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IDENTIFICATION OF COSMETIC DYES BY ION-PAIR REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

A method based on ion-pair reversed-phase high-performance liquid chromatography with detection at four wavelengths between 400 and 600 nm is reported for the separation and identification of the most common synthetic colour additives in cosmetic products. All the dyes generally employed in the U.S.A. and almost all those in current use in cosmetics in the European Community have been taken into account. The chromatography was performed on a C_8 bonded silica packed column, with a 60-min gradient changing from 10 to 95% acetonitrile in water containing 10^{-2} *M* sodium perchlorate (pH 3.0) as mobile phase (flow-rate 2.5 ml/min). Detection limits are in the range 20–100 ng for all dyes investigated. The method has been applied to the analysis of commercial lipsticks.

INTRODUCTION

In recent years concern has arisen over the extensive use of synthetic colour additives, especially in food and cosmetics. Toxicological investigations of cosmetic colours are undertaken in many countries, and the resulting data have led to repeated revisions of the number of permitted food or cosmetic dyes.

In view of the desire to restrict the indiscriminate usage of dyestuffs, likely to come into contact with the skin or mouth, the Council of the European Economic Community (EEC) has enacted Directive 76/768, modified by Amendment Directive 82/368, which lists 256 organic colouring agents permitted in cosmetic products but imposes maximum allowed concentrations only on a few of them. Unfortunately, different countries have different legislations and cosmetic products may thus be imported into a country which forbids the use of the colouring matter present in them.

Consequently, the control of cosmetics containing synthetic dyes requires methods for detection, identification and quantitation of the most of them.

Market indications suggest that at most 30–50 colouring agents are in current

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use in cosmetics in the European Community. Because lipsticks usually contain a more complex mixture of dyes than other cosmetics, their separation and identification have been the subject of much methodological development work, undertaken on the basis of the results already obtained in the closely related field of food-dye analysis. Previous qualitative methods for the identification of food colours were mostly based on paper[1,2] and thin-layer chromatography (TLC)[1,3–9]. Quantitative analysis has received comparatively little attention. After the initial extraction of the dyes, quantitation is carried out by spectrophotometry[10,11], titration with titanium(III) chloride solution[12,13] or electrophoresis on polyacrylamide[14].

In recent years, high-performance liquid chromatography (HPLC) has been shown to be more powerful for synthetic food-colour analysis, mostly for the detection of impurities in single dyes[15,16], but also for the separation of dye mixtures. Anion-exchange columns have been used for this purpose[17], but ion-pair reversed-phase HPLC has been found particularly convenient for the separation of a large number of food colours[18–24].

A recent paper[25] demonstrated the suitability of this technique, in combination with rapid-scanning visible-region spectrophotometry, for the separation and determination of 21 representative dyes used in lip cosmetics. However, TLC techniques are still widely employed in the analysis of cosmetic dyes because a good separation of all colours can often be obtained by direct application to the plate, *i.e.* without requiring a preliminary clean-up[4,26,27]. On the other hand, electrochemical techniques have been successfully applied to the determination of colouring agents, particularly for studying their degradation[28]. However, these electrochemical techniques are very useful when simple mixtures of dyes are to be analyzed and very low concentrations are to be evaluated.

We think that HPLC is a suitable way to screen complicated mixtures of synthetic dyes, particularly when the extraction of the colouring matter from the cosmetic product is complete. We report here the use of ion-pairing reversed-phase HPLC for the separation of 75 organic colours commonly used in the cosmetic industry, and its application to the analysis of two commercial lipsticks.

EXPERIMENTAL

Materials

All dyes in this study were commercial samples used as received. Throughout this paper they are referred to by their CI reference numbers[29]. We have investigated 87 dyes, 75 of which are listed in Table I; the remaining twelve dyes are not reported since they are insoluble in the mobile phase. (CI 12490, CI 51319, CI 69800, CI 69825, CI 71105, CI 73000, CI 73360, CI 73385, CI 74100, CI 74160, CI 74260 and CI 75810).

Standard solutions of individual dyes and of mixtures were prepared in dimethylformamide (DMF) containing 5% (v/v) orthophosphoric acid to give a concentration of 1 mg/10 ml or 0.4 mg/ml for each dye. These solutions were stored under subdued lighting.

All chemicals used were of analytical grade obtained from Farmitalia-Carlo Erba (Milan, Italy). Water was deionized and doubly distilled from glass apparatus. Acetonitrile was of HPLC grade. All solvents and solutions for HPLC analysis were

filtered through a filter (Millipore, Bedford, MA, U.S.A.), pore size 0.5 μm, and vacuum degassed by sonication before use. The samples of lipsticks were obtained from perfume shops.

Apparatus

A Model 5000 liquid chromatograph (Varian, Zug, Switzerland) equipped with a variable-wavelength UV–VIS detector (Varichrom UV 50), a Valco AH 60 injection valve and a Model 730 integrator recorder (Waters Assoc., Milford, MA, U.S.A.) were used. The stationary phase was octylsilane bonded to silica (silica gel 60 HPLC, C_8, 10 μm) obtained from Riedel De Haen (Seelze-Hannover, F.R.G.). This was packed into a 300 mm × 5 mm I.D. stainless-steel column at a pressure of $5.1 \cdot 10^7$ Pa, using glycerol–water (1:1, v/v) as a solvent.

HPLC conditions

The chromatographic conditions were as follows: mobile phase, acetonitrile–water containing 0.01 *M* sodium perchlorate (pH 3.0 adjusted with perchloric acid) with a linear gradient from 10 to 95% acetonitrile in 60 min; flow-rate 2.5 ml/min; column temperature, 25°C; injection volume, 10 μl; detection wavelengths, 400, 475, 525 and 600 nm; detector sensitivity, 0.64 or 0.16 a.u.f.s.; chart speed, 0.5 cm/min.

Assay of dyes in lip cosmetics

A 100-mg amount of the lipstick sample was accurately weighed and dissolved in 2 ml of a solution of orthophosphoric acid (5%, v/v) in DMF. In order to extract any fatty material, the mixture was treated five times with a few millilitres of hexane. If the combined hexane extracts were coloured, a back-extraction with 2 ml of DMF–orthophosphoric acid was needed and this extract was added to the DMF extract. The resulting solution was diluted in the mobile phase until a suitable extinction was reached. Aliquots (10 μl) of this solution were injected into the chromatograph.

RESULTS AND DISCUSSION

The chromatographic behaviour of the cosmetic dyes examined and their relative absorptions at the four detection wavelengths are summarized in Table I. The retention times are reproducible under the experimental conditions used. The mobile phase employed enables good column performance for long periods of time.

Chromatograms of some individual dye samples (not shown) clearly demonstrated the presence of colour impurities whose nature is unknown. In such cases we have put the symbols (I), (II), (III), etc., next to the CI number.

The separation obtained for a standard mixture of 20 dyes is illustrated in Fig. 1. The identification of two dyes with similar or identical retention times is aided by the fact that generally they have absorption maxima at different wavelengths in the visible region. Spectra of the dyes investigated were recorded by use of the spectrophotometer, but most of the spectra are available in the literature.

For example, the dyes CI 10020 and CI 19140 were found to have the same retention times but they can easily be distinguished, since the former is detectable at 525 or 600 nm where the latter does not absorb. The same holds true for another

TABLE I

RETENTION TIMES AND RELATIVE ABSORPTIONS, A_λ, OF THE DYES AT THE FOUR DETECTION WAVELENGTHS

Colour index No.	*Retention time (min)*	A_{400}	A_{475}	A_{525}	A_{600}
10020	1.07	100	35	15	45
10316	3.85	100	25	0	0
12075	31.30	50	100	25	5
12085	34.23	45	100	35	0
12150	30.87	35	70	100	0
13015	1.30	100	20	0	0
13065	16.91	100	65	5	0
14700	7.20	35	80	100	0
14720	6.77	25	60	100	5
15510	11.90	45	100	60	0
15585	19.40	45	100	55	0
15630	18.50	35	100	85	0
15850	15.74	50	100	100	0
15985(I)	1.35	50	100	55	0
15985(II)	2.95				
17200(I)	1.35	20	40	100	10
17200(II)	3.35				
18965	4.67	100	25	0	0
19140	1.07	100	60	0	0
26100	40.00	50	85	100	0
27755	1.16	30	60	65	100
42051(I)	12.38	20	0	5	100
42051(II)	15.74				
42053	9.98	15	5	5	100
42090(I)	10.45	20	0	5	100
42090(II)	21.08				
44045	38.80	15	0	15	100
45170	32.31	10	15	100	0
45350	19.40	15	100	5	0
45370(I)	24.71	5	60	100	0
45370(II)	25.67				
45380	25.67	5	15	100	0
45396	22.64	15	100	5	0
45410(I)	29.36	5	20	100	5
45410(II)	34.78				
47005	7.95	100	0	0	0
59040	1.03	100	0	0	0
60725	37.75	10	30	85	100
61565	44.06	45	35	55	100
75300	24.67	100	40	0	0
11920	23.55	100	20	5	0
14270	5.54	100	45	5	0
14815	5.59	40	100	10	5
15525	8.87	50	100	40	0
15800	17.95	20	80	100	0
15865(I)	18.88				
15865(II)	11.71	40	70	100	5
15865(III)	21.58				
15880(I)	17.8	40	50	100	10
15880(II)	19.05				
15980	3.95	60	100	30	0

TABLE I (*continued*)

Colour index No.	*Retention time (min)*	A_{400}	A_{475}	A_{525}	A_{600}
16035(I)	4.42	0	75	100	0
16035(II)	1.38				
16185	1.13	25	60	100	0
16230(I)	3.28	45	100	25	5
16230(II)	4.06				
16255(I)	1.39	25	80	100	10
16255(II)	2.23				
16290	0.96	20	65	100	40
20170(I)	11.51	100	60	10	0
20170(II)	4.38				
20470	11.71	20	20	35	100
21230	61.61	100	25	15	0
26105(I)	43.62	60	85	100	5
26105(II)	33.06				
28440(I)	1.44				
28440(II)	3.45	30	20	65	100
28440(III)	3.86				
42040(I)	38.36	20	0	10	100
42040(II)	34.71				
42045(I)	12.96	20	0	5	100
42045(II)	10.36				
42520(I)	23.26	5	40	100	5
42520(II)	22.33				
42555	33.58	0	5	55	100
42563(I)	40.70				
42563(II)	39.23	5	5	45	100
42563(III)	42.66				
42563(IV)	29.01				
42775(I)	6.79				
42775(II)	8.52	5	5	35	100
42775(III)	1.40				
42775(IV)	4.37				
44090(I)	8.15	0	0	5	100
44090(II)	6.90				
45425(I)	25.32	45	60	100	0
45425(II)	23.60				
45430	26.20	15	30	100	0
47000	22.29	100	0	0	0
52015(I)	16.63	0	5	10	100
52015(II)	14.98				
58000	17.88	100	50	5	0
60724	34.28	25	40	90	100
60730(I)	17.82	15	25	80	100
60730(II)	14.82				
61570(I)	14.17	65	10	30	100
61570(II)	12.60				
62045	18.66	10	35	50	100
62560(I)	13.31				
62560(II)	11.80	70	25	30	100
62560(III)	11.55				
73015(I)	1.43	5	10	20	100
73015(II)	2.57				
75470	4.95	20	70	100	5
Bromothymol blue	23.08	100	30	5	0
Bromocresol green	19.50	100	45	5	5
Bromocresol purple	16.27	100	30	0	0
Lactoflavin	4.43	75	100	0	0

pair of dyes, CI 15585 and CI 45350. The former is detectable at 525 nm where the latter absorbs very weakly. Therefore, when two dyes are not completely resolved, they can be identified and also quantitated by appropriate wavelength selection if they absorb at different wavelengths.

There are, however, some cases where dyes have coincident HPLC peaks and also exhibit very similar absorption spectra, *e.g.*, CI 45370 and CI 45380. The problem can be overcome by means of a numerical method using visible spectra and multiple regression analysis as proposed by Wegener *et al.*[25]. In the example cited, an unresolved HPLC fraction containing either CI 45370 or CI 54380 should be collected and its visible spectrum recorded and compared to the reference spectra for both dyes, recorded under similar conditions.

The applicability of this HPLC method has been demonstrated on two commercial lipstick samples of unknown composition, I and II. Fig. 2A shows the chromatogram, recorded at 475 nm obtained by injecting an extract from sample I. Similar chromatograms were obtained at 400 and 525 nm, while at 600 nm there was no appreciable absorbance. The results indicate the presence of five dyes, all fully iden-

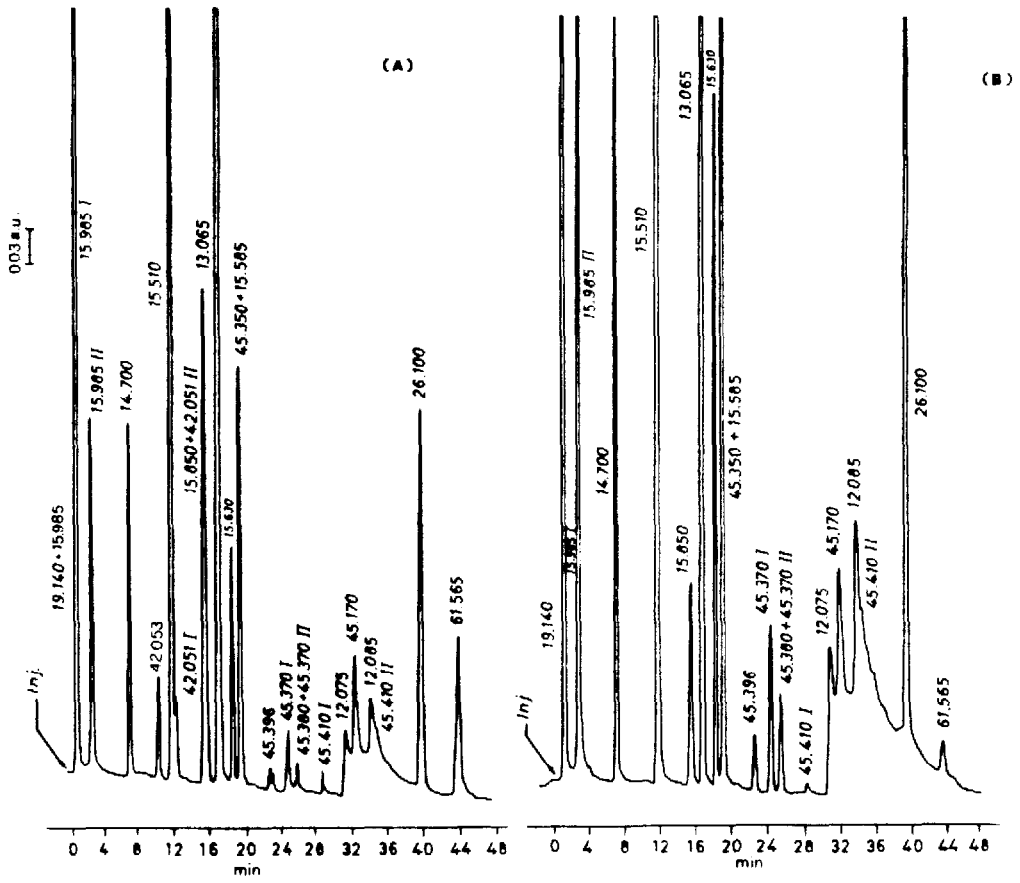

Fig. 1.

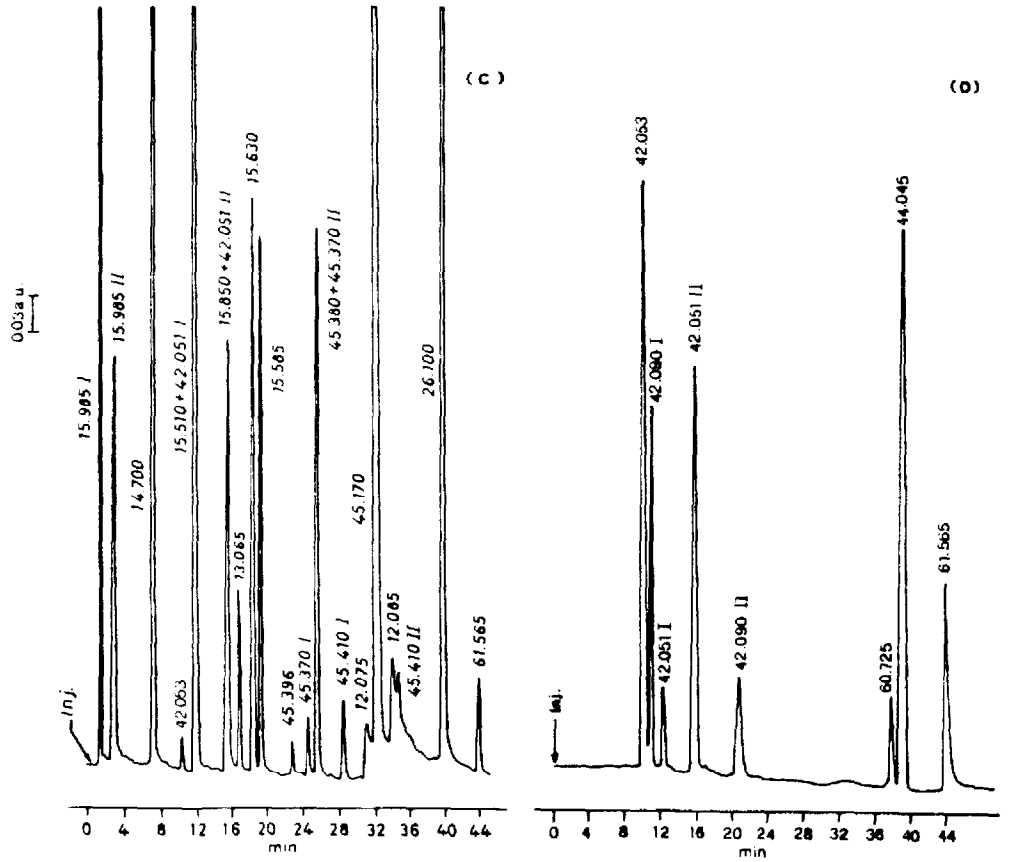

Fig. 1. Typical chromatograms of a standard mixture of 20 dyes (numbered as in Table I). Detection at 400 (A), 475 (B), 500 (C), and 600 nm (D), respectively. Chromatographic conditions as in the text.

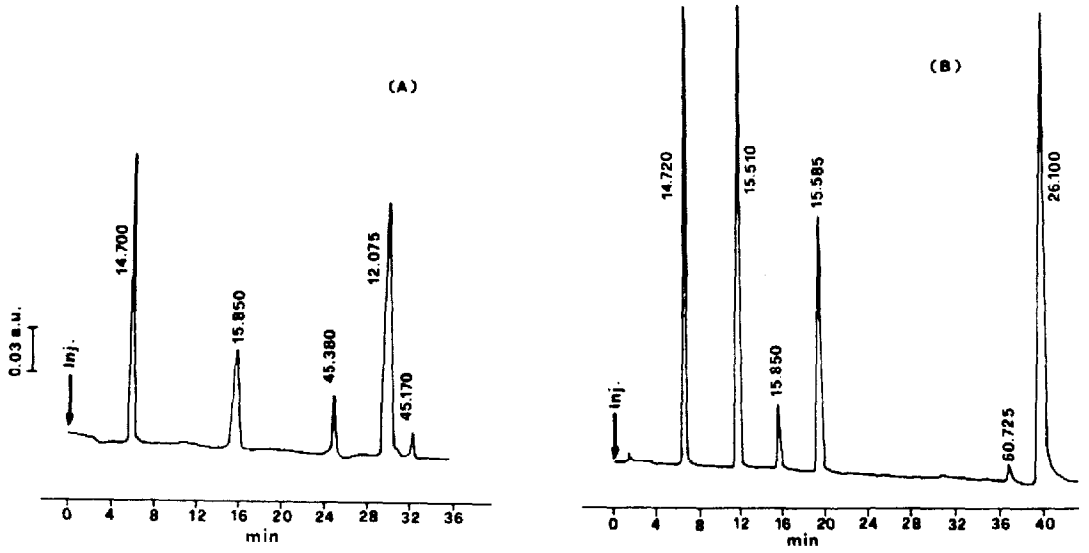

Fig. 2. Chromatograms recorded at 475 nm for an extract from lipstick samples I (A) and II (B), respectively.

tified. Similar successful results were obtained for an extract from sample II, whose chromatogram is shown in Fig. 2B. In this case, it is obvious that at least six dyes must be present, all detectable at the same wavelength.

In conclusion, the described HPLC method enables the identification, by a relatively simple procedure, of 75 representative cosmetic dyes and allows a good separation of at least 50 of them. Its application to the analysis of lipsticks has demonstrated the potential of the method for rapid screening of samples for non-permitted colours. The detection limits are of the order of 20–100 ng of pure dye in an injection volume of 10 μl and they have been estimated for a full scale absorbance.

At present the EEC's legislation on dyes in cosmetics does not generally prescribe the maximum amounts permitted; however, were this to be the case, the proposed method could be also applied to the quantitation of permitted dyes.

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