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### Identification of xanthene dyes in lipsticks by reversed-phase high-performance liquid chromatography

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(Received May 3rd, 1988)

In a previous paper<sup>1</sup> we described a method for the identification of 75 organic colours commonly used in the cosmetic industry, based on ion-pair reversed-phase high-performance liquid chromatography (HPLC), which can be used for the rapid screening of samples for non-permitted colours. The law 713|86 and subsequent adjournments which comprise the Italian legislation on cosmetics in response to the Directive 76|768 of the European Economic Community has stimulated us to study other methods of identification and quantitation of cosmetic dyes.

In particular, we have considered the series of acidic xanthene dyes. These compounds are widely employed in lipsticks in the form of aluminium lacquers or free acids, practically insoluble in the matrix used<sup>2</sup> and their analysis is generally performed by chromatographic techniques<sup>3–8</sup>. This paper reports the results of an analysis performed on 99 samples of commercial lipsticks. Since this kind of cosmetic product usually contains very complex mixtures of dyes, the samples were subjected to a preliminary extraction to isolate the class of xanthene dyes from the other colouring agents. The presence of this class of colourants in the extracts was tested by thin-layer chromatography (TLC) before performing the separation and identification of the dyes selected by reversed-phase HPLC. In particular, the dyes CI 45350 for which the law 713|86 establishes a maximum limit of 6% in the finished product and CI 45396 (maximum limit 1% as the free acid) were quantitated in order to test the compliance of the cosmetic product with the EEC legislation.

## EXPERIMENTAL

### Materials

All dyes, referred to by their CI reference numbers<sup>1</sup>, were commercial samples used as received. Standard solutions of individual dyes and mixtures were prepared in methanol. For CI 45396 and CI 45350, serial dilutions were made to obtain a set of solutions with concentrations ranging from 0.01 to 0.8 mg/ml. The samples of

lipsticks were freely available. All chemicals used were of analytical grade (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  $\mu\text{m}$ , and vacuum degassed by sonication before use.

#### *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.) was used. The analytical column was a 5- $\mu\text{m}$  Altex C<sub>18</sub> Ultrasphere (150 mm  $\times$  46 mm I.D., Beckman).

#### *Chromatographic conditions*

The HPLC conditions were as follows: mobile phase, acetonitrile-water (pH 3 adjusted with glacial acetic acid) with a linear gradient from 30 to 35% acetonitrile in 5 min and 75% acetonitrile in 30 min, and finally a purge with 100% acetonitrile for 12 min; flow-rate, 2.5 ml/min; column temperature, 25°C; injection volume, 10  $\mu\text{l}$ ; detection wavelengths, 400, 475 and 525 nm; detector sensitivity, 0.64 a.u.f.s.; chart speed, 0.5 cm/min.

#### *Sample treatment*

According to Lehmann's extraction<sup>9</sup>, a 500-mg amount of lipstick was dissolved in 10 ml of a solution of orthophosphoric acid (5%, v/v) in dimethylformamide (DMF) by heating in a water-bath. After cooling, the mixture was filtered through cotton-wool into a separatory funnel and 5 ml of water and 5 ml of hexane were added. The organic phase, if coloured, was kept for the analysis of lipophilic dyes, whereas the aqueous phase was extracted with dichloromethane. The organic phase, containing xanthene dyes, was concentrated until a detectable absorbance was obtained. If that did not occur the initial amount of lipstick was doubled. An alternative extraction was the one proposed by Etournaud and Aubort<sup>2</sup>, by which all acidic dyes, including xanthene dyes, were extracted into the ammonia-methanol fraction.

## RESULTS AND DISCUSSION

The chromatographic properties of the xanthene dyes examined and their relative absorptions at the three detection wavelengths are reported 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 most of the individual dye samples (not shown) clearly demonstrated the presence of coloured impurities whose nature was not investigated. In such cases we have put the symbols (I), (II), etc., next to the CI number for labelling the impurities. The separation obtained for a standard mixture of the seven xanthene dyes is illustrated in Fig. 1.

The extraction of the dyes from lipsticks has been performed both with the technique proposed by Etournaud and Aubort<sup>2</sup> and with that proposed by Lehmann<sup>9</sup>. Both techniques can be utilized since they are equally effective in extracting

TABLE I

RETENTION TIMES AND RELATIVE ABSORPTIONS,  $A_{\lambda}$ , OF THE XANTHENE DYES AT THE THREE DETECTION WAVELENGTHS

Colour index No.	Retention time (min)	$A_{400}$	$A_{475}$	$A_{525}$
45350	6.2	15	100	5
45396	8.4	15	100	5
45370 (I)	9.4			
45425	10.3	45	60	100
45380 (I)	11.4			
45370	11.8	5	60	100
45430 (I)	12.0			
45425 (I)	13.0			
45380	13.1	5	15	100
45430 (II)	13.8			
45430	14.4	15	30	100
45425 (II)	18.2			
45410	23.6	5	20	100

xanthene dyes from the cosmetic samples. The applicability of this HPLC method has been demonstrated on commercial lipstick samples of unknown composition. Fig. 2 gives a typical chromatogram of a lipstick extract, recorded at 400, 475 and 525 nm. Two xanthene dyes must be present: CI 45410 and CI 45380. The minor peaks at about 11 and 14 min were assigned to impurities of CI 45380.

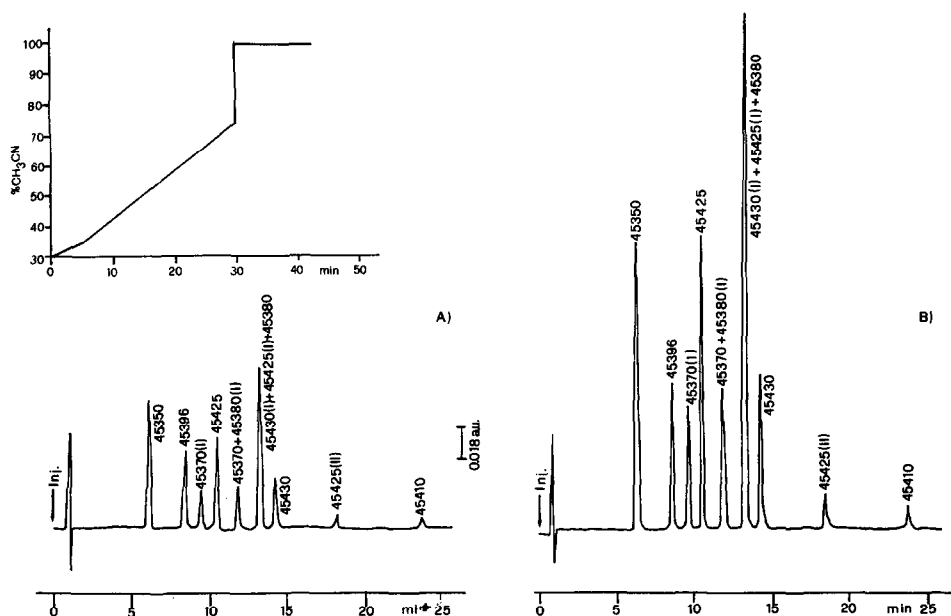


Fig. 1.

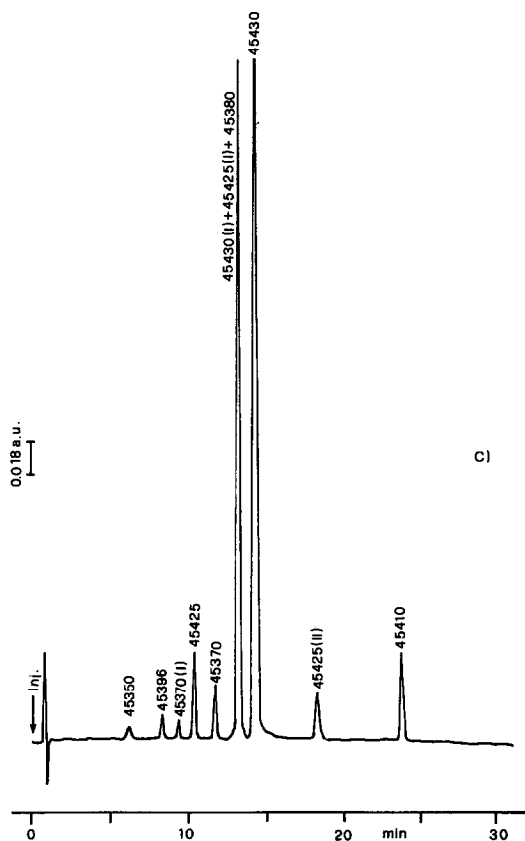


Fig. 1. Typical chromatogram of a standard mixture of the xanthene dyes. Detection at 400 (A), 475 (B) and 525 nm (C), respectively. In the upper part of (A) is reported the solvent programme for the chromatographic separation.

Ninety-nine samples of commercial lipsticks have been analyzed for the presence of the xanthene dyes and the results obtained are reported in Table II. A quantitation has been performed for the dyes CI 45430 and 45396. Standard solutions

TABLE II

FREQUENCY OF OCCURRENCE OF THE XANTHENE DYES IN THE 99 SAMPLES OF LIPSTICKS ANALYZED

<i>Colour index No.</i>	<i>Name</i>	<i>Frequency of occurrence</i>
45430	Erythrosin	21
45380	Eosin S-13	28
45410	Phloxin B	9
45370	Eosin S-10	23
45396	Dinitrofluorescein	1
45350	Fluorescein	0
45425	Diiodofluorescein	0

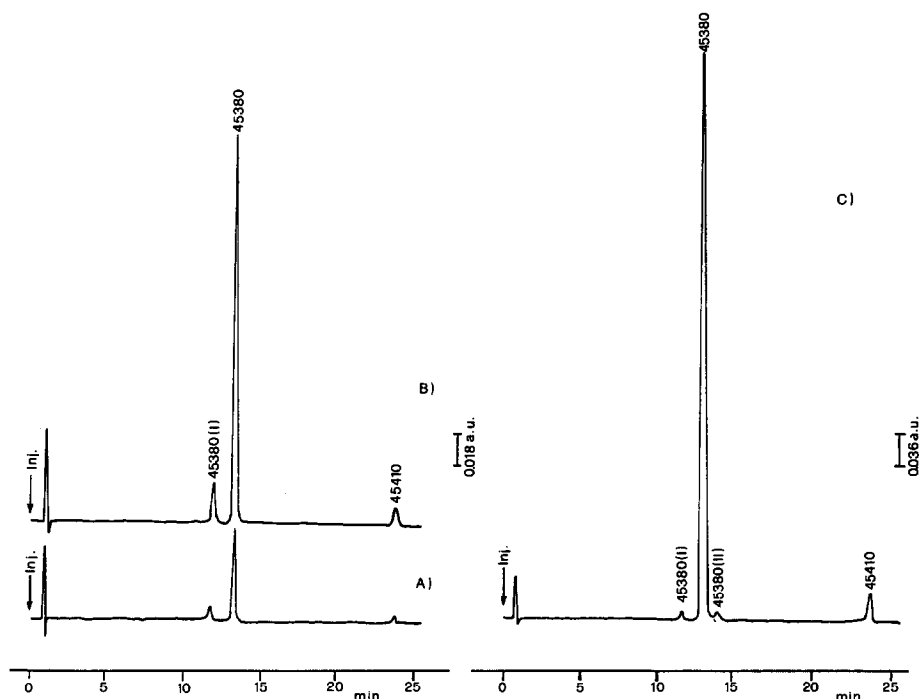


Fig. 2. Chromatogram of a lipstick extract recorded at 400 (A), 475 (B) and 525 nm (C).

were prepared by dissolving known amounts of the two standards in methanol and the solutions were processed using the HPLC conditions described above. Calibration graphs were constructed by use of the peak areas measured for the various amounts injected and were linear over the ranges of concentrations used, with regression coefficients of 0.9998. Their slopes were used in the quantitation of the two dyes in commercial lipsticks. No cosmetic sample contained the dye CI 45430 and only one contained the dye CI 45396 at a level lower than the limit imposed by the law 713 | 86.

In conclusion, the HPLC method described enables the identification, by a relatively simple procedure, of the xanthene dyes and can be used for determining those subject to legislation as well as the impurities associated with them.

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