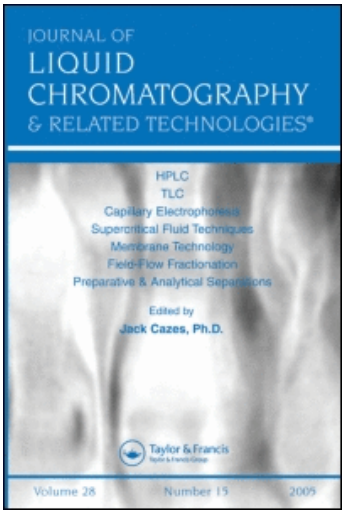

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A COMBINED HPLC-VIS SPECTROPHOTOMETRIC METHOD FOR THE IDENTIFICATION OF COSMETIC DYES

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ABSTRACT

Ion-pair reversed phase HPLC was observed to give very good separations of 20 representative cosmetic dyes whilst numerical analysis of VIS spectra provided an efficient additional means of identification when similar retention times for different dyes were encountered. The results strongly suggest that a combination of HPLC and rapid scanning VIS spectrophotometry should be very promising, especially when on-line computing facilities are available.

INTRODUCTION

As part of its programme to harmonise the European Community's legislation on cosmetics the Council of the EC has approved a list of organic dyes for use in cosmetic products (1).

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This decision and the evident need to verify adherence to the Directive led to interest in a rapid and efficient HPLC method for identification purposes. Several reports have already been published in the closely related field of food-dye analysis (2-9). Although this work has been of value to our study, its results are only partially applicable to cosmetic dyes since the vast majority of permitted food dyes are hydrophylic anionic molecules. The behaviour of other types of dyes (non-ionic and basic) under similar HPLC conditions still remained to be clarified.

Market indications suggest that around 50 organic dyestuffs are in current use in cosmetics in the European Community whilst 256 organic colouring agents are permitted by the Directive. These numbers contrast sharply with the fact that at most 29 dyes have hitherto been studied simultaneously (6). Moreover, we estimate from published compilations of retention times (6) and chromatograms (2-4) that constituents in mixtures of at most 10 different colorants can be identified by chromatography alone. The use of a secondary means of identification therefore seems inevitable. We have chosen an approach in which HPLC is coupled with VIS spectrophotometry; results obtained with a representative collection of 21 cosmetic dyes are presented in this paper.

MATERIALS AND METHODS

Chromatograph

The liquid chromatograph comprised two Waters model 6000 A pumps connected to a Valco loop injection valve and a Waters model 660 Solvent Programmer.
The detector was a Perkin-Elmer LC 55 S Spectrophotometer with a 10 mm optical pathlength flow cell.

Column

CP SpherC18 (Chrompack); 25 cm x 4.6 mm i.d.

Pairing Ion

Waters PIC^{TM} Reagent A containing $(Bu_4N)_3PO_4$ diluted with methanol according to the manufacturers specification.

Elution

45 minutes linear gradient from 50 to 100% methanol (containing PIC^{TM} Reagent A) in water at a flow rate of 1 ml/min.

Colorants

Throughout this paper, dyes are referred to by their CI reference numbers taken from the Colour Index (10).

sulfonic acids :	13065, 14700, 15510, 15585, 15630, 15850, 15985, 16035, 19140, 42051, 42090.
carboxylic acids :	45350, 45370, 45380, 45396, 45410.
basic :	45170.
non-ionic :	12075, 12085, 26100, 61565.

Sample preparation

50-100 mg of a cosmetic sample was dissolved in 2 ml of a solution of H_3PO_4 (5% v/v) in dimethylformamide (DMF). A few ml hexane was added to extract any fatty material. This extraction procedure was repeated five times. The extraction step was ommitted for non-fatty samples, e.g. powders. If the combined hexane fractions were coloured, two back-extractions with 2 ml DMF-H_3PO_4 were carried out and the DMF layers added to the DMF extract. The DMF - H_3PO_4 solution was diluted with a methanolic PIC^{TM} Reagent A solution (until a suitable extinction was reached) and was subsequently chromatographed.

Spectrophotometer

All visible spectra were recorded using an Aminco DW2a spectrophotometer equipped with a semi-automatic device for recording absorption readings at 5 nm intervals between 350 and 750 nm.

Spectrophotometric identification

In order to distinguish between colorants, with similar or identical retention times (e.g. CI 14700 and CI 15850, see Fig. 1), a generally applicable regression method has been employed. This method is based on the Lambert-Beer law :

$$\frac{A_i}{A_i^o} = \frac{c_i}{c_i^o} \qquad \ldots \quad (1a)$$

or

$$A_i = \frac{c_i}{c_i^o} \cdot A_i^o \qquad \ldots \quad (1b)$$

where the absorbance at wavelength λ_i of an "unknown" colorant solution and its reference sample are represented by A_i and A_i^o, respectively. The corresponding concentrations are denoted by c_i and c_i^o.

In the example cited, an unresolved HPLC fraction containing either CI 14700 or CI 15850 was collected and its VIS spectrum between 350 and 750 nm was recorded digitally, as described above. The validity of the Lambert-Beer law was verified at all wavelengths λ_i by computing a linear regression equation of the form:

$$y = ax + b \qquad \ldots \quad (2)$$

where the spectrum of the fraction is denoted by y and that of the reference spectrum of either CI 14700 or CI 15850 by x (11). It

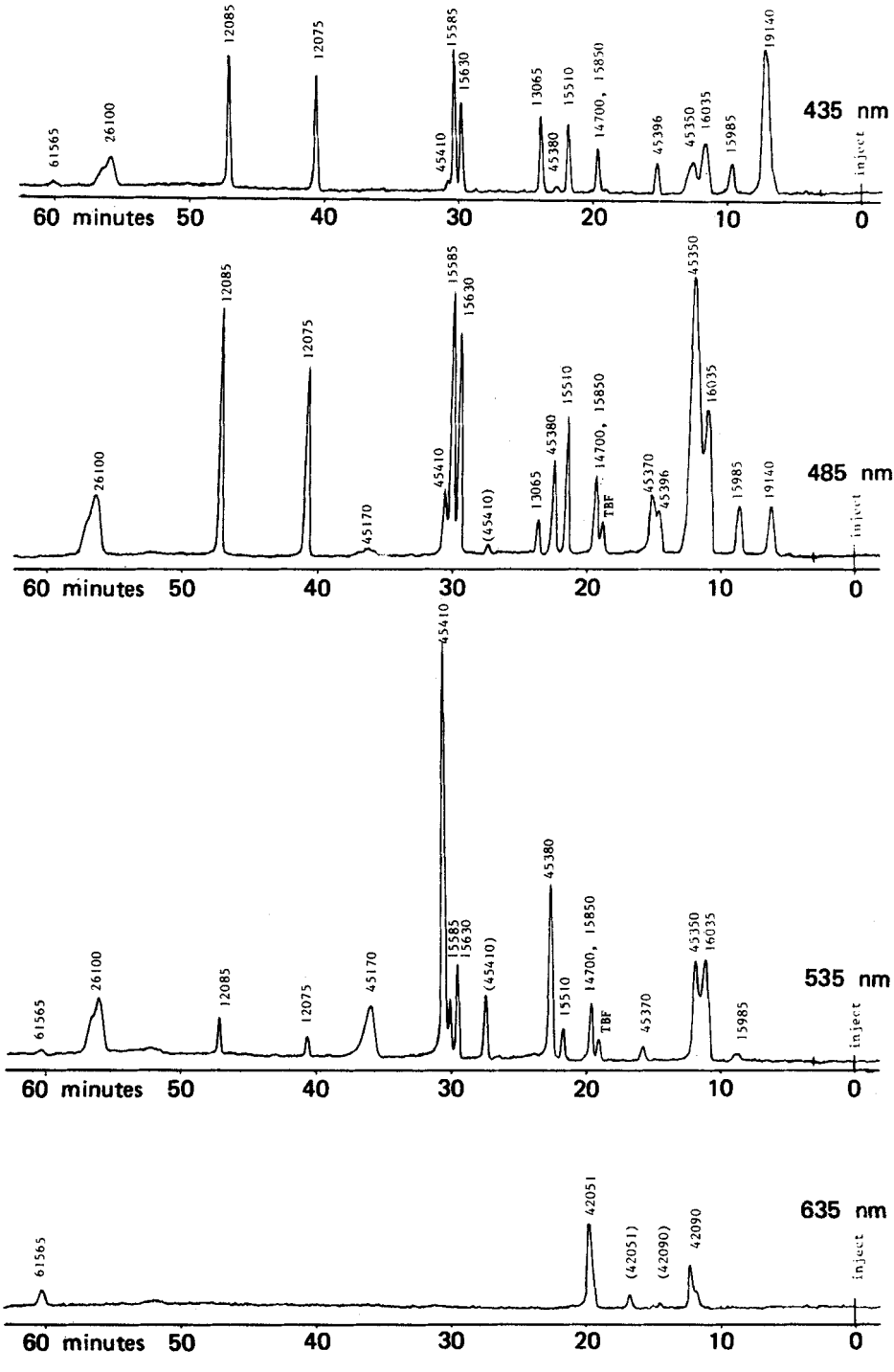
follows from equation 1b that, if the Lambert-Beer law holds, the slope a must be equal to the ratio of concentrations and the intercept b must be zero (11). The identification of the best fitting dye was based in part on the idea that calculated intercepts should be smaller than experimental error and also, in part, on the closeness of fit of the regression, as judged by several statistical test parameters (12). The parameters chosen were : Fisher's variance ratio and Student's t-test, both of which should be as high as possible; the correlation coefficient, which should be close to 1; and the standard error of estimate, which should be as small as possible.

RESULTS AND DISCUSSION

Resolution of a Mixture of 21 Colorants

Figure 1 shows chromatograms of a mixture containing all 21 colorants recorded at the following wavelengths : 435, 485, 535 and 635 nm. The general order of elution by chemical classes is : acid colorants, basic colorant(s) and finally, non-ionic colorants. This sequence is somewhat surprising as far as the behaviour of the basic colorant, CI 45170, is concerned. It was apparent in preliminary studies that this colorant was not retarded at all when unbuffered cetrimide was used as the pairing ion. In the present study, the behaviour of CI 45170 can perhaps be explained by postulating the formation of ion pairs with the phosphate buffer ions.

Two colorants were found to have identical retention times. These colorants, CI 14700 and CI 15850, also exhibit very similar spectra (see Fig. 3) : a comparison using correlation analysis (12) resulted in a correlation coefficient of 0.936 between the spectra of both colorants dissolved in methanolic PIC^{TM} Reagent A solution. This value is rather close to the limit of 1, at which



the spectra would have been identical. The problem of distinguishing between CI 14700 and CI 15850 therefore represents a sharp test of the power of the spectrophotometric method.

Chromatograms of individual colorant samples (not shown here) clearly demonstrated the presence of coloured impurities in samples of the following colorants : CI 42051, CI 42090, CI 45370, CI 45380 and CI 45410. The nature of these impurities is unknown except in CI 45370 (dibromofluorescein) and CI 45380 (tetrabromofluorescein or eosine) where tribromofluorescein (TBF) has been positively identified (7). In Figures 1 and 2, secondary peaks associated with a particular colorant are denoted by means of the CI number in parentheses with exception of tribromofluorescein, which is marked TBF.

HPLC of extracts of cosmetics

Eight different cosmetic samples supposedly containing from 1 to 4 organic colorants have been investigated. Figure 2 gives a typical chromatogram of a lipstick extract, recorded at 430, 485, 535 and 635 nm. It is obvious that at least six dyes must be present: CI 12085, CI 15850 (confirmed by its spectrum, see below), CI 45370, CI 45380, CI 45410 and TBF. Subsequent inspection of the disclosed formulation indeed showed this interpretation to be correct although it should be noticed that, according to the manufacturer, both TBF and CI 45380 should be considered as impurities of CI 45370. The small peak at about 7 minutes was assigned to an impurity of CI 45410.

Similar successful results were obtained for extracts from samples of 4 other lipsticks, a skin lotion, an after sun cream

FIGURE 1. Chromatograms of a mixture of 21 cosmetic colorants separated by ion pair reversed phase HPLC. Conditions : aqueous methanol (containing PIC^{TM} Reagent A) 50% to 100% methanol linear gradient in 45 min. at 1 ml/min. flow rate on CP Spher C18. Detection at 435 (top), 485, 535 and 635 nm (bottom).

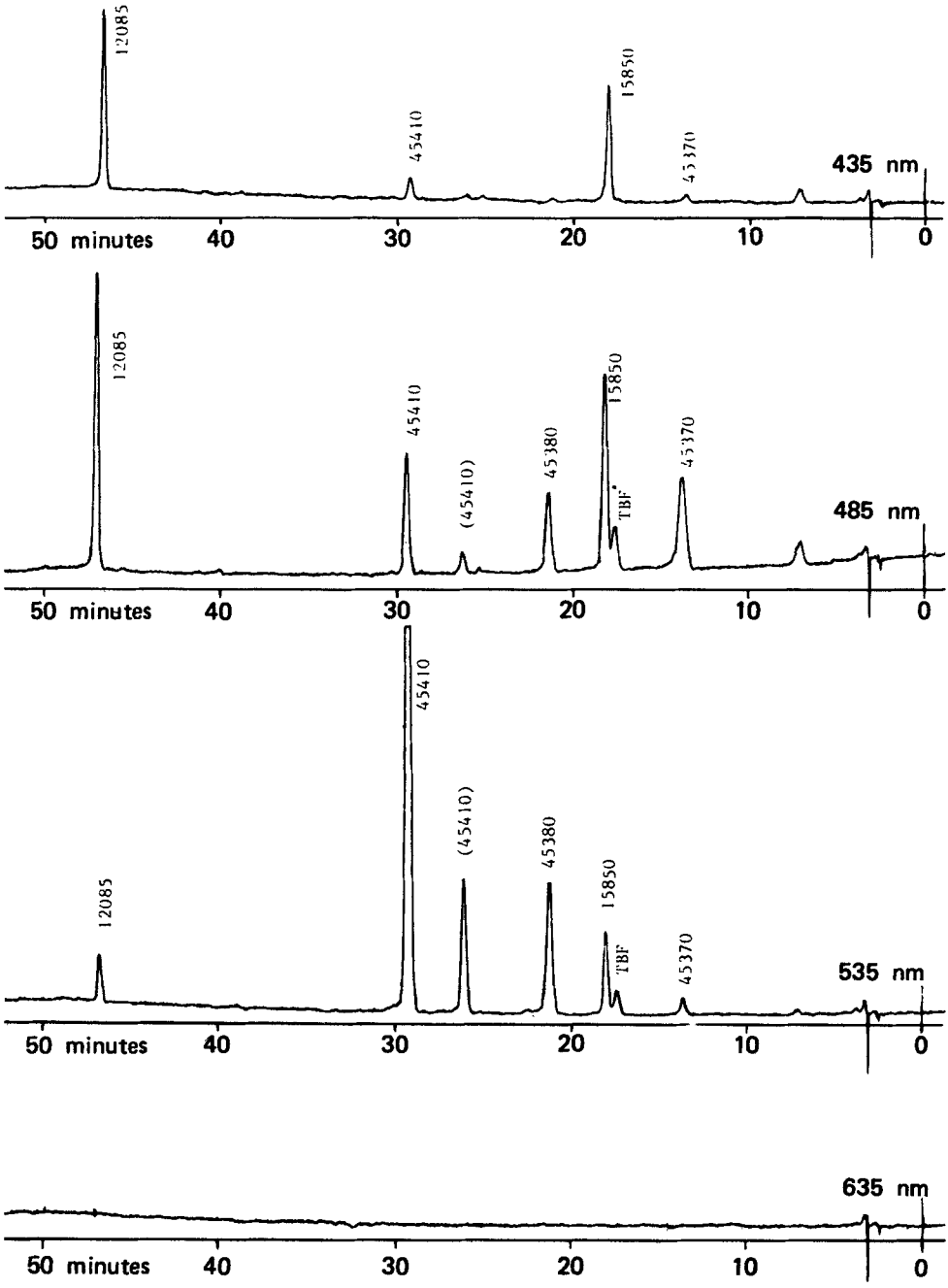


FIGURE 2. Chromatograms of an extract from a lipstick. Experimental conditions as in Fig. 1.

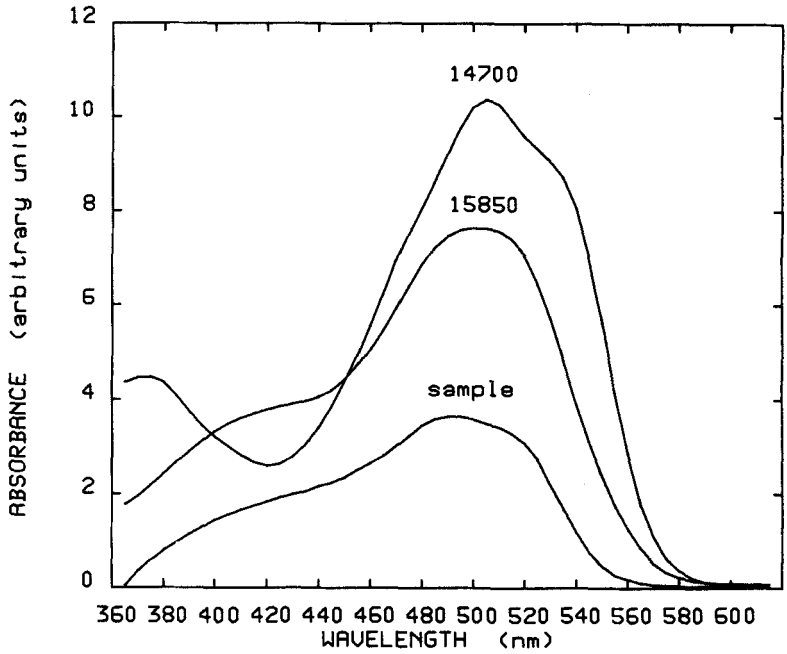


FIGURE 3: Visible absorption spectra of two colorants with identical retention characteristics in the chromatographic system used together with the spectrum of a fraction taken from the chromatograph of an unknown colorant separated from a lipstick (see text).

and a foam bath. In all cases, the right combination of colorants was correctly identified. When either CI 14700 or CI 15850 was indicated in the chromatogram, the spectrophotometric identification procedure was followed.

Spectrophotometric identification

The spectrum of the CI 14700/CI 15850 fraction of the lipstick extract of Figure 2 is shown in Figure 3, together with reference spectra of both dyes, recorded under similar conditions. Figure 3 shows clearly the similarities between the spectra which

TABLE 1

Multiple Regression Analysis of a HPLC Fraction from a Lipstick.

Regression	F	R	S	t
y versus CI 14700	174	0.845	0.007	13.2
y versus CI 15850	1280	0.974	0.003	35.8

Key: y = VIS spectrum of the HPLC fraction
F = F test for significance of fit
R = correlation coefficient
S = standard error of estimate of regression
t = Student's t-test

were confirmed by the regression analysis described earlier. Evidently, it will be very difficult to distinguish the dyes even when the full spectra are used instead of absorbance ratios at two different wavelengths.

The regression analysis of the spectra of Figure 3 is summarized in Table 1. Calculated intercepts are not shown but in both cases were smaller than the standard error of estimate. As may be seen from R in Table 1, CI 14700 can account for a fairly large part (about 70%) of the unknown spectrum, but CI 15850 leads to better results : all statistical tests are significantly better and almost 95% of the unknown spectrum is explained. This result is very satisfactory, especially when one realises that the absorbance of the unknown spectrum was very weak (a peak maximum of 0.04 absorption units). The additional 5% can be attributed to noise and CI 15850 alone is confirmed as being present in the sample.

CONCLUSIONS

1 Chromatographic conditions permitting the positive identification by a relatively simple procedure of at least 20 representative cosmetic dyes have been developed.

2 A numerical method using VIS spectra and (stepwise) multiple regression analysis is available to identify colorants with similar or identical retention time.

3 Application of both methods to representative cosmetic samples yielded satisfactory qualitative determinations of the colorant content.

As an overall conclusion, ion-pair reversed phase HPLC in combination with VIS spectrophotometric detection should be considered a very promising approach to the general problem of the identification of cosmetic dyes. In our opinion, an efficient approach to this problem could be the use of a linear photodiode array spectrophotometer coupled directly to the chromatograph. Such an arrangement, connected to an on-line computer, would permit routine identifications requiring in principle only one injection per sample in place of the four injections described here. In addition the need to collect fractions could be avoided.

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