes using reversed-phase TLC plates. As the results of various examinations, the standard solution was separated O403, R501, R505, Y204, V201, and G202 using a solvent system A; *n*-hexane/2-butanone solution (5 : 1). Although R225 and B403 showed the same Rf value, they could be distinguished because they differed in color. It was not separated into Y404 and Y405, showing the same Rf values. R215 remained at the starting point, so it was not separated. Therefore, we examined the solvent system that was able to separate these three coal tar dyes clearly. As a result, these coal tar dyes were separated clearly using the solvent system B; acetonitrile/methanol solution (5 : 1). So, it was separated that Y404 showed an Rf value of 0.64 and Y405 showed an Rf value of 0.58. R215 showed an Rf value of 0.47 and was separated from the starting point. The other coal tar dyes were also separated and showed Rf values differing from the solvent system A.

Therefore, it became possible to identify 11 standard dyes on the basis Of differences in color and Rf value by using the two solvent systems Of developing solvent A and B (Table 1).

Table 1. Rf Values of Standard Coal Tar Dyes on Reversed Phase TLC Plates

Name of dye	C.I. No.[a)]	Rf value A[b)]	Rf value B[b)]
Y404	11380	0.55	0.64
Y405	11390	0.57	0.58
Y204	47000	0.23	0.50
O403	12100	0.63	0.43
R505	12140	0.66	0.37
R225	26100	0.55	0.29
R501	26105	0.59	0.21
R215	45170	0.00	0.47
V201	60725	0.70	0.41
B403	61520	0.54	0.40
G202	61565	0.74	0.27

a) Color Index. *b*) Solvent systems: A, *n*-hexane/2-butanone solution (5 : 1); B, acetonitrile/methanol solution (5 : 1).

Measurement of Visible Absorption Spectra

Using two kinds of developing solvents, spots of 11 kinds of standard coal tar dyes were separated on the TLC plate. The visible absorption spectra of these isolated spots were measured by scanning densitometer. The visible absorption spectra and their maximal wavelengths of 11 kinds of standard coal tar dyes are shown in Fig. 1. From the results described above, we actually tried to identify these coal tar dyes in commercial cosmetics using reversed-phase TLC and scanning densitometer.

Identification of Oil-Soluble Coal Tar Dyes in Commercial Cosmetics

After the coal tar dyes were extracted from two kinds of nail enamels, a hair stick, and a po-

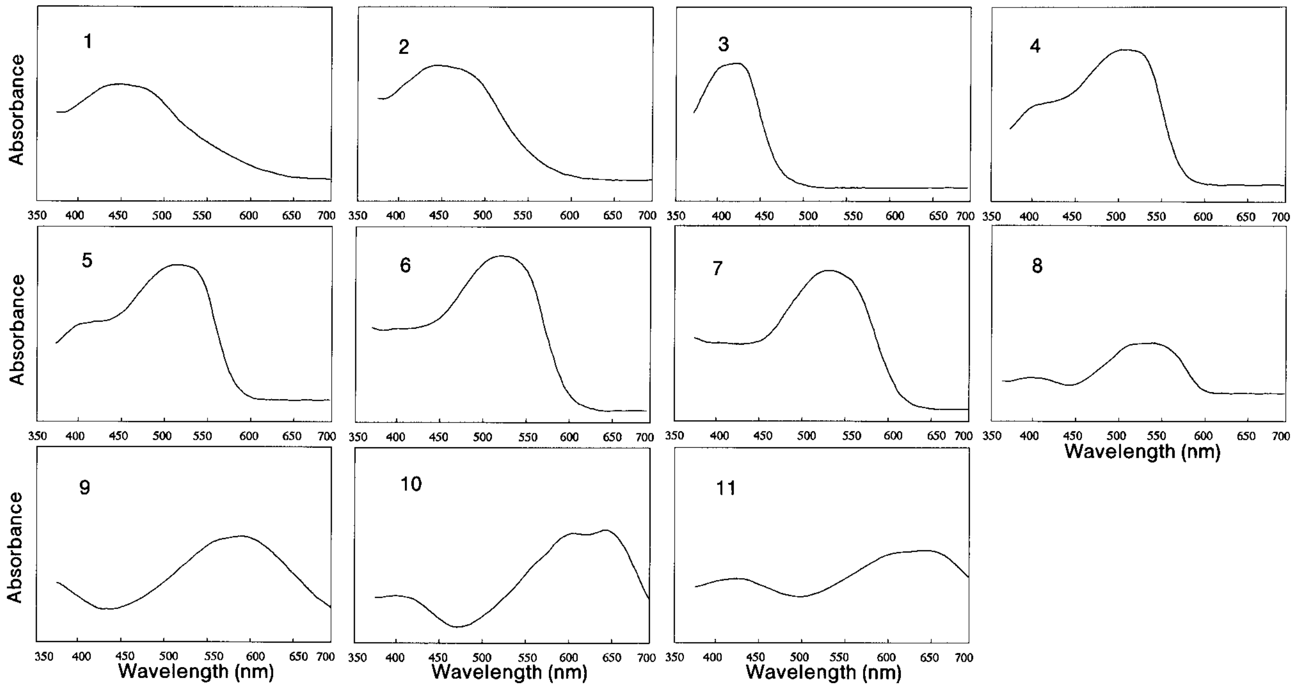

Fig. 1. Visible Absorption Spectra of Standard Coal Tar Dyes Measured by Scanning Densitometry on the Developed Reversed-Phase TLC Plates

1: Y404, 2: Y405, 3: Y204, 4: O403, 5: R505, 6: R225, 7: R501, 8: R215, 9: V201, 10: B403, 11: G202. Solvent systems: A, *n*-hexane/2-butanone solution (5 : 1); B, acetonitrile/methanol solution (5 : 1). 1. A, 2. A, 3. B, 4. B, 5. B, 6. A, 7. A, 8. B, 9. B, 10. A, 11. B. Maximum wavelengths: 1.438 nm, 2.442 nm, 3.422 nm, 4.507 nm, 5.513 nm, 6.523 nm, 7.530 nm, 8.542 nm, 9.591 nm, 10.608, 647 nm, 11.422, 650 nm.

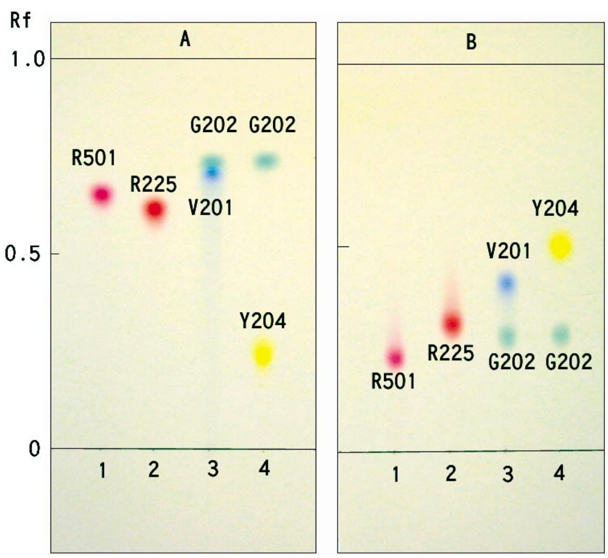

Fig. 2. Thin Layer Chromatograms of Coal Tar Dyes in Commercial Products

Commercial products: 1, nail enamel a; 2, nail enamel b; 3, hair stick; 4, pomade. Solvent systems: A, *n*-hexane/2-butanone solution (5 : 1); B, acetonitrile/methanol solution (5 : 1).

made by the method described above, the coal tar dyes were developed on the reversed-phase TLC plate using solvent systems A and B. These chromatograms are shown in Fig. 2. It was estimated that R501 was contained in the nail enamel a, R225 in the nail enamel b, V201 and G202 in the hair stick, and Y204 and G202 in the pomade by comparing Rf values of the coal tar dyes in these cosmetics with Rf values of the 11 kinds of standard coal tar dyes shown in Table 1. Furthermore, to confirm the identification, we measured visible absorption spectra of the spots of coal tar dyes developed on reversed-phase TLC plate using scanning densitometer. Then, we compared the spectra and visible absorption maximal wavelengths with the results of standard coal tar dyes. The spectra and visible absorption maximal wavelengths agreed with those of the coal tar dyes estimated by Rf values, and the coal tar dyes in the commercial cosmetics were identified.

As a result, R501 was contained in nail enamel a, R225 in nail enamel b, V201 and G202 in the hair stick, and Y204 and G202 in the pomade. By these result, the proposed method was proved to be useful and applicable to the identification of oil-soluble coal tar dyes in commercial cosmetics.

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