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Note

Determination of synthetic organic colours in lipsticks by thin-layer and high-performance liquid chromatography

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Rapid and more quantitative methods were needed for the identification and determination of synthetic colour additives in lip cosmetics. Because lipsticks usually contain a more complex mixture of colours than other cosmetics, the separation and identification of colours has been the subject of much methodological development work. The possibilities of using different thin-layer chromatographic (TLC) methods have been investigated[1–4], and some methods have been developed using spectrophotometry[1] and high-performance liquid chromatography (HPLC)[5,6]. The problematic extraction of colouring components from lipsticks has been avoided by direct application on the TLC plate[1,4].

This paper reports a new quantitative method for the direct separation and determination of organic colours in lip cosmetics by TLC and HPLC methods instead of time-consuming spectrophotometric methods. After direct application, the colours of a lipstick were separated on a TLC plate with two chromatographic solvents. Individual bands of colours were identified, scraped from the plate, dissolved and determined by HPLC.

EXPERIMENTAL

Reagents and materials

The colours were obtained from D. F. Anstead, Billericay, U.K. Standard solutions of D&C Orange 17 and D&C Red 36 (30 mg/1000 ml) were prepared in chloroform, and standard solutions of the other colours (100 mg/1000 ml) were prepared in methanol–acetic acid–tetrabutylammonium hydroxide solution (mobile phase B). The samples, eighteen lipsticks, five lip glosses and one lip cream, were obtained from perfume shops and consisted of the most commonly used lip cosmetics in 1980 in Finland, representing ten manufacturers in six European countries.

Commonly available solvents and other chemical substances, unless otherwise stated, were of analytical grade. Methanol and water for the HPLC mobile phases were of HPLC grade.

TLC conditions

Silica gel 60 plates, 0.25 mm, were obtained from E. Merck, Darmstadt, F.R.G. The TLC solvents used were (a) dichloromethane, and (b) a mixture of 15 ml of ethyl

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acetate, 3 ml of methanol and 3 ml of ammonium hydroxide–water (3:7). The latter must be freshly prepared just prior to use[1].

HPLC conditions

The liquid chromatograph used was a Waters Model 6000 A Solvent delivery system with a U6K injector. The instrument was operated isocratically and was equipped with an absorbance detector Model M 440 with fixed-wavelength detectors at 405 and 546 nm. A Waters μBondapak C_{18} column (30 cm × 3.9 mm I.D.) and a Waters guard column (5 cm × 4 mm I.D., dry packed with Bondapak C_{18}, Corasil, 37–50 μm) were used. The mobile phases used were (A) methanol–water–acetic acid (89:10:1), and (B) methanol–acetic acid–0.01 *M* tetrabutylammonium hydroxide titrant pH = 3.5, adjusted with phosphoric acid (Eastman Kodak, Rochester, NY, U.S.A.) (70:1:29). All mobile phases were filtered through a 0.45-μm Millipore filter. The flow-rate was 2 ml/min and the recorder was set for a chart speed of 1 cm/min. The injection volume was 25 μl.

Sample application

The shiny surface from the rounded end of the lipstick was removed with tissue and the lipstick was weighed. A TLC plate was warmed in an oven at 100°C for 5 min[1], and *ca.* 10–20 mg of the lipstick were applied directly to the plate with one or two overlapping streaks *ca.* 2 cm from the bottom of the plate. The lipstick was then reweighed.

Thin-layer chromatography

The plate was developed in two separate steps. Oil-soluble, unsulphonated colours (D&C Orange 17 and D&C Red 36) were separated using dichloromethane.

TABLE I

TLC OF ORGANIC COLOURS IN LIP COSMETICS

Type of colour	*Colour index no.*	*Name*	*Colour of spots*	R_F	*Frequency of occurrence in the lipsticks*
Oil-soluble unsulphonated colours	12085	D&C Red 36	Orange	0.9	2
	12075	D&C Orange 17	Orange	0.8	6
Other colours	45430	FD&C Red 3	Pink fluor*	0.25	7
	45380:2	D&C Red 21	Pink fluor*	0.22	3
	45370:1	D&C Orange 5	Orange fluor*	0.14	3
			Red fluor*	0.22	
			Red fluor*	0.25	
	45170	D&C Red 19	Pink fluor*	0.57	3
	15850:1	D&C Red 7 (Ca)	Red	0.24	15
	15510	D&C Orange 4	Orange	0.35	1
	15585:1	D&C Red 9 (Ba)	Orange	0.41	14
	42640	FD&C Violet 1	Blue	0.19	1
	69800	D&C Blue 9	Pink (weak)	0.29	1

* Fluorescence under UV light (254 nm).

The plate was developed two to four times. The bands below the waxes and oils were identified and scraped from the plate, dissolved in dichloromethane, centrifuged and filtered.

Other colours remaining at the baseline were developed with the TLC solvent b. The colours were identified tentatively on the plates by their R_F values, colours and UV absorbance (see Table I). The bands and the baseline were scraped from the plate, dissolved in the HPLC mobile phase B, centrifuged and filtered. All extractions were repeated four times with small volumes of the solvent. The volumes of the solvents were measured.

High-performance liquid chromatography

The oil-soluble colours were chromatographed with the HPLC mobile phase A and detected at 405 nm (Fig. 1), and the other colours with the HPLC mobile phase B and detected either at 405 nm or at 546 nm (Figs. 2 and 3).

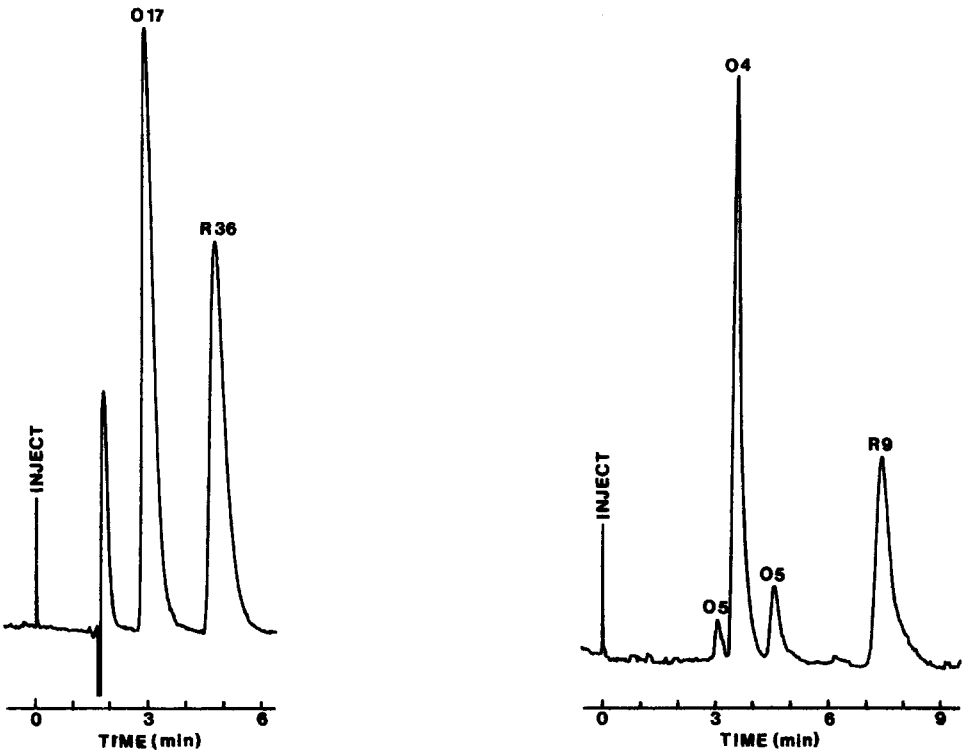

Fig. 1. HPLC separation of D&C Orange 17 (O 17) and D&C Red 36 (R 36). Column, 30 cm × 3.9 mm I.D. μBondapak C_{18} (10 μm) attached to a guard column (5 cm × 4 mm I.D.) dry packed with 37–50 μm Bondapak C_{18} Corasil; eluent, methanol–water–acetic acid (89:10:1); flow-rate, 2.0 ml/min; injection volume, 25 μl (representing 380 ng of each colour); detector, UV at 405 nm; detector sensitivity, 0.02 a.u.f.s.

Fig. 2. HPLC separation of D&C Orange 4 (O 4), D&C Orange 5 (O 5) and D&C Red 9 (R 9). Column: see Fig. 1; eluent, methanol–0.01 *M* tetrabutylammonium hydroxide titrant (pH = 3.5)–acetic acid (70:29:1); flow-rate, 2.0 ml/min; injection volume, 25 μl (representing 830 ng of each colour); detector, UV at 405 nm; detector sensitivity, 0.02 a.u.f.s.

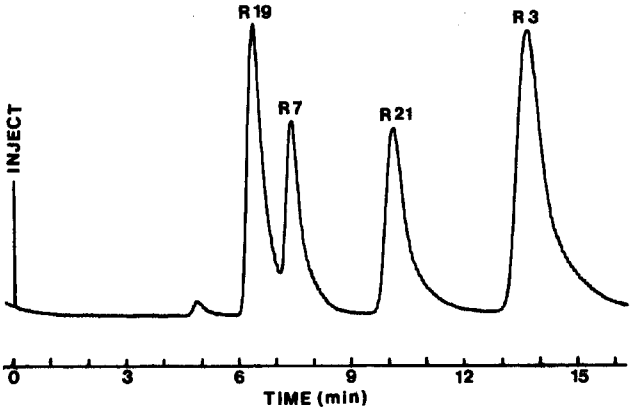

Fig. 3. HPLC separation of D&C Red 19 (R 19), D&C Red 7 (R 7), D&C Red 21 (R 21)and FD&C Red 3 (R 3). Column: see Fig. 1; eluent, methanol–0.01 *M* tetrabutylammonium hydroxide titrant (pH = 3.5)–acetic acid (70:29:1); flow-rate, 2.0 ml/min; injection volume, 25 μl. (representing 620 ng of each colour); detector, UV at 546 nm; detector sensitivity, 0.1 a.u.f.s.

RESULTS AND DISCUSSION

Even though lipsticks are used on the mouth, they usually contain much higher amounts of colouring components than foods. A typical lipstick contains *ca.* 2% of organic pigment and 10% of white and pearl pigments. If the use of a lipstick is *ca.* 40–120 mg/day, the daily intakes of organic pigments and white and pearl pigments are 0.8–2.4 mg and 4–12 mg, respectively[7]. The colour additives permitted for use in lip cosmetics are controlled by the Food and Drug Administration in the U.S.A. and by the EEC cosmetic directive in Europe[8].

The R_F values of the colours are given in Table I. By combining the results from TLC and HPLC it was possible to determine the organic colours in lip cosmetics. Some of the colours had identical R_F values, but they could be identified with the aid of their retention times in HPLC. Two colours, D&C Blue 9 and FD&C Violet 1, were analysed only by TLC. It was noticed that a small amount of undissolved colour remained at the baseline. To ascertain that none of this colour was of organic origin, the baseline was also scraped off. A correlation was observed between a brown-red baseline and the content of iron, analysed in a separate study using atomic absorption.

The proposed procedure is based on the analyses of 24 commercial lip cosmetic samples of unknown composition. Table II shows the results of these analyses. No lipsticks of known composition were available and therefre recovery data could not be obtained. However, recoveries were determined on three mixtures of colours simulating mixtures that might be found in lipsticks. It was noticed that two oil-soluble unsulphonated colours gave semiquantitative results. Small amounts of the most common organic colours were added to a lip gloss known not to contain any organic colour and the recoveries were determined (see Table III). The average recovery of four colours was 83%.

TABLE II

ANALYSES OF LIP COSMETICS FOR THEIR COLOUR COMPOSITION

*Sample No.**	*Organic colour*	*Content (%)*	*Sample No.**	*Organic colour*	*Content (%)*
1	D&C Red 9	<0.1	18	D&C Red 36**	1.7
2	D&C Red 9	0.1		FD&C Red 3	0.1
3	D&C Red 7	0.1	19	D&C Red 9	1.9
4	D&C Red 7	0.3		D&C Red 7	0.7
	D&C Orange 17**	0.4		D&C Blue 9***	
5	D&C Red 7	0.1	20	D&C Orange 17**	2.3
	D&C Red 9	0.2		D&C Red 7	1.5
6	D&C Red 7	<0.1		D&C Red 9	3.9
	D&C Red 9	<0.1		D&C Red 19	0.2
	D&C Orange 5	0.1	21	FD&C Red 3	0.2
	D&C Orange 17**	0.2		D&C Red 7	0.5
7	D&C Red 9	0.2		D&C Orange 5	1.8
8	D&C Red 7	1.8		D&C Orange 17**	0.6
9	D&C Red 7	3.2	22	D&C Red 7	0.5
10	D&C Red 9	0.7		D&C Red 36**	0.1
11	FD&C Red 3	0.1		D&C Red 21	<0.1
12	D&C Orange 4	0.4		D&C Orange 17**	0.1
	D&C Red 9	9.1	23	FD&C Red 3	<0.1
13	FD&C Red 3	0.3		D&C Red 7	0.1
	D&C Red 9	1.0		D&C Red 9	0.3
14	FD&C Red 3	<0.1		D&C Red 19	<0.1
	D&C Red 21	0.1		D&C Red 21	0.2
15	D&C Red 7	1.4	24	FD&C Violet 1***	
	D&C Red 9	5.7		FD&C Red 3	0.1
16	D&C Red 7	0.4		D&C Orange 5	0.8
	D&C Red 9	0.3		D&C Orange 17**	1.1
17	D&C Red 7	0.7		D&C Red 7	1.5
	D&C Red 9	2.4		D&C Red 9	0.5
				D&C Red 19	0.8

* Sample 1, lip cream; samples 2–6, lip glosses; samples 7–24, lipsticks.
** Analysed semiquantitatively.
*** Analysed only by TLC.

TABLE III

RECOVERIES OF ADDED COLOURS FROM A LIP GLOSS

Colour	*Number of analyses*	*Recovery (%)*	
		Average	*Limits*
D&C Red 7	11	73	(53–83)
D&C Red 9	11	85	(77–94)
FD&C Red 3	9	85	(76–97)
D&C Orange 5	11	87	(69–102)

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